INFLUENCE OF EBA AND YAM WASTE DIET ON PRE-ADULTsss DEVELOPMENTAL RATE OF *HERMITIA ILLUCENS*

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FACULTY OF LIFE SCIENCES

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCINCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMNTS FOR THE AWARD OF BACHELOR OF SCINCES (B.Sc) IN ANIMAL AND ENVIRONMENTAL BIOLOGY.

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CERTIFICATION

This is to certify that this project work was carried out by **Lydia Nmburichen ACHA** with the Matriculation number of **LSC1704825** in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City.

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DATE

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(Head of Department)

DATE

DEDICATION

This project work is dedicated to the Glory of the Almighty God who in His infinite grace and mercies has made it a huge success.

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ABSTRACT

Urban solid waste management is considered one of the most serious environmental problems confronting urban government in low and middle-income countries. However, there is a fairly novel approach of bio waste conversion by insect larvae, using the example *Hermetia illucens*. Therefore, this study was conducted to evaluate the influence of Yam and Eba waste on pre-adult developmental rate of *Hermitia illucens*. The effect of larval diets on developmental period, weight gain, survival, and sex ratio were analyzed. The mean developmental period (days) of larvae Yam, Mixture (yam/eba) and Eba diets were 40, 42 and 52 days respectively. There was no significant different in the developmental period. The Percentage survival for Eba, Yam and Mixture were 2.8%, 47.6% and 28% respectively which varied significantly. Yam and Mixture had more survival rate than Eba diet. The BSF fed with the Mixture diet had weight increase of 42.3% while the Yam diet had 26.8% and Eba diet had 21.4%. There was no significant variation in weight gain. The sex ratio of male to female varied significantly in Eba diet while Yam and Mixture varied non-significantly. Eba produced more females than male. The result of the proximate analysis carried out on the various diet revealed significant differences in diets. Yam diet had higher amount of dry matter, crude fibre and carbohydrate compared to Eba. The analysis of the results show that the type of diets play an important role in the population dynamics of *Hermitia illucens* as more larvae survived in yam diet.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Urban solid waste management is considered one of the most immediate and serious environmental problems confronting urban governments in low and middle-income countries. The severity of this challenge will increase in the future given the trends of rapid urbanization and growth in urban population.

Due to growing public pressure and environmental concerns, waste experts worldwide are being called upon to develop more sustainable methods of dealing with municipal waste that embrace the concept of a circular economy.

Recycling organic waste material (biowaste) is still fairly limited, especially in low and middle-in- come settings, although this is by far the largest fraction of all generated municipal waste. There is a fairly novel approach of biowaste conversion by insect larvae, using the example of the Black Soldier Fly (BSF), *Hermetia illucens*, an approach that has obtained much attention in the past decade. Understanding the life cycle helps one to know why Black Soldier Fly is a suitable insect for organic waste management and to learn how this cycle can be "engineered" to enhance the benefits produced in terms of waste conversion efficiency and product harvest in quantity and quality (Bram .D. *et al.,* 2017).

Waste biomass is converted into larvae and residue. The larvae consist of ±35% protein and ±30% crude fat. This insect protein is of high quality and is an important feed resource for chicken and fish farmers. Feed trials have confirmed it as being a suitable alternative to fish meal. Feeding waste to larvae has been shown to inactivate disease transmitting bacteria, such as *Salmonella spp*. This implies that the risk of disease transmission between animals and between animals and humans is reduced when using this technology at farm level or when treating waste of animal origin in general (e.g. chicken manure or slaughterhouse waste). However, risk reduction is achieved mainly through material reduction (±80%) rather than through pathogen inactivation.

Waste reduction of up to 80% on wet weight basis has been demonstrated. If treatment is applied at the source of biowaste generation, the costs for waste transport and space requirements for landfills can, thus, be reduced drastically. Such organic waste treatment could furthermore reduce open dumping, which is still an unfortunate reality in low and middle-income settings (Bram .D. *et al.,* 2017).

1.1 JUSTIFICATION OF STUDY

Due to its proven waste degradation and biotransformation capabilities, Black Soldier Fly provides a potential and economical alternative to recycling biological waste (Muhammad *et al.,* 2022).

