Adult longevity and reproductive capacity in *Cochliomyia macellaria* (Diptera: Calliphoridae) reared on an alternative diet

Longevidad del adulto y capacidad reproductiva de *Cochliomyia macellaria* (Diptera: Calliphoridae) criada con una dieta alternativa

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Abstract: The search for alternative diets that are cheap and maintain rearing stock quality is very important. The objective of the present study was to assess the adult longevity and reproductive capacity of *Cochliomyia macellaria* derived from juveniles reared on a chicken gizzard diet compared to those fed beef diet. Couples were formed shortly after adult emergence and distributed in four cages, totaling 40 couples per treatment, and maintained at 30 °C day/28 °C night, 70 ± 10% RH, anda12 hour light period. Chicken gizzards or beef were offered according to the treatment to stimulate oviposition. The mean weight of the egg mass (1.063 g; 1.12 g), mean weight of the egg mass/day (0.0658 g; 0.0698 g), and the mean weight of the egg mass/female/day (0.0118 g; 0.0125 g) did not differ significantly between the chicken gizzard and beef diets, respectively. The mean number of eggs/g (8221.23; 8569.29) and the mean viability of eggs (99%; 94%) did not differ significantly by a Student t-test, (α = 5%) for the chicken gizzard and beef diets, respectively. The Kaplan-Meier non-parametric method and the Weibull parametric regression method found no differences (in days) in the mean total longevity (37 vs. 38) and mean estimated longevity of males (41.08 vs. 40.04) and females (33.79 vs. 36.29) fed chicken gizzards and beef, respectively. The maximum longevity was 74 days for both diets. The chicken gizzard diet is an efficacious and cheap alternative for rearing *C. macellaria* in the laboratory.

Key words: Blowfly. Forensic entomology. Insect rearing. Laboratory biology.

Resumen: La búsqueda de dietas alternativas con bajo costo para mantener la calidad de la cría de insectos en laboratorio es esencial. Este estudio tuvo como objetivo evaluar la longevidad de los adultos y capacidad reproductiva de *Cochliomyia macellaria*, provenientes de formas inmaduras, criadas con dieta de mollejas de pollo en comparación con aquellas alimentadas con carne. Poco después de la aparición de los adultos, se formaron parejas, distribuidas en cuatro jaulas, para un total de 40 pares por tratamiento y se mantuvieron a 30 °C día/28 °C noche, a 70 ± 10% HR y fotoperiodo de 12 h. Fue ofrecida molleja/carne, dependiendo del tratamiento, para estimular la ovoposición. El peso promedio de la masa de huevos (1,063 g; 1,12 g), el peso promedio de la masa de huevos/día (0,0658 g; 0,0698 g) y el peso promedio de la masa de huevos/ hembra/día (0,0118 g; 0,0125 g) no difirieron significativamente entre las dietas de molleja y carne, respectivamente. Igualmente, el número promedio de huevos/g (8221,23; 8569,29) y la media de viabilidad de los huevos (99%; 94%), no difirieron de manera significativa entre las dietas (prueba t de Student α = 5%). Se demostró a través del método no paramétrico Kaplan-Meier y del método paramétrico de regresión de Weibull, que no se presentaron diferencias (en días) entre el tiempo mediano total de longevidad (37 vs. 38), el tiempo promedio estimado para la longevidad de machos (41,08 vs. 40,04) y hembras (33,79 vs. 36,29) alimentados con molleja y carne, respectivamente. El máximo de longevidad fue de 74 días para ambas dietas. Los resultados demostraron que la dieta de mollejas de pollo es una alternativa eficaz y económica de criar *C. macellaria* en laboratorio.

Palabras clave: Mosca barrenadora. Entomología forense. Cría de insectos. Biología de laboratorio.

Introduction

Establishing insect colonies in the laboratory is essential for different entomological studies, making sure to meet the biological aspects of the species while also being economically viable. For species in the Calliphoridae family, several studies have been developed seeking alternative diets that meet these two requirements (Taylor and Mangan 1987; Cunha-Silva and Milward-de-Azevedo 1994; Green *et al.* 2003; Barbosa *et al*. 2004; Silva *et al.* 2008; Mendonça 2009; Pires *et al*. 2009; Ferraz *et al.* 2011).

