

# **Fly Guys: Cultivation of Black Soldier Fly Larvae**

## **Winter 2016 Interim Report**

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# 1.0 Background

The cost of poultry feed is a growing problem for small poultry farms. Chickens need different feeds with varying nutritional values depending on their development stage. The cost of feed can add up, becoming an obstacle for farmers trying to maintain a small farm. Black Soldier Fly Larvae (BSFL) are a nutritious, low-cost supplemental feed for chickens.

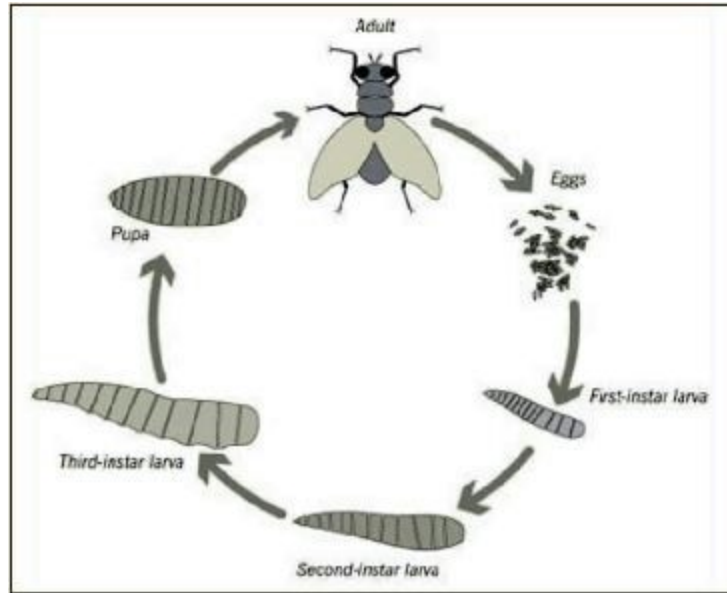
The objective of this project is to build a closed system that can breed, cultivate, and harvest the BSFL at sufficient quantities to reduce small farmer food costs. The closed system will allow farmers to control the inputs to produce the desired outputs of the system. To address potential pathogen issues, optimal temperatures will be identified. Overall, the goal of the BSFL system should be relatively sufficient, with minimal labor and cost.

The target clients are farmers who maintain small chicken farms consisting of 200-300 chickens. We want to implement a system for cultivating Black Soldier Fly Larvae (BSFL) as supplemental feed to their poultry. This alternative feed source must offset high feed costs that impact small farm budgets more than large farms. Since BSFL thrive on organic matter, the design consists of a system that will breed and cultivate BSFL using on-farm residues. Combining these aspects creates an integrated biosystem, having interdependency between the organic matter, BSFL, and chickens.

Although BSFL are a favorable substitute for chicken feed, there are a few adverse concerns farmers have to examine. Small-scale farmers need to take into consideration how much it will cost to start and maintain a BSF cultivator. To accurately estimate the cost, farmers need to consider how much their chickens eat and the costs to cultivate BSFL as a main source of food. Alternatively, the BSFL can be used in addition to chicken feed to reduce the cost of buying the standard feed. Farmers also need to take into account the labor needed to maintain the system in order to be an efficient and cost-effective process.

The amount of feed that chickens require depends on the chicken breed, flock size, and type of feed used. Chicks are typically fed commercial chick starter (found in 50 to 100 pound sacks at a feed store) for at least the first month [1]. The older they get, the greater amount of feed must be used. A single hen requires a half cup of whole grain to produce eggs and a half cup of grain for herself [2]. In the winter, chickens need to be fed twice as much per day. With all these considerations in mind, high feed costs emerge as a significant problem for small farmers who want to maintain their farm.






Preliminary research involves information on the BSFL life cycle and the duration in each cycle<sup>1</sup>. Figure 1 shows the five stages in the BSFL lifecycle.



**Figure 1.** Black Soldier Fly life cycle

The duration of each stage in the lifecycle, shown in Table 1, is critical for determining how long each step of the cultivating process should be. We also need to determine the temperatures of each stage in the life cycle that the larvae best thrive in to optimize production rate. This will be done through research and testing.

**Table 1.** Duration of each stage in the Black Soldier Fly life cycle

State	Size (mm)	Color	Picture	Duration (days)
1 instar	5	White/ yellow		15
2 instar	12	Light brown		15
3 instar	19	Dark brown		8
Pupa	19	Dark brown		10
Fly	20	Black		7

<sup>1</sup> *Assessment of Diptera: Statiomyidae, genus Hermetia illucens (L., 1758) using electron microscopy*

To reach the end goal, alternative solutions must be considered if certain components of the original project are not feasible. Other options can include changing the location of the system with respect to the coop, adding or reducing the number of sections in the larva production system, eliminating the Black Soldier Fly reproduction stage in the process, changing the shape of the prototype, and more.

## **2.0 Objectives**

Initially, our objective was to design and build a prototype containment vessel that can be used year-round in all weather conditions in which we would breed, cultivate, and harvest BSFL at a constant rate in order to provide a low cost food source for chickens in a small farm due to the high costs of chicken feed. Having an alternative feed that utilizes on-farm residues, such as chicken feces and rice hulls, for decomposition in the production of BSFL can offset costs to the farmer. Our specific objectives are to maximize production rate of BSFL and have the BSFL replace 25% of the current chicken feed for a small scale farm of about 200 chickens.

However, after conducting a feasibility analysis, it would be difficult to replace 25% of the feed. Because of this, we focused our objectives on maximizing the production of the black soldier fly larvae.

## **3.0 Design Criteria**

After brainstorming several potential designs, the team objectively analysed each using the Kepner Tregoe analysis, assessed the feasibility, and took into consideration standards to apply to each idea.

### **3.1 Kepner Tregoe Analysis**

In order to objectively decide on a final design, the team performed Kepner Tregoe analyses on the three potential designs. The results of these tests are separated into feedstock management, cultivation, and harvesting and are listed in the tables below.

Based on our KT-analysis, design 1 is our best option since it the highest weighted score and can easily be modified. Each design was ranked separately and then compared to the other options once completed. Scoring was on a 25-point basis, since our team of five members could each rank an individual aspect as being the best option (5) or the worst (1). The weight was

determined on a 1-10 scale, where we chose what was absolutely necessary (BSFL temperature control and integration) and what was optional (mesh cover). The higher the weighted score determined which out of the three was best. The score for each design ranked by the group was multiplied by the weight then summed to obtain the weighted score. For example, design 1 had a score of 21 in the size category. The score of 21 was multiplied by the weight of 8. Each category was and rank was multiplied respectively, and the products were summed. This method was used in Table 2, Table 3, and Table 4.

**Table 2.** Feedstock Management KT Analysis

<b>Properties</b>	<b>Weight</b>	<b>Design 1: Open lid on top, shovel in compost</b>	<b>Design 2: Mailbox opening</b>	<b>Design 3: Pull out drawer</b>
<i>Constraints (needs)</i>				
Size	8	21	13	18
Temperature control for BSFL	10	13	22	14
Weatherproof	9	19	20	19
Integration	10	17	18	15
<i>Features/ Attributes (wants)</i>				
Capacity for organic waste	3	20	14	17
Accessibility for organic waste	6	19	11	21
Mesh/wire cover	1	10	18	12
Durability	6	16	19	16
Cost	5	19	17	17
Easy to work with/clean	6	18	16	19
<b>Weighted Score</b>		<b>1122</b>	<b>1105</b>	<b>1089</b>

For the feedstock ensiled design, three different alternatives were considered. Design 1 is the simplest alternative with the lid on top, requiring to have just the lid on the bucket. While this design is feasible, it would be difficult to control the temperature for the larvae, and adding too much organic waste on top could cause the entire system to collapse if not built correctly. The mailbox opening considered as the second alternative would reduce the capacity of waste placed into the integrated system but would also be better sealed than the other two options. The third design would allow for easier access and visibility of the amount of organic waste in the system

but would be more difficult to integrate with the cultivator and harvester components. However, since all resulting weighted scores were relatively similar, no absolute decision could be determined. The type of feedstock chosen would ultimately depend on the design of the cultivator.

