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Survival, Fecundity and Life Cycle of the blowfly, *Chrysomya chloropyga* (Wied.) Diptera: Calliphoridae) Fed with Cow Lung, Beef and Liver in the Laboratory

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Abstract: *Adult blowfly, Chrysomya chloropyga (Wied.) (Diptera: Calliphoridae) is ubiquitous in Nigeria, particularly in abbatoirs, fish and meat markets, in restaurantsand in filthy environment around houses. C. chloropyga was reared separately on cow lung, beef and liver for a self-sustaining colony for the study of survival, fecundity and life cycle on the three diets. Survival of male and female C. chloropyga on cow lung, beef and liver at day 10 of exposure was above 95% except males maintained on cow lung at 75%. Male population trailed closely and behind female population for most period of exposure on each of the diets. Percent survival at days 20 and 30 was above 50% for males and females, except males maintained on cow lung. There was significant increase in weight of males and females from days 0 to 5. Weights of males fluctuated thereafter but female weights generally increased with age up to day 30 of exposure. Mean fecundity of females on cow lung, beef and liver was 183.00±14.22, 189.92±12.20 and 154.33±11.40 eggs respectively. Mean day of first egg laying was 9.33±1.45,9.00±0.00 and 10.50±1.50 on cow lung, beef and liver respectively. There was no significant difference in the mean duration of the life cycle stages from egg to adult stage. Total life cycle duration was approximately 10 days on each of the diets. Sex ratio of newly ecdysed adults on cow lung, beef and liver was 1:1.*

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I. Introduction

Chrysomya chloropyga is a cosmopolitan shiny blue green blowfly commonly found in the tropics and southern part of Africa (West, 1972). It is found in all parts of Nigeria, particularly in market places, refuse dumps, toilets and in the bush. It is strongly attracted to carrion and human cadaver and it is the major cause of Myiasis in sheep and cattle. Huntington and Higley (2010) demonstrated that the fresh and decomposed liver produced identical ovarian development and concluded that decomposed flesh remain a suitable protein source for carrion-feeding blowfly, *Lucilia sericata*. Linhares and Avancini (1989) compared fresh beef liver, human and chicken faeces as diets for adult *Chrysomya megacephala* and *C. putoria* and established that fresh beef liver diet completed oogenesis within 10-13 days while the chicken faeces facilitated slower oogenesis in 27-48 days after emergence. Daniels *et al*. (1991) demonstrated that protein-rich beef diet increased adult weight of *Lucilia sericata* at emergence but protein deficient diet caused a reduced rate of weight gain of *Phormia regina* larvae (Burst and Fraenkel, 1955). Clark et al. (2006) reported that larvae of Lucilia sericata developed to larger adults when reared on pig compared to cow tissue and when reared on lung and heart compared to liver. A complete meal of beef liver was sufficient to activate the endocrine cascade leading to oogenesis in Phormia regina (Yin et al.,1994) and protein diet is required for the development of male accessory reproductive gland (Stoffolano,1974). Cook (1991) reported that female Lucilia cuprina require a longer period of feeding on sheep faeces to develop mature eggs than when fed on sheep liver. Stoffolano et al. (1995) showed that females of P. regina exposed to homogenized liver for a 4-hr feeding period consumed enough protein in the crop to develop a normal compliment of eggs. There is scanty information on the blowfly, C. chloropyga and there has been no comparative study on the biology of the blowfly using cow lung, beef and liver as test diets. It is hoped that the study will further enrich the biology of the blowfly and provide information for the mass rearing of the insect, with respect to cost and choice of appropriate cow tissues.

II. Materials And Methods

Chrysomya chloropyga used for this experiment were obtained from a self-sustaining colony in the laboratory. Survival of male and female blowfly C. chloropyga was determined using twenty pairs of newly emerged blowflies. The pairs of males and females were put in wire cages (40x40x40cm) and were fed ad libitum on cow lungs, beef and liver respectively. Sugar was provided and water made available in a soaked cotton wool. Diet was replaced every 72hr for the 30-day exposure period. Weights of males and females were recorded every 5 days for 30 days on each of three diets. The first day of egg laying was recorded accordingly and egg batches deposited were also collected, recorded and counted to determine fecundity on each of the diets. The life cycle of blowfly was determined on each of the diets by recording the days of development from egg, to the first instar, second instar and third instar, pupa and adults respectively. The sex ratio of newly emerged adults was determined by counting the number of males and females accordingly. All experiments were carried out in triplicate between August, 2012 and January, 2013.

