The Effect of Antibiotic Treatment in Agriculture Production on Fly (Diptera) Attraction

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Abstract: The use of antibiotics in agricultural production has become a topic of growing concern, as these drugs have facilitated the growth of antibiotic resistant bacteria. Flies have a tendency to mechanically transmit these bacteria, causing potential health problems for humans. It is a benefit to know the demographics of such flies in order to prevent contamination. Organic livestock is typically raised without antibiotics and has some recorded health benefits. It was hypothesized that organic raised chicken breast will attract more flies than non-organic raised chicken breast. Three of each type of chicken breast were placed outside for a week. Adult Diptera were collected, killed and identified. Phormia regina, Cochliomyia macellaria, Lucilia sericata and a Musca species were identified. There was not a statistically significant difference in the number of flies collected or species breakdown between the two chicken breast types. Many variables could have skewed the results. The null hypothesis was accepted that there was no direct correlation between fly attraction and raising of chicken breast, however it was concluded that further research should be conducted with greater control over the experiment for future investigation.

Keywords: chicken, organic, non-organic, entomology, carcass, antibiotics, flies, Diptera, pathogen

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It is well known that flies are of an annoyance to most people and are therefore not wanted. The fact that flies have the potential to carry many different diseases is more of a reason why flies are so undesirable to have around. Unfortunately, they are commonly found in both houses and farms. For this reason, it is important to be aware of the type of diseases that most flies carry. A study was conducted by the Centre of Biotechnology and Fine Chemistry in Portugal to see what kind of foodborne diseases could be spread by *Musca domestica* (house fly) (Neideregger et al. 2013). The presence of *Escherichia. coli*, Enterobacteriaceae, and *Staphylococcus* were found on flies collected from a variety of different areas including rural ones (Neideregger et al. 2013). A percentage of these flies also showed evidence of antibacterial resistance, especially in flies found in close proximity to animals such as those in farms (Neideregger et al. 2013).

The increased use of antibiotics in agricultural production has resulted in some unintentional consequences. One such consequence is an increase in antimicrobial resistance among pathogens of both medical and veterinary importance. This consequence can be attributed to the overuse of antibiotics which has resulted in the development of pathogenic bacterial strains fit to survive antibiotic administration. An additional concern is the role of insects, such as flies and cockroaches, in the transmission of antibiotic-resistant zoonotic bacterial pathogens directly to agricultural products (Mohammed et al. 2016, Zurek et al. 2014). Thus, not only is it crucial to analyze the insect's role in the development and transportation of these antibiotic-resistant pathogens, but it is also imperative that these emerging drug-resistant strains of zoonotic pathogens are identified in order to develop new effective prevention methods. A crucial step to effective prevention is to survey and identify common vector Diptera in the area under concern, which can be done by collecting and analyzing Diptera in locations where food animal production is centered.

In order to test the behavior, nutrition and drug administration, the method of attraction of flies must be accounted for. Studies have shown that flies allocate strong behavioral responses in chemicals of fermenting fruit. Artificial stimulation can be used in order to see what type of pheromones the flies will be selective to. There has been evidence that flies react to a specific type of odor. Data has been found that pheromones related to food and mating are what mostly attract flies (Depettris-Chaucin et al. 2017).

As the human population grows, there is an increasing need to improve the quality of food intake. Thus, emerged antibiotic treated foods. Strict organic diets tend to diminish exposure of antibiotic-resistant bacteria and pesticides. Fertility, stress, longevity of life, and resistance to starvation levels were tested on *Drosophila melangoster* ingesting organic foods in a study done by (Bauer et al. 2013). The results indicated that there indeed were positive

benefits to the health of the flies when only fed organic diets. Likewise, a study done by Neideregger et al. claimed that both *Lucilia sericata* and *Calliphora vicina* best developed on processed meat like pork (Neideregger et al. 2013). However, *C. vicina* was more of a generalist when it came to the food substrate of preference (Neideregger et al. 2013). Overall, several Diptera were found to have a better development time on processed meats over unprocessed meats (Neideregger et al. 2013). Most importantly, the most common antibiotic food treated item is raw poultry. Although, the new treatments helped reduce bacterial related infections in the chickens, the use of these antibiotics also opened the door to new strains of drug resistant bacteria.

