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A Performance Study of Apis mellifera with Dietary and Forage Restrictions During Spring Colony Establishment in Lyon County, Kentucky

Dominique Wood

A Performance Study of Apis Mellifera with Dietary and Forage Restrictions During Spring Colony Establishment in Lyon County, Kentucky.

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Dominique Wood

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A Performance Study of Apis mellifera with Dietary and Forage Restrictions During Spring Colony Establishment in Lyon County, Kentucky.

DATE APPROVED: ______________________________

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Thesis Advisor

____
Member, Thesis Committee

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Member, Thesis Committee

____
Collegiate/School Graduate Coordinator

____
Dean of the School of Agriculture

____
Graduate Studies Coordinator

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Provost

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ABSTRACT

One-third of our worldwide fruit, nut and vegetable production is completely dependent on the existence of pollinators. Commercial honey bees, *Apis mellifera*, have an annual economic value of $15 billion in the U.S. Additionally, their honey is valued at $150 million annually. In Fall 2006, commercial beekeepers observed sudden mass disappearances of whole colonies. By Spring 2007, the condition, dubbed Colony Collapse Disorder (CCD), raised environmental and fiscal concerns while the cause(s) continued to be sought. Forensic examinations of hive samples did not reveal a singular cause of CCD. The results pointed to a collection of detrimental factors affecting the overall health and resiliency of the hives. The factors included pathogens, parasites, poor hygiene and malnutrition. Commercial colonies are particularly vulnerable to CCD due to immunosuppression caused primarily by malnutrition worsened with continuous stressors of foraging food deserts (acres and acres of monocultures), predation, parasites, exposure to chemicals (fungicides, herbicides, pesticides, neonicotinoids), and pathogens. Complete nutrition is the keystone in order to establish, maintain and expand healthy hives with resilience. A continuous diet with little diversity, as seen with monoculture crops, does not support the colony's activities (establishment, development, and colony expansion) due to limitations in essential amino acids. Soybean flour is frequently used as the base protein source for dietary substitutes due to the high protein content and it is readily available for beekeepers to make up for dearth or pollen shortages. However, dietary deficiencies occur due to the high protein content and the limiting nutrients. The colonies on a restricted protein source (soybean flour) were able to establish and sustain hives weakly for a short time but consistently underperformed and then failed in comparison with the control hives that managed their own nutrition. Performance values used in the study were weight (kg), population of brood, % of drawn comb, and % of food stores. The test colonies were not able to rear brood in generation three and all of the queens of the three test hives failed by the 11th week of the study. The singular source of protein did not support resiliency in the test colonies and the control colonies thrived with access to the same protein source but with additional self-regulated inputs.

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CHAPTER I

INTRODUCTION

Importance of the Study

In the Fall of 2006, David Hackenberg, a Pennsylvania commercial beekeeper, made an astonishing claim that his honey bees were suddenly vanishing. Dr. VanEngelsdorp of Pennsylvania State and a team of researchers conducted forensic examinations of the colony samples Hackenberg and other commercial beekeepers with the same concerns submitted. They revealed that the honey bees were very sick but not with a singular pathogen. The samples showed signs of immunosuppression and undigested pollen. The sick hives contained young workers and a queen that were infected with pests, parasites, viruses, bacterial, and fungal infections (Betz and Taggert, 2010; Johnson, 2007; Johnson, 2010; Shultz, 2007; Williams, 2008). All ailments that healthy hives could have coped with but could not because the worker bees left and did not return to support the hive to recovery. By Spring 2007, the condition had been dubbed Colony Collapse Disorder (CCD) and affected commercial colonies in 35 States (Johnson, 2010; Shultz 2007). The phenomenon was observed in Europe, Asia, and South America (Holland, 2013). The lead researchers suspected the disappearance of the older workers was a response to illness- an altruistic self-removal to save the hive (Betz and Taggert, 2010). The symptoms of CCD include rapid loss of adult worker bees, few or no dead bees found in the hive, presence of immature bees, small clusters of bees with live queen present, and pollen/honey stores in the hive (Johnson 2010).

Apis mellifera is responsible for the pollination of our fruits, nuts, vegetables, clover, and alfalfa. Over 1/3 of our U.S. food production is dependent on this insect (Johnson, 2007; Johnson, 2010). The mass disappearance of honey bees and the condition of the remaining hives proves alarming both financially and environmentally. A future without honey bees removes a variety of fruits, vegetables and nuts from our diets. The decline of the honey bee also indicates the imminent decline of other natural pollinators, 200,000 species, that help the honey bee pollinate our food supplies (Marks, 2005). Thus, potentially leaving people with deficient diets as well. We are fortunate to have the opportunity to see the ramifications of the total loss of pollinators in the Southern Sichuan Province, China. In this instance, all the pollinators including *Apis mellifera* were unintentionally eradicated in the 80s due to uncontrolled use of pesticides on agricultural crops. The main agricultural commodity in the Southern Sichuan Province, the pear, must be manually pollinated by humans with a feather duster (Shultz, 2007).

Migratory beekeepers keep their hives in stress due to the transportation; environmental, thermal, and nutritional changes; parasitic loads; and chemical and pathogenic stress. The ultimate need for hives to overcome these continuous stressors is a nutritious and palatable food source to support and maintain the size of the colony so the colonies can stay healthy, hygienic, protective, and reproductive. Inadequate feed taxes the immune system because the body will harvest body reserves in an effort to continue daily metabolic tasks. Commercial honey bees are trucked all over the country to pollinate acres of monoculture. At the end of bloom, the hives are packed up and relocated to the next monoculture crop. It too will have limiting nutrients. Each time, the workers must work harder to overcome the stress of malnutrition, travel, predation, competition for limited resources from other hives, and chemical exposure that accompany monocultures. The sick and malnourished colonies are already at a disadvantage and cannot overcome continuous stressors while living on a food desert. As populations in the colony dwindle due to stress, the colony as a superorganism grows weaker. Healthy colonies are resilient and can overcome stress. Like any animal under constant stress, adequate nutritional support will offer the greatest chance of resiliency.

Statement of Problem

The purpose of this study is to measure and record performance values of *Apis mellifera* during spring nest establishment and development while feeding on a single protein source, soy flour and 2:1 sucrose syrup.

LISTING OF SUBPROBLEMS

1. Establish and record performance values of colonies of *Apis mellifera*- weight of colony, population of brood, percentage of drawn comb and percentage of food stored.

2. Nutritional diversity and quantity enables a colony of *Apis mellifera* to develop resiliency of pathogens, parasites & environment.

3. Limiting diet creates nutritional and developmental deficiencies affecting the health or population of individuals in a colony.

4. Record observations of colonies during the study.

STATEMENT OF HYPOTHESIS AND NULL HYPOTHESIS

H_1: The test hives' performance will not exceed the performance of the control hives in this study.

H_0: The test hives will perform similarly to the control hives in this study.

