Is Insect Protein a Sustainable Alternative to Soy and Fishmeal in Poultry Feed?

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# <u>Abstract</u>

This thesis describes a research experiment examining the potential of Black Soldier Fly larvae (Hermetia illucens) (Diptera: Stratidomyidae) reared on local food waste to effectively feed poultry. Significantly less water and land is required to raise Black Soldier Fly larvae and fewer greenhouse gas emissions are generated, relative to the production of soy and fishmeal for animal feed industry. In order to account for the environmental pressure meat production puts on our environment, chickens were raised on Black Cat Farm in Longmont, CO using a more sustainable, insect-based, feed. At seven weeks of age, 127 chickens were randomly assigned to three dietary treatments (3 replicates and 14-15 birds per pen). Each chicken was weighed every three days using a fish scale and weighing basket. Feed weight was recorded using the same method; food and water were supplied *ad libitum*. Weights of chickens were averaged before analysis to overwrite inevitable variation between chicken and enclosure environments. An analysis of variance (ANOVA) was conducted on growth data to identify significant between treatments. After three weeks, there was no significant difference in growth rate, feed conversion, or mortality between the three feed types. Healthy growth was observed among all chickens, suggesting that Black Soldier Fly larvae can effectively replace soy and fishmeal in poultry feed. This confirmation of alternative feed has the potential to influence approval from the U.S. Food and Drug Administration (FDA) of insect based protein once analysis is conducted throughout the chicken's lifetime for the next 2-6 weeks.

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# **Introduction**

The objective of this Honor's Thesis project is to evaluate whether Black Soldier Fly Larvae can replace soy and fishmeal as a more sustainable protein source in animal feed for Broiler Chickens. The results of this feeding trial add to existing literature regarding insect-based feed as a way to lessen our current food system's reliance on soy and fishmeal. The experiment explored several goals of sustainable food systems including the following: food waste recycling, decreasing total water use, reducing food insecurity, and improving our current methods in meat production. I conducted my research on body mass growth, feed conversion, and mortality of Cornish Cross Broiler chickens over the course of three weeks.

The farm where the chickens were raised and measured is Black Cat Farm in Boulder, CO, under the supervision of owner Eric Skokan. This project has been funded through the proposed School for Environment and Sustainability at the University of Colorado Boulder, and has gained IACUC approval. The project contributes to research conducted by Mad Agriculture, an entrepreneurial food-system start-up that also aims to seek FDA approval for Black Soldier Fly meal, and to conduct future research with interested stakeholders. I collaborated with Peter Newton, Philip Taylor, Eric Skokan, Xavier Rojas, and Scott [last name] on the project at Black Cat Farm, and worked additionally with Dale Miller and Nancy Emery for advising on writing and data analysis. My role in this experiment was to monitor the insect hatchery and chicken enclosure located at Black Cat Farm, and analyze the effectiveness of insect protein by recording data on chicken growth rate, feed conversion ratio, and feed protein quality over a three week time frame.

The motivation of this experiment originates from the serious implications of animal agriculture on the environment, and an increasing demand for agriculture to feed a growing human population. The mass-production of animals requires quick turnover and maximum nutrient content, an energy intensive process that poses risks to biotic health and species diversity. The feed ingredients that are relied on for this kind of mass production are often soybeans, peas, fishmeal, canola, meat/bone meal and cereals (Makkar 2014). Of these ingredients, soybean and fishmeal have byproducts that directly contribute to environmental degradation (Fearnside et al. 2001, Barrows et al. 2015). Soybean harvested from Brazil, the second largest soybean producer in the world, next to the United States, has led to increased deforestation, biodiversity loss, amplified greenhouse gas emissions, and desertification (Willaarts 2011, Lathuilliere 2017). Fishmeal has lead to a decline in native fish stocks, high disease rate, and coastal habitat impairment (Henry 2015). These repercussions are not inclusive of all of the direct and indirect consequences of animal production, but serve to highlight important details concerning prominent food systems. Finding sustainable alternatives to avoid amplifying these effects will be vital as countries become more developed, expanding in population size and affluence, and demand more animal products such as poultry, cattle, and seafood (FAO 2016). To account for these problems, a feeding trial involving an alternative protein source for poultry aims to analyze a potential solution for decreasing our reliance on soybean and fishmeal, while still providing desired nutrition.

The Black Soldier Fly larvae (*Hermetia illucens*) is such an alternative. Recognized to be one of the most efficient insects for converting macronutrients of waste into viable protein (FAO 2017), the feeding trial will expand on the extent of this fly larvae to lessen the environmental impacts and fragility of standard feed operation methods. Thus, I

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hypothesize that Broiler Chickens (*Gallus gallus domesticus*), a commercial poultry species for mass production, will display an equivalent growth rate to soy and fishmeal when fed Black Soldier Fly larvae. There will be no significant difference in feed conversion or mortality between three feed types. Consequently, insect-based animal feed holds the potential to act as a more sustainable macronutrient for meat production. The results of the experiment will shed light on the effectiveness of replacing soy and fishmeal for poultry and add to the existing literature on insects as an alternative, while also utilizing one of the first commercially made insect feeds in the country. My thesis aims to analyze the quality of insect fed chicken meat in comparison to traditionally fed chickens, by answering the question: *Can insect-based protein provide an efficient alternative to soy and fishmeal in animal feed*?

# **Background**

The United Nations predicts that the global population is expected to reach approximately 9.7 billion by 2050 (UN 2016). A predicted increase of 60-70% in meat consumption is additionally expected by 2050 due to several factors including greater per capita demand and population growth (Makkar et al. 2014). There is a strong positive correlation, at a national level, between increasing affluence and demand for animal protein, inspiring a vigorous market for meat production (WHO, Nutrition 2016).

Growth in animal agriculture poses risks to human health, ecosystem diversity, and wildlife populations (Baroni et al. 2007, van Huis et al. 2013, Makkar et al. 2014). Land use change, eutrophication, and consumption of fossil fuels are a few examples of environmental externalities due to the energy intensive nature of meat production. Animal agriculture requires substantial amounts of water and land to grow and maintain crops and

aquaculture that supply the main sources of protein to animals globally. The two most common sources of protein in animal feed are soybean and fishmeal (Sapkota et al. 2007). Twenty-first century climate induced shifts will increase the cost of animal protein and feed due to an increased demand for land and water, both of which are increasingly stressed from environmental changes and food-feed-fuel competition (Makkar 2014). Addressing the problems associated with soybean and fishmeal is an essential step towards developing more sustainable production methods.