Statistical analysis: The data was subjected to one way analysis of variance test (ANOVA) using SPSS (version 16. for windows) computer software programme to assess the significance of observations on weight, egg laying, fecundity and sex ratio. Means were separated using Tukey HSD test.

III. Results

The mean percent survival of adult male C. chloropyga fed cow lung, beef and liver are shown in Table 1. Male population decreased progressively from day 0 at 100% to day 30 at 23.00±2.89, 76.67±1.67 and 51.67±10.93% respectively. Survival of males maintained on lung, beef and liver diets at day 10 were 75.00±2.89, 96.67±3.33 and 98.33±1.67 percent respectively. Population of males fed cow lung terminated at 23.00±2.89 percent at day 30 but 53.33±1.67 percent still survive at day 21 of exposure. Survival of males fed liver and beef diets at day 15 were 90.00±5.00 and 91.67±4.41% and survival at day 30 were 51.67±10.93 and 76.67±1.67% respectively. Table 2 shows the mean percent survival of adult female *C. chloropyga* fed cow lungs, beef and liver respectively for a period of 30days. Survival of females fed beef and liver were stable between 100 and 90% at 22 and 19 days respectively and at day 10 for those fed on cow lung. Percent survival at day 10 was between 90 and 96% for the three diets and 50% at day 27 of exposure. At day 30, survival was significantly high at 70.00±5.00 and 68.33±12.02 percent for females fed beef and liver respectively, while those fed on cow lung was at 40.00±2.87percent.

Mean weights of male and female *C. chloropyga* fed cow lung, beef and liver are shown in Table3. Male and female weights generally increased from days 0 to 5 of age on each of the diets. Weights of females were generally higher than male weights on the three diets and at ages 0, 5, 10, 15, 20, 25, and 30 days respectively. Mean weights of males fluctuated between ages 0-30 days of exposure. There was no significant difference in the mean weights of males fed cow beef and liver throughout the period of exposure but weights of males fed cow lungs at the different ages were significant ($F=3.63$, $p<0.02$). There was significant difference in the mean weights of females fed cow lung ($F=20.95$, $p<0.00$) and cow beef ($F=6.16$, $p<0.02$) at 0 to 30 days of age.

Table 1: Mean Percent Survival of adult Male *C. chloropyga* fed Cow Lung, Beef and Liver.

Day	Mean % Survival Male (Lung)	Mean % Survival Male (Beef)	Mean % Survival Male (Liver)
0	100.00±0.00	100.00±0.00	100.00±0.00
1	100.00±0.00	100.00±0.00	98.33±1.67
2	100.00±0.00	100.00±0.00	98.33±1.67
3	100.00±0.00	100.00±0.00	98.33±1.67
4	100.00±0.00	98.33±1.67	98.33±1.67
5	95.00±2.89	98.33±1.67	98.33±1.67
6	91.67±1.67	98.33±1.67	98.33±1.67
7	88.33±4.41	98.33±1.67	98.33±1.67
8	85.00±5.77	98.33±1.67	98.33±1.67
9	80.00±5.00	98.33±1.67	98.33±1.67
10	75.00±2.89	96.67±3.33	98.33±1.67
11	73.33±4.41	95.00±5.00	95.00±5.00
12	71.67±3.33	95.00±5.00	95.00±5.00
13	71.67±3.33	95.00±5.00	95.00±5.00
14	68.33±4.41	95.00±5.00	90.00±5.00
15	68.33±4.41	91.67±4.41	90.00±5.00
16	68.33±4.41	91.67±4.41	83.33±3.33
17	65.00±2.89	91.67±4.41	83.33±3.33
18	63.33±1.67	90.00±2.89	81.67±4.41
19	56.67±1.67	88.33±1.67	81.67±4.41
20	55.00±2.89	85.00±0.00	78.33±1.67
21	53.33±1.67	85.00±0.00	78.33±1.67
22	48.33±4.41	83.33±1.67	71.67±3.33
23	46.67±3.33	83.33±1.67	71.67±3.33
24	43.33±3.33	81.67±1.67	68.33±3.33
25	40.00±2.89	81.67±1.67	65.00±5.77
26	40.00±2.89	81.67±1.67	63.33±6.00
27	35.00±2.89	80.00±0.00	60.00±5.77
28	33.33±4.41	80.00±0.00	56.67±8.82

29	26.67±1.67	76.67±1.67	55.00±10.41
30	23.00±2.89	76.67±1.67	51.67±10.93

Table 2. Mean Percent Survival of adult Female *C. chloropyga* fed Cow Lungs, Beef and Liver.