Black Soldier Fly larvae are able to exploit a wide variety of rearing substrate, including organic side streams, thus upgrading low grade substrates into a high quality protein source; Black Soldier Fly is not considered as a pest or vector of disease and does not constitute a nuisance. These attributes make it an attractive insect species from mass production as sustainable and affordable feed ingredient (Shaphan, 2021).

This study is justified by the need to find alternative way to transform organic waste (especially Yam and Garri).

1.2 AIMS AND OBJECTIVES

This project research is aimed at determining the influence of different diet on the developmental rate of Black Soldeir Fly *(Hermitia Illuscens)* with specific objectives to determine the:

- Mean Developmental Period of *Hermitia illucens* raise from Eba and Yam diet
- Percentage survival per diet
- Weight gain per diet
- Sex ratio per diet
- Proximate analysis of diet

CHAPTER TWO

LITERATURE REVIEW

2.0 Black Soldier Fly

The Black Soldier Fly, *Hermetia illucens*, is of the dipteran family: *Stratiomyidae.* It can be encountered in nature worldwide in the tropical and sub-tropical areas between the latitudes of 40°S and 45°N. The egg starts a Black Soldier Fly's life cycle and at the same time marks the end of the previous life stage: a fly laying a cluster of eggs (also called ovipositing). The female fly lays a package of 400 to 800 eggs close to decomposing organic matter, into small, dry, sheltered cavities. Shortly after having laid the eggs, the female dies (Huang, S., *et al.,* 2012)

The closeness of the eggs to the decomposing organic matter ensures that the larvae have their first food source nearby after hatching. The sheltered cavities protect the eggs from predators and prevent dehydration of the egg packages by direct sunlight. On average, the eggs hatch after four days and the emerged larvae, which are barely a few millimetres in size, will search for food and start feeding on the organic waste nearby. The larvae feed voraciously on the decomposing organic matter and grow from a few millimetres size to around 2.5 cm length and 0.5 cm width, and are of cream-like colour (Nguyen, T.T., *et al.,* 2015).

Under optimal conditions with ideal food quality and quantity, the growth of the larvae will require a period of 14-16 days. However, the Black Soldier Fly larva is a very resilient organism and has the ability to extend its life cycle under unfavourable conditions. The larval stage is the only stage during which the Black Soldier Fly feeds and, therefore, it is during this time of larval development that enough fat reserves and protein are stored that allow the larvae to undergo pupation, emerge as flies, find mates, copulate and (as a female) lay eggs before dying (Xu, Y. P., 2019).

After having gone through five larval stages, the larvae reach the final larval stage: the pre-pupa. When transforming into a pre-pupa, the larva replaces its mouthpart with a hook-shaped structure and becomes dark brown to charcoal grey in colour. It uses this hook to easily move out and away from the food source towards a nearby dry, humus-like, shaded and protected environment that it deems safe from predators and is where the imago emerge from the pupa and fly off without significant hindrance (Liang, S. X., 2019).

The process of pupation is the transformation from a pupa into a fly. The pupation stage is initiated when the pre-pupa finds a suitable location and becomes immobile and stiff. For a successful pupation, it is best if the environmental conditions do not change too much or, in other words, that they remain warm, dry and shaded. Pupation takes around two to three weeks and ends when the fly emerges from its pupa shell. The emerging process is a very short procedure. It takes less than five minutes for the fly to break open the part of the pupa that used to be the head section, crawl out, dry and then spread its wings and fly off (Liang, S. H., 2020).

After emerging, the fly lives for about one week. During this short life, it will search for a partner, copulate and (for the female) lay eggs. As a fly, Black Soldier Fly, do not feed. Only a source of water or a humid surface is required to stay hydrated. What is important in this life stage is an abundant amount of natural light and a warm temperature (25-32°C). A humid environment may prolong the life span and, thus, enhance the chance for successful reproduction. It has been observed that the flies prefer to copulate in the light of the morning. After copulation, the females then search for an ideal location to lay their eggs as explained above.

