THE IMPACT OF DIET PROTEIN AND CARBOHYDRATE ON SELECT LIFE-HISTORY TRAITS OF THE HOUSEFLY *MUSCA DOMESTICA* LINNAEUS, 1758 (DIPTERA: MUSCIDAE)

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[Kökdener, M. & Kiper, F. 2020. The impact of diet protein and carbohydrate on select life-history traits of the housefly *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Munis Entomology & Zoology, 15 (1): 171-179**]**

ABSTRACT: This study examined the impact of ten diet with different protein and carbohydrate percentages on the immature development, survivorship, pupal and adult weight of the house fly, *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Different ratio of protein and carbonhydrate diet impacted development and corresponding life-history traits. Survival from the pupal to adult stages was also found to produce *significantly different* between different diet ($F_{9,30}$=768.251, p=0.01). High pupal mortality were seen in blood meal diet (92%) and chicken meal diet (96 %). Percent pupation was significantly higher on the wheat bran diet (P:C= 1:3.4). Larvae reared on the wheat bran diet developed the slowly but had the greatest survivorship to the pupal and adult stage. It is important to know the effect resource has on development *M. domestica* Linnaeus, 1758 helps to understant population dynamics.

KEY WORDS: Artificial diet, development, nutrition ecology

Nutrition is the process in which an organism obtains from its diet are essential for growth and development. Availability of key nutrients, such as sugars, amino acids, lipid during the developmental stages affects life history traits of insect (Nash & Chapman, 2014; Runagall-McNaull, Bonduriansky, & Crean, 2015; Florez-Cuadros, 2017) such as animal survival, longevity (Lee et al., 2008; Nash & Chapman, 2014; Krams et al., 2015; Florez-Cuadros, 2017), size (Diamond & Kingsolver, 2010; Florez-Cuadros, 2017) growth and reproduction (Simpson & Raubenheimer, 2012). Insects undergo remerkable morphological and physiological changes during development stages. Nutritional *requirements* can vary within a group of insects depending on what the insects have fed on many resources (Hochuli, 2001). The major component of most nutrients are protein and carbohydrates. The best sources of essential amino acids are proteins and crucial for life (Nash & Chapman, 2014). Protein and carbohydrate are species-specific that leads to in optimal performance (Raubenheimer & Simpson, 2003; Lee, Behmer, & Simpson, 2006; Behmer & Joern, 2008; Behmer, 2009; Simpson & Raubenheimer, 2012). Carbohydrates are a source of energy and the main fuel used for development. Organisms use the stored energy in all living processes (Nash & Chapman, 2014). The balance of diet consisting of both carbohydrates and protein is very important to growth (Nguyen, Tomberlin, & Vanlaerhoven, 2013) and successfully mature reproductive system of insect and produce eggs (Pastor et al., 2011; Nguyen et al., 2013). Larvae reared on poor protein diet, can cause larval mortality (Green, Simmonds, & Blaney, 2003) delay larval development, reduce body size (Gebhardt & Stearns, 1988; Tu & Tatar, 2003; Bonduriansky, 2006; Parker & Johnston, 2006; Colasurdo, Gélinas, & Despland, 2009; Chown & Gaston, 2010; Sentinella, Crean, & Bonduriansky,

2013; Nash & Chapman, 2014). Small body size is associated with reduced male mating success (Nash & Chapman, 2014).

The species *Musca domestica* Linnaeus, 1758 which belongs to the family Muscidae, popularly known as house fly, (Srinivasan, Jambulingam, Gunasekaran, & Boopathidoss, 2008). The house fly, *Musca domestica* Linnaeus, 1758, is a well-known cosmopolitan pest of livestock and poultry and play an important role as vector of some diseases (Al-Shami et al., 2016; Firoozfar, Moosa-Kazemi, Bahrami & Ahmed Yusuf, 2017). It is known for the medical and economic veterinary importance (Al-Shami et al., 2016). *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) has also been used in maggot therapy (Al-Ghamdi et al., 2014). *M. domestica* Linnaeus, 1758 (Diptera: Muscidae) can be used as a suitable species in a different ecological, biological, agricultural and medical investigation because of a short life cycle and high growth rate (Black & Krafsur, 1985; Axtell & Arends, 1990; Asiri, 2017).