The natural diets used in there a ring of Calliphoridae in the laboratory include beef, horse meat, fish, sheep meat, and other foods with high protein values (Greenberg and Szyska 1984; Marckenko 1985; Queiroz and Milward-de-Azevedo 1991; Cunha-Silva and Milward-de-Azevedo 1994; Chaudhury *et al.* 2000; Day and Wallman 2006; Barbosa *et al*. 2008). Chicken gizzards have similar nutritional characteristics to beef (Esposito *et al.* 2009), are readily available in the market, cost approximately 40% less than beef, and are easy to handle. This has triggered the interest of the scientific community, as shown by studies on rearing juvenile calliphorids, such as *Chrysomya albiceps* (Wiedemann, 1819) and *Cochliomyia macellaria* (Fabricius, 1775), with promising results in terms of post-embryonic development (Ferraz *et al.* 2012; Silva *et al.* 2012).

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Cochliomyia macellaria is an important species from a medical and veterinary point of view because it can cause secondary myiasis in cows, sheep, horses, dogs, pigs, and chickens (Bermudez *et al.* 2007) and can be associated with human myiasis, making it an agent of a neglected disease (Marquez *et al.* 2007; Ferraz *et al.* 2011). This species can be a vector of *Dermatobia hominis* (Linnaeus Jr., 1781) eggs, which are responsible for cutaneous furuncular myiasis (Guimarães and Papavero 1999; Moya-Borja 2003), and it is also a pathogen carrier in humans (Thyssen *et al.* 2004; Graczyk *et al.* 2005; Ribeiro *et al.* 2011) and animals (Greenberg 1971). This species has also been mentioned in studies on ecological succession in animal carcasses (Batan *et al.* 2007; Gomes *et al.*2009; Biavati *et al.* 2010), and it has been found invading human corpses, allowing it to be used to estimate the post death interval (IPM) (Byrd and Butler 1996; Barreto *et al.*2002; Oliveira-Costa and Mello-Patiu 2004).

Females necrobiontophageous blow flies are anautogenous, *i.e*., they require a protein meal in order to produce eggs. They require regular ingestion of amino acids, vitamin C, and mineral salts for normal ovule production (Zucoloto 2000). Observed feeding habits of adult *C. macellaria* include necrophagy and ingestion of body exudates and urban food residues (Laake *et al.*1936; Ferreira1983; Guimarães and Papavero 1999). They have been observed to feed on flower nectar and attracted to plants that emit odors similar to putrefied meat, like *Aristolochia* sp. and *Iris foetidissima*, Linnaeus (Greenberg 1971; Tompkins and Bird 1988). However, it is the quantity and quality of food consumed in the larval stage that may affect growth and development, resulting in a reproductively competitive adult (Parra *et al.*2009).

Thus the objective of the present study was to compare the reproductive potential and longevity of *C. macellaria* adults from juveniles reared on natural chicken gizzards to those reared on beef, under controlled conditions.

Materials and methods

The experimental part was carried out in the Fly Study Laboratory of the Department of Microbiology and Parasitology at the Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Brazil.To establish a colony, adults were collected in the municipality of Seropédica, Rio de Janeiro (22°45’48”S 43°41’23”W). The trap used followed the model by Mello *et al.* (2007), baited with fresh fish (*Lycengraulis grossidens*) and exposed for about six hours during the day. Adults were identified using the taxonomic key of Mello (2003). The rearing methodology followed the orientation developed by Cunha-e-Silva and Milward-de-Azevedo (1996) and Barbosa *et al.* (2008).

The adults (third-generation) used in the present study came from juveniles reared on two natural diets, chicken gizzards and beef (rump steak). The larvae rearing methodology was described by Silva *et al.* (2012) and Aguiar-Coelho and Milward-de-Azevedo (1996). Shortly after abandoning the diet, the larvae were transferred to test tubes containing sterilized wood chips that served as a pupation substrate, sealed with nylon fabric and fastened with elastic. After emergence, the adults from each diet (chicken gizzard and beef) were sexed and randomly formed couples, which were transferred to1L polyethylene cages with nylon screens on the sides (Barbosa *et al.* 2004). Ten couples were used per cage, with four cages per treatment, totaling 40 couples. The protein source, 30 g chicken gizzard or 30 g beef, according to the treatment, was offered until the fourth day of age to stimulate oogenesis. After the 12th day, to standardize the start of the oviposition phase, the protein source was offered again, kept for a period of 24 hours, and reintroduced every two days until all the females died. A 50% honey and water solution was offered without interruption throughout the experiment. The experiment was carried out in a rearing chamber regulated to 30 °C day/28 °C night, 70 ± 10% RH, and 12 hour light period. The observations were made daily at 09:00 hours until the death of all the adults.

