

Settlement *Alphitobius diaperinus* Panzer, 1779 (Coleoptera: Tenebrionidae) on Different Diets in the Laboratory

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Abstract *Alphitobius diaperinus* is an insect from the order Coleoptera, commonly known as the lesser mealworm, which has adapted well to poultry-sheds due to the availability of food and level of humidity they offer. These factors increase its reproductive capacity, causing significant losses for poultry farmers. The present study was carried out with the intention of establishing a protocol for settlement *A. diaperinus* in large numbers in the laboratory, with a view to supplying the insect for future research into their prospective use as the object of biological control strategies. For this experiment two different types of diet were used: diet 1 (rabbit-feed) and diet 2 (chicken-feed), both autoclaved. After sexing, ten repetitions with five couples were isolated in a transparent plastic receptacle with lid (capacity 500 mL) for each diet tested. The receptacles were kept in a room at temperature of (28±2)°C, humidity of (80±10)% and light-phase of 14 hours, and were observed daily. All the data generated underwent statistical analysis, using Kruskal-Wallis analysis of variance in the Sigma Stat program.

Keywords Lesser mealworm, Poultry farming, Insect pest

Background

The lesser mealworm, *Alphitobius diaperinus* (Panzer, 1779) is a cosmopolite species, originally from Africa (Vaughan et al., 1984), commonly found infesting residues of damp stored products, which probably migrated to poultry-farms in foodstuffs or from neighboring farms where food was stored (Wallace et al., 1985). This insect feeds on excrement, animal feed and the viscera of dead birds (Chernaki and Almeida, 2001).

In a poultry farm, the lesser mealworm population presents high heterogeneity. The larvae in the last stages of the lifecycle, pupae and adults all live in the ground, at a depth of about 10 cm, preferably under feeding troughs, where the substrate is dense, compacted and low in humidity. In low temperatures and/or stressful situations these insects do not present diapause, like other insects, instead migrating vertically downwards into the ground (Salin et al., 2000).

The mealworm's biological cycle is directly related to temperature. A temperature of 30°C is considered the most suitable for development of immature phases, with high survival rates. Low temperatures (below 16.5°C) can contribute efficiently to controlling these insects, since development of immature phases does not then occur, reducing the population (Chernaki and Almeida, 2001).

High populations of mealworm are one of the main problems in Brazil's poultry-farming industry, because in direct contact with poultry litter it is a vehicle for various pathogens, such as enterobacteria (Chernaki-Leffer et al., 2002), Eimeria protozoa (Goodwin and Waltman, 1996) and fungi (De Las Casas et al., 1968). The insect has also been noted as a potential carrier of avian viruses, such as Newcastle Disease and Gumboro Disease (McAllister et al., 1995), which cause immuno-suppression in poultry and can lead to their death.

Mealworms not only transmit diseases to poultry, but also damage farm installations, destroying thermal insulation in sheds in cold climates. This type of damage is caused by the action of larvae (Vaughan et al., 1984; Despins et al., 1987).

Another factor that harms poultry is that they stop eating balanced feed and eat larvae and adults of the lesser mealworm, particularly younger birds, which tend to eat anything that moves. Chernaki-Leffer et al., (2001) evaluated feeding behavior and growth in broiler chickens fed on lesser mealworm larvae. The mean body weight difference of larvae-fed chickens was significantly lower than that of birds that only received feed. Furthermore, they showed signs of stress when fed with larvae, presenting runny feces and larval cuticle.

Control of this pest is difficult and its natural enemies are little known. No safe and efficient method of control has been found so far. Chemical insecticides used in control are difficult to apply, due to the nature of the environments inhabited by the insect, such as earth, litter containing a high quantity of organic matter (excreta, feed, sawdust, grass), and the crevices and gaps in sheds. Another disadvantage is that chemical insecticides leave residues in the carcass of birds, creating a barrier to export trade and endangering the health of those who apply the insecticide (Japp et al., 2010).

This work aimed to establish a protocol for settlement *A. diaperinus* in large numbers in the laboratory, with a view to supplying the insect for future research into its use as the object of prospective biological control strategies.

