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Household Bio Waste, Chicken Manure and Dog Stool Consumption of Various Fly Species

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Abstract

Bio-waste conversion is an important global environmental issue. Household bio-waste, poultry manure and dog stool constitute a considerable percentage of the produced bio-waste. Flies such as *Hermetia illucens* (L.) and *Musca domestica* (L.) are largely used in the conversion of household bio-waste and poultry manure. So far, no flies have been used in conversion of dog stool. In this study, effectiveness of conversion of such biowastes of different flies, namely *Calliphora vicina*, *Lucilia sericata*, *Musca domestica, Fannia canicularis* (L.), and *Sarcophaga argyrostoma* are investigated and contrasted against the reference fly, *Hermetia illucens*. In household bio-waste conversion, *Hermetia illucens* and *Sarcophaga argyrostoma*, and in dog stool conversion *Hermetia illucens* and *Musca domestica* have been found to be effective. Conversion of poultry manure mixed with cattle lung by four fly species has been found to be moderate.

Keywords: Conversion • Household bio waste • Chicken manure • Dog stool • Fly maggot

Introduction

Nutrition need of the humans increase in correlation with the population growth and the bio-waste problem stemming from the increasing nutrition production aiming to cover the increased need is also growing. The elimination of the accumulating bio-wastes has become one of the primary environmental problems of the World. According to various resources, the waste produced per year is 1.5-2 billion tons and 0.74 kg/person-day and 30% percent of this waste are bio wastes which is estimated to be duplicated by the year 2050 [1]. Even though it is a renowned method, fly usage in bio-waste control has not become a widespread implementation. Each year over 60 billion chicken is being slaughtered to be consumed and poultry production has reached 125 billion metric tons in the year 2018. Poultry production necessitates regular waste control and the production should conform to environmental measures and legal regulations [2]. The dog, *Canis lupus familiaris*, is a mammalian species which exists in all the geographies where humans live. The dog, as a result of its over ten-thousand-years relationship with the humans, is being elevated for different purposes and depends on humans for nutrition and elimination of its stool [3,4]. A 30 kg dog produces approximately 123-250 kg of stool yearly. Even though there are products such as biodegradable poop bags, flushable poop bags, outdoor flushing, waste digesters, composting and worm farming for the dogs owned by humans, there are no methods for the elimination of the stools of stray dogs and those which live in animal shelters and stool conversion through fly larva consumption has not been used for dog stool management [5-10].

The order Diptera is one of the largest within the class Insecta and one of the most widespread insect group in the World; more than 350 fly species dispersed in 29 families are related to food borne diseases and more than 50 synantropic fly species are related with unhygienic conditions [11]. Some fly species, apart from disturbing the humans, are held responsible for being the transmitters of various diseases as mechanic and biologic vectors. In spite of this bad reputation, the fly which is sensible to ecological changes have an “environment friendly” role in the elimination of human biowastes and animal carcass [12,13]. The view that flies transmit disease-causing microorganisms to humans is losing its accuracy; recent researches have shown that *Musca domestica* maggots (L.) render the animal faeces and household wastes contaminated with harmful pathogens aseptic [14]. It has also been reported *that Hermetia illucens* maggots clean the common *Escherischia coli*, *E. coli* O157:H7, *Salmonella enterica, Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in animal excrement. Another characteristic of this fly is that it consumes almost all the volatile emission in organic wastes [15,16].

There are adequate publications on the household waste conversion by *Musca domestica* and *Hermetia illucens*, however no study has been carried concerning the *Sarcophagidae* species [17]. No other fly species have been reported to consume chicken manure apart from *M. domestica* and *H. illucens*. There are no publications

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on dog stool conversion either [18-21]. This study aims to determine the ratio of; household waste consumption of *H. illucens* (*Diptera*: *Stratiomyidae*) used in biowaste and poultry manure control and also the consumption by *S. argyrostoma* species (*Diptera*: *Sarcophagidae*) maggots; 5% and 15% cattle lung mixed chicken manure consumption of *S. argyrostoma, M. domestica* (*Diptera: Muscidae*), *L. sericata* (*Diptera*: *Calliphoridae*) and *C. vicina* (*Diptera*: *Calliphoridae*) in comparison with *H. illucens*; and pure dog stool consumption of *M. domestica, L. sericata* and *C. vicina* in comparison with *H. illucens*.