## I. The Environmental Consequences of Soybean Production

Soybean is the most abundant source of protein in animal feed across the world (FAO 2015). Eighty five percent of the world's soy production is used for oil and meal, and 98% of this is used directly for feeding animals (Soyatech 2016). Using cereal-based foods such as soybean for animal feed is an inefficient use of land; more human food can be produced from the same amount of land being used for soybean than can meat/dairy products (Pimental 1997). An estimated 800 million people could be fed with the grain currently used for livestock (Pimental 1997).

Despite this assessment, there is an increasing global demand for soy, instigating the conversion of large expanses of land worldwide to pasture and cropland from its natural vegetative state. Brazil is the second largest producer of soybean (after the US), with a 37.5% megaton growth in production from 2000 and 2014 (Lathuillere 2017). Land conversion for industrial soy agriculture instigates tropical deforestation; cattle ranching and international demand for feed/soybean oil are the driving factors (Barona 2010). Deforestation prompts the release of additional carbon into the atmosphere and alters rates of evapotranspiration (Fearnside 2001). Currently cultivated areas of the Amazon reveal the repercussions of land conversion for soy production. The state of Mato Grosso, the

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largest soybean producer in Brazil, has exhibited a significant increase in the predicted "potentially disappeared fraction of species" within the area (Lathuilliere 2017). Reduced local evapotranspiration, as a result of land-use change, decreases regional precipitation rates and poses a long-term risk to atmospheric water balance and ecosystem function (Lathuilliere 2017). The long-term effects of soybean production on water tables in the biomes of Amazon and Cerrado, are yet to be explicitly defined, and will require research as global markets for soy and meat expand. Despite gaps in water table research for soybean production, there is an explicitly defined loss in biodiversity and forest cover leading to significant effects on global atmospheric balance (Fearnside 2001).

# **II. The Environmental Consequences of Fishmeal Production**

Commercial fisheries and aquaculture currently supply about 20 kg of sustenance per capita, earning their place as one of the most traded commodities in the world (FAO 2016). An increasing global population will put more pressure on the use of aquaculture, which currently supplies about half of all fish consumed (FAO 2016). Fishmeal requires a significant proportion of global fish production to sustain; about 15.8 million tons was allocated to fishmeal in 2014. Fishmeal in feed is produced from the crude flour that results from milling and drying fish parts and fish by-products (FAO 2016). Though these fish byproducts supply a significant nutrition source for both fish and livestock, the need for optimizing feed production is incredibly prevalent to reduce costs and maintain a sufficient supply for a growing demand. As oceans become less resourceful due to overfishing, bycatch, and acidification, more efficient and sustainable systems need to be evaluated (FAO 2016).

Weakened fish stocks in maritime regions are the result of a high demand for fish oil, fish, and fishmeal in feed across the globe. The world fish stock acts as a stable income for communities and helps improve food security and employment sectors as fisheries management has improved and aquaculture techniques modify. The use of fish for feed, food, and trade has had beneficial impacts on nutritional stability of communities and total output for fish stocks. However, 31.4 percent more fisheries were overfished in 2013 than 1974 (FAO 2016). Stocks of the ten most productive species, which supplies 27% of global fish, are fully fished with no potential for increases in production. The remaining stocks are overfished and require restoration plans to reach healthy population levels (FAO 2016).

Overfishing has instigated biodiversity loss, habitat destruction, and has altered the state of the ocean's health (Okamoto 2017). This anthropogenically-driven change has resulted in the acceleration of high density fish farming which results in high disease risk due to overcrowding and an increased use in antibiotics and growth hormones to increase supply for a hungry economy (Jackson 2001). Detrimental effects are evident when examining the export values of countries globally; for example, in 2014 Thailand dropped from its status of the third major exporter due to disease outbreak in shrimp production (FAO 2016). Though other exporters can step in to meet demand, the risks associated with mass rearing of animals of any kind outline the potential for future risks and the need for much more stable production methods.

Fish farming requires resources from the ocean such as habitat space, biogeochemical cycling, and micronutrients, despite the common misconception that aquaculture is decreasing our reliance on wild fish (Naylor 2000). The more common species harvested from fish farming are finfish, shellfish, giant clams, anchoveta, Chilean jack mackerel, Atlantic herring, chub mackerel, Japanese anchovy, round sardinella, Atlantic mackerel and European anchovy (Naylor 2000). These particular species are used for feed production and livestock industry and characterized as small pelagic species (Naylor 2000). In their life cycles, they require nutrient dense resources, and farming is often supplemented with materials to stimulate the growth of algae as they are at too high of a concentration to receive it from their immediate surroundings (Naylor 2000). The combined requirement for space, nutrient addition, and feed made generally of soy, pea and fishmeal, make fish farming a costly resource that is advancing the exploitation of our ocean ecosystems.

## **III. Reducing Environmental Impacts of Soy and Fishmeal Production**

The most efficient way to reduce the environmental impacts of soy and fishmeal based animal feed would be to develop integrative and sustainable systems specific to geographical location, societal demand, and economic stability. Effectively installing and sharing new technological advancements in agricultural production could reduce the environmental impact of our current production system. Wide ranges of technological developments currently exist, though few are adopted as new global systems. A few examples include genetically modified crops resistant to drought-prone areas, vertical farming for urban food production, livestock and agricultural sensors to detect optimize water usage, and in-vitro meat (Parry 2010). Each of these advancements could potentially decrease the need to expand aquaculture harvest and soybean crops by providing steady, additional sources of nutrition, thereby decreasing reliance on water and land in full (Parry 2010). Implementation of these new procedures would require thorough research of production systems to create an incentive for large agricultural companies to change supply processes. Shifting the demand away from unsustainable methods would ideally trigger a market response for companies who would find it more beneficial to change current food system processes.

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The 2016 proposal for aligning the future of fisheries and aquaculture with the 2030 agenda for Sustainable Development in the FAO proposes an integrative solution to account for food security, current stocks, global hunger, trade and economic stability (FAO 2016: *State of World Fisheries and Aquaculture*). In order to restore and manage coastal ecosystems at their current state, this proposal broadly summarizes the need for a long-term shift in our ability to produce, distribute, and dispose of food in our current agricultural systems. A few examples of this are outlined in the most recent development for alternatives on the basis of World Fisheries:

- 1) aquaculture zoning to minimize risks (for new aquaculture), and relocation to less exposed areas (existing farms)
- 2) appropriate fish health management
- 3) increasing efficiency of water use, water recycling, aquaponics, etc.
- 4) increasing feeding efficiency to reduce pressure and reliance on feed resources developing better-adapted seed stock (e.g. tolerance to lower pH, broader salinity resistance, faster-growing strains and species, and other attributes)
- 5) ensuring high-quality, reliable hatchery production to facilitate outgrow in more stressful conditions, and to facilitate rehabilitation of production after disasters
- 6) improvement of monitoring and early warning systems
- 7) strengthening farming systems, including better holding structures (e.g. sturdier cages, depth-adjustable cages [for fluctuating water levels], deeper ponds) and management practices
- 8) improving harvesting methods and value addition.