1 Results and Discussion

All the pupae selected and used in the assay generated adults, with no mortality observed. Adults started to emerge three days after pupae were collected (Figure 1). It was noted that the number of females emerging was higher than the number of males on the third day; that the number of males and females that emerged on the fourth day was similar; and that on the fifth day

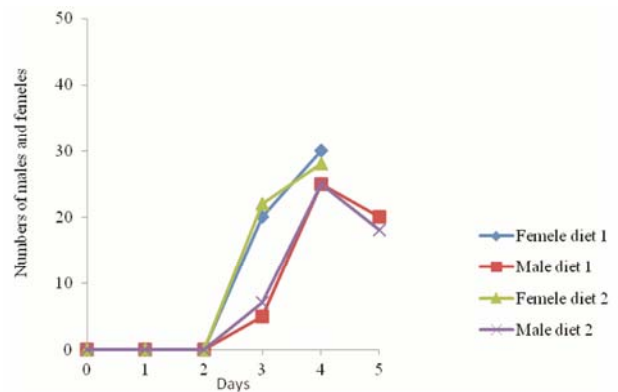


Figure 1 Emergence from pupae in adults

only males emerged. These data, however, differed statistically in the two diets used ($p=0.986$). According to data in the literature, the mean time for adult emergence is four to seven days.

Mating started to occur when the adults raised on the two diets were 14 days old, and all were mating on the fifteenth day. Two days after mating started, the first eggs were found in the grooves of the corrugated paper, which initially presented a spherical shape, changing to elliptical after two days. The presence of eggs was observed in other parts of the receptacles, but for this study only the eggs found in the corrugated paper were analyzed.

The mean number of eggs collected was 106.4 in each collection for the rabbit-feed and 118.8 eggs in each collection for the chicken-feed (Figure 2). A peak in egg-laying was observed at the first collection carried out for the two diets. The number of eggs produced

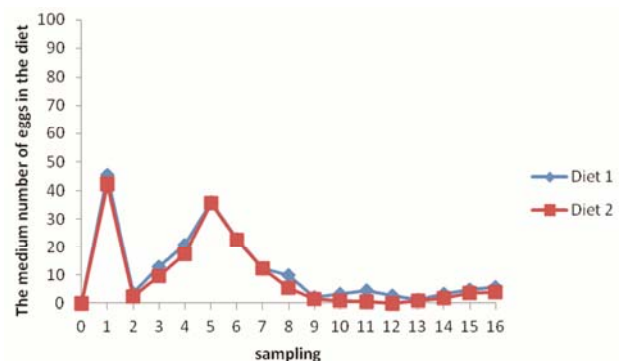


Figure 2 Percentage of eggs of *A. diaperinus* produced in the diets 1 and 2

fell appreciably on the second evaluation, increasing gradually until the fourth collection and falling slightly until the ninth collection, when it stabilized at a low quantity. This part of the assay finished at the sixteenth collection.

There was no statistically significant difference between the number of eggs obtained in the two diets ($p=0.092$).

The emergence of larvae took place on average three days after egg-laying in the two diets. The total number of larvae obtained was 1 068 on rabbit-feed (diet 2) and 1131 on chicken-feed (diet 1), with 81% and 72%, respectively, hatched. There was a statistically significant difference ($p=0.009$) in emergence of larvae between the two diets.

The highest mortality was seen between the first and second stage, and was 174 larvae for diet 1 and 46 larvae for diet 2. From the second to fifth stage mortality was lower, with 18 larvae from second to third stage, 12 from third to fourth, 7 from fourth to fifth for diet 1, while for diet 2 mortality was 5 larvae from the second to fifth stage, larvae from second to third stage, 3 from third to fourth, 1 from fourth to fifth. From the fifth stage onwards, there was no mortality (Figure 3).

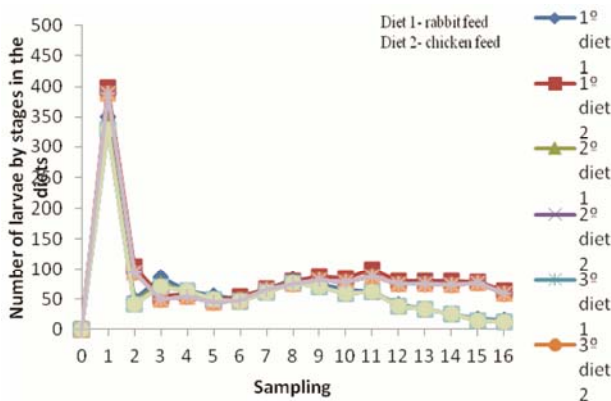


Figure 3 Total number of larvae in each stage during evaluations

It was observed that the total larval period was 35 days for the two diets, and eight larval stages were seen. The first to fifth stages lasted on average three

days, and the sixth to eighth lasted from five to seven days on both diets, with a statistically significance difference between the two diets ($p<0.001$).