DELIMITATIONS OF THIS STUDY

1. Due to cost, the study is limited to six colonies that will be divided into three control hives and three test hives
2. The study utilizes genetically identical, proven, mated queens for colony establishment.
3. The study will occur in one location, Lyon County, KY.
4. The study will have randomized selection of control or test group.
5. The study is limited to a control group that has access to polyfloral pollen naturally present in the environment.
6. The colonies in the control groups are exposed and susceptible to environmental hazards within the area not limited to animal harassment, predation, weather, herbicides, pesticides and fungicide applications in surrounding farms.
7. The colonies in the test groups are caged.
8. The colonies in the control groups are expected to travel up to three miles to locate pollen and carbohydrate sources.
9. The study is limited to an identical single source of queens and packages.
10. The study is established with the installation of 3 lb. package bees with queens on new wax foundations of five-frame nucs for each colony.
11. All nucs received carbohydrates in the form of 2:1 ratio of sugar water with feeding stimulant, “Honey-B-Healthy”, and offered identical protein source of soy flour via dry pollen feeders.
12. Feeders are filled daily to ensure fresh foodstuffs.

DEFINITIONS OF TERMS

1. Abdomen: The posterior segment of the bee containing the honey stomach, stomach, intestines, reproductive organs, and stinger.
2. Acini: small, sac-like cavities in a gland surrounded by secretory cells
3. Amino Acid: an organic compound of an amine group and a carboxyl group. Building blocks of proteins.
4. Apiary: a place where bee hives are kept and managed.
5. *Apis mellifera*: scientific name for the European honey bee.
6. Bee bread: pollen mixed with nectar and bee secretions and stored in the comb for later use for brood food.
7. Beeswax: a substance secreted by bees from four pairs of glands under the abdomen, used by worker bees for constructing honeycomb, covering brood, and food stores. Dull yellow solid plastic when warm. Composed primarily of a mixture of esters, hydrocarbons, and fatty acids.
8. Biodiversity: the relative abundance and variety of plant and animal species and ecosystems within a particular habitat.
9. Bloom: produce flowers; be in flower.
10. Brood: all immature bees in the hive, including egg, larvae and pupae.
11. Brood Comb: any comb in the hive in which brood is found.
12. Brood Food: glandular secretions of nurse bees that are used to feed all larvae, the queen, drones and foragers.
13. Caged: to confine
14. Capped Brood: pupae sealed in the brood comb.

15. Carbohydrate: sugars and starches, together constituting one of the three principal types of nutrients used as energy sources (calories) by the body.
16. Cell: a hexagonal compartment in a honey bee comb used for rearing of brood and storage of pollen and honey.
17. Colony: a community of bees composed of one queen, workers, and drones.
18. Colony Collapse Disorder (CCD): a disorder of honey bees (*Apis mellifera*) that is characterized by sudden colony death due to the disappearance of all adult worker bees in a hive while immature bees, the queen bee, and the honey remain
19. Comb: an interconnected group of wax cells
20. Dearth: a lack of availability usually referring to pollen or nectar.
21. Deep: the largest sized hive body commonly used for the brood nest and beebread.
22. Drone: haploid male member of the colony that develops from unfertilized eggs.
23. Eclosion: The emergence of an adult insect from a pupal case or an insect larva from an egg.
24. Egg: ovule produced by the queen. Occasionally a worker may produce unfertilized eggs.
25. Essential Amino Acid: any amino acid that cannot be made by the body but must be obtained by diet.
26. Fertile: capable of producing offspring
27. Fertilize: introduction of male genetic material with female genetic material to develop offspring
28. Floral: of flowers

29. Food desert: area devoid of healthy food
30. Forage: search widely for food and provisions
31. Foul Brood: a fatal disease of larval honey bees
32. Foundation: a commercial product made from beeswax that is used as a starter substrate for bees to build comb. Its use ensures straight, uniform and parallel combs.
33. Frame: a rectangular structure with or without foundation, in which bees build comb. Frames allow combs to be removed for inspection or harvest without damaging the colony.
34. Fungicide: agent used to destroy or inhibit fungi growth
35. Guttation: loss of water in the form of water droplets that form on the uninjured margins of leaves, takes place due to root pressure and contains dissolved substances from the plant
36. Herbicide: agent used to destroy or inhibit plant growth
37. Hive: See Colony
38. Honey: a sweet viscid material elaborated out of the nectar of flowers in the honey sac of various bees, stored in cells and allowed to desiccate and capped
39. Hypophyrangeal gland: gland located within the head of the worker bee, most developed between 3-16 days post emergence and atrophies after
40. Instar: period of time between molting in the development of insect larva or other invertebrate

41. Israeli Acute Paralysis Virus (IAPV): a virus of Dicistroviridae family that is transmitted by the Varroa destructor mite and leads to honey bee death and colony collapse
42. Larvae: newly hatched wormlike individual that precedes pupa in the process of metamorphosis
43. Liebig's Law, also The Law of Limiting Factors: a law in physiology: when a process is conditioned by several factors, rate is limited by the factor present in the minimum
44. Lipids: any of a class of organic compounds that are fatty acids or their derivatives and are insoluble in water but soluble in organic solvents, includes many natural oils, waxes, and steroids
45. Mandibles: mouth parts used for biting or crushing, used in self defense
46. Mandibular gland: sac like glands located at the base of each mandible, largest in queens, smallest in drones
47. Mid gut: Also ventriculus, the functional stomach of bees and is the largest part of the intestine
48. Monoculture: agricultural practice of producing or growing a single crop in a field farming system at one time
49. Monoflora pollen: pollen from one plant source
50. Nectar: sweet liquid that is secreted by the nectaries of a plant and is the chief raw material of honey

51. Neonicotinoids: any of a class of broad-spectrum insecticides having a chemical structure similar to that of nicotine, acts on the central nervous system of insects selectively binding to nicotinic acetylcholine receptors
52. *Nosema apis*: a microsporidian, a small, unicellular parasite recently reclassified as a fungus that mainly affects honey bees, causes nosemosis, also called nosema
53. *Nosema ceranae*: a microsporidian, a small, unicellular parasite that mainly affects *Apis cerana*, the Asiatic honey bee
54. Nucs or nucleus colony: a small colony made up of larger colonies
55. Nurse Bee: usually about a week old (after their hyperpharyngeal glands have developed, they feed larvae. Nurse bees secrete bee milk or royal jelly.
56. Pesticide: an agent used to destroy pests
57. Pollen: a fine powdery substance, typically yellow, consisting of microscopic grains discharged from the male part of a flower or from a male cone. Each grain contains a male gamete that can fertilize the female ovule, pollen is transported by the wind, insects, or other animals.
58. Pollination: the transfer of pollen from an anther to the stigma in angiosperms, aided by pollinators
59. Pollinator: any insect or animal that forages blooms for nectar and spreads pollen to other flowers
60. Polyflora pollen: pollens from multiple species of plants
61. Propolis: brownish resinous material of waxy consistency collected by bees from the buds of trees and used as a cement in repairing, sealing and maintaining the hive

62. Protein: any of a class of nitrogenous organic compounds that consist of large molecules composed of one or more long chains of amino acids and are an essential part of all living organisms, especially as structural components of body tissues such as muscle, hair, collagen, etc., and as enzymes and antibodies
63. Proven queen: a queen that has demonstrated the ability to produce offspring
64. Pupa: an insect in the non-feeding stage between the larva and adult, during which it typically undergoes complete transformation a cocoon
65. Queen: fertile female member of a colony that lays eggs
66. Resiliency: ability to withstand stress and pressures, the capacity to recover quickly from difficulties; toughness
67. Royal jelly: protein and lipid rich secretions of the nurse bee's hypophayngeal and mandibular glands fed to larvae, the queen and workers
68. Small Hive Beetle: *Aethina tumida*. Small beetle introduced in the U.S. in 1996 and spread by migatory beekeepers. A pest in hives that consume resources and spoil food stores.
69. Soy bean flour also soyflour: high protein, gluten- free, hull-free flour made from toasted soybeans
70. Worker: sterile female member of colony that specializes in all activities of the hive except sexual reproduction
71. Varroa Mite: honey bee parasite that attaches itself to bee larvae, responsible for spreading viruses
72. Wax: see beeswax

73. Wax moth: A moth *(Galleria mellonella)* that lays its eggs in beehives, where the larvae feed on the wax and debris of the honeycombs, an indicator of a failing hive
74. Wheast: combination of whey and yeast used to feed insects

ASSUMPTIONS OF THIS STUDY

1. 3 lb packages of workers and queens are healthy.
2. The queens are proven.
3. Soybean flour (soy flour), the protein source, is consistent in each package.
4. The soybean flour is organic and free of herbicides and pesticides.
5. The tested individuals will consume the carbohydrate and protein diet provided.

