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Alternative natural diet for the creation of immature oriental latrine flies under controlled conditions

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Abstract. An evaluation was made of the post-embryonic development of *Chrysomya megacephala* (Fabricius, 1794) with an alternative natural diet of chicken liver under controlled conditions (27ºC during daytime, 25ºC at night). Beef was used as the control diet. The diets were defrosted in a refrigerator for 24 hours. Forty neolarvae were placed on each 100 grams of diet, with four replications per treatment. The body mass of mature larvae were recorded in lots of five, and each lot was placed in a test tube containing sawdust. The average duration of each stage did not differ significantly from one treatment to the other: larval (3.04 liver/3.05 beef), prepupal (1.65/1.53), pupal (3.63/4.06) and neolarval to adult (8.20/8.97). The pace of abandonment of the substrate by the mature larvae and the emergence of *C. megacephala* showed a similar pattern in both treatments. The mature larvae that abandoned the beef diet (average weight: 80.0 mg) did not differ significantly from those that abandoned the chicken liver diet (average weight: 72.7 mg). The survival rate of the larval (85.8% liver/98.8% beef) and neolarval to adult stages (62.8%/ 83.1%) reached significantly lower indices in the tested diet. However, the prepupal and pupal stages were not affected. The larval chicken liver substrate demonstrated a potential for use as a viable alternative for breeding these dipterans in the laboratory.

Keywords: chicken liver, *Chrysomya megacephala*, post-embryonic development.

Resumo. Dieta natural alternativa para a criação de larvas imaturas de *Chrysomya megacephala* **(Fabricius, 1794), sob condições controladas** Objetivou-se avaliar o desenvolvimento pós-embrionário de *Chrysomya megacephala* (Fabricius, 1794) na dieta natural alternativa fígado de frango, sob condições controladas (27°C/dia, 25°C/noite). Como dieta controle utilizou-se carne bovina. As dietas foram descongeladas em refrigerador por 24 horas. Acondicionou-se 40 neolarvas/100 gramas de dieta realizando-se quatro repetições/tratamento. O registro da massa corporal das larvas maduras foi realizado em lotes de cinco, as quais foram acondicionadas em tubo de ensaio contendo serragem. A duração média dos estágios larvais (3,04 fígado/3,05 carne), pré-pupal (1,65/1,53), pupal (3,63/4,06) e de neolarvas a adulto (8,20/8,97) não diferiu significativamente entre os tratamentos. O ritmo de abandono de larvas maduras e o ritmo de emergência de *C. megacephala* mostraram padrão similar em ambos os tratamentos. Com relação à massa corporal, As larvas maduras que abandonaram a dieta à base de carne bovina (massa corporal média: 80,0 mg) não diferiram significativamente daquelas da dieta fígado de frango (massa corporal média: 72,7 mg). A taxa de sobrevivência dos estágios larvais (85,8% fígado/ 98,8% carne) e de neolarvas a adulto (62,8%/ 83,1%) atingiram índices significativamente inferiores na dieta testada. Porém, o estágio pré-pupal e pupal não foram afetados. O substrato larval fígado de frango apresenta potencial para ser utilizado como uma alternativa viável para a manutenção destes dípteros em laboratório.

Palavras-chave: *Chrysomya megacephala*, desenvolvimento pós-embrionário, fígado de frango.

Introduction

Chrysomya megacephala (Fabricius, 1974) (Diptera: Calliphoridae), commonly known as the oriental latrine fly, was introduced in Brazil in the 1970s and can now be found throughout the country (Guimarães *et al*., 1978). According to Sukontason *et al.* (2005), this fly was responsible for human cases of myiasis in Thailand. Myiasis especially affects cattle, sheep, horses, goats and dogs (Moya-Borja, 2003). In Brazil, the disease accounts for $150 million in annual losses for the cattle-breeding industry (Grisi *et al.*, 2002).

This species shows high synanthropy and is frequently associated with environments modified by humans. Compared with muscoid species, its occurrence, distribution and predominance increase public health risks in metropolitan areas (D'Almeida & Lopes, 1985). *Chrysomya megacephala* is responsible for the mechanical transmission of pathogens, such as protozoans, enteric bacteria and helminths (Parallupi *et al*., 1996; Oliveira *et al*., 2002). According to Maldonado & Centeno (2003), individuals of this species show one of the greatest pathogen-transmitting abilities due to their large body area.

Chrysomya megacephala adults may be attracted by a variety of substances: those undergoing fermentation and decomposition, foodstuffs, animal or human feces, sanitary dumps and landfills, animal carcasses, and vertebrate blood and wounds (Guimarães & Papavero, 1999). Their larvae develop in decomposing organic animal material, making them of fundamental importance in forensic entomology for crime investigations (Harvey *et al*., 2003; Oliveira-Costa, 2003; Sukontason *et al.,* 2003).