Not only is this a problem for the general public, but it is also a rising concern for public health officials. In response, there have been many studies conducted to pinpoint exactly what the problem is with antibiotic treated substances (Landers et al. 2012). As the study by Landers et al. mentions, antibiotic use on livestock production remains commonplace resulting the prevalence of antibiotic resistant bacteria to raise concern. In addition, Graham JP et al.

discovered that both enterococcal and staphylococcal antibiotic resistant isolates were identified in flies near broiler poultry operations (Graham et al. 2008). This raises suspicion that these bacteria may spread and increase exposure to humans, resulting in undesired health effects. However, in another study performed by Sapkota et al., it was suggested that chickens raised by organic practices had lower levels of incidence of antibiotic resistance enterococcus (Sapkota et al. 2011). Salmonella, a pathogenic bacterium was found carried by sarcophagi dipterans in a study done by Russel et al. (Depettris-Chaucin et al. 2017). It is helpful to discern which flies may be carrying the bacteria, and whether organic poultry tends to attract less of these disease-carrying agents.

The following research sought to determine the effect of organic raised and non-organic raised chickens on fly attraction. It is important to discover the effect of antibiotic treatment in agriculture production in order to see the most efficient turnout on fly attraction. The data from this study could reveal whether or not the use of antibiotics has an effect on the level of attraction in flies or specific fly species, thus determining if there will be a difference in pathogen transmission due to the level of contact with vectors. From the literature on antibiotic resistance, treatment, and the effect of diet on fly development, it was hypothesized that organic raised chicken will have a higher yield of fly attraction than non-organic raised chicken.

Materials and Methods

Three organic raised chicken breast (Great Value Bentonville, AR) and three non-organic chicken breasts (Great Value Bentonville, AR) were brought from a grocery store. These were weighed (Ozeri San Diego, CA) to make sure they were approximately the same size, about 0.6 to 0.8 ounces. These chickens were approximately a couple days old before placement. The chicken was kept in a cooler until placed in a fielded area behind the Easterwood Airport in College Station, Texas. The fielded area had some pine trees around it that may provide shade and opened nearby a dirt path. However, environmental data was not needed as all the pigs were placed in the same open area. There was no testing that would require the presence of environmental data. Chicken placement was randomly decided by a random number generator (www.random.org). The chickens were placed in a 2 by 3 grid. Each cage of chickens was 20 feet apart from the next one from the center of the cage. The cages were made using chicken wire (Acorn International Memphis, TN). Each cage had a top, bottom, and sides with dimensions of 2 by 3 feet. Each chicken had a wire mesh cage placed on top of it with either bricks or large stones to hold it down. Bricks were placed in the non-organic chicken cages while stones were placed in the organic cages as markers. This was used to prevent any scavengers or predators from taking the chicken.

The chickens were then left out for one week before returning. Before removing the cages, a sweep net (BioQuip Rancho Dominguez, CA) was performed 5 times in succession over the cage. Afterwards the cage was removed and more sweep nets were performed until 45 flies were captured from each type of chicken (organic or non-organic).

Any captured Diptera were placed in plastic bags or vials and were then later frozen to kill. Once dead, the rest of the flying insects were placed into vials. Each vial was labeled with the cage it was taken from. No other information was added as the samples were all taken at the same time with equal help from the team. There were no larvae to be collected at any cage. The insects were taken back to the lab where an Olympus AZ51 microscope was used. Using the Keys to the genera and species of blow flies (Diptera: Calliphoridae) (Whitworth 2006), the flies and larvae were identified down to species. Results were then determined. These analyzed results would be what species were present, the number of present species, and any significant differences between the two chicken types. This was performed by doing a two-way ANOVA test using Microsoft Excel. In order to perform this data analysis, the abundance data was changed into Log # + 1.

Figure 1: The view of the chicken and cages as seen from the back end looking towards the site entrance.