CHAPTER II

REVIEW OF LITERATURE

Honey Bee History

Honey bees have been ever present as long as flowers have been around. The honey bee evolved from hunting wasps that developed the taste for nectar and pollen (Millner, n.d.). 40 million year old specimens of Apini fossils were found in amber (Winston, 2010). Culliney reported very little morphological change of the honey bee in the past 30 million years and comparisons to modern worker bees indicated that the complex social behavior was developed 27 million years ago (Winston, 2010). The modern honey bee are in the genus Apis and includes five species- *A. mellifera* (the common honey bee), *A. dorsata & A. laboriosa* (the giant honey bees), *A. cerana* (the Indian honey bee), and *A. florea* (dwarf honey bee) (Winston, 2010; Milner, 1996). Michener observed that *A. mellifera* did not populate the western hemisphere until modern times when European settlers introduced the bees for beekeeping. *A. mellifera* was thought to have originated in the African tropics or subtropics during the Tertiary period (Winston, 2010). Hybridization of the races of *A. mellifera* by beekeepers for traits has interrupted the natural distribution of the races. Beekeepers now stock hives with hybridized bees in order to achieve preferred traits such as hygienics, early brood development, large brood development, docility, disease resistance, and honey production.

Honey Bee Life Cycle

The honey bee goes through metamorphous just like most insects. The egg is laid and adhered to the bottom of the brood cell by a single female queen. The eggs vary in sizes and weights in a queen; however, the variations have no impact on the hatch rate or larval size. During the three days in the egg stage, the egg loses 30% of its weight due to water loss. The egg membrane dissolves rather than ruptures in a process called ecolsion. (Winston, 2010) The larvae does not have any external features. It has a simple mouth equipped to lap up royal jelly, a mid gut and hind gut. The larvae will molt five times in the larval stage. This larval stage lasts nine days. It will gorge itself and is considered pre-pupa (Waller, 1980). The pupa stage starts with the larvae stretching out the length of the cell with the head towards the opening. The larvae excretes its lasts meal. The cell is closed by young worker bees (Winston, 2010). During this time, the pupa will molt a sixth time. The sixth and final time will result in the darkening of the pupa. The pupa uses its newly acquired mandibles to release itself from the cell.

Divisions of Labor in the Honey Bee Hive

The honey bee hive contains three castes-worker, queen and drone. The worker caste has several divisions of labor. The honey bee worker graduates in to these divisions of labor based on their age and the requirements of the hive. The various duties in the hive are all essential to the health of the hive as a superorganism. As the young worker honey bee emerges from the cell, it will feed heavily on bee bread (pollen and honey mixture) in order to complete its own development. The first task that the newly emerged bee assumes are cleaning activities, cell preparation and nest sanitation. The house bee is responsible for cleaning and hygiene. They remove dead bees and waste. They also clean out cells and smooth down the cell walls to prepare them for the laying of new eggs. The honey bee becomes a nurse bee when the mandibular and hypopharyngeal glands are well developed at about day three post emergence. The nurse bee will secrete royal jelly from the hypopharyngeal and mandibular glands in its head. Royal jelly is a milky substance imperative to the growth and development of the larvae into pupa. Adequate protein consumption is necessary for the full development of these glands and production of royal jelly. Insufficient pollen consumption early in life leads to poor glandular development and shorter life span (Winston, 2010).

Most studies indicated that the brood food glands peak production at 6 days old to 16 days old. The acini of the hypophyrangeal glands begin to reduce in conjunction with the increase of juvenile hormone (Winston, 2010). Toth and Robinson (2004) found in their study of worker nutrition and division of labor, that nurse bees had greater fat bodies that are important in the production of vitellogenin, a lipoprotein in royal jelly.

The nurse bee does not feed the larvae directly in the mouth but rather deposits the royal jelly along the cell wall or bottom. Six to ten nurse bees that are less than 12 days of age feed the queen directly mouth to mouth every 20-30 minutes during peak egg production (Winston, 2010).

The older nurse bee will alternate from nursing duties to comb building and cell capping. The alternating of duties allows for the wax glands to produce more wax for construction and the hypopharyngeal glands to produce more royal jelly. The older workers that build combs also participate in food handling. Receiving nectar from foragers, they expose the nectar to the air in the hive and evaporating water from it. She then deposits the partially evaporated nectar with enzymes into a cell where it is fanned until it is less than 18% water. The foragers deposit the pollen in the cells. The loose pollen pellets are moistened with regurgitated honey and saliva. Pollen is packed into the bottom of the cells. The first outdoor task the young workers take on is ventilation. They position themselves at the entrance with her abdomen down and fan to cool the hive, evaporate honey, reduce humidity and carbon dioxide in the hive. While all the workers can ventilate, this activity peaks when the worker is 18 days old. Guarding occurs between ages 12 and 25 days of age usually before they assume foraging duties. The time threshold is hive-need dependent. Guards are recognizable at the entrance with forelegs and antennae in the air and standing of their four hind legs. They are responsible for inspecting all incoming workers.

Pre-foraging workers conduct orientation flights around the hive. The first orientation flight can last up to five minutes and then successive orientation flights last longer in duration and distance. Coss and Brandon observed that the stem length of interneurons shorten during the first flight possibly preparing the worker's nervous system to record and remember orientation stimuli (Winston 2010).

The final job held by the worker bee is that of the forager. The foragers' fat bodies are depleted at this point in development. The onset of starvation and the level of juvenile hormone correlated with the onset of foraging activity. Older workers or foragers consumed carbohydrates as their energy source for foraging (Toth and Robinson, 2004). However, she will occasionally revert back to the previous jobs held in order to satisfy the needs of the hive but she no longer produces royal jelly to support the queen or larvae. Older workers that revert back to the nurse role due to hive need have substantial larvae mortality thus indicating that reverted nurse bees are less effective either by physiological or behavioral changes (Toth and Robinson, 2004). Foragers collect nectar, pollen, water, and propolis. The brood food and wax gland degenerate and the foragers begin to show visible wear. Workers tend to die after flying around 800 km regardless of age but averages 4 to 5 days. This is due to the breakdown of the enzymatic mechanism used to metabolize carbohydrates into glycogen in older workers (Neukirch, 1982).