#### (FAO 2016)

These changes would require significant alterations to our current governmental bodies, but also demand more research for long-term installment of unconventional aquaculture practices (FAO 2016). Appropriate policy modification and application would have to be involved in this process, as well as any other method to reduce the environmental impact of fishmeal.

Another effort to reduce the environmental impacts of soy and fishmeal is to effectively assess the impact of various dietary patterns on the environment, and use this knowledge to install a multifaceted food system according to location, public health, and local economy. These would entail efficient life-cycle assessments of different diets, few of which exist for every climate and location due to the natural variation of human consumption and accessibility to resources across the world (Baroni 2001). The motivation for these assessments can be derived from the increasingly problematic use of water for food production. These life cycle assessments can effectively summarize the water use of different diets, and which is more/less probable in areas considering irrigation demands, population size, and current eating habits. "The planet's freshwater reserves will no longer be sufficient to feed our descendants with the present western diet" (Baroni 2001). Water, combined with projected land use and the byproducts of livestock (methane, manure, fertilizer buildup) display the statistic of animal farming and agriculture consuming approximately 70% of the freshwater consumption on the planet (World Watch Institute 2004). Given this figure, and the total water required for a meatreliant diet, it is evident that our consumption habits require significant alteration if we expect to feed a growing population sustainably; that is, nutrition that supports healthy individuals, economy and the environment. A barrier in implementing this solution is convincing the public to change dietary habits; a task often met with extreme reluctance (Baroni et al 2001). The pressure of climate change, which will increase drought frequency, desertification and lower food security at an increasing rate, will most likely be the driving factor in widespread consumption change (van Hius et al. 2013). Freshwater implications of feed origin and composition are important variables that need to be understood for future environmental policymaking (Mekonnen 2010). This will

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remain as a top priority for reducing the deleterious effects of concentrated feeding operations and expanding agricultural sectors. Initial changes can be made in the food industry by confronting production processes.

In order to decrease the reliance on soy and fishmeal to feed in our meat industry, food industries should encourage the expansion of alternative calorie production and consumption. This could be met by using insects as feed, which requires fewer resources compared to traditional soy, corn, pea, and fishmeal based feed (FAO 2016). Using insects as a source of livestock feed for indirect consumption by humans could significantly increase food security and bypass social barriers of insects for human consumption (Shadreck 2014). A survey of poultry farmers in the states of New South Wales and Queensland Australia concluded through the details of rearing, feeding, and producing insects as feed commercially, that insect based feed is economically feasible if the correct utilization is used per geographic area (Shadreck 2014). Multiple insects show potential to replace feed for humans and animals. An exemplary effort to reduce the environmental impact of standard meat production was the EU FP7 "Proteinsect" insect initiative started in late 2013 by the European Commission. This assembly of multiple research projects demonstrated efforts to increase efficiency in production and display a concern for the long term sustainability of our current food production systems. These efforts are outlined below and display motivations to increase the use of creative, unconventional processes such as insect feed into our food systems.

- *i.* Awareness and collaboration among relevant ministries, such as agriculture, health and the environment;
- *ii. The implementation of existing policies and the creation of new policies, such as food and feed regulations;*
- *iii.* The creation of incentives aimed at knowledge centers for research, development and graduate and post-graduate training; **10** of 47

- *iv. The creation of incentives aimed at the private sector for investment and technical development;*
- *v.* The provision of technical assistance in sustainable insect harvesting and insect farming.

(van Huis et al. 2013)

Below I examine studies across a range of bird species, insect feed, and geographical location, to reinforce these predictions and encourage FDA approval of alternative animal production methods. These are certainly not inclusive of all research conducted rearing livestock on insects but serve to highlight the important conclusions and suggested areas for future research.

# **IV. Previous Evidence of Insect-Based Feed Potential**

Insects are part of the natural poultry diet, and can be reared on a variety of different diets such as manure and food waste (Diener 2011). When raised in backyard settings, poultry have access to insects, but this is not the case with concentrated feeding operations. Feeding trials have been conducted regarding insect feed as a sustainable alternative to soy and fishmeal and its effectiveness has been positive as early as 1977 (Newton 1977). Additionally, it has proven a versatile ingredient when fed to fish with no significant change in growth or taste quality (Bondari 1980). It is necessary to see the extent of these successes through a culmination of different feed trials and analysis of Black Soldier Fly for poultry and other livestock. The diversity of insects on earth is higher than any other, and the potential for just a few to replace soy and fishmeal is entirely feasible (De Marco et al. 2014).

Crickets are similar to Black Soldier Fly, in their ability to provide a steady nutrient source for multiple animals, particularly broiler chickens. Feeding trials examining the amino acid profile of crickets when fed to broiler chickens has demonstrated that there is no adverse effect on meat quality or chicken growth as a result (Finke et.al. 1984). The quality of the Mormon cricket is equally as viable as soymeal for chicken body weight and feed ratios. This was proved using a "taste trial" with 26 panelists who would attempt to detect the difference in taste, as well as a closer inspection of the meat using amino acid analysis (Finke et al. 1984). Both demonstrated no significant change. A limitation of this specific experiment was revealed to be the absence of sufficient arginine and methionine in a cricket based diet. By supplementing these amino acids to the diet, there was an increase in body weight and protein conversion in general (Finke et al. 1984). The utilization of soy-cricket, corn-cricket, vs. corn-soymeal trials in this publication demonstrates a similar method that will be performed in this 2017 feeding trial at Black Cat Farm. This variation demonstrates that different ratios of traditional and non-traditional feed can work in tandem to promote poultry health. This may be beneficial when allocating the most efficient feedtype for different animals at different locations across the globe.

A Zimbabwean experiment demonstrated that using insects for feed was not only equal to standard feed, but economically competitive with it (Shadreck et al. 2014). This experimental design used *Macrotermes falciger* (termite) and *Encosternum delegorguei* (edible stinkbug) as insect feed, and saw a regular steady increase in growth (doubling of weight) every five days. The food utilization did not differ for either feed-type and there was no evidence of malabsorption for hens or cocks. This was evident from a closer inspection of internal organs. Insects supply a sufficient amount of proteins which help the animal select building blocks for growth, but the chitin present in insect exoskeleton may pose a problem for chicken digestibility (Shadreck et al. 2014). Supplying an aid for digestion in tandem with insect protein is a topic of upcoming research for further implementation.