After oviposition, the egg masses were weighed and each egg mass was divided randomly. One part continued in the rearing chamber for a further 12 hours, and, after eclosion, egg viability was observed using a stereoscopic microscope from the eclosion of the larva. The other part of the egg mass was again weighed and taken to the refrigerator (-5 °C) in a Petri dish for later egg counting. A solution of sodium hypochlorite solution (0.5%) diluted in 50% distilled water was used to help separate the egg mass. After removing from the refrigerator, about 3 ml of the solution was placed on the mass and observed under the microscope, where a stiletto was used to loosen the eggs from the mass to be counted.

The reproductive potential results were analyzed by a Student t-test ($P \leq 0.05$). The longevity was described by the Kaplan-Meier non-parametric method and the Weibull parametric regression method. A complete randomized experimental design was used.

Results and discussion

The mean weight of the *C. macellaria* eggs from the females fed chicken gizzards was 1.06 g and 1.12 g for those fed beef (Table 1), and there was no significant difference ($P = 0.774$). Cunha-e-Silva and Milward-de-Azevedo (1996) worked with groups of 20 and 40 couples per replication fed with horsemeat and obtained a mean of 1.11 g and 1.68 g of eggs, respectively, suggesting that there was not necessarily a tendency for exponential growth in egg production with increasing couples. Paes *et al.* (2005) analyzed the reproductive performance of *Lucilia cuprina* (Wiedemann,

Table 1. Reproductive capacity of *Cochliomyia macellaria* females, reared on chicken gizzard and beef diets under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period).

Diet	Mean of egg mass (g)	Egg mass (g)/Day	Egg mass (g)/female/day	Number of egg masses/g
		X ± dp	X ± dp	X ± dp
Gizzard	1.06[a]	0.0658 ± 0.0038[a]	0.0118 ± 0.0025[a]	8221.23 ± 318.45[a]
Beef	1.12[a]	0.0698 ± 0.0073[a]	0.0125 ± 0.0021[a]	8569.29 ± 494.58[a]

X = mean, dp = standard deviation; values followed by the same letter in the same column do not differ significantly by the t-test at a level of 5%.

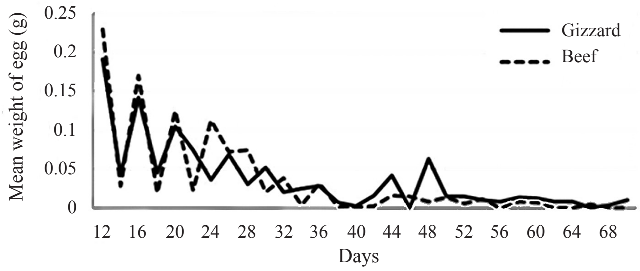

Figure 1. Oviposition rhythm of *Cochliomyia macellaria* reared on chicken gizzard and beef diets under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period).

1830) fed horsemeat and observed that females of isolated couples laid more eggs than grouped females.

There was no significant difference in the mean weight of eggs laid per day (P = 0.02) between the chicken gizzard and beef treatments (0.0658 g and 0.0698 g, respectively), the same was true for the mean daily weight of eggs/female, 0.0118 g and 0.0125, respectively (P = 0.9582) (Table 1). According to Hall (1948), *C. macellaria* lays 49-250 eggs/female, while Greenberg and Skyska (1984) observed 75-150 eggs/female using fish to stimulate oviposition, but these authors did not record the oviposition weights. Cunha-e-Silva and Milward-de-Azevedo (1996) used horsemeat to stimulate oviposition and observed that the egg mass/female varied from 0.017 to 0.027 g, corroborating the data of the present study.