Nutrition is important factor affecting population dynamics (Han & Dingemanse, 2015). It is important to know how nutritional resources impact longevity, development, reproductive potentials of insect. An understanding of different diet affect on insect development would allow us to increase the effectiveness of the rearing process under laboratory conditions. Previous experiments in the different insects showed that diet affect a range of different life-history (Sutherland, 1978; Kaneshrajah & Turner, 2004; Clark, Evans, & Wall, 2006; Day & Wallman, 2006; Ireland & Turner, 2006). An important deficiency from existing studies of diet is the effect of nutrient quality and quantity on different developmental stages.

Less is known about the effects of carbohydrate (C) and protein (P) content in the larval diet on life history of *M. domestica* Linnaeus, 1758. We addressed this deficincy by testing the effect of novel protein and carbohydrate components on the developmental life history of *M. domestica* Linnaeus, 1758. We altered diet components to provide variation on survival, development, size and life-history traits of of the *M. domestica* Linnaeus, 1758. Information from this study is important for improving rearing methods necessary for future investigation.

MATERIALS AND METHODS

Breeding of *M. domestica* Linnaeus, 1758

The house fly colony used in these experiments was established in January 2017 from larvae received from Ankara municipality investigation laboratories, Ankara, TURKEY, which was initiated from a laboratory colony at the University of Ondokuz Mayıs, also located in Samsun, TURKEY. This colony has been maintained for 2 years and supplemented periodically with wild-caught material.

Adult house flies were kept in 50× 50× 50 cm screen cages at 61.2 ± 1.5% relative humidity (RH), 25.2 ± 0.5° C and a photoperiod of 12:12 (L:D) h and were provided with water, sugar cubes *ad libitum* in open containers as food for the flies (Hogsette, Farkas, & Coler, 2002). Males and females were held together in the same cages. The eggs collected were used to sustain the colony.

From approximately 6 days after emergence, flies were provided with oviposition substrate in the form of 25 g of larval diet (a mixture of the 20 g wheat bran and 50 ml milk) in a 100 mm petri dish. Flies were allowed to oviposit for a 24-hour period every 72 hours over 3 weeks. Newly hatched (every 8 h) larvae were used for the experiment. All the replicates were used from same generation of colony to reduce the genetic variability among the sample. Eggs were transferred aseptically to a sterile petri dish and maintained in a SANYO MIR 252

growth chamber set at 25ºC, 70% RH, and a photoperiod of 12:12 (L:D) h. Eggs were monitored hourly for hatch. Resulting larvae were used in the subsequent experiments. After egg hatch, the groups of first-instar larvae were randomized by mixing with a fine paintbrush (Faber-Castell Soft Touch size 4, Faber-Castell Manufacturing, Stein, Germany). With the brush, 20 first-instar larvae were collected and placed onto 20 g of different a feeding substrate composed of 66% moisture inside a 200 ml polypropylene containers (Hobby Life Products, Demirel Plastic,TR). Each plastic cup had a tight-fitting cheesecloth covered to allow for air circulation (Fig. 1C).

Life cycle of *M. domestica* Linnaeus, 1758

Larvae that hatched from the eggs and passed from one instar to another were counted and maintained under the same environmental conditions as the adult organisms. The following aspects were taken into account when analysing each diet's efficacy: developmental stage duration (in days; the I, II and III larval instars, puparia and adult), sex ratio, adult and pupal weight. The size of immature and adult stages was also determined using a stereomicroscope (Leica MZ 12.5, LAS Version 3.8.0, Leica Microsystems, Switzerland) linked to a high-resolution digital camera (DC 100).

Experimental design

Each diet mixture, 20 g, was combined with 47 mL of water, but 20 g wheat bran was combined with 47 ml milk and placed in a 200 ml polypropylene clear plastic cups. Protein and carbohydrate components in the diets were varied (Table 1).

Each cup was seeded. The experiment was replicated five times wheat bran diet mixed with milk had initial moisture levels of 66%, whereas the chicken meal and fish wheat meal diet moisture was slightly lower at 60%. For each diet during an experiment, 20 first instar larvae were placed in each 200 ml polypropylene clear plastic cups.