The use of live fly larvae to remove necrotic tissue in chronic or infected wounds is called biotherapy or larval therapy. This procedure has reappeared in several countries, such as the United States, England, Germany, Belgium and Israel, to treat necrotic, chronic, post-surgical or diabetes-related skin wounds, ulcers, traumatic lesions, gangrene, and tumors (Neves, 2005). On the other hand, *C. megacephala* larvae may be associated with cutaneous myiasis in domestic animals, leading to great economic losses (Guimarães & Papavero, 1999).

Chrysomya megacephala has been used as an alternative host for the maintenance of the pteromalid *Nasonia vitripennis* (Walker, 1836) in laboratory conditions. The use of microhymenopters in control programs to develop flies of veterinary and sanitary importance is considered to be an efficient and ecological alternative (Cardoso & Milward-de-Azevedo, 1996). Therefore, it is important to test new substrates for the breeding of *C. megacephala* immature forms in order to find less expensive options that enable the production of insects with reproductive capabilities. This would make medium- and large-scale breeding possible by reducing operational costs.

The objective of the present trial was to evaluate the post-embryonic development of *C. megacephala* under controlled conditions using an alternative diet based on chicken liver.

Material and Methods

The trial was conducted in the Laboratório de Estudo de Dípteros (LED), Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro (UNIRIO).

The *C. megacephala* stock colony was collected under field conditions at Fundação Rio Zoo in the

São Cristóvão neighborhood, a metropolitan area of Rio de Janeiro. Taxonomic identification was carried out according to Mello (2003). Adults were kept in cages with wooden edges and nylon sidings (30.0 cm x 30.0 cm x 35.0 cm) and fed a daily diet of equal parts honey and water. They were offered bovine meat in plastic containers during the first five days after their emergence in order to aid the maturation of ovarian follicles in females. From the 15th day on, the same substrate was used to stimulate oviposition.

The experimental trial was conducted in an acclimatized chamber regulated at 27°C during the day and 25°C at night, with relative humidity (RH) 60± 10% and a 14-hour photoperiod, beginning at 6 a.m.

Eggs masses from females of the third generation of the stock colony were transferred to Petri dishes coated with filter paper imbibed in distilled water. Dishes were sealed with PVC film and were kept in the acclimatized chamber for 24 hours. After this period, 40 neolarvae were transferred to plastic containers (8 cm x 5 cm) with 100 grams of chicken liver that had been previously thawed in a refrigerator for 24 hours. These containers were placed into larger ones (10 cm x 10 cm) with sterilized sawdust as the substrate for pupariation. The control diet was the same amount of bovine meat that had been previously thawed in a refrigerator and cut into 3 cm^3 cubes. Each treatment had four replicates.

After larvae left the substrate, they were weighed in batches of five, transferred to test tubes containing sawdust, and sealed with hydrophobic cotton. After emergence, adults were sexed. These biological observations were carried out daily, in the morning.

The following parameters were evaluated in order to analyze post-embryonic development: duration and viability of larvae, pre-pupas, pupas, neolarvae and adults; body mass of mature larvae; leaving rate of larvae in the diet; emergence rate of adults; and sex ratio.

Results obtained were submitted to variance analysis. Means were compared by Turkey post-test analysis at a 5% significance level using GraphPad Sofware, version 2.05a Instat 2.

Results

The mean duration of *Chrysomya megacephala* larva stage bred in the liver-based diet did not differ significantly from the control group, as assessed by the variance analysis (p= 0.07398). This was also observed for pre-pupa (p=0.03362), pupa (p= 0.0948) neolarvae and adult stages (p=0.3886) (Tab. 1).

Table 1. Duration (days) of *Chrysomya megacephala* post-embryonic stages bred in chicken liver and bovine meat diets (T: 27°C/day and 25°C/night, R.H.: 60± 10%, 14 h photoperiod).