Also, the size to which an individual insect grows is affected by both genetic and environmental factors that operate through complex molecular and physiological mechanisms (Nijhout, 2003). Growth rate varies substantially in response to various stimuli, including resource availability, competition, predator presence, time of season, humidity, and temperature (Harnden & Tomberlin, 2016). Moreover, food quality affects many life-history traits such as larval and adult performance (Moreau *et al.,* 2006).

High food quality enhances the rate of development and increases survival in some insect species (De Haas *et al*., 2006). For example, Nguyen *et al*. (2013) and Oonincx *et al.* (2015b) observed that the detritivorous larvae of the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), when fed diets of vegetable by-products high in protein, had a shorter development time (21 days) than larvae fed low-protein diets (37 days). Conversely, Simon *et al*. (2011) suggested that diets with a higher proportion of protein increase development time and survival rate of some predatory fly species.

Nguyen *et al.* (2013) established an increase in growth rate and decrease in developmental duration of BSF larvae on both high-protein and high-fat diets. However, indications have been found that high levels of fat – 20–36% crude fat based on dry matter (DM) – may be detrimental for both larval and adult survival, lifespan, and reproductive output (Ujvari *et al.,* 2009; Nguyen *et al*., 2013). It seems that availability of balanced amounts of calories, fat, and protein may be more important for fast development and higher larval weight than only a high-protein content (Nguyen *et al*., 2013).

Although BSF larvae on average contain both a high-protein and a high-fat level compared to other edible insect species (Zheng *et al*., 2012a; Barragan-Fonseca *et al.,* 2017), body composition of the larvae depends on the quality and quantity of ingested food (Nguyen *et al*., 2015; Oonincx *et al*., 2015a,b). Consequently, larvae fed on different substrates had varying body protein (ranging from 37.0 to 62.7% DM) and fat content (6.6–39.2% DM) (Barragan-Fonseca *et al*., 2017).

Also physical factors may influence insect performance. For example, if the layer of food substrate consisting of meat meal, swine meat, fish, or liver is too thick, larval food intake is reduced resulting in lower survival and longer developmental time (Nguyen *et al*., 2013). Moisture levels of 60–70% in manure and chicken feed have been found adequate for BSF larvae (Tomberlin *et al*., 2002; Myers *et al.,* 2008; Holmes, 2010). However, due to the variable composition of organic waste, food moisture is difficult to control and needs to be evaluated not only under laboratory but also under field conditions where evaporation rate fluctuates.

Biotic factors may also affect BSF performance. For example, larval density can be a major factor affecting the rate of development (Tomberlin *et al.,* 2002; Diener *et al.,* 2009). Parra Paz *et al*. (2015) demonstrated that larval density has a significant influence on bioconversion of residual organic matter into body mass by BSF. BSF larvae tend to aggregate and overcrowding slows larval development due to competition for feed (Rivers & Dahlem, 2013). Moreover, high larval densities may result in decreased substrate quality by accumulation of larval waste products (Green & Popa, 2012) and may generate direct energetic costs if larvae spend extra energy interacting with each other (Jannat & Roitberg, 2013).

Compensatory mechanisms are activated in response to crowding and nutritional deficiencies. Insects have a tendency to prolong the larval period, or to increase either the rate of ingestion or the total amount of food ingested during larval development (Green *et al*., 2003). Sullivan & Sokal proposed two basic types of responses to crowding: (1) a reduction in the number of individuals able to complete their life cycles, with the emerging adults maintaining normal body size, and (2) sustaining survival accompanied by reduction in body weight, as has been reported for the dipterans *Phormia regina* (Meigen) (Calliphoridae) (Green *et al.,* 2002), *Drosophila melanogaster* Meigen, *Drosophila simulans* Sturtevant, and *Aedes albopictus* Skuse (Yoshioka *et al.,* 2012).

Lower larval are not always better to maximise growth rate. In some insect species, larval aggregations provide adaptive benefits to individuals due to heat generation, which might enhance food assimilation (Green *et al.,* 2002) and provide protection from low environmental temperature and possibly predators (Rivers & Dahlem, 2013). BSF larval weight gain is also affected because of their potential dependence on bacteria as food (Liu *et al.,* 2008). Higher larval are associated with higher bacterial densities which might allow larvae to have better access to bacterially recycled nutrients, thereby resulting in more effective nutrient absorption. Therefore, optimising density may benefit the productivity of insect rearing.