**Table 3.** Cultivator KT-Analysis

<b>Properties (Features, Attributes, Constraints)</b>	<b>Weight (1-10)</b>	<b>Design 1</b>	<b>Design 2*</b>	<b>Design 3</b>
		FilterBug	Wood, rectangular prism, translucent box, multi-sections, auxiliary ramp	Wood, rectangular prism, single harvesting area, 1 ramp, breeding area, (similar to Design 2) [Manual larvae reproduction]
<i>Constraints (needs)</i>				
Size	5	15	19	18
Weight	7	18	16	16
Temperature for BSFL	10	13	19	19
Weatherproof	6	13	16	17
Humidity	8	9	18	17
Integration	10	3	20	20
8-9 inches deep of organic waste	8	15	22	23
Light for Breeding	7	6	19	21
<i>Features/ Attributes (wants)</i>				
Durability	4	17	17	18
Cost	3	18	12	15
Ease of Maintenance	6	15	12	13
Drainage system	2	22	13	14
<b>Weighted Score</b>		<b>929</b>	<b>1348</b>	<b>1384</b>

For the cultivator design, three different designs were considered. The FilterBug is a homemade device that relies on the capture of wild Black Soldier Flies to maintain its BSFL population. The second design is a simple wooden box, featuring an attached translucent plastic breeding container into which the BSFL would crawl upon maturation, while some larvae would crawl into a harvesting area. The third design is similar to the second design, however, the BSFL would not crawl into the breeding chamber, but into one harvesting area, where they would be manually separated into groups for feed and for reproduction to maintain the BSFL population.



The second and third designs scored very similarly, as they shared the same basic principles. Based on whether there was an addition breeding chamber attached (design two), the durability, ability to control humidity, cost, ease of maintenance, and other aspects changed slightly. While the FilterBug was better than the other designs in some aspects, such as drainage and cost, its difficulty both to control temperature and to integrate with the rest of our designs ultimately caused us to reject it. As for the choice between the remaining two designs, because they were fundamentally very similar, the difference in available light for breeding and the slight advantage in cost and ease of maintenance played a large role in selecting design three.

**Table 4.** Harvester KT-Analysis

Properties (Features, Attributes, Constraints)	Weight (1-10)	Design 1	Design 2	Design 3
		Removable closed collection tray (not open for chickens to feed at)	Open trough (chickens can openly eat at the trough)	Manual harvesting of larvae
<i>Constraints (needs)</i>				
Size	4	18	19	10
Weight	5	16	14	17
Brightness	8	13	22	19
Dryness	8	12	19	14
<i>Features/ Attributes (wants)</i>				
Ease of user collection	6	22	22	5
Not Labor Intensive	5	16	24	6
Durability	8	17	16	15
Cost	3	16	16	14
Ease of Maintenance	6	19	17	10
Portability	6	22	13	7
Preservation	2	19	9	5
Weatherproof	4	20	10	6
<b>Weighted Score</b>		<b>1112</b>	<b>1140</b>	<b>747</b>

The designs for the harvesting portion of the cultivator are straight-forward. All three options were created with the purpose of being added to the above cultivator designs described in Table 3. The first design was a removable tray or drawer in which the black soldier fly larvae would fall into once they were ready to pupate. This design would be closed off so the chickens are unable to feed from, thus giving more control to the farmer. The second harvesting design consisted of an open trough in which the chickens can freely feed from the container. The third design would rely on the owner to manually sort through the organic waste in order to harvest the larvae. Obviously, this design is the least favorable in terms of effort needed on the part of the farmer, but if the BSFL do not crawl up the ramp, it may become the only viable option for the above cultivators. The choice between the remaining two options is a difficult one that ultimately requires the choice of one of the above cultivator designs. Both the first and second designs excel in different aspects: the open trough provides more light and a lower humidity, which would likely attract more BSFL to the harvesting area, while the tray design is easier to

maintain, more portable, more weather resistant, and provides the farmer with greater choice over what to do with the harvested BSFL.

### 3.2 Feasibility

One of our initial objectives for this project was to replace 25% of chicken feed with the black soldier larvae. In order to determine whether this would be possible, a feasibility analysis was conducted. The chicken farm that would be utilizing our system has 145 chickens that eat three to four 50 pound bags of feed per week.

$$3.5 \text{ bags} \times 50 \frac{\text{lb}}{\text{bag} \cdot \text{week}} \cdot \frac{1 \text{ week}}{7 \text{ days}} \cdot \frac{453.592 \text{ g}}{1 \text{ lb}} = 11339.8 \frac{\text{grams of feed}}{\text{day}} \text{ for 145 chickens}$$

$$11339.8 \frac{\text{grams of feed}}{\text{day}} \div 145 \text{ chickens} = 78.2 \frac{\text{grams of feed}}{\text{day} \cdot \text{chicken}}$$

1 chicken eats 78.2 grams of feed per day.

According to an article by Diener<sup>2</sup>, larvae average 48 mg (dry weight).

$$78.2 \frac{\text{grams of feed}}{\text{day} \cdot \text{chicken}} \cdot \frac{1 \text{ larvae}}{0.048 \text{ grams of DW larvae}} = 1629.17 \frac{\text{larvae}}{\text{day} \cdot \text{chicken}}$$

To replace 25% of chicken feed with larvae:

$$0.25 \times 1629.17 \frac{\text{larvae}}{\text{day} \cdot \text{chicken}} = 407.29 \frac{\text{larvae}}{\text{day} \cdot \text{chicken}}$$

$$407.29 \frac{\text{larvae}}{\text{day} \cdot \text{chicken}} \cdot 0.048 \frac{\text{gram DW}}{\text{larvae}} = 19.55 \frac{\text{grams DW of larvae}}{\text{day} \cdot \text{chicken}}$$

$$407.29 \frac{\text{larvae}}{\text{day} \cdot \text{chicken}} \cdot 145 \text{ chickens} = 59057.05 \frac{\text{larvae}}{\text{day}} \text{ for 145 chickens}$$

Because we would also need to grow and continuously breed larvae in staggered batches, 59000 larvae per day for 145 chickens plus more seems rather high. Therefore, we have changed our objectives to focusing on maximizing our BSFL population.

### 3.3 Standards and Performance

A major concern in poultry is the spread of disease amongst birds and to humans. The ensiling process in the feedstock management of this project aims to sterilize the larvae such that *Salmonella* and *Escherichia coli* levels are low enough to reduce disease and contamination to the birds.

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<sup>2</sup> Diener, S., Zurbrügg, C., & Tocknera, K. (2009) [Conversion of organic material by black soldier fly larvae – Establishing optimal feeding rates]

For the Black Soldier Fly larvae cultivator/ harvester contraption to be successful, it must produce larvae that comply with the FDA's regulations for *E. coli* and *Salmonella* levels. If the system produces larvae with contamination levels below acceptable levels for human consumption, the design's performance can be considered successful. According to fao.org<sup>3</sup>, *Escherichia coli* can be killed with adequate heat or pH levels, which can be achieved by ensiling feed products.