Diet	Larval Stage (days)		Pre-Pupa Stage (days)		Pupa Stage (days)		Neolarvae Stage Until Emergence (days)	
	X ± sd	IV	X ± sd	IV	X ± sd	IV	X ± sd	IV
Chicken liver	3,04^{ns} ± 0,05	3,00-3,11	1,65^{ns} ± 0,21	1,36-1,86	3,63^{ns} ± 0,19	3,35-3,76	8,20^{ns} ± 0,13	8,12-8,40
Bovine meat	3,05^{ns} ± 0,07	3,00-3,15	1,53^{ns} ± 0,07	1,48-1,63	4,06^{ns} ± 0,38	3,65-4,47	8,97^{ns} ± 1,65	8,95-11,44

X = mean; sd = standard deviation; IV = variation interval; ^{ns} did not differ significantly in the T test with 5% significance; * differ significantly in the T test with 5% significance

Both treatments showed a peak in the leaving rate of mature larvae on the third day after the beginning of the trial, 96.4% and 94.3% of the larvae bred in liver and bovine meat, respectively. On the fourth day, leaving of mature larvae in the meat-based diet stopped. This period lasted longer for larvae bred in the liver-based diet (Fig. 1). Mean weight of immature larvae in liver and bovine meat diets did not show significant differences (p= 0.0674) (Tab. 2).

About 79.0% and 73.6% *C. megacephala* bred in liver and bovine meat, respectively, emerged on the eighth day after the beginning of the trial. About 20% of the adults emerged on the ninth day in both treatments. This period was longer, until the 10th day, for larvae bred in the chicken liver diet (Fig. 2). There was a significant difference on the viability of larvae (p= 0.0240), neolarvae and adults (p= 0.0287). Pre-pupas and pupas (p= 0.2279) did not show differences between treatments. Around 62.8% and 83.1% of the larvae inoculated in chicken liver and bovine meat, respectively, produced adults (Tab. 2).

Discussion

Duration of the developmental stages – larva, pre-pupa, pupa, and from neolarva to adults – of *C. megacephala* bred in chicken liver diets did not differ significantly from those bred in the control

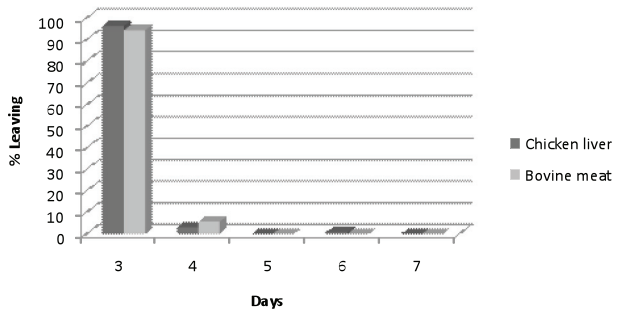

Figure 1. Leaving rate of *Chrysomya megacephala* mature larvae in the different diets as a function of time in days (T: 27°C/day and 25°C/night, R.H.: 60± 10%, 14 h photoperiod).

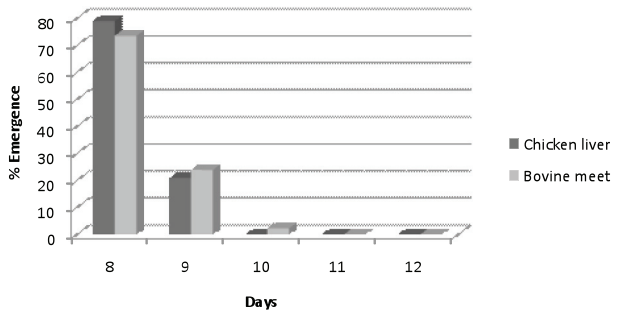

Figure 2. Emergence rate of *Chrysomya megacephala* in the different diets as a function of time in days (T: 27°C/day and 25°C night, R.H.: 60± 10%, 14 h photoperiod).

Table 2. Body mass (mg) of mature larvae and mean viability of the development stages of *Chrysomya megacephala* bred in chicken liver and bovine meat diets (T: 27°C/day and 25°C/night, R.H.: 60± 10%, 14 h photoperiod).

Diet	Body mass		Larvae (%)	Pre-Pupa (%)	Pupa (%)	Neolarvae Stage Until Emergence (%)	Sex Ratio
	X ± ds	IV					
Chicken liver	72,7 ^{ns} ± 7,3	56,6 - 72,7	85,8 *	100 ^{ns}	73,5 ^{ns}	62,8 *	0,53
Bovine meat	80,0 ^{ns} ± 4,5	71,0 - 80,7	98,8 *	100 ^{ns}	84,1 ^{ns}	83,1 *	0,40

X = mean; sd = standard deviation; IV = variation interval; ^{ns} did not differ significantly in the T test with 5% significance; * differ significantly in the T test with 5% significance

diet. D'Almeida & Oliveira (2002) compared *C. megacephala* bred in a diet based on brewer's yeast and ornamental fish feed with another diet containing whole milk, brewer's yeast and casein, and also with a bovine meat control diet. These authors observed that the meat-based diet was more efficient for all parameters tested. The larval stage was significantly shorter when meat was used. However, during the pupa stage, there were no significant differences between artificial diets and meat diets.