The mean numbers of eggs/g were 8,221.23 and 8,569.2 with chicken gizzard and beef diets, respectively, which were not significantly different (P = 0.5981) (Table 1). Similar data were reported by Cunha-e-Silva and Milward-de-Azevedo (1994), who observed that 1 g of eggs corresponded to an average of 8,457.5 eggs. A lack of micronutrients can affect egg production without damaging insect body mass and size (Colegrave 1993; Vamosi 2005).Thus, experimental confirmation is important for analyzing the reproductive potential of adiet.

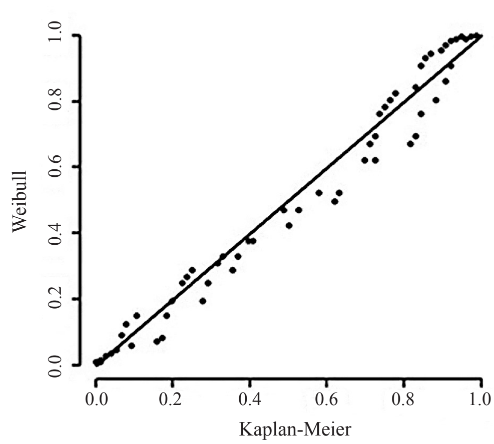

Figure 3. Longevity of *Cochliomyia macellaria* adults reared on chicken gizzard and beef diets under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period) estimated by the Kaplan-Meier non-parametric method versus Weibull parametric regression method.

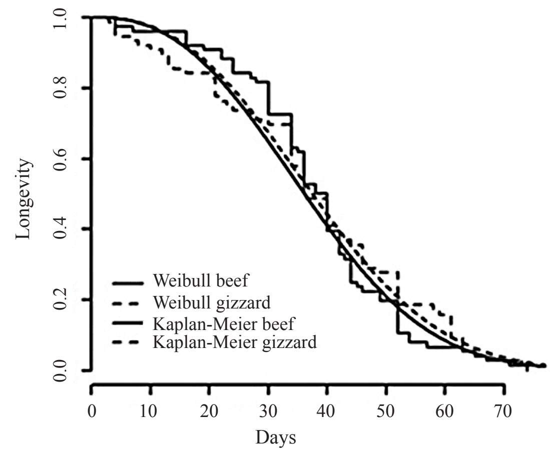

Figure 2. Longevity curves of adult *Cochliomyia macellaria* reared on chicken gizzard and beef diets under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period) estimated by the Kaplan-Meier non-parametric method and Weibull parametric regression method.

The mean egg viability with the chicken gizzard diet was 99%, while that with the beef diet was 94%, which were not significantly different (P = 0.3805). The protein substrate is important for ovocyte maturation (Wall *et al.* 2002) and the stimulation of mating and oviposition (Barton-Browne *et al.* 1976) in necrobiontphageous blowflies, so the gizzard substrate was shown to be efficient.

C. macellaria oviposition was the highest between the 12th and 20th day and the females made intermittent ovipositions, exceeding 68 days after emergence, on both diets (Fig. 1). Cunha-e-Silva and Milward-de-Azevedo (1996) analyzed *C.macellaria* fed with horsemeat where the females were stimulated to daily oviposition twice a day, and they observed peaks on the 11th and 22nd day, similar to the present study. However, oviposition occurred within 40 days, after which there was no oviposition, shorter than the oviposition time in this study. This may have occurred because, in the present study, the stimulus to oviposition may have been late, after the 12th day.

The estimated longevity curves were presented by the Kaplan-Meier non-parametric method and Weibull parametric

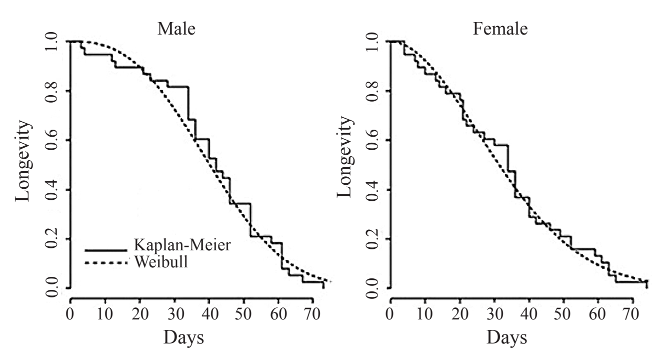

Figure 4. *Cochliomyia macellaria* longevity estimated by the Kaplan-Meier non-parametric method and Weibull parametric regression method for males and females reared on a chicken gizzard diet under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period).

regression method (Fig. 2). In both cases, the chicken gizzard and beef diets were considered co-variables.