## 4.0 Current Status

The work achieved by the team during winter quarter is described below. It is split into two categories: feedstock management and behavior, which combines the cultivator and harvester portion of the project.

### 4.1 Ensiled Feedstock Test

The goal of the feedstock testing was to identify and quantify the amounts of pathogens in the larvae after given different foods.

#### 4.1.1 Testing Procedures

In the feedstock component, the growth of larvae using various feeds with different ratios of green material, kale, fresh rice hulls, and rice hulls with manure or ensiled rice hulls with material was tested. The results can be used to determine the minimum feed needed for the maximum production and growth of larvae. With the selected feed and larvae, pathogens will be tested to determine if there is any sign of horizontal contamination, pathogens from the chicken manure that will be transferred to chickens when larvae are used as feed.

Ensiling material is a method used to kill pathogens and prolong plant life for storage through fermentation by anaerobic bacteria. The efficiency of this process is dictated by the ability to maintain anaerobic conditions in the environment with the material, the capacity and form of bacteria present in the material, the type of sugars that can be fermented, and the variety of acids that are produced through the fermentation process. Typically farmers will create silages with materials such as crop residue, grass, and corn. They store the material in silos, a large bundle of material, compressed together to reduce the presence of oxygen. The pH of the ensiled material can be tested where a lower pH signifies that fermentation has occurred. Farmers would ensile their crop residue at the end of harvest provided that it would last through the winter to be used as feed for their livestock.

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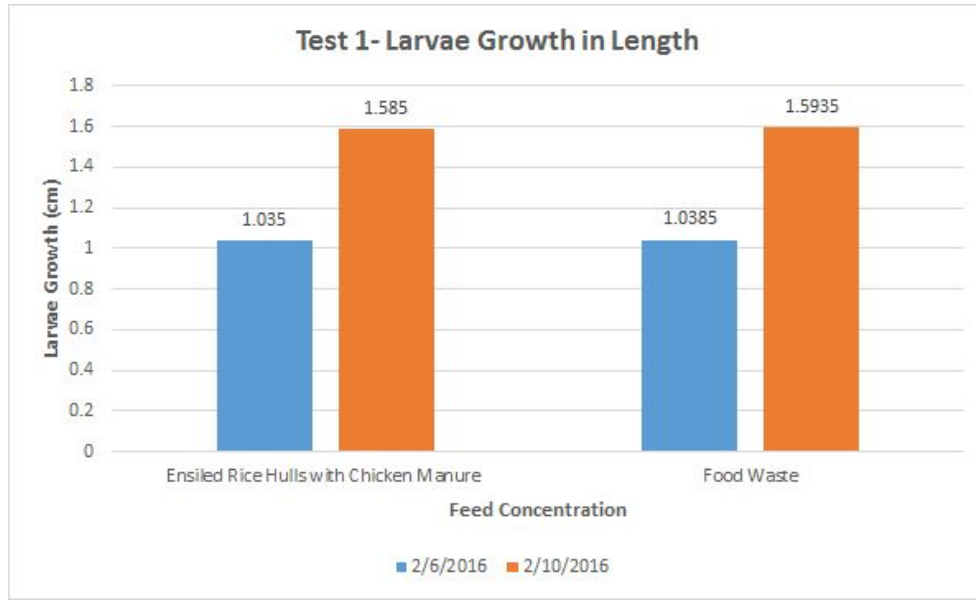
<sup>3</sup> [http://www.fao.org/docrep/article/agrippa/x9500e04.htm#P93\\_28540](http://www.fao.org/docrep/article/agrippa/x9500e04.htm#P93_28540)

Our ensiling process consisted of a small scale production instead of using silos. Plastic air tight zip lock bags were used to create the anaerobic condition. Rice hulls with chicken manure were first weighed and placed into the ziplock bag. Water was also added to assist in the ensiling process with around 60% in overall moisture content. All air was compressed out of the zip lock bag and was placed into another zip lock bag to ensure the anaerobic environment. The bag was set aside for one week then checked for pH. The material had a resulting pH of 6.4.

Using the ensiled material, Test 1 was performed. Ensiled rice hulls with chicken manure was utilized as one testing feed with another feed with food waste comprised of apple cores, banana peels, and orange peels. Ten larvae were accounted for their weight and length then put into each respective feed concentration. The pH of each environment was also recorded as shown in Table 5. After incubating a total of 5 grams of material in each reactor at a temperature of 30 °C for 4 days, the weight and length of the ten larvae in each respective feed were recorded as well as the pH for their residential environment.

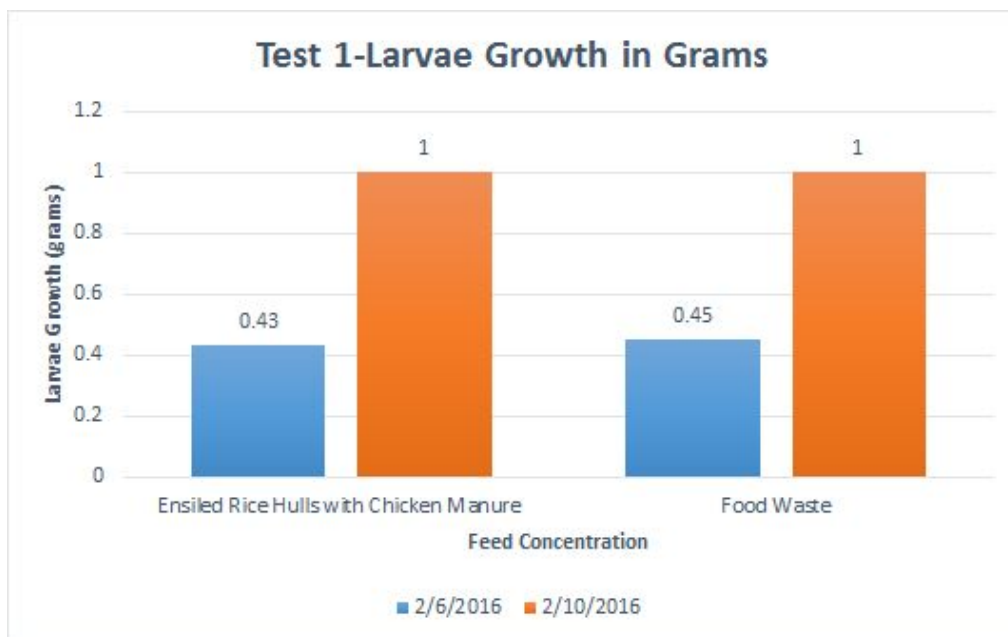
**Table 5.** Environmental pH in a 4-day Span for Ensiled Feedstock Test 1

Larvae Testing	Day 0		Day 4	
	Silage	Food Compost	Silage	Food Compost
pH level	6.6	>6.0	6.6	6



**Figure 2.** Larvae Length for Ensiled Feedstock Test 1

The initial average length, over ten larvae, for the ensiled rice hulls with chicken manure was 1.035 cm. The resulting average length after four days was 1.585 cm with a difference of 0.55 cm. The initial average length of food waste was 1.0385 cm while the resulting average length after four days was 1.5935. The overall difference was 0.555. This demonstrates that the ensiled rice hulls with chicken manure compared to food waste had relatively equal growth making both feed concentrations desirable to the larvae.



**Figure 3.** Larvae Weight for Ensiled Feedstock Test 1

The initial overall weight of ten larvae for the ensiled rice hulls with chicken manure was 0.43 grams. After four days, the resulting weight was recorded to be 1 gram. The difference was 0.57 grams. The initial overall weight of ten larvae for the food waste was 0.45 grams, while the resulting weight after four days was 1 gram. The difference was calculated to be 0.55 grams. The difference between these two food concentrations is not significant which was primarily caused by the scale we used. The original scale that was used was not as sensitive as the scale that would be used for testing two, thus slight differences in weight could not be detected. Overall, this test could not be used to determine the desirability of larvae.