Figure 2: The view of the chicken and cages as seen from the dirt path.

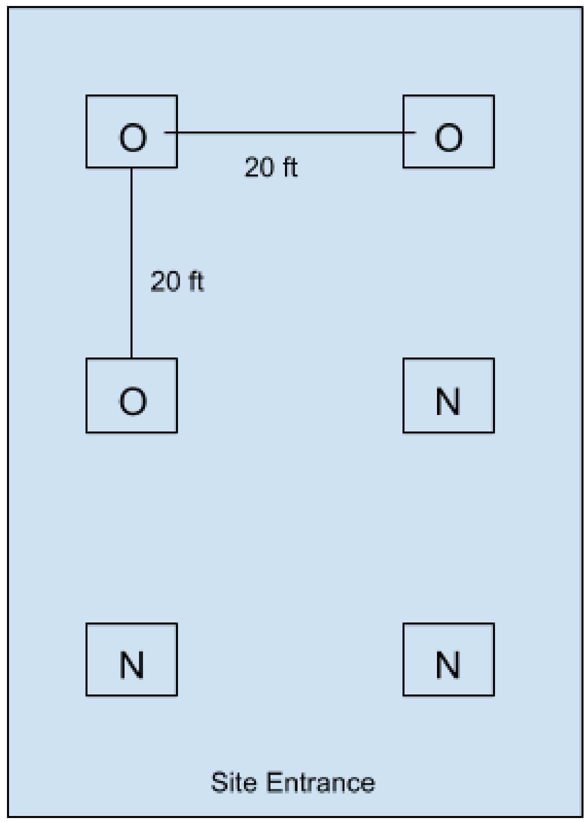

Figure 3: Where each of the different cages were placed, how far, and what type of raw chicken they contained. N is non-organic and O is organic. The dirt path is to the left.

Results

Of the 51 flies collected from the organic type of chicken, 3 species of diptera were identified. This consisted of 41 *Phormia* *regina*, 8 *Cochliomyia macellaria*, and 2 *Lucilia sericata*. These same species were identified in the non-organic type chicken collection where 43 flies were collected. However, an additional species of diptera was also identified. This collection was made up of 34 *Phormia regina*, 7 *Cochliomyia macellaria*, 1 *Lucilia sericata*, and 1 *Musca* species. *Phormia Regina*, the black blow fly, was evidently the most encountered dipteran species in both collections with a higher occurrence of this species found on or in close proximity of each chicken.

Only dipteran adults were trapped due to the absence of fly larvae in both the organic and non-organic chicken breasts. Coleopteran adults were seen to be occupying both types of chicken, which could be a reason for the lack of fly larvae seen as the various coleopterans were using the chicken for their development and so they did not allow competition internally. The strong colonization of coleopteran adults and immatures did not prevent adult dipterans from feeding and congregating on the chicken breasts provided, enabling the collections to be completed.

Figure 4: The flies identified from the brick/non-organic chicken (top half) and stone/organic chicken collections (bottom half). Top: Starting from the top, rows 1-3 are Phormia regina, row 4 is Cochliomyia macellaria, and row 5 is Lucilia sericata. Bottom: Starting from the very bottom, rows 1-3 are Phormia regina, row 4 is Cochliomyia macellaria, row 5 is Lucilia sericata, and row 6 is Musca species.

Due to the close nature of the experiment the (ANOVA) Analysis of Variance test was decided to be used. The test provides a further accurate grasp on the data by allowing for comparison of the data that was discovered from the species of Diptera that were found on the two different types of chicken breast. As show in the figure below the rows represents the non-organic and the columns represent the organic chicken. What was discovered was the difference between the abundance of different species was calculated to be the p value of (0.0376). In order for are data to be significant the p value had to be lower that (0.05). This means that there was significant difference in the abundance of different species between the types of chicken. This just points to the additional species collected from the organic type chicken. However, the p value (0.19588) determined between the types of chicken showed no significance.