Materials and Methods

This study has entirely been carried in Hacettepe University, Biology Department, Biocriminal Entomology Laboratories in Ankara, Turkey, between February-December 2019. The fly species belonging to the order *Diptera* used in the study are, *Hermetia illucens, Musca domestica, Calliphora vicina, Lucilia sericata* and *Sarcophaga argyrostoma*. These species, except the *Hermetia illucens*, have been captured with bait traps in the Hacettepe University Beytepe Campus. The Hermetia illucens species have been elevated in the laboratory from the eggs procured from a producer in Kocaeli province [22-25].

Bait traps made out of plastic bottles have been used to capture the flies used in the study. Two plastic bottles one of which is 1.5 lt and the other ½ lt were attached mouth to mouth to one another with scotch tape and the bigger bottle was cut lengthwise in the shape of “I”, then the slit was slightly bended inwards [26]. The yellow scotch tapes stuck on the two sides of the slit was used as a means of visual orientation. A cube-sugar was stuck on the bottom of the smaller bottle (the top of the trap) with water based wood glue, and attractant substances on which different fly species prefer to lay eggs on such as cattle liver, cattle lung, dog stool, blood, *Homo sapiens* urine and rotten egg were placed on the bottom of the trap (the bottom of the bigger bottle) [27]. The fly which entered the bigger (bottom) bottle through the slit flew towards the sugar and was trapped in the upper bottle [28].

The collected flies were brought to the laboratory for identification, anesthetized by being kept in a deep freezer in -18ºC for not more than 1 minute (e.g. *C. vicina*) and have been identified using the appropriate keys. Male genitalia and chaetotaxy (distribution of the setae) were also used in the identification of *Sarcophagidae* spp.[29]. The fly rearing was made in cages of 60 × 60 × 60 cm inside fly mesh. A 15 cm diameter hole was opened on one side of the cage and a 50 cm long, 15 cm diameter cylindrical fly mesh was stuck around the hole in order to keep and maintain the flies inside the cage. The adult and larvae rearing of *Calliphora vicina* started up at February 2nd inside the Sanyo MIR-253 incubator at 22 ± 1°C, and the rearing of the entire fly species were made in a room temperature of 27 ± 5°C controlled with an air conditioner during summer and egg hatching was made possible [30]. Because adult flies were captured in their natural apparition period, it was possible to rear and reproduce them under the above mentioned room temperatures. Adult flies were fed with a mixture of 50% milk powder +50%granulated sugar prepared in petri dishes [31,32].

These mixtures were weekly renewed. The adults were continued to be fed after the oviposition. 1.5 lt plastic bottles were cut into half and the bottom parts were used as brood containers. The brims were left open to avoid the larvae from suffocating. Glycerine was applied on the brims of the containers in order to prevent the hyperactive larvae to escape [33]. The forage amount placed in the brood containers were recorded with a Sartorius TE214S microbalance, prior to and following the consumption [34]. The forage was daily humidified with a water spray. In order to prevent over-humidity, litter was laid under the forage. Household wastes of maximum one-day old, pure chicken manure or manure+cattle lung or dog stool were weighed and placed inside the brood containers. The dog stool was obtained from the authors’ own dogs, fed on dry dog pellet [35,36]. Chicken manure was obtained from a small-scale farmer family rearing open air broiler and hen (fed on standard chicken food). Cattle liver and lung were bought from the authors’ neighborhood butcher. Household waste was produced by the authors (rice mixed with other kitchen wastes) [37]. These materials were collected after being excreted by the respective animals, kept in hermetically closed plastic boxes and were used the following day. The egg numbers oviposited in brood containers, were considered to be self-balanced ecologically, corresponding to the amount of food to be consumed in these containers [38]. More eggs were laid in the containers which contained more food. Supposedly the laid egg amounts vary from one species to another. Therefore, the observations were assumed to be done according to optimal larval numbers, depending on the species then the broods were assumed comparable [39]. The observations were initiated with the appearance of the first instar larvae and ended when the third instar larvae entered the post-feeding or wandering phase. A random sample group selected within these postfeeding larvae which were drained in tap water in room temperature and killed by being left in boiling water for 30 seconds and a second species identification with key was done [40]. A Leica zoom 2000 stereo microscope was used for species identification. When the observations were completed, the remaining larvae were either released to nature in the appropriate season or transferred to cages following the pupation or adult emergence.