Clutches of eggs laid by a single female were divided amongst several replicate containers, randomly alternating among treatments to control for genetic variation, parental age, and environment. The replicate containers were maintained at 25ºC, 70% RH in an incubator. Cups were each covered with a paper towel and held in the incubator. When needed for feeding to the larvae, batches of diet were mixed with water (except of wheat bran diet) and distributed among each container receiving that diet-moisture treatment to ensure each replicate container received the same diet. Feeding was terminated in a treatment when a cumulative 40% of the larvae in the five cups reached the prepupal stage. However, daily observations continued until all larvae had entered the prepupal stage or died. Prepupae were identifed by a change in integument color from larval white to black. Prepupae were removed daily from each container and weighed, then placed in appropriately-labeled 500-mL rearing containers, containing approximately 15 g of vermiculite, which provided a 3 cm-deep pupation substrate. Prepupae/pupae were held in the same incubator in which the larvae were reared and were monitored daily for adult emergence.

Larval and pupal development time

The number of pupae emerging each day was recorded allowed for pupation, allowing calculation of mean larval development time. The daily cohort of emerging pupae was sieved from the sand and transferred to a petri dish. These petri dishes were then checked daily for adult emergence. Adults were counted and their sex recorded, allowing mean pupal development time to be calculated for each replicate. Individuals that only partially emerged were discarded from

the experiment. Overall, development time was calculated by summing the mean larval and pupal development time of each replicate.

Larval and pupal survival

The total number of pupae present allowed for pupation was recorded as a measure of larval survival. The total number of fully emerging adults was used as the measure of pupal survival.

Pupal weight

Each treatment of pupae were weighed pupal weight was used as a proxy for adult size.

Statistical analysis

The average size of the immature and adult stages was calculated, as well as *M. domestica* Linnaeus, 1758 life cycle duration regarding each of the ten diets during five replications. Analysis of variance (ANOVA) was used for evaluating comparisons; such results were analyzed using a 95% confidence interval life-cycle data were analysed according to descriptive statistical parameters; the data derived from some biological phases and life-table variables were also recorded. An analysis of variance (ANOVA) for multiple factors was used to assess differences in time, size and survival amongst several treatment groups. Amount of diet provided, time (days) required for 40% prepupation, prepupal size, adult longevity, and egg production were compared across all diet-moisture treatments using analysis of variance followed by the Tukey–Kramer HSD test, and trial differences (significance set at $p < 0.05$) were tested using the paired t-test. Interactions between diet protein:carbohydrate, and trial on each life-history parameter were also examined. Kruskal–Wallis tests were performed when assumptions of normality and/or homoscedasticity were not met. IBM SPSS Statistics v.21.0 was used for all analyses (IBM, Armonk,NY, USA). Two-way ANOVA was conducted, including the interaction between sex and weight.

RESULTS

Development time

There was a significant difference in development time for larvae to reach pupal, and adult stages of development because of the different diet (Table 2). Diet had a significant effect on larval development (i.e. duration of development from egg to pupa; $F_{9,40}$ =2.177, P=0.045). The development time of larvae reared on mix of poultry feed and wheat bran meal was significantly shorter than other diet. Larvae reared on chicken meal, meat bone meal, mix of wheat bran and milk had the longest time to reach the pupal stage. The duration of the pupal period (time from pupa to adult eclosion) was affected by either diet ($F_{9,40}$ =5.580, P = 0.000). When measuring time to adult emergence, larvae reared on soybean meal, mix of wheat bran and blood meal and blood meal once again had the shortest time needed to reach adult emergence. The longest maximum time to adult emergence was for those reared on fish meal, followed by meat bone meal. When measuring total time from egg hatching to adult emergence, larvae reared on soybean meal (P:C= 1:0.15), mix of wheat bran and blood meal (P:C= 1:0.16), blood meal (P:C= 1:0.012), mix of poultry feed and wheat bran meal (P:C= 1:2.7) again had the shortest time needed to reach adult emergence (Table 2).

Survavial

Diet had a significant effect on larval survival ($F_{9,40}$=1804.469, p=0.000). Larvae feeding on rich carbonhydrate diet (wheat bran diet) showed lowest mortality compared to the other diet treatment. Other the lowest larval mortality occurred on the soybean meal diet. High larval mortality were seen in blood meal

(92%) and chicken meal diet (96%) (Table 3). The third highest rates of mortality occurred on diet meat bone meal. Survival from the pupal to adult stages differed significantly between different diet ($F_{9,30}$=768.251, p=0.01). Percent pupation was significantly higher on the (P:C= 1:3.4) wheat bran diet. The highest emergence rate was observed in wheat bran diet and soybean meal. The lowest emergence rate was observed in blood meal, (50%) mix of poultry feed and wheat bran diet (53%) (Table 3).