The digestibility coefficients of the nutrients and metabolic energy supplied from two different insect larval meals can be analyzed for broiler chickens to detect dry matter (g/kg diet), crude protein, ether extract, gross energy, indispensable amino acids, and dispensable amino acids (DeMarco 2015). These characteristics demonstrate a more indepth analysis of the extent to which insect feed may or may not affect chicken growth and development. The chemical composition, digestibility coefficients and amino acid profiles have previously revealed that insect larval meal "could be used as both a protein and an energy ingredient for feeds" (Sauvant et.al., 2004). The two different insect feeds, Tenebrio molitor (mealworm) and Hermetia illucens (Black Soldier Fly), indicated no significant statistical change in digestibility and only small variation in the absorbance of ether, with *H. illucens* absorbing it more effectively. *T.molitor* indicated a higher ileal absorbance of amino acids, but this was not significant enough to discount the positive correlation between chicken growth and insect ratio in feed. This study, being the first of its kind, demonstrated a meticulous investigation of digestible Amino Acids for characterizing the nutritional value of *T.molitor* and *H.illucens*. This will be essential in any long term implementation of insect based poultry due to the extensive research that will need to be done for feed companies to sell insect raised meat to the public (DeMarco 2015). Given the successes of this feeding trial, it is feasible to assume chicken meat grown on insect feed will ultimately provide an appealing and effective alternative to consumers and producers without any significant reduction in digestibility.

A microbiological analysis of quails raised on *H. illucens* was performed to observe specialized bacterial growth and any significant change to the microbial load

with alternating diets (Cullere 2016). Quails ultimately demonstrated "the same final slaughter weight, body weight gain, feed intake, feed conversion ratio, and mortality in all dietary groups" (Cullere 2016). Limitations arose with the effective digestion of chitin, similar to that of DeMarco, 2015. However, the polysaccharide was also suggested as a prebiotic for bird immune systems "by increasing the caecal production of butyric acid, the prime energy source for enterocytes" (Cullere 2016). This would imply increased nutrient absorption, blood flow, and tissue oxygenation (Mahdavi and Torki, 2009). Continuous evidence of poor absorption of chitin, resulting in nutrient limitation, suggests that its presence may pose more of a burden than effective prebiotic (DeMarco 2015, Cullere 2016). There is a proposed suggestion for high-pressure processing to remove this chitin (Cullere 2016), a process that may be necessary if agricultural companies adopted mass assembly of insects for meat production.

Given insect feed research is still in its infancy, a few insects have been specifically favored and recognized as realistic replacements. As previously discussed, preceding research endeavors feature mealworm, Black Soldier Fly, and crickets. All three, among many others, present possible alternatives for animal growth. Black Soldier Fly is chiefly recognized due to its characteristics of waste conversion that significantly add to the ability of this feed to decrease its total water, land, and resource usage (Salamone et al. 2016). Black Soldier Fly based feed could provide a sustainable farm system by creating a closed cycle of nutrient use and conversion, especially for smallholder farms (Salamone et al. 2016). Implementing this method on a larger scale to meet commercial demand for fish and soy, requires a more in-depth analysis of its conversion ratio, life cycle, and nutritional limitations. This thesis utilized slow growing Cornish Cross Broiler species to determine the viability of insect feed for larger feeding operations, which often utilize this species of chicken. Additionally, the U.S. per capita availability of broiler species is currently surpassing beef for the first time since 2010 (Bentley: USDA, 2014); making them favorable for research endeavors such as this 2017 feeding trial. Examining characteristics of this species during and post-trial will help to gain approval by the FDA and USDA for wide-scale adoption by meat manufacturers.

## V. Black Soldier Fly Potential as Animal Feed

Black Soldier Fly (*Hermetia illucens*) is notorious in its ability to break down complex macromolecules and convert them into viable nutrients for surrounding soil and environments. Among the many different potential benefits from Black Soldier Fly, their ability to mitigate two huge problems, food waste and food insecurity, has made them subject to high debate and prospective research. There is still a novel understanding of the ability for these insects to replace feed for all livestock, but its success thus far has proven extremely efficient (Bondari et.al. 1981, Newton et.al. 2005, Cullere 2016).

The fly life cycle is comprised of a four day hatching period, by which larvae begin to grow from 1mm to about 27mm in length. Full development is reached around 14 days, and pupation begins when the exoskeleton of the larvae darkens, this stage takes approximately two weeks, and when hatched the adult can mate within 2 days and lay about 500 eggs soon after (DiCarlo 2009). The stage in which the larvae grow (the 14 day segment) is when they require the heaviest input of nutrients (DiCarlo 2009). This may include livestock waste, food waste, or animal feed, but they prefer to feed on substances with ubiquitous microbial loads (Jeon et al. 2011). 45,000 larvae can effectively consume about 24 kg of feed in 14 days (Newton et al. 2005) while simultaneously reducing the microbial load of animal waste (Sorkin 2015). There is a lot of potential regarding public health and the Black Soldier Fly, particularly concerning their ability to reduce the amount of E.Coli. present in waste (Erickson 2004), and their potential to lower the concentration of Salmonella spp. present (Lalander 2015). The high content of lauric acid in H.illucens acts as an antimicrobial agent, disrupting the cell membrane, and maintaining a potential to control various foodborne pathogens (Kim and Rhee, 2016). In addition to this, the peptide production secreted in the haemolymph due to insect immune response can result in antimicrobial secretions (Cullere 2016). Each of these naturally occurring antimicrobial properties could ultimately prove useful for food production and lower the risk of bacterial outbreaks resulting from meat consumption.

Once the larvae have grown for 14 days and matured to the point of pupa growth, there are two proposed uses. (1) To be collected and fed to livestock and (2) the remaining soil and excrement can be utilized as a fertilizer. The Black Soldier fly is proficient at converting waste due to its diet-dependent microbiota (Jeon et al. 2011). Different feed for the larval stage creates different ratios of gut microbiome consisting generally of Proteobacteria, Firmicutes, and Bacteriodetes (Jeon et al. 2011). Due to this variation it is suggested that the fly has evolved an incredibly adaptive gut biota, which explains its uniquely efficient waste conversion characteristics. Regardless of feed types, the waste is broken down within the gut of the fly through enteric fermentation that releases carbon dioxide and water; this process of biodegradation involves "the temperature of the substrate rising, pH changes from neutral to alkaline, ammonia release increases and moisture decreases" (Cickova 2015). Understanding the microbiota of Black Soldier Fly will become increasingly important as more alternatives for animal feed are considered because little is known about the interactions between insect gut microbiota and livestock microbiome.