The pH level stayed relatively the same over the course of four days in the silage food composition and the food compost or waste composition. The silage stayed a constant 6.6 and the food compost stayed around 6.0.

In order to test for pathogens, a sample from each of the five components (food compost, ensiled rice hulls, larvae that fed on food compost, larvae that fed on the ensiled rice hulls, and fresh rice hulls) were placed into Whirlpak bags and sent to the UC Davis Veterinary School of Medicine for pathogen testing. Pathogen testing was done to determine the amount of *Escherichia Coli* and *Salmonella* present in each of the five samples. LB agar was used in order to test for these pathogens. 1 gram of each sample is placed into their own buffer solutions and allowed to grow over a period of 4 days. After 4 days it was concluded that the plate counts were higher than the amount guessed on the non-selective media used. Selective media and MPN (most probable number) were then used to further analyze the samples. Due to complications with the testing, the samples sat in the fridge for about 10 days, causing the number of bacteria collected to not be as much as expected.



**Figure 4.** Comparison of Larvae Used for Pathogen Testing

#### 4.1.2 Experimental Results

Unfortunately, not all our test samples were analyzed due to procedural complications. The pathologists did not know how many and exactly what types of pathogens should be expected. In result, it took them several weeks to order and reorder medias to perfect the process. By the time the procedure was perfected, our test samples could not be properly analyzed without giving false results. However, two samples were still tested.

Clean rice hulls resulted in a growth for *Salmonella* or *Pseudomonas* at a range of  $10^3 - 10^4$  Colony Forming Units (CFU/ gram of material). A latex agglutination test is needed to differentiate the two pathogens. No growth of *E. Coli* was found.

The ensiled rice hulls with chicken manure had  $10^4$  CFU/ gram of *E. Coli*. *Salmonella* and *Pseudomonas* were also present at a range of  $10^5$  CFU/gram.


Overall the colonies on the plates looked “clonal” meaning that most that were dealt with had one species making pathogens simpler to detect.

In the second trial, larvae are being tested with green material (kale), fresh rice hulls and rice hulls with manure or ensiled rice hulls with green material. An additional batch of ensiled rice hulls with chicken manure was produced. The ensiling procedure was the same as the ensiling process for the first test, however, this ensiled material was set aside for two weeks instead of one week. The pH was tested after two weeks and recorded at 6.4, the same pH as the rice hulls with chicken manure ensiled for one week. This exhibits that the duration of ensiling, the one week versus two weeks of ensiling, does affect the pH level.




Table 6 below displays the 9 testing samples used for the feedstock preference test, with two additional tests of 100% green material and 100% rice hulls with manure. In total, there are 21 tests. The green material cell corresponds with the respective fresh rice hulls percentage as well as the respective rice hulls with manure percentage. For example, one test contains 0% fresh rice hulls, 30% rice hulls with manure, and 70% with green material. Table 7 behaves in a similar fashion however ensiled rice hulls with manure was used instead of rice hulls with manure. Table 7 is used for the ensiling testing which corresponds to Figure 5.

**Table 6.** Material Composition for Larvae Feedstock Preference Test 2


Test 2 - Rice Hulls with Manure				
Percentages  Green Material		Fresh Rice Hulls (%)		
		0	10	20
Rice Hulls w/ Manure (%)	30	70	60	50
	40	60	50	40
	50	50	40	30

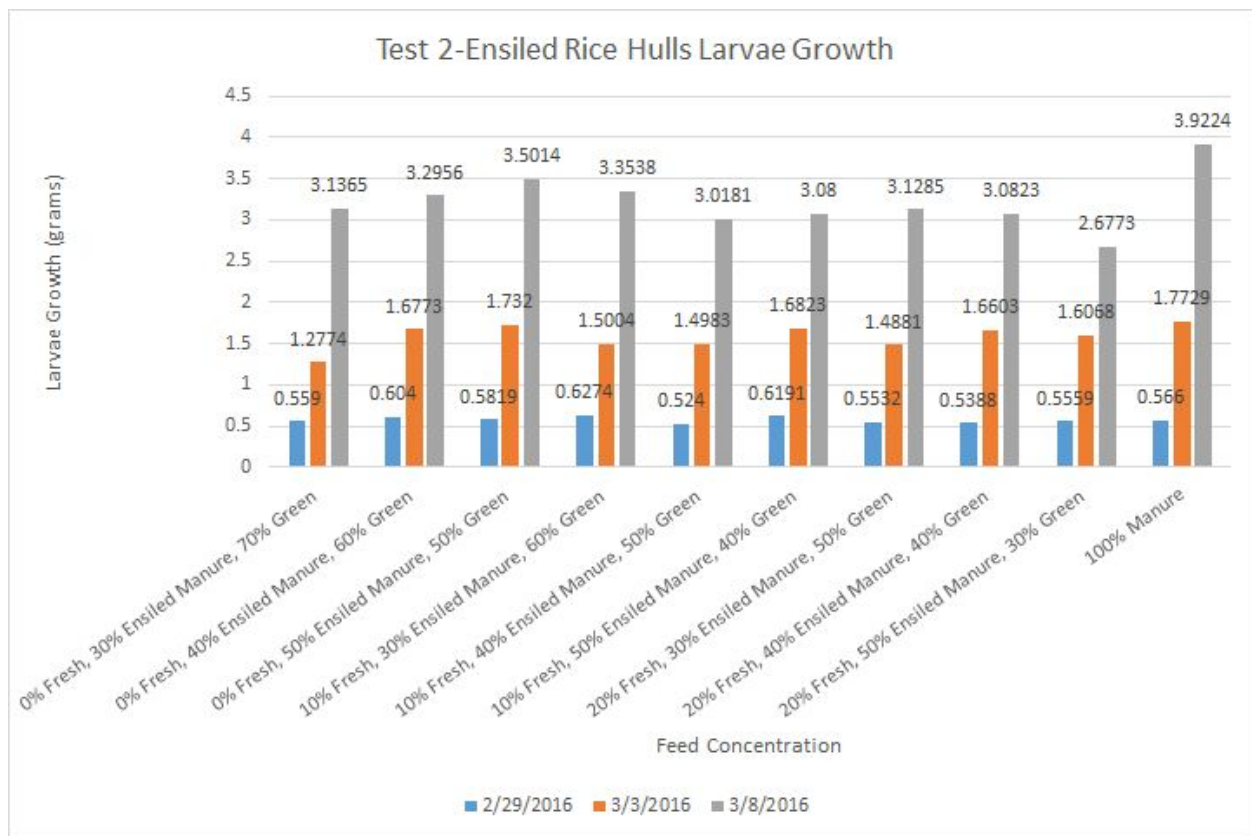
**Table 7.** Ensiled Material Composition for Test 2

Test 2 - Ensiled Rice Hulls with Manure				
Percentages  Green Material		Fresh Rice Hulls (%)		
		0	10	20
Ensiled Rice Hulls w/ Manure (%)	30	70	60	50
	40	60	50	40
	50	50	40	30

**Table 8.** Ensiled Material Composition for Test 2 in Grams

Test 2 - Ensiled Rice Hulls with Manure				
---	--	--	--	--

Percentages  Green Material		Fresh Rice Hulls (%)		
		0	1	2
Ensiled Rice Hulls w/ Manure (%)	3	7	6	5
	4	6	5	4
	5	5	4	3