Pupal and adult weight

The nutrient content of diets affected the pupal weights. Pupal weight was significantly different between the rearing diets $F_{9,591}$ = 8.679, P,0.001). The highest mean pupal weight was recorded at mix of blood meal and wheat bran (0.202 g) and blood meal diet (0.200 g) while the lowest weight was recorded at chicken meal diet (0.59 g) and mix of poultry feed and wheat bran diet (0.96 g) (Table 4).

The weight of adult body related to the content of diet. This effect is significant between the ten dietary groups. The smallest mean weight of adult were found in mix of chicken meal and the highest mean adult weight was found at blood meal, mix of blood meal and wheat bran diet (0.305) and soybean diet (0.262 g) (Table 4). There was significant difference in adult weight between diets ($F_{59,600}$ = 12.874, P = 0.000). There was significant differences observed in the weight of adult sex reared on different diets (F=35.226, p=0.000).

DISCUSSION

The ecology, physiology, behaviour, performance and the life-history traits of insect are influenced by nutrition or more specifically by the quantity and quality of food (Barragan & Fonseca, Dicke & van Loon, 2018). Diet is crucial determinant of key life-history traits (Barragan & Fonseca et al., 2018; Lee et al., 2008). Our study results provide extremely important highlights into the lifestyle of the *M. domestica* Linnaeus, 1758 and its ability to utilize a variety of resources for larval development. This is the first study to investigate how varying the composition of protein and carbohydrate in an artificial diet effects the life-history of the *M. domestica* Linnaeus, 1758.

Development rate and time

There was a signifcant difference in development time for larvae to reach pupal and adult stages of development because of the different diet (Table 2). The results confirm that the house fly can develop successfully on a wide range of different protein and carbohydrate sources. Protein:carbohydrate content *had no effect* on development duration in our study. Cammack & Tomberlin (2017) found that larval development rate was faster on the protein:carbohydrate balanced diet. Hogsette & Washington (1995) observed that the development and survival of the *O. aenescens* larvae were higher in more protein diets. Nash & Chapman (2014) showed that the larvae developed significantly slower when reared on a low-protein diet.

Survavial

Larval nutrition influences pupal mortality in our studies. In this study, percent emergence for adults and larval survavial produced from the wheat bran diet was higher than other diet but the development time of larvae reared on wheat bran diet was longer than other diet. Larval survival was greatest on the high carbohydrate diet. Our result show that carbohydrate is important component housefly larval and pupal survavial. Wheat bran diet is most effective nutritients for *M. domestica* Linnaeus, 1758 development. Larval and pupal

survavial are high with wheat bran diet. Other effective nutritients for *M. domestica* Linnaeus, 1758 are soybean meal, blood meal with wheat bran diet and poultry feed with wheat bran diet. In this study, percent emergence for adults 88% produced from the wheat bran diet was higher than other diet. Hogsette & Washington, 1995; Simon, Krüger, & Ribeiro, 2011; Van Broekhoven, Oonincx, Van Huis, & Van Loon, 2015 showed that the survival of the larvae was higher in diets with more protein.

We record fastest development and second greatest survival rate when larvae of *M. domestica* Linnaeus, 1758 were reared on a soybean diet.

Weight

The results from the present study also demonstrate that impact of diet affects the pupal and adult weight. Pupae reared on high protein diet (blood meal and mix of blood meal and wheat bran) had significantly higher mean weights than all other treatments. Both diets had a significant effect upon mean adult weight. Adult weight from larvae reared at fish meal diet (other high protein diet) and soybean (high carbonhydrate diet) were high (Table 4). Chicken meal diet were negatively effect on pupal weight. Hogsette, 1992 showed that pupal weight from larvae reared at high protein diet heavier than from larvae reared at low protein diet. Nash and Chapman (2014) showed that carbohydrate also had a significant effect on pupal weight. Green et al., (2003) show that high protein contect affect pupal weight. Other high pupal and adult weight are recorded at larva reared on wheat bran diet. The adult body size is positively correlated with adult fecundity. larger bodies of females had the largest ovaries (Gobbi, Martinez-Sanchez, & Rojo, 2013).