In statistical analysis, a non-parametric statistical test was used in order to compare the nutrition consumption ratio of each observed fly species with the nutrition consumption ratio of the reference fly [41]. For all statistical analysis, EpiInfo 7.2.0.1. v2016 StatCalc and Windows 2010 calculator programs were used. The results of the statistical analysis were presented with *Chi-square*, Degree of Freedom (df) and p values. The consumption percentages were given in two digits after the comma, *Chi-square* and p-values were given in three digits after the comma. In the tables, Weight Prior to Consumption and Weight Following Consumption were represented as gram; the consumption rates as percentage were given in two digits after the comma [42].

Results

Household biowaste consumption by *Sarcophaga argyrostoma*, 5% and 15% cattle lung mixed chicken manure consumption by *Sarcophaga argyrostoma*, *Musca domestica, Lucilia sericata* and *Calliphoravicina*, and dog stool consumption by *Calliphora vicina, Lucilia sericata* and *Musca domestica* have been observed. These observations were contrasted one by one with *Hermetia illucens* consumption rates of each item [43].

Species	S. argyrostoma	H. illucens
Before consumption weight (g)	80	150
After consumption weight (g)	12	0
Consumption(%)	85	100

Table 1. Household bio waste consumption by the *S. argyrostoma* and the reference fly *H. illucens*.

Table 1 demonstrates that biowaste consumption of *S. argyrostoma* (85.00%) is lower than the reference fly *H. illucens*' consumption (100%) in a statistically significant manner (*Chi*-square=17,911 p<0.001 for df=1).

Species	S. argyrostoma	M. domestica	L. sericata	C. vicina	H. illucens
Before consumption weight (g)	80	80	80	80	150
After consumption weight (g)	52	50	49	53	35
Consumption (%)	35	37.5	38.75	33.75	76.66

Table 2. 5% cattlelung+95% chicken manure mix consumption (Reference fly *H. illucens* consumed 100% chicken manure).

Table 2 demonstrates that 5% cattle lung mixed chicken manure consumption of S. *argyrostoma* (35.00%), *M. domestica* (37.50%), *L.sericata (38.75%) and C. vicina (33.75%) is lower than the 100%* chicken manure consumption of the reference fly *H. illucens* (76.66%) in statistically significant manner (respectively *Chi-squares*=16.218, 14.799, 14.098, and 16.935, df=1 and p<0.001 for all).

Species	S. argyrostoma	M. domestica	L. sericata	C. vicina	H. illucens
Before consumption weight (g)	48	48	48	48	150
After consumption weight (g)	18	19	20	18	35
Consumption (%)	62.5	60.41	58.33	62.5	76.66

Table 3. 15% cattle lung+85% chicken manure mix consumption (Reference fly, *H. illucens* consumed 100% chicken manure).