Detailed knowledge of the conditions required for optimal growth, development, and nutrient allocation of BSF is necessary for implementation of large scale production systems (Coelho *et al*., 2013). The effect of nutrient density of the ingested food on development, growth, and body composition of BSF in interaction with larval has not been investigated systematically before.

Optimal environmental conditions and food sources for the larvae can be summarized as: ·

Warm climate: the ideal temperature is between 24 and 30°C. If too hot, the larvae will crawl away from the food in search of a cooler location. If too cold, the larvae will slow down their metabolism, eat less and develop slower. ·

Shaded environment: larvae avoid light and will always search for a shaded environment, away from sunlight. If their food source is exposed to light, they will move deeper into the layer of food to escape the light.

Water content of the food: the food source has to be quite moist with a water content between 60% and 90% so that the larvae can ingest the substance.

Nutrient requirements of the food: substrates rich in protein and easily available carbohydrates result in good larval growth. Ongoing research indicates that waste may be more easily consumed by the larvae if it has already undergone some bacterial or fungal decomposition process.

Particle size of the food: as the larvae have no chewing mouthparts, access to nutrients is easier if the substrate comes in small pieces or even in a liquid or pasty form (Bram, D. *et al.*, 2017).

The adult black soldier fly is not usually considered a pest (Newton L., 2005) and because the larvae have been shown to be effective manure recyclers, a "Black Soldier Fly Manure Management System" has been proposed to not only reduce livestock waste, but also generate a food source for fish and other animals. In a program outlined in Newton L. (2005) swine manure was fed to black soldier fly larvae, which greatly reduced the waste material.

The manure was transferred into a basin containing black soldier fly larvae. As the larvae developed they reduced the manure by 50%. Approximately 45,000 larvae will consume 24 kg of swine manure in 14 days (Newton L., 2005). Larval and bacterial activities, not only reduce the dry matter, but also other components such as nitrogen or phosphorus. Experiments with cow manure showed a reduction of 43% nitrogen and 67% phosphorus (Gobbi F. P., 2012).

The combination of waste treatment capacity along the generation of a product of economic value makes *Hermetia illucens,* a promising tool for organic waste management. As the larvae mature they crawl out of the basin, thereby self-harvesting themselves, and are subsequently available as livestock feed. In addition to being a good source of oil and protein for animal feed, black soldier fly larvae have the potential of improving organic waste into a rich fertilizer (Diclaro *et al.*, 2010). Larvae feed on the chicken manure and can convert it to 42% protein and

35% fat. Black soldier flies management of manure offers many advantages as a reduction of 25% waste annually, a high quality feedstuff will be produced, and house fly populations will be controlled (Eco waste Solutions, 2015).

Furthermore, the pupae of black soldier flies can be used as additives for animal feed because they are high in protein and fat (Stamer A., 2015). Adult black soldier flies can be used to produce biodiesel because they contain a high amount of fat (Zhengi, L., 2012).

2.1 Factors affecting the growth and development of Black Soldier Fly Larvae

The growth and development of Black Soldier Fly Larvae are affected by several environmental parameters like temperature, humidity, sunlight, moisture contents, pH, etc.

2.1.1. Humidity and temperature

Black Soldier Fly Larvae is a eurythermic species that can tolerate a wide temperature range (15–47 °C), but at the same time, it is tremendously sensitive (Park, 2016). The different stages of Black Soldier Fly Larvae are affected by increasing or decreasing temperature. Hatching eggs from Black Soldier Fly larvae incubated at high temperature takes less time than at low temperature. The development time of the larvae differed significantly between temperatures and between rearing substrates. There is a significant interaction between the effect of temperature and the brood substrate on the development time of the larvae. The longevity of adult Black Soldier Fly larvae is significantly affected by temperature in both females and males. Adult flies live longer at intermediate temperatures than at higher extreme temperatures (Chia *et al.*, 2018a, Chia *et al.*, 2018b).