CONCLUSION

Results for larvae reared on the ten different diet revealed that additional information on the life history of this species. This study might not be representative of the response of wild house fly colonies or colonies established from different populations around the world. Variety diet significantly affected *M. domestica* Linnaeus, 1758 ability to develop with respect to developmental rate, size of larvae, and mortality. In conclusion Wheat bran diet is most efficient for mass rearing of *M. domestica* Linnaeus, 1758 and reduce the cost.

Soybean meal, Mix of poultry feed and wheat bran and blood meal and wheat bran diet are other affective diet. Further research is necessary to understand the nutritients contributions of adult lifespan and fitness and optimizes black soldier fly production.

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Table 1. Nutrient content of ten different diet.

Quantity/100 g	Protein (g)	Carbohydrate (g)	Protein:carbohydrate
Wheat bran and milk	21.8	75	1:3.4
Fish meal	65	1	1:0.015
Poultry feed and wheat bran	10.71	29.31	1:2.7
Fish meal and wheat bran	57.4	11	1;0.19
Fermented	18.4	60.5	1:3.2
Blood meal and wheat bran	67.8	11	1:0.16
Soybean meal	44	24.4	1:1.8
Blood meal	80	1	1:0.012
Meat bone meal	45	2	1:0.044
Chicken meal	58	2.5	1:0.043

Table 2. Larval and pupal development.

Diet	Larval Development (Mean ± Std. Error)	Pupal Development (Mean ± Std. Error)
Wheat bran and milk	6.0 ± 0.44	5.0 ± 0.15
Fish meal	5.5 ± 0.44	6.5 ± 0.44
Poultry feed and wheat bran	4.0 ± 0.22	5.0 ± 0.35
Fish meal and wheat bran	5.0 ± 0.44	5.0± 0.31
Fermented feed	5.0 ± 0.44	5.0 ± 0.31
Blood meal and wheat bran	5.0 ± 0.44	4.0 ± 0.31
Soybean meal	5.0 ± 0.44	4.2 ± 0.25
Blood meal	5.0 ± 0.44	4.0± 0.15
Meat bone meal	6.0 ± 0.44	5.0 ± 0.31
Chicken meal	6.0 ± 0.44	5.0 ± 0.31

Table 3. Pupal and larval survavial.

Diet	Larval Survavial		Pupal Survavial	
	n	%	n	%
Wheat bran and milk	118	94	110	93
Fish meal	70	56	57	81
Poultry feed and wheat bran	84	67	45	53
Fish meal and wheat bran	50	40	40	80
Fermented feed	71	57	63	88
Blood meal and wheat bran	85	68	77	90
Soybean meal	91	73	78	85
Blood meal	10	8	5	50
Meat bone meal	21	17	19	90
Chicken meal	5	4	4	80

Table 4. Pupal and adult weight.

Diet	Pupal Weight (Mean ± Std. Error)	Adult Weight (Mean ± Std. Dev.)	
		Male	Female
Wheat bran and milk	177.00 ± 15.6622	24.1667 ± 11.25022	25.0645 ± 8.89072
Fish meal	174.51 ± 9.4070	32.1304 ± 7.70067	28.2059 ± 12.30861
Poultry feed and wheat bran	96.714 ± 5.05147	23.1071 ± 8.76161	21.5294 ± 9.44138
Fish meal and wheat bran	129.47 ± 8.38310	20.2083 ± 7.18077	25.9375 ± 3.21390
Fermented feed	145.436 ± 6.10369	21.8750 ± 9.53586	19.6522 ± 8.66869
Blood meal and wheat bran	202.816 ± 7.69256	32.7576 ± 8.09718	35.7500 ± 12.06595
Soybean meal	169.61 ± 7.54490	29.4474 ± 8.18931	32.6250 ± 7.66506
Blood meal	200.500 ± 6.18466	35.3333 ± 3.05505	38.0000 ± 1.41421
Meat bone meal	129.28 ± 8.98097	20.4545 ± 10.38618	20.3750 ± 9.08590
Chicken meal	59.000 ± 14.97665	9.3333 ± 7.37111	14.0000 ± -