According to Colosimo and Giolo (2006), the suitability of a model can be verified by a graph comparing the estimates obtained by the Kaplan-Meier non-parametric method with the estimates obtained by the model. If the fit is good, the curve should follow the x = y line. In this sense, figure 3 presents the estimated longevity by the Kaplan-Meier non-parametric method versus the estimates obtained by the Weibull parametric regression method. As the curve follows a straight line, the Weibull parametric regression method was a good fit to the data.

According to the Kaplan-Meier estimator, which expresses a graphic representation of the real data (Carvalho *et al.* 2011), the average longevity for flies on the beef diet was 38 days, while for the chicken gizzard diet it was around 37 days. It was also observed that, for both diets, all the flies were dead within74 days. A *log-rank* test found no difference between the longevity curves for the chicken gizzard and beef diets, considering a confidence level of 95% (P = 0.573). Cunha-e-Silva and Milward-de-Azevedo (1996) worked with groups of 20 and 40 couples and observed a maximum longevity of 52 days for females and 49 for males and 65 days for females and 70 days for males, respectively.

From the Weibull regression (Fig. 2) (P = 0.58) it was concluded that there was no difference between the diets. The estimates of maximum likelihood for the form and scale parameters were 2.51 and 40.37, respectively. According to Reis and Haddad (1997), the survival curve was type I, that is, the mortality rate increased with time as the shape parameter was greater than 1.

The *log-rank* test (P = 0.21) showed that there was no difference between the mean time estimated for male longevity (41.08 days) and female longevity (33.79 days) when fed a chicken gizzard diet (Fig. 4). The shape and scale parameters for the males were 2.63 and 46.23, respectively, and for the females, 1.89 and 38.08, respectively. The same occurred for the beef diet, where there was no significant difference between the sexes (P = 0.36). The estimated mean longevity was 40.04 days for males and 36.29 days for females (Fig. 5). The values of the estimates obtained for the fit of the Weibull parametric regression method were 3.67 for the shape parameter and 44.38 for the scale parameter. The estimated values were 2.48 and 40.91 for the females for the

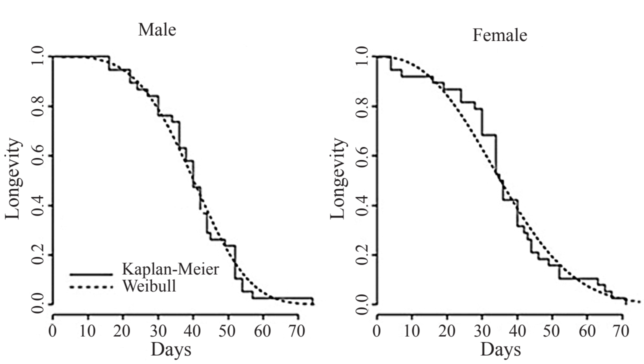

Figure 5. *Cochliomyia macellaria* longevity estimated by the Kaplan-Meier non-parametric method and Weibull parametric regression method for males and females reared on a beef diet under controlled conditions (30 °C day/28 °C night, 70 ± 10% RH, and 12 hours light period).

form and shape parameters, respectively. This showed that the survival curve was type I for both sexes on each diet. Cunha-e-Silva and Milward-de-Azevedo (1996), observed a mean longevity of 30 and 35 days for females and males of *C. macellaria*, respectively.

The function of the adult, in many cases, is related to dispersion and, especially, reproduction. These functions depend on the interaction of physiological and behavioral processes that are related to food intake and use. Egg or progeny production involves energy and nutrient accumulation by females, which makes them consume more and gain more weight, affecting egg production (Parra *et al.* 2009). In this sense, an efficient alternative diet should have the nutritional aspects that supply the needs of the insect, resulting in individuals with the same or greater longevity and/or reproductive capacity as those reared on a traditional natural diet.

From the observed results, chicken gizzards are an efficient natural diet for *C. macellaria* rearing. Furthermore, chicken gizzards are advantageous because they cost less than beef, are easy to buy in the market, and are easier to handle than beef.

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