Table 3 demonstrates that 15% cattle lung mixed chicken consumption manure by *S. argyrostoma* (62.50%), *M. domestica* (60.41%), *L. sericata* (58.33%), and *C. vicina* (62.50%) is slightly lower than 100% chicken manure consumption by *H. illucens* (76.66%) at the borders of statistical significance (respectively *Chi-squares*=2.038, 2.066, 3.217, and 2.038, and p=0.081, 0.058, 0.040, and 0.081; and df=1).

Species	C. vicina	L. sericata	M. domestica	H. illucens
Before consumption weight (g.)	167	90	207	622
After consumption weight (g.)	167	51	81	42
Consumption %	0	43.33	60.86	93.24

Table 4. *C. vicina, L. sericata, and M. domestica pure dogstool consumption versus H. illucens* pure dog stool consumption.

Table 4 demonstrates that pure dog stool consumption by *C. vicina* (00.00%), *L. sericata* (43.33%), and *M. domestica* (60.86%) is lower than pure dog stool consumption by the reference fly *H. illucens* (93.24%) in statistically significant manner (respectively *Chi-squares*=256.024, 101.382, and 84.847, and df=1 and p<0.001 for all).

Discussion

The main goal of our study was to find out whether the selected fly species consumed household waste, pure chicken manure, cattle lung-chicken manure mix and dog stool or not and if so, to identify their consumption rates [44]. Two of the wastes used in our study, namely household waste and chicken manure, were previously tested with some of the fly species which were also used in our study. However, effective consumption of household wastes by a *Sarcophagidae* species was observed for the first time in our study

[45]. Consumption of the third waste, namely the dog stool, used in our study, by *Hermetia illucens, Musca domestica, Lucilia sericata*, and *Calliphora vicina* species was again observed for the first time in our study [46]. As demonstrated in Table 1, the observations began with 80 gr of household waste per *Hermetia illucens* and *Sarcophaga argyrostoma*. Because the *Hermetia illucens* consumed all types of wastes without having completing their development, the amount of nutrition was augmented, reaching up to 150 gr. When the consumption rates of these two species were compared it has been observed that *Hermetia illucens* consume 100% of the household waste, whereas *Sarcophaga argyrostrama* consume 85% of the waste [47,48].

The consumption difference between the two species is statistically significant ($p<0.001$), yet the consumption rates of both species can be considered high. The consumption rate of *Sarcophaga argyrostoma* is almost as high as the *Hermetia illucens*' and it has a short larval development duration; these factors can be a significant advantage and might increase the potential to widely use the *Sarcophaga argyrostoma* in household waste conversion [49,50]. Cattle lung and chicken manure mix have been given to 4 different fly species in 2 different mixture percentages. The reference fly *Hermetia illucens* were fed on pure chicken manure. The first of these mixtures is 5% cattle lung +95% chicken manure mix (Table 2), and the second is 15% cattle lung +85% chicken manure mix (Table 3) [51]. Both mixtures have been given to *Calliphora vicina, Lucilia sericata, Musca domestica* and *Sarcophaga argyrostoma* species. The mixture containing a higher percentage of chicken manure, which can be seen in Table 2, was consumed by all the species mentioned above [52]. For each species 80 gr of nutrition was prepared initially. With the exception of *Hermetia illucens*, the other four species' consumption rates were similar and varied between 33.75% and 38.75%. Whereas the *Hermetia illucens* consumed 76.66% of the pure chicken manure, which is almost two times the consumption of other four species. In the second phase, 48 gr of mixture was given to the same species (Table 3) [53]. This time again, it has been observed that the consumption of four species, with the exception of *Hermetia illucens*, were similar and varied between 58.33% and 62.50%. Whereas pure chicken manure consumption rate of *Hermetia iluucens* is 76.66% as mentioned above. Augmentation of the cattle lung percentage by 10% or reduction of chicken manure percentage by 10% has almost doubled the consumption rate of the four species. This study has proven for the first time that two *Calliphoridae* and one *Sarcophagidae* species consume cattle lung+chicken manure mixture. It has been concluded that these species are successful in consumption of cattle lung mixed chicken manure.