Relative humidity and temperature significantly affect the development, mating, and oviposition of species (Tomberlin *et al.,* 2009). Several authors carried out related studies and concluded that most (approximately up to 99.6%) oviposition occurs in a temperature range of 27.5 to 37.5 °C and relative humidity of 60% (Holmes, 2010 and Sheppard et al., 2002). Other studies by many authors have shown that maintaining a relative humidity of around 60% and a temperature of 27 °C at the site are perfect conditions for egg-laying and mating (Holmes, 2010). The research results are also positively correlated with each other.

2.1.2. Sunlight

Direct sunlight plays a significant role in mating, survival, growth, and development in the natural environment. Artificial light sources are essential for indoor experiments. Sunlight with an intensity of 110µmol m^2 s^{-1} is required for almost 85% of mating events (Park, 2016). In one study, artificial light sources were reported to impact species' breeding events. This precise method is beneficial and effective for raising species outside their natural habitat, where the primary source of influence is the sunlight. The mating and oviposition of the species are positively correlated with quartz iodide lamps (135µmol m^2 s^{-1}) as sources of artificial light (Zhang *et al.,* 2010). Insects cannot see the light beyond 700 nm, so the wavelength range between 450 and 700 nm is best suited for adult breeding events (Zhang *et al.,* 2010).

2.1.3. Moisture content

Moisture contents of the waste significantly influence the survival and growth of Black Soldier Fly larvae (Cheng *et al.,* 2017). Excessive moisture content obstructs the decomposition rate and is agglomerated, and thick materials can accompany the subsequent residue, making further processing difficult (Diener *et al.,* 2011b). Proper control of moisture contents can help overcome these issues. Similarly, Banks (2014) revealed in his study that the growth rate of pupae is higher when 85% of the water in the fecal sludge is present.

2.1.4. pH

The value of pH is an essential parameter that influences the survival and life span of Black Soldier Fly larvae. Various research studies on black soldier flies have revealed that the optimum condition for larval development and growth is a pH higher than 6 (Meneguz *et al.,* 2018). Green and Popa (2012) revealed that the larvae of BSF can adjust the liquid organic leachate pH up to 9. Alattar (2012) also determined that the ability of the organic medium to adjust the pH strictly depends on the density of the larvae. Ma *et al.,* 2018a, Ma *et al.,* 2018b further studied the influence of different pH levels on the development of larvae and illustrated that the larval growth performance is high at pH values above 6 to 10. The larval body weight is increased at pH levels of 4 or 2. It was also determined that the larvae in alkaline substrates could adjust their pH value from 8 to 8.5 but could not adjust the pH value of strongly acidic substrates.

2.2. Optimal conditions for the survival of Black Solider Fly

Like other flies, Black Soldier Fly larvae is sensitive to several environmental parameters, predominantly temperature, which is the most important non-biological factor. Not only can it affect the development rate, season, and insects' daily cycle, but it has a significant effect on the different features of insect biology, such as adult life expectancy, larvae survival, fertility, growth, sex ratio, and population growth parameters (Chia *et al.,* 2018a, Chia *et al*., 2018b). The two fundamental factors which affect the black soldier fly's foraging, development, and life cycle are temperature and humidity.

The Black Soldier Fly larvae had been used for waste management purposes in low latitude areas, where the temperature and sunlight are very suitable for the species to reproduce throughout the year (Alvarez, 2012). Moisture content plays an essential role in composting because it is a significant parameter for the survival of microorganisms, especially for the growth and development of Black Soldier Fly. The optimal water content of the feed varies between 65% and 90%, as shown in Table 1 (Liu *et al.*, 2021).

The pH value is an inherent parameter that affects the life cycle and survival of Black Soldier Fly.

Many studies on the black soldier fly revealed that a pH higher than 6 is the best condition for the survival, growth, and development of larvae. It is recommended that Black Soldier Fly larvae be used for organic waste biotransformation with an initial pH of 6.0 to 8.0 (Ma *et al*., 2018a, Ma *et al*., 2018b). The initial pH effect may be favorable to beneficial bacteria, thereby contributing to the survival, growth, and development time of the larvae; the gut microbiome of insects promotes weight gain, growth, and egg production of larvae (Ma *et al.*, 2018a, Ma *et al.*, 2018b). Sunlight plays a key role in the mating events of the species in the natural environment. The study results demonstrated that sunlight with an intensity of 110μmol m^2 s^{-1} is required for almost 85% of mating events, which is the leading cause why mating in the winter season is highly restricted (Singh and Kumari, 2019).