The quantity and quality of food is the determining factor in the development of a female larva into a queen. The feeding of the worker and queen larvae are the same until day 3. The size of the cell determines the caste. A queen cell is significantly larger than the worker cell. A queen takes 16 days to develop from egg to emergence. The queen larvae receives royal jelly containing more mandibular secretions than worker brood food for a longer period of time (Winston 2010). At emergence, the queen will conduct a mating flight and egg laying will take place 2-4 days after mating. She will lay both fertilized and unfertilized eggs in the cell constructed by workers. She will lay up to 1500-2500 eggs daily during the summer (Betz and Taggert, 2010; Shultz, 2010; Winston, 2010).

The drone results from an unfertilized egg. His only purpose in the hive is to deliver his sperm into a virgin queen. The drone does not do any work in the hive. The workers control the quantity of drones through the construction of drone cells in the nest. Workers will build drone cells when the queen fails or shows signs of failing and before swarming. The drone cells are larger than worker cells and therefore receive more brood food than worker larvae. The workers are stimulated to produce drone comb around four weeks before swarming. The drone cells are oriented on the periphery of the nest or hive. The older drone larvae receive increased quantities of honey and pollen. When adult drones emerge from cells in 24 days, they are entirely dependent on workers for food for the first week. They will gradually learn to feed on honey (Winston, 2010). The drone requires a short amount of time to mature sexually and physically before he is able to mate with a virgin queen. Equipped with large eyes and large flight muscles, he will leave the hive to fly in common areas waiting for an opportunity to mate. The act of mating kills the drone. She will mate with up to a dozen drones. In autumn, any remaining drones are evicted from the hives by workers as they will drain the hives limited resources during the winter.

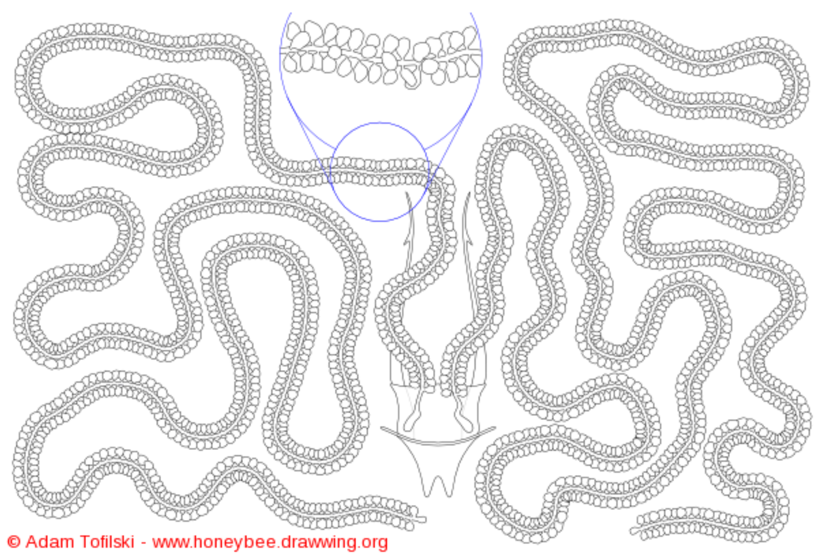
Honey Bee Nutrition & Diet

The honey bee diet requires proteins, carbohydrates, lipids, vitamins, sterols, and minerals. All nutrients are derived from flowering plants. However, very few flowering plants meet the nutritional requirements completely. Deficient nutrition can impair immune function and increase the susceptibility of individuals to disease (Aluax et al, 2010). The nutritive capability of the colony as a whole all falls on the ability of the nurse bees to process the pollen gathered into royal jelly.

The honey bee forager gathers two components, pollen and nectar, from varieties flowering plants to provide the colony with these nutrients. As a polyletic forager, she will collect pollen and nectar from different species of flowering plants. This way, the hive ensures it has a variety of nutrients and the best chance to bolster the immunity of the hive. In a study of diet related immunocompetence, Aluax et al (2010) determined that the polyfloral diets enhanced immune functions, glucose oxidase activity, when compared with monofloral diets. Diversity in floral resources confers bees with better in-hive antiseptic protection. Schmidt conducted a series of studies and convincingly showed that in general, mixed pollen given to caged bees had greater longevity than those on a single species of pollen (Huang, 2012, February 20).

The foragers or older worker honey bees are not able to consume or digest pollen. That job is left to the nurse bees (3 day-16 days post emergence). The nurse bees eat the pollen in the form of beebread that is stored in the cells. It is converted into royal jelly using the vitogellin it does not use in the development of eggs. The hypopharyngeal gland and mandibular glands are essential in the production of royal jelly. The jelly or bee milk is composed of proteins, lipids, and vitamins. The queen and drones do not possess a hypopharyngeal gland (Rahman 2014). The worker loses her ability to produce royal jelly around 16 days post emergence due to the shrinking of the hypopharyngeal gland.

Table 1. Dissected Hypopharyngeal gland of a worker honey bee



Royal jelly is also fed to the queen and older workers by nurse bees. The foragers beg for royal jelly from the nurse bees. In this way, the foragers are able to detect nutritional needs by consuming the royal jelly. The foragers will set out to gather more of the needed components, pollen, or nectar for the production of sufficient royal jelly (Oliver, 2017). In the instance of dearth, the eggs and uncapped larvae can also serve as protein reserves. The nurse bees will consume those reserves to keep the colony surviving. She will not consume the capped brood as they do not require any more inputs and will benefit the colony at emergence (Oliver, 2016). The protein, lipids, and sterols come from pollen and the carbohydrates comes in the form of nectar or honeydew. Through a series of dietary experiments, de Groot (1953) determined the ten essential amino acids required for growth and development. He set the ratio with tryptophan set as 1, as seen on Table 1. Randy Oliver, of Scientific Beekeeping.com, revised deGroot's ratio because pollens are not usually analyzed for tryptophan and requires an additional test (Somerville, 2010). Oliver set histidine as 1 and revised the suggested ratio as listed on Table 2.

Protein is the most important nutrient for population recovery as it is essential for the development of brood (Oliver, 2016). Ten of 21 amino acids, the building blocks of proteins, are essential for rapid growth of larvae and development of worker bees (Zheng et al, 2014). The highest requirement is of l-leucine, l-isoleucine and l-valine, and limitations of at least one of the essential amino acids in the food protein limits colony development (Stace, 1996). Pollens from different plants have different nutritive values (Brodschneider and Crailsheim, 2010). Honey bees can adjust their consumption of inadequate pollens to achieve nutritive requirements (Somerville, 2000) through a diverse diet. Oliver noted that honey bees cannot synthesize cholesterols and must find the source in their diet. The sterol, 24-methylenecholesterol, is particularly important to honey bees and may be a critical limiting nutrient because bees cannot synthesize it. 24-methylenecholesterol can be found in egg yolk, some raw vegetable, almond, borage, or corn (Oliver 2016).

Commercial migratory beekeepers expose their colonies to monocultures during bloom to pollinate commodity crops around the country. Some will move their colonies up to five times in one year. This creates a situation in which the colonies are living in a "food desert" due to the monocultural pollens, coping with limiting nutrients and reduced colony developments. Any beneficial flowering weeds have been stripped away to maximize production yields.

Table 2. Essential Amino-Acid Requirements for Honey Bees (de Groot 1953)

Amino-acid	Minimum required ratio of amino-acid in protein digested
Threonine	3.0
Valine	4.0
Methionine	1.5
Leucine	4.5
Iso-leucine	4.0
Phenylalanine	2.5
Lysine	3.0
Histidine	1.5
Arginine	3.0
Tryptophan	1.0

Table 3 shows Randy Oliver's revised de Groot's ratio. Oliver found it easier to interpret the pollen qualities with histidine set to 1. Most pollen analysis do not include tryptophan due to complexity of testing (Oliver 2016).