Despite gaps in our understanding of insects, this thesis explores the following question: *Can insect-based protein provide an efficient alternative to soy and fishmeal in poultry feed?* In an experimental trial, feeding Black Soldier Fly larvae to Broiler chickens will demonstrate how effectively birds can develop on a less resource intensive protein source; I hypothesize that their growth rate will not be stunted and there will be no significant change in feed intake or mortality. This process demonstrated a method that could be scaled up for future implementation into our agricultural systems.

# **Methods**

To compare the efficacy of Black Soldier Fly feed to traditional soy and fishmeal on poultry development, 150 Cornish Cross Broiler chickens (Gallus gallus domesticus) were raised at Black Cat Farm in Boulder, CO. Broiler chickens are known to be the most efficient for meat production, with an input of 4 kcal of fossil energy for each 1 kcal of broiler protein produced (Pimentel 2003). One-day old vaccinated chicks were purchased and delivered to Philip Taylor in Boulder, Colorado on February 3<sup>rd</sup> 2017. For the first three weeks of the feeding trial, the chickens were fed chicken starter feed which broadly consisted of corn, soy, pea, barley, wheat, and various vitamins to ensure consistent early growth. The chicks were initially raised in a small 'brooder' enclosure within a garage to reduce mortality given climate variation and freezing night-time temperatures. This brooder was composed of a cardboard fence enclosure approximately 4ft x 4ft. This expanded to 4ft x 6ft for the second week of growth, and continued to expand every week of growth until the enclosure reached 4ft X 8ft. The brooder was supplied with two infrared lights for heat, these did not disturb the chicken's circadian rhythm. There were two insulator heaters that provided chickens with warmth and additional footing for perching. To maintain

temperature for the first 3 weeks within the brooder, a space heater was used. New chicks thrive in temperatures of 90+ degrees at the start of their growth. The temperature thus dropped from an initial 90+ degrees down to 60-70 degrees as they aged into the fourth week. After four weeks, the chicks were continuously exposed to outdoor temperatures by opening windows and temporarily exposing the brooder. This encouraged adaptation to a colder climate as they were eventually moved to a colder outdoor enclosure. Potential risk of stress due to different heat exposure and consequential oxidative trauma could temporarily suppress feed conversion efficiency or lead to the risk of immunosuppression (Akbarian et al. 2016). Therefore, the temperature was continuously monitored throughout the duration of the experiment, especially for the first two weeks of the chick's life.

#### **Experimental design**

After six weeks, the chickens were moved from the brooder to Black Cat Farm chicken pens. This enclosure consisted of a barn retrofitted into nine pens. The nine chicken "pens" were 3ft x 10ft, each with a 8in x 8in trapezoid shaped door that exits into a chicken run, and a 25ft long outdoor open area extension from each pen. This extension is protected from predators with snow fencing secured by T posts and enclosed by low voltage electricity fencing. Each pen had lodgepole pine bark bedding, a roosting bar, one food bucket, and one water basin. Food and water were fed to chickens *ad libitum* throughout the experiment.

At week seven, 127 chickens were divided, counted and labeled between the nine experimental blocks (pens). Each chicken within the group was tagged using a uniquely numbered poultry leg tag. Three replicates of each feed group (insect, soy, fish) were represented in each of the three pens (A,B,C). This helped to account for the variation between each pen such as the presence of a tree and/or patches of red clover.

#### Feed preparation and composition

All three feeds were mixed and prepared by John Fehringer of Fehringer Farms, a professional feed manufacturer. Each feed was be supplemented with vitamins and other ingredients such as pea protein and wheat; none of which alter the effect of the independent variable (soy vs. fishmeal vs. insect). These ingredients are necessary to create a healthy and complete grower mix for poultry that includes vitamins not otherwise specified. Prior to milling, the insect feed was defatted due to its high fat content using a mechanic press. The ingredients of each feed are (1) dominant protein source (soy vs. fishmeal vs. insect),

(2) pea protein (3) wheat base, and (4) various vitamins and minerals to supplement.

The three feed types were mixed with standard supplementary constituents to create equitable nutrient composition. All ingredients independent of the main protein variable, remained constant to accentuate the differences between soy, fishmeal, and insect feed. The insect-based feed was produced using the larvae of Black Soldier Fly (Hermetia Illucens) reared on local food waste. These insects were grown in a hatchery located on Black Cat Farm previous to the start of the feeding trial. They were fed local brewer's grains, food waste, and fish offal, which is stated to be a more effective diet for rearing Black Soldier Fly larvae (Anankware et al. 2014). The final preparation of the insect feed was defatting larvae by mechanical press to increase crude protein percentage and remove unnecessary fat that prevents optimal growth (FAO 2016). This will also aid in the digestibility by increasing the ratio of available crude protein in the larvae that can be consumed, converted, and stored for chicken growth. 1 kg dry weight of insect meal contains approximately 200,000 housefly larvae House fly larvae meal has a reported protein content of 37.5 -63.1% (Proteinsect 2017).

#### Feeding trial duration

The trial took place for 3 weeks, observing chicken growth from 7-10 weeks of age. Nine weeks is the estimated length of time for Cornish Cross chickens to approach maturity and this feeding trial provided evidence for how well the chickens transition into adulthood. The remainder of the experiment will test whether these different feeds affect chicken growth and survival from weeks 10 onwards. Slow growing Cornish Cross Broiler Chickens (*Gallus gallus domesticus*) generally reach maturity around 12-16 weeks depending on environmental conditions and feed accessibility (CIWF 2013). After the chickens have reached an optimal age of 12-16 weeks, the optimal age for meat production, they will be euthanized and analyzed for Amino Acid content and protein digestibility. The weeks following this thesis' time frame will entail the same method of data collection.

#### Data collection

Throughout the trial, the weight of each chicken was measured every three days to track three variables: 1) chicken standard growth rate (defined as the proportion of food consumed converted into meat, and measured as *100 x* ((*final body weight - initial* (*g*))/ *days of trial*); 2) feed conversion ratio (defined as the proportion of food consumed converted into meat, and measured as *feed intake/ wet weight gain (mass units)*); and 3) mortality (defined as death due to experimental condition factors and measured as *Total deaths per feed/ total number of chickens*). Weight was measured using an American Weigh Scales AMW-SR-20 Yellow Digital HanGinG fishing Scale, and an attached plastic hanging basket to place/hold the chicken in. The weight of this hanging basket was recalibrated between each measurement so it wasn't included in the chicken weight. Units of the fish scale were in pounds (lbs). Occasionally, specific weights were repeated on the fish scale for different chickens, and when this occurred, the scale was restarted,

recalibrated and the chicken was measured again. If different numbers appeared, the average of these numbers was recorded as the final weight. This was not a continuous occurrence, but this procedure helped to outweigh additional bias of the scale.