**Figure 5.** Periodic Ensiled Feedstock Test for Larvae Growth for Test 2

For the second feedstock ensiled testing, Tables 7 and 8 were used as a references. Each testing sample were placed into reactors and incubated at a temperature of 30 °C for 8 days. The weight of twenty larvae in each respective feed were recorded as used for comparison. Each sample had different material mixtures (fresh rice hulls, dried kale, ensiled rice hulls with manure) placed into reactors based on the percentages calculated from Table 7 to obtain the weight in grams in

Table 8. For instance the first material, containing 0% fresh rice hulls, 30% ensiled rice hulls with manure, and 70% green material, had 0 grams of fresh rice hulls, 3 grams of rice hulls with manure, and 7 grams of ensiled rice hulls. After testing the larvae every three to four days, we can determine the best ensiled feed for the larvae to maximize growth with a minimum amount of feed. The 100% ensiled rice hulls with chicken manure ranked the highest growth with an increase weight of 3.3564 grams. The next highest growth was 0% Fresh, 50% Ensiled Manure, 50% Green with an increase of weight of 2.9195. The third highest growth was 10% Fresh, 30% Ensiled Manure, 60% Green with an increase of 2.7264 grams. 0% Fresh, 40% Ensiled Manure, 60% Green closely followed with an increase of weight of 2.6916 grams. All other feed concentration had an increase of weight in grams less than 2.6 grams.



Figure 6. Larvae after 8 days from Test 2-Rice Hulls with Ensiled Manure Front View



Figure 7. Larvae after 8 days from Test 2-Rice Hulls with Ensiled Manure Back View

### 4.1.3 Analysis of Results

Samples of larvae from different feed concentrations in ensiled and non-ensiled rice hulls with chicken manure will be used to test for pathogens. The feed material the larvae resided in will also be used to test for pathogens. With this, it can be determined if pathogens were killed when comparing the ensiled versus non-ensiled larvae. Comparing the larvae to the material can also help determine if there was a transfer of pathogens between the two and whether the larvae decrease the number of pathogens.

In addition, entomophagy will also be researched more extensively. Entomophagy is the practice of humans eating insects. In preparation of entomophagy, insects are removed from the food source and set aside to fast for at least twenty four hours. With this process, pathogens are purged resulting with insects that have little to no pathogens. Unfortunately, there is not much literature and data to support this process. Larvae from our tests will be utilized to investigate this process. After placed in isolation to fast for twenty four hours, the larvae will also be sent to test for pathogens.

## 4.2 Larvae Feedstock Preference Behavior

The behavior portion combines the cultivator and harvester portions of this project. The goal of these preliminary tests were to understand how the larvae behaved, namely how quickly they grew when given different foods.

### 4.2.1 Larvae Feedstock Preference

Before the construction of a working prototype, tests were performed in order to determine what type of feedstock the BSFL grow best in. Each test used small, plastic containers as bioreactors, filled with different feedstock types and ten larvae. The plastic containers were estimated to hold a volume of about  $2.38 \text{ cm}^3$  using the mass-volume density equation,

$$\rho = \frac{M}{V}$$

along with the mass and density<sup>4</sup> of tap water at 20 °C. Currently, two different rounds of testing have been performed using these reactors.

The first test was concerned with whether the larvae would consume the rice hulls (both soiled and fresh) used as bedding on poultry farms. Seven rice hull mixtures were tested, with 10 grams of each being placed into a separate bioreactor. One reactor containing crushed, dry dog food, and one containing 10 grams of banana were used as controls. To increase the water content to

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<sup>4</sup> Based on tap water density data from [http://www.simetric.co.uk/si\\_water.htm](http://www.simetric.co.uk/si_water.htm)

60 percent, 15 grams of water were added to each container. Next, after being cleaned and massed, 10 larvae were added to each bioreactor and left for a week to develop. After a week, the larvae were removed from their containers, cleaned, and weighed. The level of activity of the larvae was also checked. Two one-week trials were performed with the same larvae.



**Figure 8.** Growth Behavior Larvae Feedstock Preference Test 1

The second test added green waste (kale) to the mixtures in order to better simulate the type of organic waste found on poultry farms. In this test, three variables were changed: the amount in each mixture of green waste, soiled rice hulls, and fresh rice hulls. It followed the percentages of Table 6. This test used a total mass of 20 grams, rather than 10 grams as the previous test used. 10 new larvae were used. In order to find the moisture content of each component, samples of each were taken, weighed, and then dried in an incubator at 45°C. The dried samples were weighed, and the moisture content was found by subtracting the dry mass from the wet mass. We found that the moisture content of fresh rice hulls were 11.53%, rice hulls with manure were 26.25%, and the kale was 80.87%. We used a 40% moisture content level for this test, thus different amounts of water were calculated for each reactor. After 7 days, the larvae were cleaned and weighed. In order to calculate how much water to add for each reactor to achieve a certain moisture content, the following equation was used:

$$MG = \frac{(m_1 \times MC_1) + (m_2 \times MC_2) + (m_3 \times MC_3) + \dots + (m_n \times MC_n)}{m_1 + m_2 + m_3 + \dots + m_n}$$

in which:

$MG = \text{moisture goal}$

$m_n = \text{mass of material}$

$MC_n = \text{moisture content of material}$



**Figure 9.** Larvae Feedstock Preference Test 1 Reactors with Green Material

We currently have a new test that utilizes the same ratios as the second test. However, the total mass used reduced back to 10 grams due to lack of headspace, and we used the green material as is rather than dry. We also decided to place the reactors on its side in the incubators rather than upright in order to replicate the conditions that the larvae would be in the actual system.



**Figure 10.** Larvae Feedstock Preference Test 2 Reactors with Green Material

#### **4.2.2 Larvae Migration**

Before large-scale prototypes were constructed, tests were performed to confirm that the larvae behaved as they did in other, similar BSFL-cultivating processes. The most crucial behavior to test was that the larvae, once ready to pupate, would crawl upwards toward a source of light in order to commence pupation. To observe this behavior, three small-scale prototypes were constructed.

The first prototype created consisted of an 18 gallon plastic storage tote with a lid. Into a short side of the container, two one-inch holes were drilled, and two equal lengths of PVC pipe were

inserted at a 30° angle. The ends that stuck into the plastic tote had the first two inches cut as shown. The other ends of the PVC pipes were stuck into a cardboard box that served as the holding container for the larvae, should they climb up the pipes. Both the cardboard box and the plastic tote were covered with mosquito netting in case any larvae pupated and became flies. In the plastic tote, a mixture of fresh rice hulls, dry dog food, and water was placed until a depth of about six inches was achieved. Then, about 800 BSFL were placed into the plastic tote, and the container was covered with the lid, to prevent light from entering.



**Figure 11.** Initial behavior testing bin Prototype 1

The second prototype consisted of a two gallon bucket, lid, and wooden frame. A 4"x1/4" slit was cut roughly 8 inches from the bottom of the bucket, and a plastic bag was affixed completely over the hole. The bucket rested in a wooden frame at around 40°, with the slit and plastic bag facing downward. Toward the bottom of the bucket, near the point where the wooden frame and bucket make contact, small pinholes were poked to release excess water from the feed mixture. A sponge was placed below these holes. Small holes were also made near the top of the bucket, in order to allow airflow through the container. Twenty grams of soiled rice hulls were placed in the container along with ten BSFL. The container was then sealed to block excess light and to prevent larval escape.