Table 3. Essential amino acid requirements of honey bees (Oliver 2016)

Essential Amino-acid	Minimum required ratio of of amino-acid in protein digested
Threonine	2.0
Valine	2.5
Methionine + cysteine	1.0
Leucine	3.0
Iso-leucine	2.0
Phenylalanine +	2.0
Lysine	2.5
Histidine	1.0
Arginine	2.0
Tryptophan	.5

Honey Bee Health and Immunocompetence

Researchers at the French National Institute for Apicultural Research found a correlation between pollen diversity and a healthy immune system. Carbohydrates provide energy for metabolic process that have innate humoral and cellular immune responses and may provide plant based metabolites that may have antimicrobial attributes (Degrandi-Hoffman and Chen, 2015). The result of feeding a polyfloral pollen showed higher glucose oxidase level than the bees that fed on a monofloral pollen regardless of the protein level. Glucose oxidase is produced in the hypopharyngeal glands. Bees use the glucose oxidase to preserve honey and protect the hive of pathogens (Alaux et al. 2010). Additionally, it is important in the development of gluconic acid and hydrogen peroxide secreted in larval food for sterilization and preservation (Alaux et al. 2010). Alaux et al concluded the polyfloral diet increased the antiseptic protection of the hive. Having a large diversity of pollen stored, the bees increase the probability of balancing vitamins, minerals and proteins while decreasing the probability of toxic pollen poisoning (Burlew, 2010).

Parasitic infections weaken colonies regardless of the quality or quantity of pollen sources (Degrandi-Hoffman and Chen, 2015; Huang, 2012; Pasquale, G. D. et al). As the colonies continue to feed on the single source protein, they grow deficient in the limiting amino acid or nutrient of that pollen. Dietary protein deficiency reduces most amino acids in plasma and compromises the immune system (Alaux, 2010). Liebig's Law, also known as The Law of Limiting Factors, derives from a discovery made by a German botanist named Carl Sprengel and later popularized by German chemist Justus Von Liebig. This law states that performance is affected not by the most abundant resource, but by the most deficient one. This is illustrated in the honey bee when they will over-feed on proteins in order to accommodate their carbohydrate needs or by consuming larger quantities of essential amino acids (EAA) in order to meet their need of the most limiting EAA. This results in reduced lifespans of bees supplemented with high protein diets (Paoli et al., 2014). Many beekeepers rely on weight as an indicator of bee health. Weight includes quantity of food stores, quantity of brood, quantity of workers and quantity of comb. A light-weight hive will need further management such as feeding, inspecting, adding more bees or replacing a queen.

The intensification of agriculture has changed the honey bee's landscape and has made foraging more stressful. The common kitchen garden producing a variety of fruits and vegetables that was available to bees in every homestead home has turned into hundreds of acres of commercial monocultures. Vast acres upon acres of single crop production. This advancement is due to the increase demand for food to feed our ever-growing world population with the greatest amount of efficiency. The challenges of monoculture farming include controlling pests, disease, fungus and weed associated with high-density populations. The expectation of meeting high yield demands at reduced expenses has forced the producer to treat their crops with fungicides, pesticides, fertilizers, and herbicides. The farmer will even rent thousands of hives to pollinate the crop for three weeks from migratory beekeepers to ensure max yield production at the expense of the honey bee. Huang (2012) conducted a study that revealed that the acini in the hypopharyngeal glands in migratory hives were significantly smaller than those that remained stationary.

One systemic pesticide, neonicotinodes, are currently being researched for their sub lethal effects on honey bees. Pisa et al (2014) reported that field studies to investigate the exposure of pesticides face major difficulties. Imidacloprid, a neonictoinode, disrupts the development of hypopharyngeal glands by decreasing the size of the acini in adult honey bees that were exposed to contaminated brood food as larvae. Additionally, neonicotinodes have shown to induce flight muscle paralysis when guttation droplets were consumed from contaminated plants (Pisa et al, 2014). The already weak hives come away weaker and hungrier.

Pollen and Pollen Substitutes

During dearth, many beekeepers supplement the dietary shortfalls with pollen substitutes or pollen supplements (Standifer, 1977). Pollen substitutes contain no pollen while pollen supplements contain 5-25% pollen. The most common protein substitutes are based in soyflour or soybean flour, wheast (whey-yeast blend), or brewer's yeast or a combination of those proteins. Soybean flour meets the minimal requirements to keep a honeybee alive but does not meet the nutritional requirements needed in the production of royal jelly or brood. It is important to note that Huang found reduced worker longevity with the use of soybean flour and lupin flour probably due to the presence of stachyose. Stachyose is toxic to honey bees unless diluted to below 4% of total sugar using 50% sucrose (Huang, 2012). DeGroot (1953) ration experiments on honey bees identified potential artificial food sources. Pollen substitutes are made of a base flour with additional inputs of phagostimulants, vitamins, minerals, and sugars. Pollen substitutes work well to supplement diet during dearth rather than substitute the diet completely. The only exception to polyfloral pollen feeding would be with rape seed *(Brassica napus* L.), which is a very nutrious pollen when fed alone (Huang, 2012). Beekeepers will often collect pollen from their hives in the spring and summer to use during dearth.

CHAPTER III

MATERIALS and METHOD

Purpose of the Study

The purpose of this study is to determine the effects or impacts of a single source protein on the establishment, development, and performance on new colonies using three pound packages and proven queens. The single source protein is designed to replicate a dietary situation that a monoculture would create. Soybean flour was chosen for the single source protein because it is the base for many pollen substitutes and it is readily available. While that choice of protein is toxic at high levels, it met the intent to limit protein source for the study. The protein source was available to the control group as well.

Table 4. Analysis of Essential Amino Acids of Soybean Flours (Stace, 2010)

Essential Amino-acid	Minimum ratio of Amino-Acid from De Groot (1953)	WAI	Continental grain	Riverland oil seeds
Threonine	3	4.2	3.9	4
Valine	4	4.5	4.5	5.1
Methionine	1.5	1.6	1.3	1.4
Leucine	4.5	7.7	7.4	7.6
Iso-leucine	4	4.5**	4.4**	5.6**
Phenylalanine	2.5	5	4.8	5.1
Lysine	3	6.2	6.1	6.4
Histidine	1.5	2.8	2.8	2.4
Arginine	3	8.2	7.1	7.1
Tryptophan	1	-	-	-
Crude protein		42.8%	47.9%	50%

** Beneficial excess of Iso-leucine.

Materials and Methods

The design of the study was limited due to expense. The EZ NUC corrugated plastic box was chosen to establish the colonies. Each NUC was fitted with five wooden deep frames with wired wax foundation. Jar feeders were installed on top of the nucs. The frames were sprayed with sugar water and Honey-B-Healthy to initiate comb building. The queens that came with the packages were removed and replaced with mated Italian honey bee queens purchased from Gardner Apiary of Baxley, GA. The queens were introduced into the packages in sealed cages for 24 hours and then released into the nucs. Each queen had a minimum of three active nurse bees with her upon inspection. The control hives were placed parallel to the test hives. The test hives were placed in a hive tunnel that restricted the foraging of the workers. The hive tunnel is a screen covered wire tunnel that was 20 feet long by 6 feet wide and 8 feet tall. The tunnel was designed specifically for this study. The nucs were fed defatted soybean meal in pollen feeders set in front of each nuc exit/entrance. The nucs were supplied with 2:1 sucrose syrup with Honey-B- Healthy feeding stimulant. The frames of each nuc were inspected weekly and measured for four factors, weight, percentage of drawn wax comb, percentage of food stores and population of brood. The data was collected weekly until the final test hive failed at 11 weeks in the study. The data was compiled and examined using statistical analysis using Excel 2016.