Day	Mean % Survival Female (Lungs)	Mean % Survival Female (Beef)	Mean %Survival Female (Liver)
0	100.00±0.00	100.00±0.00	100.00±0.00
1	100.00±0.00	96.67±3.33	98.33±1.67
2	100.00±0.00	95.00±2.89	98.33±1.67
3	100.00±0.00	93.33±3.33	98.33±1.67
4	100.00±0.00	93.33±3.33	98.33±1.67
5	98.33±1.67	93.33±3.33	98.33±1.67
6	93.33±1.67	93.33±3.33	98.33±1.67
7	93.33±1.67	93.33±3.33	98.33±1.67
8	91.67±3.33	93.33±3.33	98.33±1.67
9	91.67±3.33	93.33±3.33	98.33±1.67
10	90.00±2.89	93.33±3.33	96.67±1.67
11	86.67±4.41	93.33±3.33	96.67±1.67
12	86.67±4.41	93.33±3.33	96.67±1.67
13	83.33±3.33	93.33±3.33	96.67±1.67
14	83.33±3.33	93.33±3.33	96.67±1.67
15	78.33±6.01	93.33±3.33	95.00±2.89
16	76.67±6.67	93.33±3.33	93.33±4.41
17	75.00±5.00	93.33±3.33	93.33±4.41
18	75.00±6.00	93.33±3.33	91.67±3.33
19	68.33±1.67	93.33±3.33	90.00±2.89
20	63.33±1.67	93.33±3.33	83.33±7.26
21	63.33±1.67	90.00±2.89	83.33±7.26
22	63.33±1.67	90.00±2.89	80.00±8.66
23	60.00±2.89	85.00±7.64	80.00±8.66
24	60.00±2.89	83.33±6.67	78.33±8.82
25	56.67±4.41	78.33±6.67	76.67±10.14
26	53.33±7.26	78.33±6.67	76.67±10.14
27	57.67±7.6	78.33±6.67	76.67±10.14
28	50.00±5.77	78.33±6.67	70.00±13.23
29	46.67±3.33	73.33±4.41	70.00±13.23
30	40.00±2.89	70.00±5.00	68.33±12.02

Table 3. Mean weight (mg.) of male and female *C. chloropyga* fed cow lung, beef and liver at different ages of the adult.

		Male		Female		
Day	Lung	Beef	Liver	Lung	Beef	Liver
0	26.67±3.33a	36.67±3.33a	43.33±8.81a	26.67±3.33a	36.67±6.67a	56.67±12.02a
5	50.00±0.00ab	60.00±5.77a	60.00±5.77a	63.33±3.33b	40.00±5.67ab	86.67±12.02a
10	60.00±0.00b	56.67±3.33a	50.00±5.77a	73.33±3.33b	63.33±6.67abc	75.00±2.89a
15	50.00±0.00ab	66.67±8.81a	56.67±6.67a	66.67±3.33b	76.67±8.81c	63.33±3.33a
20	50.00±11.55ab	40.00±5.77a	48.33±4.41a	73.33±6.67b	66.67±3.33bc	73.33±3.33a
25	46.67±3.33ab	36.67±6.67a	55.00±5.00a	70.00±0.00b	53.33±3.33abc	70.00±5.77a
30	43.33±6.67ab	46.67±12.01a	45.00±2.89a	60.00±0.00b	56.67±3.33abc	58.33±1.67a
F value	3.63	2.90	1.19	20.95	6.16	2.18
P value	0.02	0.47	0.40	0.00	0.02	0.11

Mean fecundity of females maintained on cow lungs, beef and liver respectively is shown in Table 5. Beef supported the highest mean fecundity of 189.9±12.20 eggs while cow liver fed females had a mean fecundity of 154.22±11.40 eggs. Mean total number of eggs laid during the 30-day exposure of females on cow lungs, beef and liver were 2196.33±272.34, 2710.67±233.71 and 1848.67±237.39 eggs respectively. Females fed cow lung, beef and liver deposited first egg batch at 9.33±1.45, 9.00±000 and10.50±1.50 days respectively (Table4).

Table 5. Fecundity of *C. chloropyga* fed on cow lungs beef and liver

Diet	Mean total number of eggs	Mean fecundity
Lungs	3075.33±472.34a	194.90±5.95a
Beef	2710.67±233.71a	188.68±6.13b
Liver	2000.00±156.57a	157.33±3.80b
F-value	2.97	13.71

P-value	0.13	0.006

Life cycle duration of *C. chloropyga* fed cow lungs, beef and liver is shown in Table 6. Eggs hatched on the three diets between 18 and 48 hr. There was no significant difference in the mean duration of the first, second and third larval instars maintained on cow lungs, beef and liver respectively. Pupa duration in each of the diets was 4.33±0.33 days. Total life cycle Duration was approximately 10 days on each of the diets. The total number of adults that emerged vary with the diet but the number of males and females from each of the diets were not significantly different from each other as shown in Table 7, giving a mean sex ratio of 1:1 for each of the diets.