2.3 YAM

Yam is the common name for some plant species in the genus *Dioscorea* (family: Dioscoreaceae) that form edible tubers. Yams are perennial herbaceous vines cultivated for the consumption of their starchy tubers in many temperate and tropical regions, especially in West Africa, South America and the Caribbean, Asia, and Oceania. The tubers themselves, also called “yams”, come in a variety of forms owing to numerous cultivars and related species. Yams were independently domesticated on three different continents: Africa (*Dioscorea rotundata*), Asia (*Dioscorea alata*), and the Americas (*Dioscorea trifida*).Yam crop begins when whole seed tubers or tuber portions are planted into mounds or ridges, at the beginning of the rainy season (Nora *et al.,* 2019).

The crop yield depends on how and where the sets are planted, sizes of mounds, interplant spacing, provision of stakes for the resultant plants, yam species, and tuber sizes desired at harvest. Small-scale farmers in West and Central Africa often intercrop yams with cereals and vegetables. The seed yams are perishable and bulky to transport. Farmers who do not buy new seed yams usually set aside up to 30% of their harvest for planting the next year. Yam crops face pressure from a range of insect pests and fungal and viral diseases, as well as nematode (Nutrition value, 2020).

Their growth and dormant phases correspond respectively to the wet season and the dry season. For maximum yield, the yams require a humid tropical environment, with an annual rainfall over 1500 mm distributed uniformly throughout the growing season. White, yellow, and water yams typically produce a single large tuber per year, generally weighing 5 to 10 kg (11 to 22 lb). Despite the high labor requirements and production costs, consumer demand for yam is high in certain sub-regions of Africa, making yam cultivation quite profitable to certain farmers. Many cultivated species of Dioscorea yams are found throughout the humid tropics.

The most economically important are discussed below:

Non-Dioscorea tubers that were historically important in Africa include *Plectranthus rotundifolius* (the Hausa potato) and *Plectranthus esculentus* (the Livingstone potato); these two tuber crops have now been largely displaced by the introduction of cassava. Raw yam has only moderate nutrient density, with appreciable content (10% or more of the Daily Value, DV) limited to potassium, vitamin B6, manganese, thiamin, dietary fiber, and vitamin C. But raw yam has the highest potassium levels amongst the 10 major staple foods of the world. Yam supplies 118 calories per 100 grams. Yam generally has a lower glycemic index, about 54% of glucose per 150 gram serving, compared to potato products. The protein content and quality of roots and tubers is lower than other food staples, with the content of yam and potato being around 2% on a fresh-weight basis. Yams, with cassava, provide a much greater proportion of the protein intake in Africa, ranging from 5.9% in East and South Africa to about 15.9% in humid West Africa (Zulu, D. *et al*., 2020).

2.5 EBA (GARRI)

In West Africa, garri is the creamy granular flour obtained by processing the starchy tuberous roots of fleshy harvested cassava. Cassava, the root from which garri is produced is rich in fiber, copper and magnesium (Nwokolo, 2022).

Eba ia an essential food mainly eaten in West African sub-region and other African countries. It is a cooked starchy vegetable food made from dried grated cassava.

100g of Roasted garri has 94.3kcal of energy. Most of this energy is from carbohydrate (23.28g). for the yellow garri (Eba) that has palm oil added during the processing, a 100g will give you, 117kcals. However, yellow Garri contains more fiber and sugar content compared to the white garri and the beta carotene (Vitamin A) is higher (Amaechi, 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Area

This study was conducted at Prof. Aigbodion Entomological laboratory of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin City, Nigeria. Latitude: 6°20’1.32”N and Longitude: 5°36’0.53”E.

3.1 Experimental Insect

Hermetia illucens eggs were obtained from a colony maintained in the laboratory at LD12;12h photoperiod 28±2℃ and 70±5% RH relative humidity.