**Figure 12.** Behavior testing Prototype 2

The third prototype consisted of a 5-gallon bucket, laid on its side. A five inch ramp was secured along the side such that the edge was flush with the lip of the bucket. An 8"x1/4" slit was cut into the lid of the bucket and aligned with the ramp on the inside. Hot glue was used to affixed the ramp to the bucket and bridge the gap between the edge of the ramp and the slit in the lid. The entire outside of the bucket was spray-painted black in order to reduce light penetration onto the surface of the organic material.

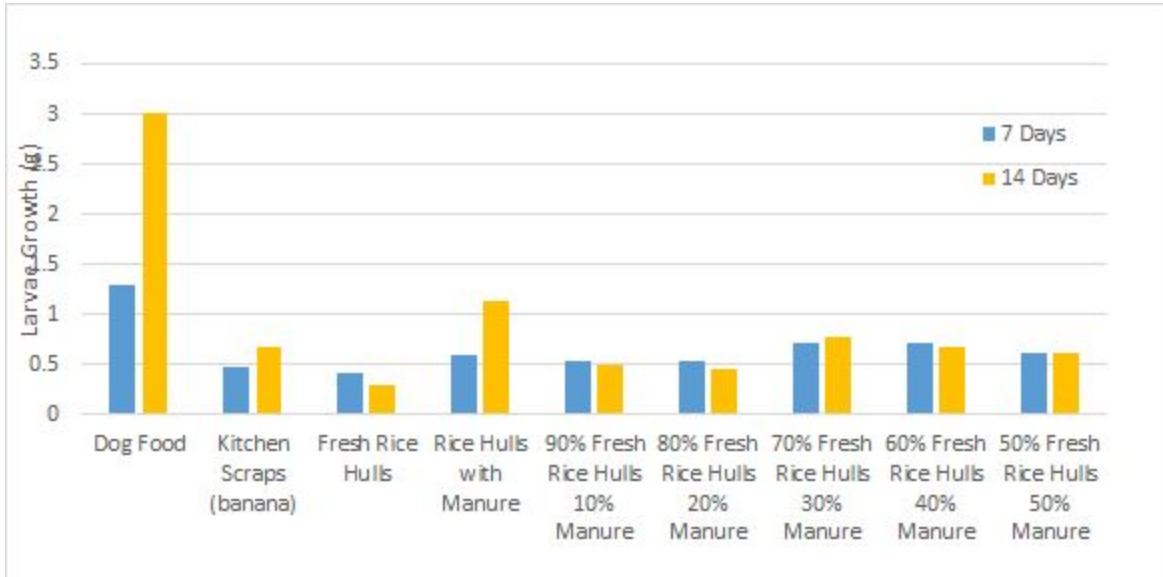


**Figure 13.** Behavior testing Prototype 3



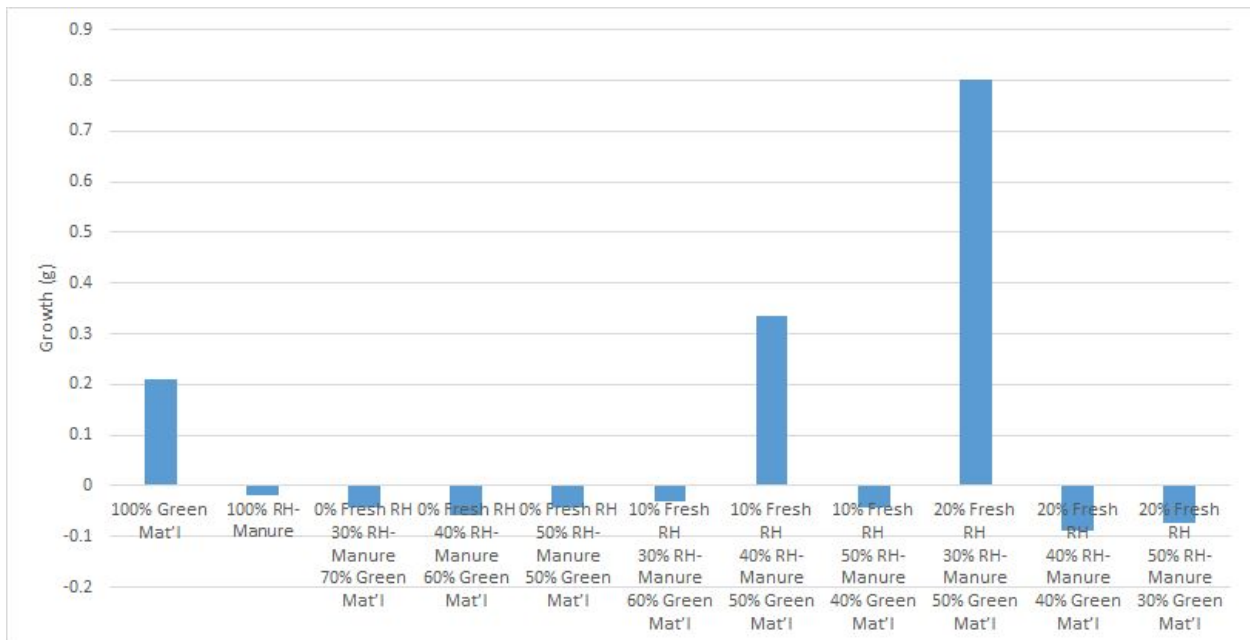
### 4.2.3 Experimental Results

Results for feedstock preference test 1 are shown in figure 12 below:



**Figure 14.** Larvae Feedstock Preference growth between 7 days & 14 days for Test 1

Results for feedstock preference test 2 are shown in figure 13 below:



**Figure 15.** Larvae Feedstock Preference growth over 7 days for Test 2

In the first behavior test with the PVC pipes in the bin, the dog food ended up molding and there was a buildup of ammonia, due to poor ventilation and drainage. Although the larvae grew, they did not seem to enjoy the mixture because they were crawling up the sides of the bin rather than the PVC pipe even after removing excess liquid. This resulted in about 200 escaped larvae.

Behavior prototypes 2 and 3 are currently housing young larvae, therefore we have not obtained any results, yet.

#### **4.2.4 Analysis of Results**

For the first feedstock preference test, our results showed that after 14 days, the larvae grew the most in dog food, as expected. As for the mixtures, larvae grown in 100% rice hulls soiled with manure grew the most. What is surprising is that the larvae in the 70/30 and 60/40 mixtures of fresh rice hulls and soiled rice hulls grew second greatest. We did not expect that such a high amount of fresh rice hulls would generate much larvae growth. The data also showed that after 14 days, some larvae in the mixtures decreased in size from its 7 day size. This is possibly due to water loss from dead or pupated larvae in the reactors. We also noticed an ammonia smell when working with this test, which is an indication that there was an excessive amount of water for the material to absorb and lack of sufficient mixing. We realized that our 60% calculation did not take into account the moisture contents of the material, and that the material was compacted.

In the second feedstock preference test that included 3 changing variables, the results were disappointing. We expected more larval growth than the first test, but many of the larvae ended up dead. A mistake we made was that, although we determined the moisture content of the material beforehand, we used a 40% moisture content goal rather than the recommended 60% moisture content. This resulted in the feed being too dry rather than too wet. Because we used a total of 20 grams in the reactor, there was less headspace for the larvae. We decided many larvae died because the moisture conditions were not ideal and there was not enough oxygen in the system with the amount of feed used. The negative growths possibly resulted in the water loss from the dead larvae. However, there was tremendous amount of larvae growth, about 0.8 g larger, in the 20/30/50 mixture. We do not know why this had happened while the mixtures similar to it did not grow like that.