Population of Study

The population for this study included six three-pound packages of Apis mellifera and six mated queens. The packages of *,Apis mellifera lingusta,* Italian honey bee were acquired from the same source, The Bee Barn in Lone Oak, KY. The proven queens were acquired from Gardner Spell Bee Apiary. The genetics of the queens were identical. A three-pound package of bees contains 10,500 individuals. The individuals in the study are made up of one queen, foragers, house bees and nurse bees. The packages were received with one quart of 1:1 sucrose syrup each.

Data Collection

Performance measures will include nuc weight (kg), percentage of drawn comb, percentage of food stores and population of brood (inclusive of eggs, larvae, pupae, and capped brood). Weights were measured on a digital scale. Initial weights of each Nuc with bees, five frames and the new queen were taken to set the baseline at week 0. Percentages of drawn combs, percentage of food stores and populations of nucs were measured using a frame grid developed for the study. The grid is a transparent film that is positioned over the frame. The grid is divided into 128 units. Each unit is equivalent to 36 cells or .078125% of a nuc. See design in Appendix A. Each nuc has 10 frame sides to quantify or 46,080 cells at 100% capacity. This does not account for bridge comb, burr comb, or drone cells which were culled from the colony at weekly inspections. The nuc is never allowed to reach 100% capacity because that would place the colony at risk of swarming and stress. The performance measurements recorded weekly until the last of the test hives failed.

Data Analysis

Statistical tests were performed on the data collected. The data was analyzed using Microsoft Excel 2016 with Analysis of Variance (ANOVA) testing and two sample t-Test on Weight (kg), % of Drawn Comb, % of Food Stored, and Population of Brood (eggs, larvae, pupae, and capped cells). All were chosen as a measure of performance with respect to Spring Nuc establishment for the test and control groups.

CHAPTER IV

Results

Weight of Hives

Data was collected, analyzed, and entered into Microsoft Excel spreadsheet for analysis and comparison. The weekly weights of the nucs were recorded.

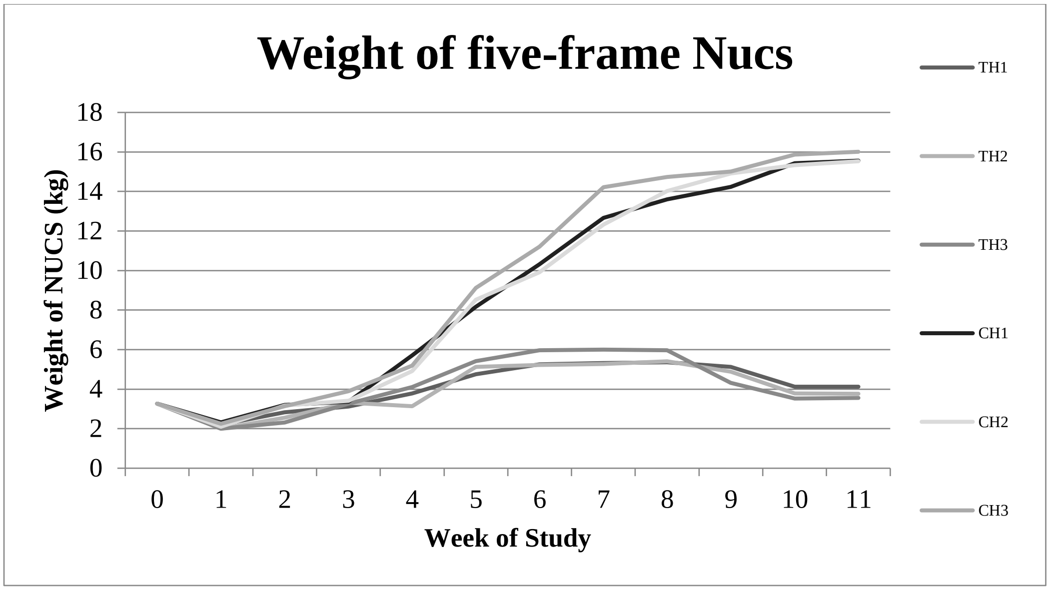
Table 5 contains the recorded weights for the Control Hives and the Test Hives for each week of the study.

Table 5

Weights (kg) of Control and Test Hives

Week	0	1	2	3	4	5	6	7	8	9	10	11
CH1	3.27	2.32	3.20	3.36	5.71	8.16	10.32	12.67	13.6	14.23	15.43	15.56
CH2	3.27	2.10	3.17	3.42	4.92	8.53	9.91	12.32	14.02	14.92	15.33	15.53
CH3	3.27	2.25	3.13	3.89	5.19	9.12	11.21	14.21	14.73	15.01	15.87	16.01
TH1	3.27	2.2	2.83	3.12	3.79	4.75	5.26	5.32	5.36	5.12	4.12	4.12
TH2	3.27	2.0	2.56	3.32	3.13	5.13	5.23	5.27	5.41	4.89	3.78	3.77
TH3	3.27	2.0	2.32	3.27	4.11	5.42	5.97	6.0	5.97	4.32	3.53	3.56

Note, CH- Control Hive, TH- Test Hive



Graph 1- Weight of Nucs during study

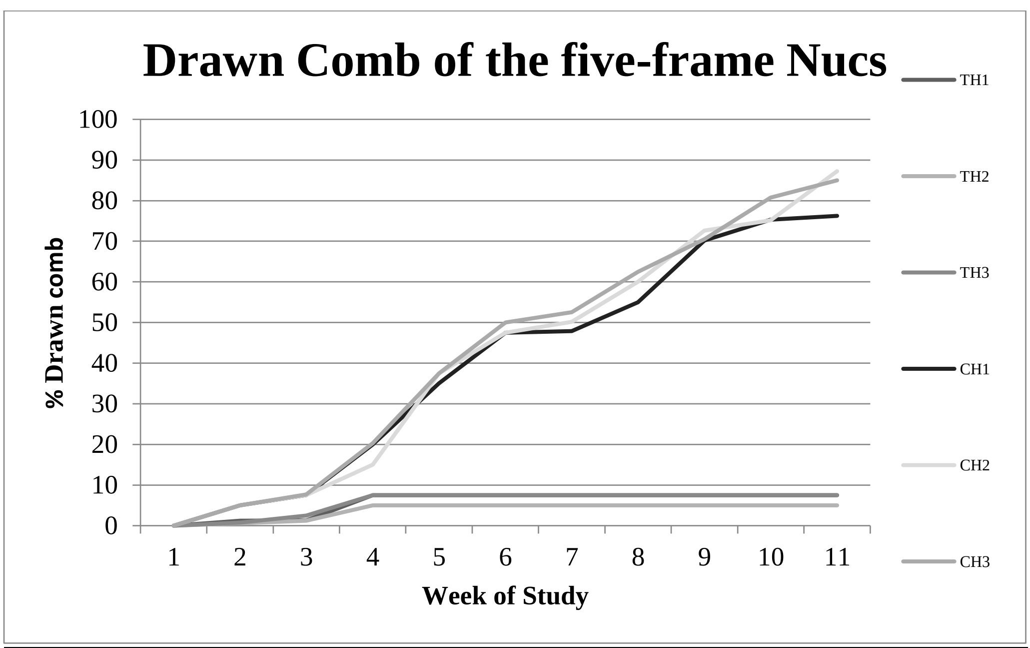
Graph 1 is a visual representation of the recorded weights taken weekly for each nuc. The control nucs had obvious increase in weights and began to outperform the test nucs at week 3 of installation. This is plausible with the emergence of the first generation of juvenile bees and/or an increase of food stores. The test nucs also had an emergence of juvenile bees with lesser quantity and/or lesser food stores. An important observation made on the 2nd day of installation was that the foragers in the caged nucs were all gathered at the ceiling of the hive tunnel and initial mortality of those foragers may have been a factor in the lesser weights at week 2 and 3.