#### Collaborators

The experiment involved several different collaborators. The project took place within a hatchery and refinery run by Dr. Phillip Taylor of MadAgriculture, where Black Soldier Fly Larvae were bred and harvested using organic food waste from local restaurant sources. The chickens were raised at Black Cat Farm, within a garage for the first six weeks, and a barn/run enclosure for the remainder of their growth. Black Cat Farm is owned by Eric and Jill Skokan, who helped advise and oversee the management of chickens pre and post euthanasia. Feeds were prepared and nutritionally balanced by John Fehringer of Fehringer Farms, a commercial feed manufacturer in Nebraska who supplies 30 tons of feed to Front Range Farms. The Amino Acid profile and ileal digesta will be sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories for analysis post euthanasia. The project has met the guidelines for the ethical treatment of animals from the Institutional Animal Care and Use Committee (IACUC) at the University of Colorado, Boulder. An on-site inspection and project analysis permitted this authorization.

#### Data analysis

Data was recorded in a notebook and transferred to Excel datasheets. Chicken growth measurements were entered into a MAC statistical program for analysis, Aabel NG Data Analysis Software. Various types of the general linear ANOVA models were utilized to account for variation and identify significance between each feed type (soy, fishmeal, insect). This included *between subjects* ANOVA, D'Agostino-Pearson test on Regression Residuals, and a Scheffé Test. I set significance levels of p<0.05. *Between subjects* ANOVA explained variance of mean weight due to the independent variable (feed type). Comparisons within levels of feed were examined using a Scheffé test; standard growth rate (SGR) and Feed intake / day were analyzed (separately) to observe chicken growth patterns. Mortality was defined within this feeding trial as *Total deaths per feed/ total number of chickens*. Of the original 150 chickens, 23 individuals died before the start of the feeding trial. The factors that led to these losses were unrelated to the insect feed, and were due to shipment conditions and environmental stress for juveniles. There were no instances of mortality for the 127 chickens during this 3-week examination concluding that feed type did not influence mortality within this thesis.

### <u>Results</u>

The observed 127 Broiler Chickens displayed an equivalent growth rate to soy and fishmeal based feed when fed Black Soldier Fly larvae. Figures (1-4) analyze how soy, fishmeal and insect compare on the basis of standard growth rate (SGR), feed intake, and feed conversion ratio (FCR).

#### Chicken growth rate

There was no statistically significant difference in growth rate for chickens among each of the three feed groups (ANOVA: p = 0.3448, 0.2082, 0.5950; Fig 1). Comparisons between each feed group (soy, fishmeal, insect), as outlined in the following table, generate a non-significant statistical relationship.

Scheffé Test ( $\alpha = 0.05$ )							
. Comparisons Within Levels of Feed							
Groups	Difference	Statistic	р	Significant			
Fishmeal vs. Insect	-0.007	1.089	<mark>J.344と</mark>	No			
Fishmeal <i>vs.</i> Soy	-0.011	1.621	J.2082	No			
Insect vs. Soy	-0.004	0.525	J.595C	No			
Dependent: SGR2	Γ'	1	•				

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The standard growth rate (SGR) between each treatment revealed p > 0.05 and is thus, not

significant. SGR was calculated as (final weight-initial weight (lbs))/(days).



Weight was recorded every three days, and the growth rate between these three days was averaged with the initial and final weight all chickens within the **same** treatment. In doing so, variation between individual chickens within the same treatment is accounted for. Outliers (*Figure 2*) show deviation from the mean of the results, but do not cause a significant difference in growth rate (lbs)/ bird/ day. Distribution varies for each feed type due to the natural variation of chicken consumption and conversion between pens. This had no effect on the calculated rate of growth.

# Feed Intake Per Group

Feed intake per feed group was calculated as *[(total feed consumed/days)/(# chickens in Group)]*. Groups A-C for each treatment (feed type) were then averaged. In the same approach as calculating Broiler SGR, the feed intake per group was calculated using a Scheffé multiple comparison procedure.



Figure 3

Scheffé Test (α = 0.05) Comparisons Within Levels of Feed						
Groups	Difference	Statistic	р	Significant		
Fishmeal vs. Insect	0.010	0.472	0.6265	No		
Fishmeal <i>vs.</i> Soy	0.018	0.862	0.4286	No		
Insect vs. Soy	0.008	0.384	0.6830	No		
Dependent: Feed In/Day2						

Adjusting significance levels in this linear regression analysis accounted for multiple comparisons, and resulted in p-values > 0.05 for soy, insect, and fishmeal. Despite a slightly higher intake of fishmeal feed for three weeks, the lbs./group consumed by chickens was relatively the same, averaging at about 0.2 - 0.23 lbs/group.

#### **Feed Conversion Ratio**

Feed conversion ratio is the amount of food consumed that is converted into body mass. Cornish Cross have a range of known conversions, as it is dependent on food availability, environmental pressures, and feed type. "Good" conversion numbers for commercial production are generally 1.42-1.85 FCR (University of New England 2017).



FCR stayed within typical ranges for commercial chicken production. Variation between feed intake and weight was insignificant. Distribution of weights was greater for soy. Soy group in Pen A, demonstrated a decrease in weight compared to the other eight groups between the dates of March 28<sup>th</sup> and April 1<sup>st</sup>; lost weight is discussed further on page 31. Despite this decrease, soy vs. insect and soy vs. fishmeal was still insignificant for feed



conversion. Not only were their conversion ratios within standard levels for commercial chickens, but they did not vary significantly for Black Soldier Fly based food. The margin for Insect (Figure 4) is also smaller, demonstrating less variability in feed conversion ratio. This can be interpreted as a more consistent intake and conversion for chickens eating insect based feed.

#### Temperature

Given the occurrence of rain and snow, and a significant drop in temperature between March 28<sup>th</sup> and April 1<sup>st</sup>, mid-way through the feeding trial, temperature for the given time frame was analyzed and compared to all three characteristics of growth to identify prominent trends or explanations for outliers within my data.







Linear Regression: Temperature vs. SGR & Feed Intake

There is a direct relationship between temperature and feed intake (ANOVA: p=0.0498) and temperature and standard growth rate (ANOVA: p=0.0498). The decrease in growth rate and feed intake followed a similar pattern to temperature (Figure 5); this synonymous decrease helps to explain a temporary weight loss of chickens between the dates of March 28<sup>th</sup> and April 1<sup>st</sup>. Long term affects of this drop in feed intake may be

seen post euthanasia in 2-6 weeks time. The decrease in growth rate and feed intake was shared among each treatment; feed type was not correlated to weather resistance.