Under the conditions offered in the room, with constant temperature and humidity and abundant food, the larval cycle lasted 35 days with 8 stages. Duration of larval stages depends on temperature, varying from 35 to 70 days with up to 13 stages.

In the larval phase, from the seventh stage onwards, pieces of card were placed in boxes for the larvae to pupate. Pupae presented whitish coloring and their shape resembled that of the adult. The larvae reached their last stage and entered the grooves, where light was low and there was more safety, since pupae, unlike larvae, cannot move to avoid predation.

The adults emerged with white coloring and without chitinization. It was only after the fourth day that the process of chitinization was observed, and around the 20th day the adult reached the reproductive phase. The insect's reproductive cycle was approximately 45 days.

The pupal period lasted on average 4 to 5 days for diets 1 and 2. Low mortality was observed for insects at this stage in both diets, with 12 pupas for diet 1 and 26 pupas for diet 2, thus differing statistically ($p=0.009$) (Figure 4).

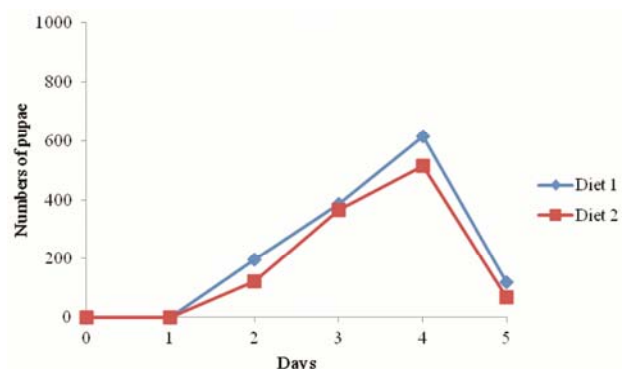


Figure 4 Time development of the pupal stage

To evaluate the period before F1 laid eggs, five couples and ten repetitions were separated, obtaining a mean of 15 days for both diets.

The biological cycle of *A. diaperinus* that corresponds to the period of sexual maturity until reaching adulthood was 55 days (Table 1).

Table 1 Biological cycle of *A. diaperinus*

| Development phases | Duration of each phase |
|------------------------------|------------------------|
| Sexual maturity | |
| Before egg-laying | |
| Eggs | |
| Larvae | |
| Pupae | |
| Adults without chitinization | |

The differences cited above may be due to stress resulting from manipulation at all stages. Adult coleoptera may live for more than a year in poultry litter, which makes control difficult: Using efficient chemical products is not advisable, because these would remain in the meat of birds.

The assays did not show significant differences in development of the lesser mealworm in the two diets tested. As this insect is a pest in poultry farms and develops in poultry litter, the chicken-feed diet was chosen. Currently this method of settlement the insect is providing lesser mealworms to carry out studies using entomopathogens and pheromones for their control.

According to data from the literature, the average time for adults to emerge is four to seven days, and no difference is reported between time for males and females to emerge (Chernaki, et al., 2001).

Between the first and second stage the greatest mortality was observed, with 174 larvae dying in diet 1 and 46 in diet 2, corroborating data from Silva et al., (2005).

It was confirmed that that highest mortality of larvae took place in the first 15 days after hatching, and this is an important fact for biological control, also corroborating data from Silva et al. (2005).

In poultry houses the larvae burrowed into the litter, near the floor or in crevices, for the development of

pupae (Paiva, 2000).

In work carried out previously, by Dass et al., 1984 and Silva et al., 2005, the period of incubation took place in temperatures of 25°C, 28°C and 31°C, and it was noted that at 28°C the incubation period was 3 days. At 25°C the incubation period varied, and the first larvae emerged after four to seven days, while at 31°C this took over seven days.