3.2 Type of diet

The following diets were used:

Yam, Eba and Mixture of (Yam and Eba) as control. The experiment was replicated of 50, 100, 200 and 400 larva.

3.3 Diet preparation

A measurement of 150g of Yam and Eba diets were used separately for the experiment and the Control diet was composed of mixture (Yam and Eba) of 75g each making 150g. These were added to each larvae containing 50, 100, 200 and 400. The Yam and Eba used for the experiment were purchased at Usele market along Lagos express way, Benin City. The Yam and Eba were prepared separately and allowed to cook until a very tender and soft texture.

3.4 Experimental Design

The larvae were divided into three groups based on the different three (3) experimental diets of Yam, Eba (garri) and Yam/Eba mixture. Each group had four replicates consisting of 50larvae, 100larvae, 200larvae and 400larvae (hatched from the egg, 5 days per diameter × depth. Larva were fed 150g per diet while the mixed diet has 75g each summing up to 150g. Water was added to each diet to make them moist but not waterlogged. Also, the larvae were kept in a labeled plastic containers of 10.5 × 17.0 × 7.5cm capped with perforated lids. After pupal stage, they were transferred into transparent bowls of 15.5cm diameter placed in a love net cage of 3.1 × 2.7 × 3.1ft until emerged for easy identification of the sex ratio of adult.

3.2 Pre-Pupal

3.2 Storage facility for adult *Hermetia illucens*

3.5 Experimental Procedure

During dietary treatment 3 groups making a total of three replicates; one group fed with a diet consisting of only boiled Yam, second group fed with a diet consisting of only Eba (processed Garri) and the third group fed with a diet consisting of yam/Eba mixture and larvae were harvested at the pre-pupal stage as indicated by the characteristic melanized caticle of pre-pupal (Karol B. *et al.,* 2019).

Development time was considered to be the number of days between the start of the experiment and the day of harvesting. All larvae from each container were harvested with forceps, counted and weighed collectively. The initial and final weight were registered as *Hermetia illucens* larval yield on a precision balance.

To determine survival, the number of live *H. illucens* larvae at the day of harvesting that were prepupal were picked out and placed into transparent bucket with holes on it for ventilation. The pupae were subsequently counted as they transformed while the adults were separated by sex.

The development time until the adult stage was considered as the number of days between the start of the experiment and the median day of adult emergence.

3.6 Proximate Composition of diets

3.6.1 Moisture Content Determination (Oven Drying Method)

The moisture content of the food is an important factor in food quality, resistance to determination, preservation which is determined with the following procedure;

A clean crucible was weighed and 5g of the sample was added and weighed. The crucible was placed in an oven dryer at 105°C for one hour. The crucible was removed from the oven and placed in a desiccator. Furthermore, the crucible was removed from the desiccators and the weight was taken. This process was repeated for 30mins until a constant weight was achieved.

$$\text{Moisture \%} = \frac{\text{weight of crucible and fresh sample} - \text{weight of crucible and dried sample}}{\text{Weight if fresh sample}} \times \frac{100}{1}$$

3.6.2 Crude Protein Content Determination

The protein content of foods is determined using the kjeldahl procedure which measures the nitrogen content of a sample. The determination of nitrogen in any sample involves complete digestion of sample in hot concentrated acid and in the presence of an appropriate catalyst which covert all nitrogenous material in the sample into ammonia that is released and then distilled out of the aliquot and determined by simple acid-base titration which is determined with the following procedure; 5g of sample was weighed into kejldahl flask. 10ml of concentrated tetraoxosulphate (vi) acid was added and the flask was placed on a heating mantle. After digestion, 5ml of the digest was measured and 5ml of sodium hydroxide (NAOH) solution was introduced. While on the receiving flask, 10ml of Boric acid and Bromoceresol indicator was added. After distillation, the sample collected at the receiving flask was titrated with 0.1ml of normal hydrochloric acid (HCl) and the solution changed from blue colour to purple colour.

$$\text{Protein \%} = \frac{\text{Molarity} \times \text{Titre value} \times 0.014}{\text{Weight}} \times \frac{V1}{V2} \times 100$$

3.6.3 Crude Fibre Content Determination

Crude fibre is that portion of the plant material which is not ash or dissolves in boiling solution of 1.25% H_2SO_4 or 1.25% NaOH. Fibre consists of cellulose which can be digested to a considerable extent by both ruminants and non-ruminants.