#### **Discussion**

The purpose of this feeding trial was to determine whether Black Soldier Fly larvae (*Hermetia illucens*) is a viable alternative to soy and fishmeal in poultry feed. I hypothesized that the Cornish Cross Broiler chickens would show no significant difference in growth rate, feed conversion ratio, feed intake, or mortality. My hypothesis was provisionally confirmed on the basis of three weeks of data analyzed within this experiment. Black Soldier Fly larvae, according to these results, is therefore a potentially viable alternative to soy and fishmeal in poultry feed. This supports previous research that has also identified BSF larvae as completely viable for some livestock. This growing consensus indicates that there is potential to manufacture insects and raise chickens on Black Soldier Fly reared on food waste.

Figures 1-4 demonstrate that standard growth measurements (SGR, FCR, and feed intake) were not inhibited. Outliers within the graph represent natural variation in chicken species, and each of their ability to consume, convert, and grow. The variation that exists between each individual chicken also exists between each pen. Pens were within the same size, but slight differences (2in less wide, etc.) would have the potential to decrease sq. ft. per bird, and change growth patterns. As chickens approached 3.5 pounds, they were more likely to jump around the pen and attempt to escape. If this occurred, smaller weight gain for a few chickens could explain outliers (*figure 2*). These variables still had no effect on the overall health of the chickens. Not only did they grow healthy, but feed type did not act

as an inhibitor to growth. The results of the Scheffé test confirmed my hypothesis (*ANOVA:* p>0.05, *Fig 1-4*), and revealed the versatility of potential feed ingredients.

Due to unexplained deviation from normal weight gain, specifically during a time frame of March 28<sup>th</sup> and April 1<sup>st</sup>, the temperature (F) of each day of data collection was recorded using local weather reports, and plotted against SGR and feed intake. A linear regression revealed whether the drop in temperature could account for the drop in weight and and increase in specific growth rate (lbs/bird/day). Temperature and feed intake follow along a similar pattern (Figure 5 A, B, C) a Y on X Linear Regression ANOVA revealed a significant correlation between temperature and feed intake per day (ANOVA: p=0.0488, *Figure 6*). Specific growth rate and temperature were also significantly correlated (ANOVA: p=0.0488, Figure 6) though they did not share a direct relationship with one another. Where temperature dropped on day 13, specific growth rate increased, opposite to the pattern represented by feed intake. This may be explained by the fact that within a small time frame (3 days), the growth rate is still continuous as chickens are maturing and growing larger, but the amount of feed consumed was less overall. This would imply that (lbs/bird/day) would increase because less food was consumed, so conversion of any feed intake would appear larger and more significant relative to the usual rate of consumption.

# Limitations

There were several limitations to the completion of this feeding trial. Climate variability caused temporary expenditure of the on-site cistern, restricting access to water for a day, which could have stressed the chickens growth. As previously stated in this thesis, *potential risk of stress due to different heat exposure and consequential oxidative trauma could temporarily suppress feed conversion efficiency or lead to the threat of immunosuppression* (Akbarian et al. 2016). A temperature dip between March 28<sup>th</sup> and

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April 1<sup>st</sup> (*figure 5, C*) reached a temperature of 38 degrees Fahrenheit. This drop was accompanied with rain and snow. The combined cistern drought and succeeding cold weather may have led to a slight decrease in water weight of the chickens, or caused stress that could have temporarily suppressed feed conversion efficiency. Cold weather can stress poultry immunity and instigate a temporary weight reduction; there was evidence suggestive of this on April 1<sup>st</sup> for soy feed group (A). This variation is described by the bottom heavy distribution of soy feed intake compared to other feeds (*figure 4*). This weight loss was temporary, but may have caused longer-term damage that may be seen in later data collection. This particular pen (Soy A) was located on the north-most side of the barn, where it was subjected to the most contact to incoming wind. This may explain the drop in weight of the chickens between March 28<sup>th</sup> and April 1<sup>st</sup>.

Due to freezing overnight temperatures and snow/rain exposure mid-day, chickens were occasionally kept inside for longer periods of time than usual. Given that each pen is 3ft x 10 ft, each chicken would have about 2ft x 2ft given 14-15 chickens per pen. More square footage is better for chicken health (Pandurang et al. 2011), but any crowding increases risk of disease exposure, stress and aggressiveness. The general recommendation for space is about 2.1-2.7 sq ft. per bird (FAO, *Ch 4.1*, 2016), which is within the space allocated. There is still a risk of overcrowding due to the variation between pen sizes, so extended periods inside remain a variable in growth outliers of these chickens.

Additionally, a few chickens briefly escaped from their assigned pens, either removing their access to food and water (if they left the enclosures completely), or providing them with access to a different food, until they could be reassigned to their correct enclosure. Either eventuality could have affected the weight of the chicken over the short term. This was not a significant occurrence, and chickens were checked at least twice a day to account for movement out of or between pens. The proportion of chickens that stayed within their assigned pens greatly outnumbered these random instances of variance. Further, growth rate over a three-week period was reflected as an averaged weight of each of three replicates within each three feed to overwrite inconsistencies between pens and chickens. The use of a fish scale and hanging bucket to record chicken weights every three days may have presented additional limitations in accuracy of data. All of these limitations were relatively minor disruptions, and the lack of significant difference between the feeds inspires a justifiable conclusion that insect based feed is a viable alternative for raising chickens.

The last limitation to be specifically recognized is the absence of data for the first seven weeks of growth within this trial. A feeding trial that began from the start of growth and compared all three feeds from birth to death of the chickens could show a more detailed account of long-term growth or impairment. This was not explored in this feeding trial as it would require insect based starter, which would consist of different vitamins and minerals, separate from the feeds prepared for this feeding trial starting at Week 7.

#### **Barriers to Scaling up Insect-based Animal Feed**

Despite research on the viability of insect based feed to replace soy and fishmeal, there are multiple barriers to the wide-scale adoption and implementation of insects into our food systems. These barriers include: legal and economic barriers, and a general gap in our knowledge of insects as feed which places an additional obstacle in universal adoption of alternative feed. Overcoming these obstacles is important for long-term change within our food systems to shift agriculture into alternative, more sustainable methods of production. Observing past successes of experiments, such as those mentioned in section (IV, page 14), will help to approximate the scale of each of these barriers in an effort to overcome them.

#### Legal barriers

Feed acts as a potential source of contamination and must go through rigorous assessment to ensure consumer safety and longevity (FDA 2015). Common contamination includes "non-Typhi serotypes of Salmonella enterica (that can) infect animal carcasses and cross contaminate other foods" (Crump 2002). Due to significant knowledge gaps in our understanding of insect interactions with humans and animals, transition into alternative feed will require additional inquiry. This inquiry would need to assess the following risks; insects as a source of new transmission of diseases in varying environments, allergies and preservation of storage for mass production (van Huis et al. 2013), and environmental or plant pest issues (Khusro 2012). Given the methods conducted within this experimental feeding trial, ecosystem damage was avoided by maintaining a hatchery and refinery at Black Cat Farm.