Rodrigueiro (2008) was not successful in settlement the insects in the laboratory; that work used plastic receptacles of 3 liters containing sawdust and chicken-feed in the proportion of 1:2. This medium was moistened three times a week with water sprayed on the surface. The plastic boxes had lids with a central opening, covered in organza, which allowed air to enter, and were maintained in a climatized chamber, with controlled temperature (26±1) °C, relative humidity (60±1)% and photoperiod (12L:12D). There was an attempt to obtain adults (F1) in the laboratory, but it was not possible, as not enough adults were obtained to carry out bioassays, due to high mortality throughout development and the variation in the emergence time of individuals.

According to Silva et al. (2005), for insects maintained at (25±2)°C, the larval period was 67 days, and it was observed that the larvae needed high humidity for development. In the larval phase, from the seventh stage onwards, pieces of cardboard were also used for larvae to pupate in the grooves.

2 Conclusion

Alphitobius diaperinus leads to sanitary problems in poultry farms and interferes in broiler chicken development, causing serious financial losses. This work made it possible to know more about the lesser mealworm's evolutionary cycle, and the establishment of a colony in the laboratory will allow tests to be implemented to determine strategies for the control of this pest.

3 Material and Methods

3.1 Collection of insects

To collect and maintain a colony of the insects, a request was made to IBAMA for authorization of activities for scientific purposes, which was granted to

our research group on September 9th, 2010, under number 25038-1.

The insects were collected together with chicken litter and feed from sheds at Pito Aceso Farm, in Brazil's Federal District, and sent in hermetically sealed plastic bags to the Entomopathogenic Bacteria Laboratory at Embrapa Recursos Genéticos e Biotecnologia. There, material underwent triage, separating adult insects from larvae and removing all litter that came from the farm to prevent contamination of the insect colony.

After triage, the insects were put in plastic boxes measuring 41 cm × 23 cm × 13.5 cm with voile covering them, and were then fed on commercial rabbit-feed. Autoclaved sawdust was added to the diet to maintain humidity. The insects were kept in a room at temperature of (28±2)°C, humidity of (80±10)% and light-phase of 14 hours.

These boxes were observed daily and moistened with water; all phases of the insect's biological cycle were observed. For egg-laying, pieces of corrugated cardboard with grooves were put in place. This was necessary because the boxes had no grooves, and the corrugated card simulated the gaps found in the floor and structures of poultry sheds, so that the insect would lay eggs as usual.

After three days of incubation at controlled temperature, the larvae hatched. The corrugated card was changed every 48h and stored in boxes separated according to larval stage.

3.2 Evaluation of *A. diaperinus* development on different diets

For this experiment two different diets were used: Diet 1 (commercial Nutrina rabbit-feed triturated in an electrical triturator, brand Arbel) and diet 2 (chicken-feed produced by Asa Alimentos), both autoclaved.

The insects were separated at pupa phase and sexed, using a magnifying glass to see if a genital appendix was present (females) or absent (males), according to the methodology of Barke and Davis (1967).

After sexing, five couples were isolated in a transparent plastic receptacle with a lid (capacity of 500 mL). For each diet tested, 10 repetitions were

carried out. Within each receptacle 200 g of diet were placed, with a piece of corrugated card the size of the circumference of the receptacle, and a hole in the lid was filled with cotton wool to maintain humidity. The receptacles were kept in a room at temperature of (28±2)°C, humidity of (80±10)% and light-phase of 14 hours. Sexed insects in the pupal phase were observed daily to verify their emergence as adults.

After adult emergence, the receptacles were observed in the early morning and late afternoon, to check the presence of eggs. When eggs were observed, the corrugated card was removed from receptacles to count eggs, with the help of a stereoscopic microscope. The procedure was repeated every 48 hours until the number of eggs was constant. The corrugated card with eggs was placed in other plastic receptacles of 500 mL with 100 g of diet and these were observed daily to watch for larvae hatching. After hatching, the larvae were counted daily and selected by stage, in accordance with the presence of ecdysis. The survival rate and duration of each stage were then determined.

At the end of the larval phase, pieces of corrugated card were again added, so that the larvae could hide in the grooves to form a pupa. Two hundred were randomly selected and of these 50 couples were conditioned in plastic receptacles of 500 mL with 200 g of diet, a piece of corrugated card the size of the circumference of the receptacle, and a hole in the lid filled with cotton wool to maintain humidity. The pupae were observed twice a day (early morning and late afternoon) to check the emergence of adults.

All the data generated were statistically evaluated using Kruskal-Wallis analysis of variance in the Sigma Stat program.

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