Consumption of dog stool by fly larvae was observed for the first time in our study. Ali Khan et al. and Patricia and Claudio explored the effect of different livestock manures-including dog stool on the fitness, development and mortality of *Musca domestica*. Table 4 shows the dog stool consumption rate of the 4 selected fly species *Calliphora vicina, Hermetia illucens, Lucilia sericata* and *Musca domestica*, from egg hatching to pupation. Because the *Sarcophaga argyrostoma* colony collapsed in the course of the observation, the study has been carried on with the four remaining species [54]. The observation began with the equal amount of nutrition, then reached to the final consumption amount by augmentation in different amounts for each species. *Calliphora vicina* eggs were hatched on pure dog stool however their larvae did not develop. The consumption amount was considered as zero and it has been concluded that dog stool is not appropriate for this species (Table 4). No comparison was made because there are no studies in the literature in regards to the dog stool consumption rates of different fly species. *Lucilia sericata* have consumed 39 gr out of 90 gr during its development. In another study *Lucilia sericata* larvae have been reported effective in chicken manure consumption, however no records have been found regarding their consumption of dog stool [55].

We evaluated their 43.33% dog stool consumption as a significant data. Even though this is the species which consumed less the dog stool within the species selected in our study, the consumed amount is worth consideration. *Musca domestica*, consuming 126 gr of the dog stool out of 207 gr with a consumption rate of 60.86%. This figure is one of the two highest figures observed. Finally, *Hermetia illucens* brood was added in intervals and 622 gr of dog stool was used in total. 580 gr of this amount was consumed by *Hermetia illucens* larvae and reached a very high consumption rate of 93.24%. With the mentioned dog stool consumption rate, *Hermetia illucens* was identified as the most successful species between the four observed species. According to the findings of our study, *Hermetia illucens* and *Musca domestica* are successful dog stool consumers. In our study, *M. domestica* consistently laid eggs on dog stool. There are numerous studies on the biowaste conversion capacity of *M. domestica*. It has been reported that it degrades 75% of raw livestock manure; and between 35% and 18%-65% of dry manure and produce high quality protein [56]. Other studies have reported *M. domestica* appropriate for use in livestock farms due to its livestock manure conversion capacity. We concluded that *M. domestica* is an efficient biowaste convertor. *Hermetia illucens* have been reported to consume 33-58% of cattle manure, 50% of any kinds of excrements, and 50% of the chicken and swan excrement weight. Our study has reported a value much higher than these figures reported in the literature in regards to chicken manure consumption. *Hermetia illucens* is a useful fly which colonizes the decaying organic substances in all temperatures and tropic climates all around the World and plays an important role in dissolving the organic substances. This fly, as an efficient biowaste conversion agent, apart from consuming animal excrements with the condition of being fresh, is also known for suppressing the *Musca domestica* (*Diptera: Muscidae*) population by rivalry, therefore has been used as a biologic agent for the control of house flies. *Hermetia illucens* larvae are mainly elevated as manure convertors and livestock forage. According to the findings of our study, *Hermetia illucens* was reported for the first time as consumers of dog stool in big amounts.

Conclusion

Hermetia illucens efficiently consumed all types of bio wastes used in our study. As has been proven for the first time in this study, it is also an efficient consumer of dog stool with the sole disadvantage of having a long life cycle. Again, as has been shown for the first time in this study, *Sarcophaga aryrostoma* species have also been reported as an efficient household bio waste convertor. Our study has revealed that *Musca domestica* is also an efficient dog stool consumer, however the nutritional habits of the adult members of this fly species and its potential as a pathogen transmitter, might limit its span of use. The aim of this study was to qualitatively determine whether the above mentioned fly species consume dog stool, household waste and chicken manure or not. Several other ecological and fly fitness parameters such as the type of nutrition, room temperature, can be analyzed in further studies.

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