Table 6

% of Nuc Drawn Comb of Control and Test Hives

Week	0	1	2	3	4	5	6	7	8	9	10	11
CH1	0	5	7.5	20	35	47.5	47.9	55	70.16	75.32	76.25	80
CH2	0	5	7.5	15	37.5	47.5	50.2	60	72.63	75.16	87.25	90
CH3	0	5	7.65	20.31	37.5	50	52.5	62.5	70.47	80.78	85	92.5
TH1	0	1.25	1.33	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
TH2	0	.625	1.25	5	5	5	5	5	5	5	5	5
TH3	0	.78	2.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5

Note, CH- Control Hive, TH- Test Hive



Graph 2- Drawn Comb of Nucs

Graph 2 is a visual representation of the percentage of drawn comb measured weekly for each nuc. The control nucs drew significantly more comb than the test hives from the onset of the study. Test hive drawn comb ever increased past week 4 of the study. This is significant and may be due to nutritional deficiencies created by the singular source of protein or the depletion of fat bodies due to the limited nutritional resources.

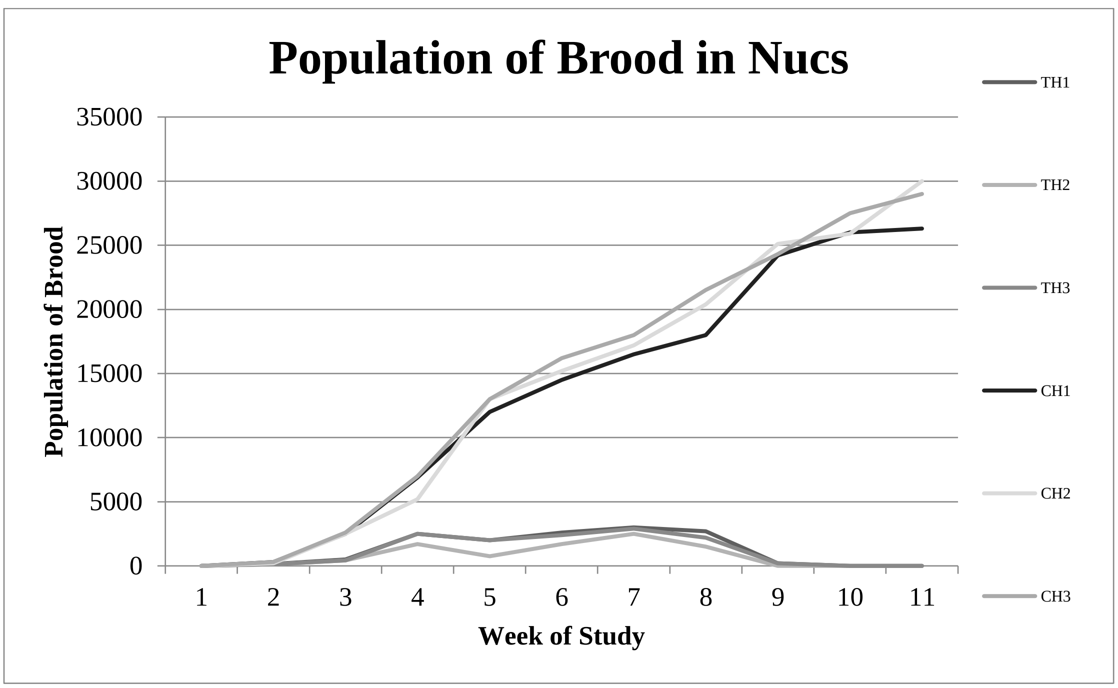
Graph 3 is a visual representation of the population of brood measured weekly for each nuc. The control nucs had greater success in rearing brood into adulthood. Test hives were successful in rearing brood until generation 3. An important observation made on week 6 of the study showed that removal of uncapped brood from cells. This most likely occurred due to the limiting nutrients in the diet of the test hives. The nurse bees may have consumed the brood in an effort to sustain the queen and foragers.

Table 7

Population of Brood of Control and Test Hives

Week	0	1	2	3	4	5	6	7	8	9	10	11
CH1	0	300	2500	6900	12000	14500	16500	18000	24200	26000	26300	27600
CH2	0	200	2500	5200	13000	15200	17200	20400	25100	25900	30000	31100
CH3	0	300	2600	7000	13000	16200	18000	21500	24300	27500	29000	31900
TH1	0	150	500	2500	2000	2600	3000	2700	200	0	0	0
TH2	0	140	430	1700	750	1700	2500	1500	5	0	0	0
TH3	0	140	430	2500	2000	2400	2900	2200	200	0	0	0

Note, CH- Control Hive, TH- Test Hive



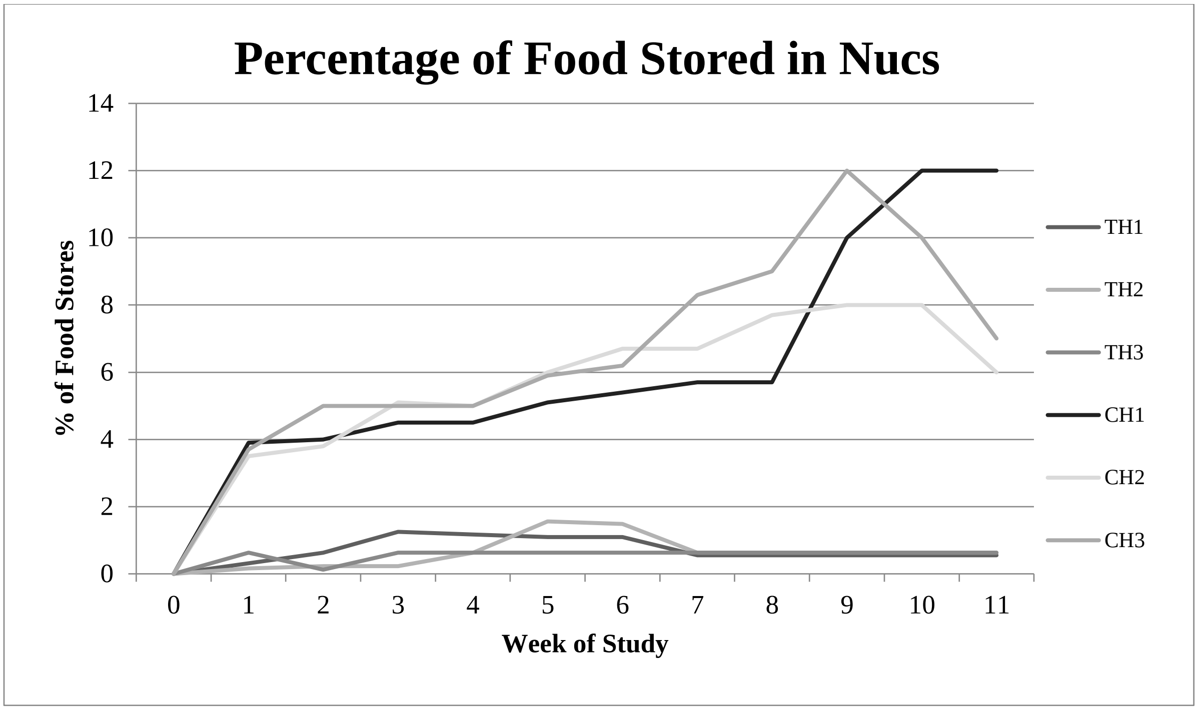
Graph 3- Population of Brood in Nucs during study