In conclusion, the data further shows that there is no significant difference between the preference of most of the Diptera species identified. The only considerable finding was that there was one extra Diptera species that was found to prefer the non-organic chicken. These results could possibly show that there are species that show preference which could lead to the foundation for a future experiment to find out why.

	Phormia regina	Cochliomyia macellaria	Lucilia sericata	Musca spp.	Total Number of Dipterans Collected
Non-organic Type Chicken	34	7	1	1	43
Organic Type Chicken	41	8	2	0	51

Table a: Summary of results. The rows indicate the different treatments while the columns indicate the different Diptera species.

Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
Row 1	4	3.049218023	0.7623045	0.35217438		
Row 2	4	4.054613055	1.0136533	0.22106332		
Column 1	2	3.167317335	1.5836587	0.00313483		
Column 2	2	1.857332496	0.9286662	0.00130829		
Column 3	2	0.77815125	0.3890756	0.01550407		
Column 4	2	1.301029996	0.650515	0.24427953		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.126352396	1	0.1263524	2.74929491	0.19587759	10.12796449
Columns	1.581838763	3	0.5272796	11.4730478	0.03759746	9.276628153
Error	0.137874328	3	0.0459581			
Total	1.846065487	7				

Table b: The rows indicate the different treatments while the columns indicate the different Diptera species. As noted by the p value, the species are significantly different but not the treatments.

Discussion

Despite the difference in substrate treatment, the abundance of Dipteran collected really only varied in the total quantity that was able to be collected and not the species composition. As depicted in Table 2, the ANOVA analysis indicates that there is not a significant difference in species composition between the two treatments. This is apparent by the p-value not being less than 0.05, indicating that there is a lack of significant variance between the organic and non-organic treatments. Therefore, the null hypothesis is accepted: there is no significant difference between Dipteran species attraction to non-antibiotic treatment (organic) or antibiotic treatment (non-organic) chicken breast.

The absence of any significant variance between the treatment groups may be due to the proximity of each chicken breast. Due to the limited space available to set up and run the experiment, each treatment unit (scavenger prevention cage + chicken breast in a tray) was placed with a 20 feet radius from each other. Refer to Figure 3 for a graphic demonstration of the field set up. As such, this restricted space may have resulted in each treatment being too close to each other for sufficient measurement of Dipteran organic or non-organic treatment preference. Thus, the results could be due to the limited set-up and trial space rather than the Dipteran species actual preference or in this case, indifference. When performing this experiment, it is suggested that each treatment unit is placed farther away (at least 40 feet radius) from each other to determine if the distance between each chicken breast is what caused the flies to show no preference for a specific treatment. An adjustment like this would have improved the performed experiment.

Furthermore, the lack of variance could be a result of nonoptimal weather that occurred at the start of the experiment. As discussed in the methods, each chicken breast was placed in an open top tray for ease of set up. Although it was known that these open top trays did not impede diptera colonization, since colonization was observed soon after they were removed from their sealed containers, the trays did present an issue later on day 1. Approximately 3 hours after set up, a severe rain and hail storm occurred flooding the trays in a few inches of water. Thus, initial colonization that occurred earlier that afternoon may have been washed out by the flooding and heavy rainstorm the night of day 1 potentially skewing the results.

Since there were no statistical difference in Diptera colonization between the treatments, it can be inferred that there was no observable difference in the pheromones and/or odors expelled from the decaying organic and nonorganic chicken breast which could attract different species of Diptera. However, as stated earlier, the unexpected initial weather and the proximity of the treatment units could have contributed to the observed indifference. In addition, the results suggest that although there was not a type of treatment that a specific fly species was attracted to the most, the study indicates that there are many different species of flies that may be attracted to the possible chemicals or smells found in chicken breast.

Despite these events and potential disturbances to the study, the results remain far from complete insignificance. There may in fact be no difference in Diptera species attraction between inorganic or organic chicken breast as the results showed, but the results cannot be challenged unless similar studies are done. The methods for this study, as mentioned earlier, can easily be replicated for similar studies to repeat this study in the future. Then the findings can be compared to these replicate studies as a reference to determine if there is in fact no correlation between treatment groups.

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