Table 6. Life cycle duration of *C. chloropyga* fed on cow lung, beef and liver

	Means duration of stages of life cycle (days)		
Life cycle stage	**Lungs**	**Beef**	**Liver**
Egg	18h	2.00±0.00	18h
First Instar	1.00±0.00	1.00±0.00	1.00±0.00
Second instar	2.00±0.00	1.00±0.00	1.33±0.33
Thirdinstar	2.00±0.00	1.67±0.33	2.00±0.00
Pupa	4.33±0.33	4.33±0.33	4.33±0.33
Approx. Total (days)	9.33	10.00	8.66

Table 7. Mean sex ratio of newly ecdysed *C. chloropyga* adults

Diet	Sex ratio (Male: Female)
Lungs	31.67±4.33:33.00±6.08 (1:1)
Beef	16.67±0.33:14.67±0.33 (1:1)
Liver	20.33±18.6:18.67±1.53 (1:1)

IV. Discussion

The three protein diets, cow lung, beef and liver evidently supported the survival of male and female *C. chloropyga* for the 30-day exposure, with over 80% of female still surviving at day 30. Male population trailed closely behind female population, thereby ensuring contact between the two sexes for the period of exposure. Survival of female *C. chloropyga* maintained on poultry waste at day 30 was 60% (Ajala, 2012 unpubli.) demonstrating the superiority of animal diet compared with other types of diets. The performance females on the three diets are a demonstration of the high fecundity of the blowfly which is probably responsible for their persistence in then environment.

The pronounced increase in weight of males at day 5 of exposure compared with the weight of newly emerged males on cow lung, beef and liver suggests the need for newly ecdysed blowflies to quickly access food for their survival. Newly emerged males and females are obviously deprived of energy since energy acquired at the larval stage has been used for the formation of pupae and adult body parts. Male weights fluctuated from day 10 to day 30, demonstrating continuous acquisition and dissipation of energy, probably used for searching and mating females. Female weights however continued to increase at days 10, 15 and 20 compared to weight of newly ecdysed females owing to the need to acquire protein for ovarian development. The ovaries of newly emerged females are undeveloped, therefore the need for protein for egg development (Rasso and Frankel, 1954. Daniels *et al*. (1991) demonstrated that protein rich diets increase adult weight of *Lucilia sericata* at emergence. Stoffolano *et al*. (1995) reported that protein source is necessary for stimulating the nervous system for mating activity and also implicated protein diet for the development of the male and female accessory reproductive glands (Stoffolano, 1974). Cook (1991) showed that *Lucilia cuprina* fed sheep faeces require longer time tomature eggsthan when fed on sheep liver. The first egg laying by females fed cow lung, beef and liver was approximately 10days, it is therefore obvious that *C. chloropyga* require protein meal to initiate vitellogenesis as shown for *L. sericata* (Hayes *et al*., 1999). The total fecundity of 3075, 2710 and 2000 eggs from females maintained for 30days on cow lung, beef and liver respectively demonstrated a high reproductive potential of female *C. chloropyga* on the test diets. The three protein diets clearly supported embryonic and post-embryonic development of *C. chloropyga,* which is why the females are strongly attracted to carrion and dead bodies. The high fecundity of *C. chloropyga* indicates the need for its control. It is therefore important to regularly and properly dispose of dead human and animal bodies in the environment.

The duration of life cycle of *C. chloropyga* fed cow beef, from egg to adult was 10.00 days, which was shorter than 13.89 days for *L. sericata* (Rueda *et al*., 2010). The life cycle duration of *C. chloropyga* fed cow liver and cow lung were 8.66 and 9.33 days respectively, indicating the possibility of several generations throughout their lifespan and consistent with Stoffolano *et al.* (1989) who reported that cow liver was the best for rapid egg maturation in *Phormia regina*.In addition to cow liver, the present study has further demonstrated that cow lung and beef are good alternatives for similar investigations. In this study we have been able to prove that cow lung, beef and liver are highly suitable for the reproduction of the blowfly, *C. chloropyga*, supporting larval development, pupal formation and the emergence of adult males and females of same sex ratio.

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