Despite risk of contaminants, there is noteworthy potential of insects and their microbial properties to actually decrease the occurrence of bacterial colonization and foodborne illnesses. Insects as natural bio converters and will only be safely used and applied with extensive microbiological research. However, using insect based feed as a staple protein source for livestock results in the following benefits; lower use of antibiotics (less crops are required), natural fertilizers (less chemical exposure to worker and consumer), and improving resistance to pathogens. Widespread use of alternative feed must meet sanitary safety and risk guidelines for any realistic consideration in food production, but due to it's lower environmental cost and beneficial characteristics, Black Soldier Fly larvae as feed would strengthen the safety of animal feed within the industry.

## Economic barriers

Individuals cannot solve proper installation of edible insect sectors; this shift in production will require stakeholders to work towards a common agenda and strengthen their bargaining power (van Huis et al. 2013). Overcoming economic barriers requires efforts of research, advertisement and archetypal sustainable systems. Combined research efforts could consequently demonstrate the opportunity for insects to produce income at the household level and industrial scale. The European Commission research initiative is such an example, and recognizes specific barriers to over come before scaling up in the market and making their "Proteinsect" initiative applicable.

- *i.* The current lack of robust safety data that is holding up progress on the development and discussion of appropriate legislation within Europe.
- *ii.* Additional nutritional quality data is required to show the potential of the use of insect protein for feed and added value products.
- *iii.* Consumer acceptance of insect protein in animal feed has not been fully evaluated.
- *iv.* The current production processes are labor intensive and require further development of semi-automated systems.

#### (Melzur-Venturi, *Proteinsect* 2015)

Approval from the FDA and USDA follows similar barriers like those of Proteinsect. Working towards mass adoption of insects as feed requires recognition of these barriers; observing the ability of the European Commission to approve insect use can serve as a framework for future planning of U.S. agricultural modifications.

Scaling up will ultimately be required to lower the costs of insect-based animal feed. Processing and mechanization is not well established for insect products making the replication of large-scale industrial systems economically inefficient to explore (van Huis et al. 2013). The potential for our food industry to become less resource intensive and

environmentally degrading can be met with sustainable protein sources with high volume, competitive prices, and approval from food industries. Using multiple species of insects to replace standard ingredients and create another market would require extensive knowledge on structure, pH, taste, and water holding capacity of each of these insects (van Huis et al. 2013). Diverse knowledge of insects and their interactions with livestock is required for FDA and USDA approval. Encouraging partnerships between shareholders and small companies would create a viable source of economy to slowly scale up in the market and shift our meat industries to be a bit more effective. Examples of this include various insectbased startups such as Enterra, Enviroflight, Agroprotein, Ynsect, and large scale research initiatives such as those undertaken by Proteinsect to spread knowledge across different sectors and determine market feasibility.

# **Conclusion**

My research suggests that insect feed can viably replace soy and fishmeal as the dominant protein source for animal feed. There are still no significant limitations to chicken growth as demonstrated using an ANOVA to reveal non-significant distinctions between feeds. Differences in chicken growth rate, feed conversion ratio, and mortality were all non-significant within this three week trial. Additional data will be collected throughout the entire lifetime of the chicken to ensure that the trends observed during these first three weeks hold true for the lifetime of the chickens. A longer feeding trial will also create a more stable comparison of the long-term impacts of insect based feed on chicken growth, which are expected to be minimal to nonexistent. Post-euthanasia analysis of the ileum, may confirm this conclusion if it demonstrates zero variation in the apparent protein digestibility (Lemme et al. 2004). Ultimately, this 2017 feeding trial, in

addition to further investigation, aims to help facilitate FDA approval of insect based animal feed. FDA and USDA approval will require a conjoined effort to convey this success, and market its effectiveness to meat producers that would benefit from such a product.

# **Further Research**

In regard to this feeding trial with MadAgriculture and Black Cat Farm, data collection will continue for approximately 2-6 more weeks, during which period all of the same measurements will continue to be recorded. Euthanasia by "cone method" will take place at 2.5-3.5 months of age and an in-depth analysis of amino acid content will be analyzed from the ileum after dissection. This will further confirm/deny initial findings of the study and provide a closer inspection of growth characteristics for the chickens. This will demonstrate the effectiveness of the feeds prepared by John Fehringer, and identify supplements of the feed mixes that may need to be adjusted for commercial use. A more long-term collection of data will also be a realistic depiction of raising poultry for consumption on smallholder farms with insects. The use of Black Soldier Fly on farms outlines that, as this experiment demonstrated, insects can be reared on local food waste, and used to support another source of income (poultry). This closed system has a significant reduction in carbon emissions and ecological degradation due to the collective reuse of food waste, conversion of micronutrients to macronutrients, less reliance on intensive resources such as soy and fishmeal, and the creation of a well-round diet for raising healthy poultry.

Limitations recognized within this experiment served to create the framework for a larger-scale feeding comparison, where feed could be created for each stage of chicken growth, rather than only grower. This would require the manufacturing of separate starter and grower mixes, but could serve to highlight realistic potential of depending on insects to feed and raise poultry without substantial necessity of a removed agricultural system, such as aquaculture or monoculture soy crops. Each of which leads to severe externalities. To further this exploration, feeding insects to other livestock could lessen the effect of meat consumption on the environment significantly. Previous research has identified varying levels of effectiveness for poultry, pigs, and fish. There has yet to be a study examining whether feed can be manufactured for cattle consumption, or for larger systems similar to concentrated feeding operations that currently dictate our agricultural systems. Both of these investigations into Black Soldier Fly potential could serve to be useful in the commercial production and manufacturing for insect based feed. Overcoming barriers to FDA and USDA approval would require investigations into the extent of BSF to substitute soy and fishmeal for various livestock on a large scale to prove/disprove its absolute effectiveness.

Investigations and future research in insects as food and feed will become increasingly necessary to restructure our agricultural systems to be sustainable for nutrition, economy, and the environment. Due to the novel understanding of insects and how they interact with both humans and animals in terms of consumption, a closer inspection of microbiological characteristics could open many doors for innovative thinking. Specific investigations include insect usage to decrease bacterial outbreak, unused insect matter as natural fertilizer, and harboring a healthy microbiome in livestock to promote nutritional value. Understanding more species of edible insects and their microbial/infectious/nutritional interactions with livestock will aid in creating and installing sustainable agriculture techniques that are diverse, dependent on region, and create a less fragile method in an increasingly unstable climate.

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