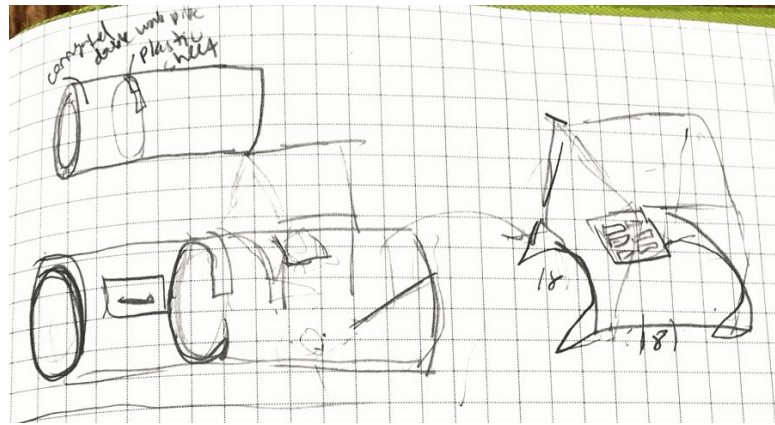
From the initial behavior bin that used PVC pipe, we noticed that the larvae are able to crawl up sides of walls, so they are perpendicular to the floor. However, the surface needs to have enough traction for it to crawl on, which usually occurs when the surface is wet.

### **4.3 Prototype**

Our current design that we want to construct consists of a main cylindrical body that will be split in different sections. The first section is where the feedstock will be placed via the mailbox

opening. In order to incorporate the ensiling process, a plunger system would be installed which would push the feedstock against a removable pane in the first chamber to ensure an anaerobic condition. Once the organic waste is ensiled, the pane is removed and the compressor pushes the waste to the next chamber. This second section is the feeding area, where the black soldier fly larvae would be eating the ensiled waste. Once the larvae are ready to pupate, they would crawl up a ramp within the same chamber to the closed-off harvesting bin. The farmer will be able to then control how much pupated larvae will be used in the breeding area. The breeding area will be a clear container that sits above the feeding section. A section of the cylindrical body that the container will sit above will be cut out and stuffed with corrugated cardboard. Once the pupae have become adult flies and mated, the flies will lay their eggs in the corrugated cardboard. When the eggs hatch, the larvae will fall into the feeding chamber, ready to begin the cycle again.

We currently do not have the dimensions and scale of our design, yet. We will model the design in SolidWorks by the beginning of April.



**Figure 16.** Initial sketches of prototype designs

## 5.0 Progress

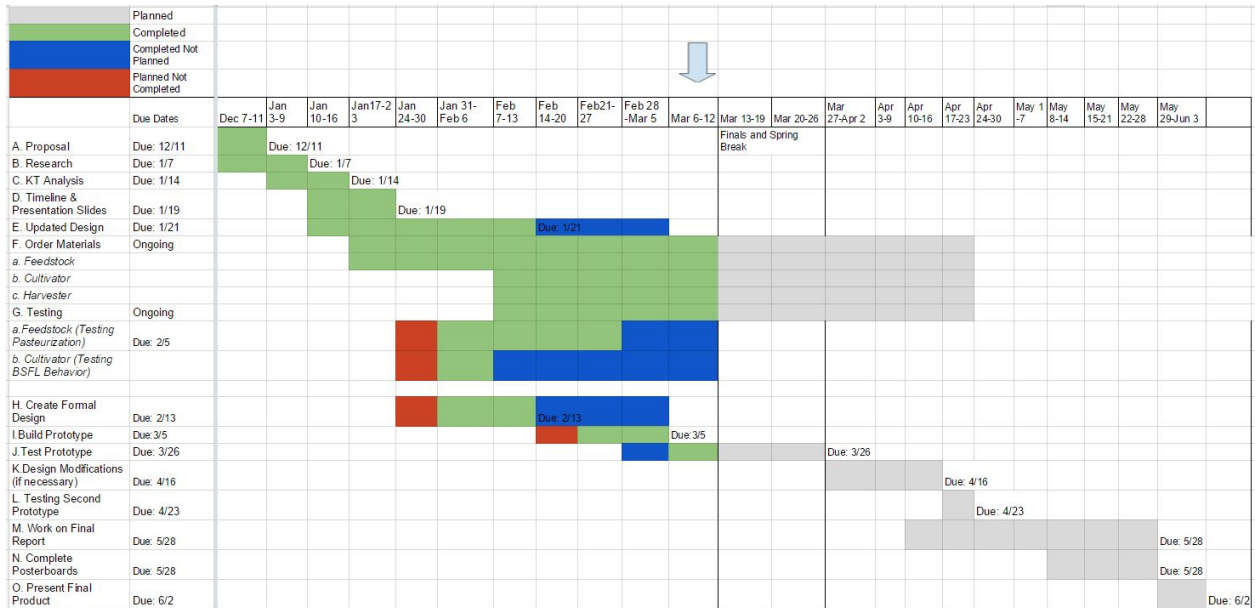
The progress of this project is assessed in this section by comparing the initial timeline to what has been accomplished and by comparing the budget to what has been purchased.

### 5.1 Timeline Comparison

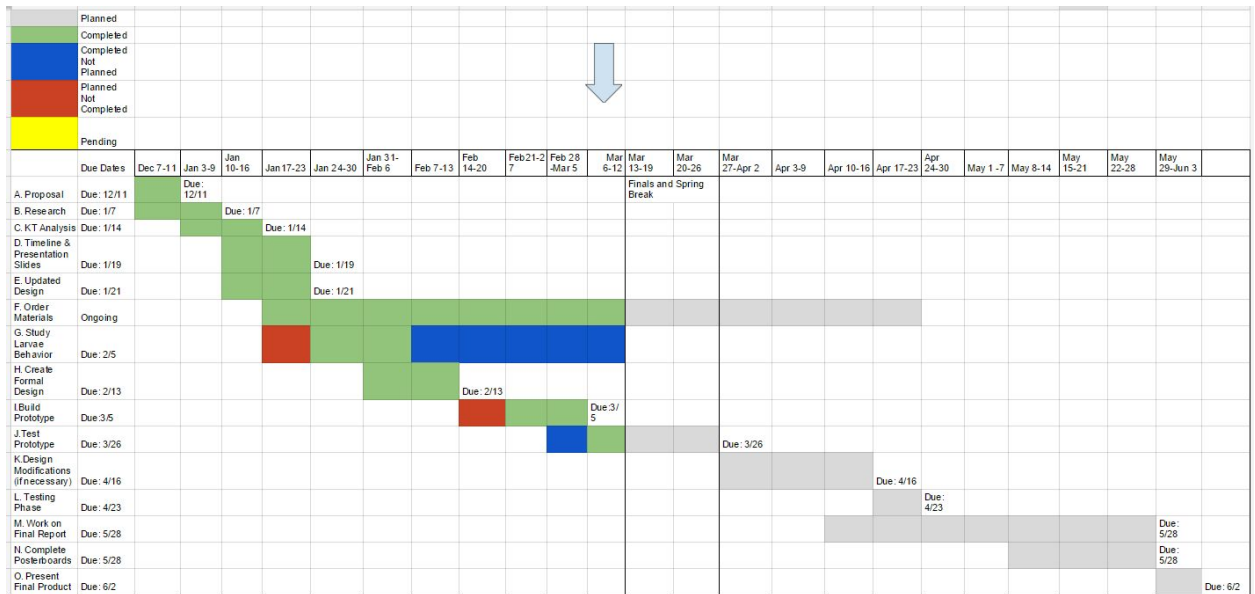
Our original timeline, depicted in Table 9, was created at the beginning of Winter Quarter in hopes of completing a prototype and testing it before spring break. While we had a broad idea of what we wanted to do for the entire project, we had to make more detailed timelines for each

respective component to be more realistic of deadlines. The changes made to the timeline can be seen in Table 9.