The leaving rate of *C. megacephala* adult larvae was similar in the two substrates, with a peak on the third day after the beginning of the trial. This was also observed by Santos *et al*. (1996) for *C. megacephala* larvae bred in a diet based on sardines previously kept for 2 and 24 hours at 30°C. Therefore, it was suggested that the sardine diet in the experimental conditions tested by these authors, as well as bovine meat and chicken liver evaluated in the present trial, did not affect the speed of post-embryonic development in immature forms of this species. There was no significant late leaving by larvae in these substrates.

Emergence rhythms of *C. megacephala* were similar in both treatments. Adults from both diets emerged between the eighth and the ninth day, with a peak on the eighth day. Only few adults from the control diet emerged on the tenth day. According to D'Almeida & Oliveira (2002), *C. megacephala* adults from larvae bred in two artificial diets emerged between the ninth and the tenth day after the beginning of the study.

D'Almeida & Mello (1996) carried out assays with muscoid flies using six kinds of substrates for oviposition - sardines, bovine liver, ground bovine meat, shrimp, squid, fermented banana and fresh human feces. They showed that meat was generally the substrate where the greatest number of eggs and larvae were found, and it was also the substrate most frequently used for oviposition. In the same study, it was also observed that *C. megacephala* laid virtually all eggs in meat (99.9%), and that human feces and banana did not stimulate oviposition. This was also observed by Mendonça & D'Almeida (2004), who showed that meat was the most efficient diet for breeding *C. megacephala* larvae, compared with artificial diets made up of milk, agar, brewer's yeast and distilled water. The authors emphasized that in natural conditions, larvae of this species are necrophages and will prefer meat.

In the present study, there was no significant difference between mean weights of mature *C. megacephala* larvae bred in the two diets. The weight of immature forms directly affects the development parameters of adults, such as size, survival rate, dispersion rate, and reproductive ability (Aguiar-Coelho & Milward-de-Azevedo, 1998; D'Almeida & Oliveira, 2002; Barbosa *et al.,* 2004). In *Hemipyrellia ligurriens* (Wiedemann, 1830) (Diptera: Calliphoridae), larger individuals showed greater mortality in later stages, whereas mortality rates of smaller individuals were greater in younger stages, suggesting that larger individuals lived longer. Studies by Zuben *et al.* (1996) showed that the survival curve for *C. megacephala* males and females was similar to the one observed for larger *H. ligurriens* individuals.

As for the viability of immature forms, the *C. megacephala* larva stage was significantly less viable in the liver-based diet than in the control diet. This was also observed in relation to the viability from neolarvae to adults. On the other hand, pre-

pupa and pupa viabilities did not differ between the diets. According to Cunha-e-Silva & Milward-de-Azevedo (1994), the physical consistency of the diet makes it difficult for excretion products of the larvae to disperse and evaporate. Besides, the inadequate quality of the diet, among other biological factors, may contribute for the reduction in viability of the different developmental phases.

Due to the softer consistency of the chicken liver diet, it decomposes faster than bovine meat. The decomposition of the diet by the action of proteolytic enzymes produced by immature forms, together with metabolic heat of the group of larvae (Goodbrod & Goff, 1990; Aguiar-Coelho & Milward-de-Azevedo, 1998), liquefied the liver at the end of larval development. This fact, along with the probable lack of some nutritional component in the liver diet, may have contributed for the reduction in the viability of larva and pupa stages.

Santos *et al.* (1996) bred this species on a diet based on sardines previously stored at 30°C for 24 hours, and observed total viability equal to 87.0%, showing that this kind of substrate may be efficient in a given temperature and storage time range. However, when sardines were used after longer storage times (48 h/72 h), total viability was reduced, showing that degradation of the diet affected the development of *C. megacephala* immature forms.

Sex ratio in the experimental diet was close to the normal 50% pattern. This was also observed by Milward-de-Azevedo *et al.* (2000) when comparing two diets: one based on carrots, yeast and eggs and the other based on putrefied meat and blood. Mendonça & D'Almeida (2004) also reported the same findings for the following diets tested on *C. megacephala*: whole powdered milk, brewer's yeast and agar; lactose-free powdered milk, brewer's yeast and agar; and powdered milk, brewer's yeast and agar; the control diet was ground bovine meat.

Although there were significant differences in relation to the survival rates of larva stages and neolarvae-to-adult stages when compared with the control group, the diet tested here may be a viable alternative to decrease operational costs in dipter breeding in laboratory conditions. Although chicken liver requires refrigeration due to its primary nature, it costs less than bovine meat, which may be an advantage in breeding these insects.

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