Table 8

% of Nuc Food Stores of Control and Test Hives

Week	0	1	2	3	4	5	6	7	8	9	10	11
CH1	0	1	2	3	4	5	6	7	8	9	10	11
CH2	0	3.9	4.0	4.5	4.5	5.1	5.4	5.7	5.7	10	12	12
CH3	0	3.5	3.8	5.1	5.	6.0	6.7	6.7	7.7	8	8	6
TH1	0	3.7	5	5	5	5.9	6.2	8.3	9	12	10	7.0
TH2	0	.3125	.63	1.25	1.17	1.09	1.09	.55	.55	.55	.55	.55
TH3	0	.16	.23	.23	.63	1.56	1.48	.63	.63	.63	.63	.63

Note, CH- Control Hive, TH- Test Hive



Graph 4- % of Food Stored

Graph 4 is a visual representation of the percentage of food stored measured weekly for each nuc. The control nucs had greater success in drawing comb and increasing populations. Therefore, the control hives were able to successfully store food. Between 9 to 11 week of the study, the nurse bees increased consumption of food stores which correlates with increased brood populations. Test hives were able to store food but with the limited populations and comb drawn. The test hives were not able to perform similarly to the control hives.

ANOVA

ANOVA two factor with replication weight resulted in the F-citical value of 1.99 for interaction. The F-statistic value is 211.91. Any number greater than the F critical causes rejection of the H_0 with regard to weight performance. The P-value <.05 means that there is statistical significance with the weight data.

ANOVA two factor with replication data with regard to percentage of comb drawn in the 6 nucs revealed that F-critical value of 1.99. The F-statistic value was 221.9. Any number greater than the F-critical causes the rejection of H_0. The P-value <.05 confirm statistical difference in the data sets and statistical significance.

ANOVA two factor with replication data with regard to percentage of food stores in the 6 nucs revealed that F-critical value of 1.99. The F-statistic value was 10.48. Any number greater than the F-critical causes the rejection of H_0. The P-value <.05 confirm statistical difference in the food stores data sets and statistical significance.

ANOVA two factor with replication data with regard to population of brood in the 6 nucs revealed that F-critical value of 1.99. The F-statistic value was 263.5. Any number greater than the F-critical causes the rejection of H_0. The P-value <.05 confirm statistical difference in the data sets and statistical significance.

T-tests

The three test nucs had a mean weight of 4.08 kg. The three control hives had a mean weight of 9.13 kg. Therefore, with respect to weight, the test hives did not perform equal to or better than the control hives. This t-test indicated that the t-statistic value of 3.15 exceeds than the t-critical value of 2.18. Due to these values, there was a significant difference (P<.05) and I can reject null hypothesis with regard to the performance factor of weight.

The three test nucs had a mean comb draw of 5.21%. The three control hives had a mean comb draw of 45.32%. Therefore, with respect to drawn comb, the test hives did not perform equal to or better than the control hives. This t-test indicated that the t-statistic value of 3.15 exceeds than the t-critical value of 2.18. Due to these values, there was a significant difference (P<.05) and I can reject null hypothesis with regard to the performance factor of drawn comb

The three test nucs had a mean population of 976.25. The three control hives had a mean population of 15,330.56. Therefore, with respect to population, the test hives did not perform equal to or better than the control hives. This t-test indicated that the t-statistic value of 4.48 exceeds than the t-critical value of 2.20. Due to these values, there was a significant difference (P<.05) and I can reject null hypothesis with regard to the performance factor of population.

The three test nucs had a mean food storage of 0.62 %. The three control hives had a mean food storage of 6.01 %. Therefore, with respect to food stores, the test hives did not perform equal to or better than the control hives. This t-test indicated that the t-statistic value of 6.59 exceeds than the t-critical value of 2.20. Due to these values, there was a significant difference (P<.05) and I can reject null hypothesis with regard to the performance factor of food stores.

The three test nucs had a mean comb draw of 5.21%. The three control hives had a mean comb draw of 45.32 %. Therefore, with respect to comb draw performance, the test hives did not perform equal to or better than the control hives. This t-test indicated that the t-statistic value of 4.38 exceeds than the t-critical value of 1.79. Due to these values, there was a significant difference (P<.05) and I can reject null hypothesis with regard to the performance factor of comb draw.

CHAPTER V

Conclusion

In western Kentucky, vast acres of monoculture is not as problematic as it maybe in the Great Plains but it does occur. Colony collapse disorder has been identified in 35 states, including Kentucky. A limiting diet will cause developmental delays in the nurse bees that will affect the entire colony attrition and recruitment; reduced hive populations; immunosuppression; and vulnerability to parasitic infections. In this study, the colony in test hives showed signs of population decline initially due to the loss of foragers at the onset of the study. The house bees assumed the role of precocious foraging and began feeding on the pollen substitute of soybean flour. Immediately into the study, comb building began but was limited with the population of individuals present. Evidence of food storage occurred in both the control and test hives. If the stores are from a single protein source, in this case, soybean flour, developmental delays in colony recruitment occur due to inhibited glandular developments in the hypopharyngeal glands in the nurse bees (Pasquale et al, 2013). Interestingly, it is highly possible that the success of the nurse bees in production of brood in the first and second generation were a result of the reserved fat bodies within the nurse bees from the start of the study. While the restricted diet of soybean flour allowed the bees to live (forage, conduct hive building, caring for the queen, and hive hygiene) the diet's limiting nutrients caused developmental delays in the rearing of brood as a result of reduced hypopharyngeal and mandibular gland development of emerging nurse bees. As a result, the nurse bees were unable to develop the eggs laid by the queens into larvae after eclosion in the third generation. The inability of the nurse bees to reproduce brood after generation three left the hives vulnerable to predation, starvation, parasitic infections, and pathogens.   In this study, the dietary and forage restrictions of the test nucs of *Apis mellifera linguista* did not perform similarly to the control nucs. The test nucs each displayed low performance measurements of weight, quantity of drawn comb, quantity of stored food and quantity of brood compared to no forage and dietary restrictions.

Appendix

Deep Frame Grid- 1 unit=.078125% of Nuc

Each unit = 36 cells

Estimate of 4,600 cells per frame side

1,280 units=46,080 cells=100% of Nuc

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