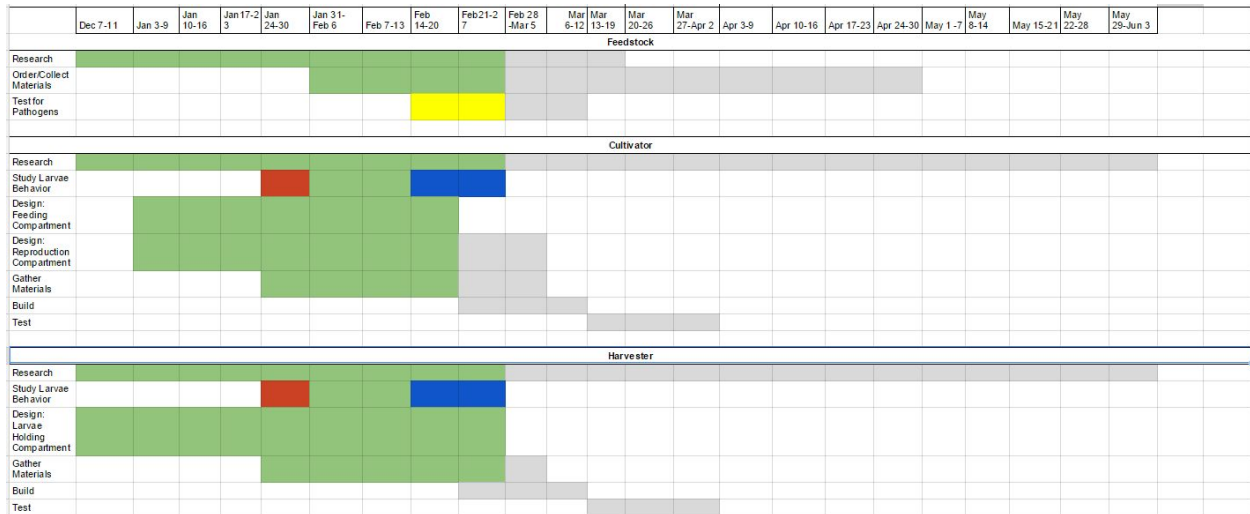
**Table 9. Original Timeline**



**Table 10. New Timeline Split into Three Parts: Feedstock, Cultivator, and Harvesting Components**



**Table 11. New Timeline Comprised of Three Parts: Feedstock, Cultivator, and Harvesting Components**



Overall, we have mostly followed the deadlines on our timeline. Delays in the development of our project were due to not having access to a more precise scale for weighing larvae in the initial testings, additional testing required by the pathologists for the results of the feedstock component’s first testing, retrieving the escaped larvae from the behavior bin, and not having a sufficient amount of green material for the second testing. These unexpected delays have slowed us down in our process, but we will still be able to achieve our goals in a reasonable time.

## 5.2 Budget

This section displays the initial planned budget and lists the expenses incurred throughout the last three months.

### 5.2.1 Initial Budget

**Table 12. Initial list of materials for budget**

<b>Budget</b>	
Winter Quarter	<ul style="list-style-type: none"> <li>● Larvae eggs (1 batch)</li> <li>● Organic material</li> <li>● Materials (wood, insulation, metal, mirrors, plexiglass, screws, cardboard, netting, aluminum, thermometers)</li> </ul>
Spring Quarter	<ul style="list-style-type: none"> <li>● Materials for modified prototype (wood, insulation, metal, mirrors, plexiglass, screws, cardboard, netting, aluminum, thermometers)</li> <li>● Poster boards</li> </ul>

## 5.2.2 Total Expenses

**Table 13.** Total expenses incurred up to this point

Item	Quantity	Cost per Unit	Total Cost
1000 BSF Larvae	3	\$ 20.99	\$ 62.97
Fresh rice hulls	1	\$ 7.00	\$ 7.00
Plastic storage bin	3	\$ 4.99	\$ 14.97
5-gal plastic bucket	2	\$ 4.49	\$ 8.98
5-gal plastic bucket lid	2	\$ 2.99	\$ 5.98
2-gal plastic bucket	1	\$ 3.49	\$ 3.49
2-gal plastic bucket lid	1	\$ 2.49	\$ 2.49
Kale large	2	\$ 5.00	\$ 10.00
Kale small	1	\$ 3.99	\$ 3.99
<b>Total Expenses (with tax)</b>			<b>\$ 123.93</b>

## 6.0 Future Steps

The next steps in the project are outlined below along with a brief summary of the project's success and recommended changes for future projects.

### 6.1 Steps to Completion

For spring quarter, our main concern is integrating all three project components, evaluating the results of the first prototype, and making the necessary modifications. We plan to have a second prototype that meets our integrated system expectations.

The final report, poster boards, and presentation will also be an important aspect of spring quarter. We plan on creating our final report based on all the recorded data, the proposal from fall quarter, and this interim report.

### 6.2 Success

This quarter we successfully collected some data on how the larvae react to different foods and combinations of fresh rice hulls, manure-soiled rice hulls, kitchen waste in the form of a banana, and green material in the form of kale. The process of how to send out samples to test for contamination was better understood, as well as the nature of the larvae themselves. This knowledge is critical for planning and implementing the next steps in building more prototypes.

### 6.3 Changes

In the future, this project should try to start working with larvae in late October or early November, as that is the end of their naturally active season. It is also important to have a clear plan to implement in the event that there is a larvae or fly escape.

## 7.0 Group Contribution Summary

Since we had two main subdivisions (feedstock management and cultivator) this quarter, certain tasks were divided only amongst team members in each respective division.

<b>Team Meetings</b>					
<b>Meetings</b>	<b>Tasks</b>				
	David	Emily	Lisa	Marianelly	Sara
January	-Research -Read advisor's assigned winter reading -Cultivator and Harvester KT Analysis -Designed cultivator prototypes	-Research -Read advisor's assigned winter reading -Cultivator and Harvester KT Analysis -Designed cultivator prototypes	-Research -Read advisor's assigned winter reading -Cultivator and Harvester KT Analysis -Designed cultivator prototypes	-Research: Pasteurization & Mixing Methods -Feedstock KT Analysis -Read advisor's assigned winter reading	-Research: Pasteurization & Mixing Methods -Feedstock KT Analysis -Ordered BSFL -Read advisor's assigned winter reading
February	-Assembled prototype 1 -Researched prototype materials	-Ordered BSFL -Researched prototype materials	- Larvae Feedstock preference Test 1 -Researched	-Prepared silage -Ensiled Feedstock: Test 1	-Prepared silage -Ensiled Feedstock: Test 1

	-Larvae Feedstock preference test 1	-Larvae Feedstock preference test 1	prototype materials	-Pathogen Testing	-Pathogen Testing
March	-Fabricated prototypes 2 & 3 -Larvae Feedstock preference test 2	-Fabricated prototypes 2 & 3 -Larvae Feedstock preference test 2	-Fabricated prototypes 2 & 3 -Larvae Feedstock preference test 2	-Ensiled Feedstock: Test 2 -Pathogen Testing -Research: Entomophagy	-Ensiled Feedstock: Test 2 -Pathogen Testing -Ordered BSFL -Research: Entomophagy
<b>Grading</b>	0.2	0.2	0.2	0.2	0.2

By signing below, we agree with the scoring.

1. Lisa Wolbert
2. Emily Quan
3. David Barraza
4. Sara Wat
5. Marianelly Lopez



## 8.0 References

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