Five (5g) grammes of defatted sample and 200ml of 1.25% of tetraoxosulphate (vi) acid (H_2SO_4) was added on to a conical flask and weighed. The flask was boiled for 30mins and the sample was filtered and rinsed with hot distilled water. 200ml of 1.25% of sodium hydroxide was introduced and boiled for 30mins. The sample was also filtered and rinsed with hot distilled water. The sample was rinsed again with 10 % hydrochloric acid and the followed with methylated spirit to rinse. The residue was drain and oven dry at 105°C, cool in a desiccators and the weight was taken.

$$\text{Fibre \%} = \frac{\text{Initial sample mass} - \text{Final sample mass}}{\text{Initial sample mass}} \times \frac{100}{1}$$

3.6.4 Carbohydrate Content Determination

Carbohydrate was calculated by method of difference.

Carbohydrate % = [100 – (Moisture % + Crude fibre % + Crude lipid % + Crude protein %)]

3.6.5 Ash Content Determination (Dry Ashing)

The ash content gives an idea of the amount of mineral elements present and content of organic matter in the sample which is determined with the following procedure;

A clean dried crucible was weighed and 5g of the dried sample was added and weighed. The crucible was placed in the muffle furnace at 550°C for four hours. The muffle furnace was turned off and allowed to cool to 200°C before taking out the crucible into desiccators. Furthermore, after cooling in the desiccators, the weight of the crucible is taken after ash.

Ash % = Weight after ashing - First weight of crucible × 10

3.6.6 Crude Lipid Content Determination

Lipids are referred to group of compounds that are sparingly soluble in water but show variable solubility in a number of organic solvents which is determined with the following procedure;

The weight of the sample was taken and introduced into a fat extraction flask, Petroleum ether solvent was introduced into the extracting flask. After extraction, the petroleum is distilled out of the extract and the residue is weighed.

$$\text{Crude lipid \%} = \frac{\text{Dish weight and weight of fat} - \text{weight of dish}}{\text{Weight of sample extract}} \times \frac{100}{1}$$

3.7 Analysis of data

Larvae in various diets containers were monitored up to the adult emergence and the mean developmental period (days), sex ratio, survival rate and percentage weight gain were calculated. Kolmogorov-Smirnov one sample test was used to test for significant difference among diets.

CHAPTER FOUR

RESULTS

4.0 Mean Developmental Period (Days)

The pre- adult developmental period of *Hermetia illucens* is shown in Figure 4.0.The pre-adult mean developmental period (days) of *Hermetia illucens* raised in Yam and Mixture (Yam/Eba) and Eba diets were 40, 42 and 52 days respectively. There was no significant difference ($P>0.05$) among the diets. The Yam had the shortest developmental period follow by Mixture and Eba had the longest developmental rate. The larvae feed Yam developed faster than Mixture and Eba.

4.1 Survival rate

The different diets had a significant effect ($P<0.05$) on the survival rate of *Hermitia illucens* in Figure 4.1. The percentage survival of Eba, Yam and Mixture (Yam/Eba) were 2.8%, 47.6% and 28%. There was significant difference ($P<0.05$) among diets. The Yam and Mixture (Yam/Eba) diet as had more survival than the Eba diet as the Eba diet had the lowest survival rate.

4.2 Percentage Weight Gain

The percentage weight gain per diet is shown in Figure 4.2. The *Hermetia illucens* fed with Eba diet exclusively were found to have a weight increase of 21.42% on the average. When the Yam diet was administered, it showed that there was a 26.78% increase in weight. Finally the weight differed also when the flies were fed a combination of Eba and Yam with weight increase of 42.33%. There was no significant variation ($P>0.05$) in weight gain among diets. The Yam and Mixture (Yam/Eba) diets produced more weight than Eba diet.

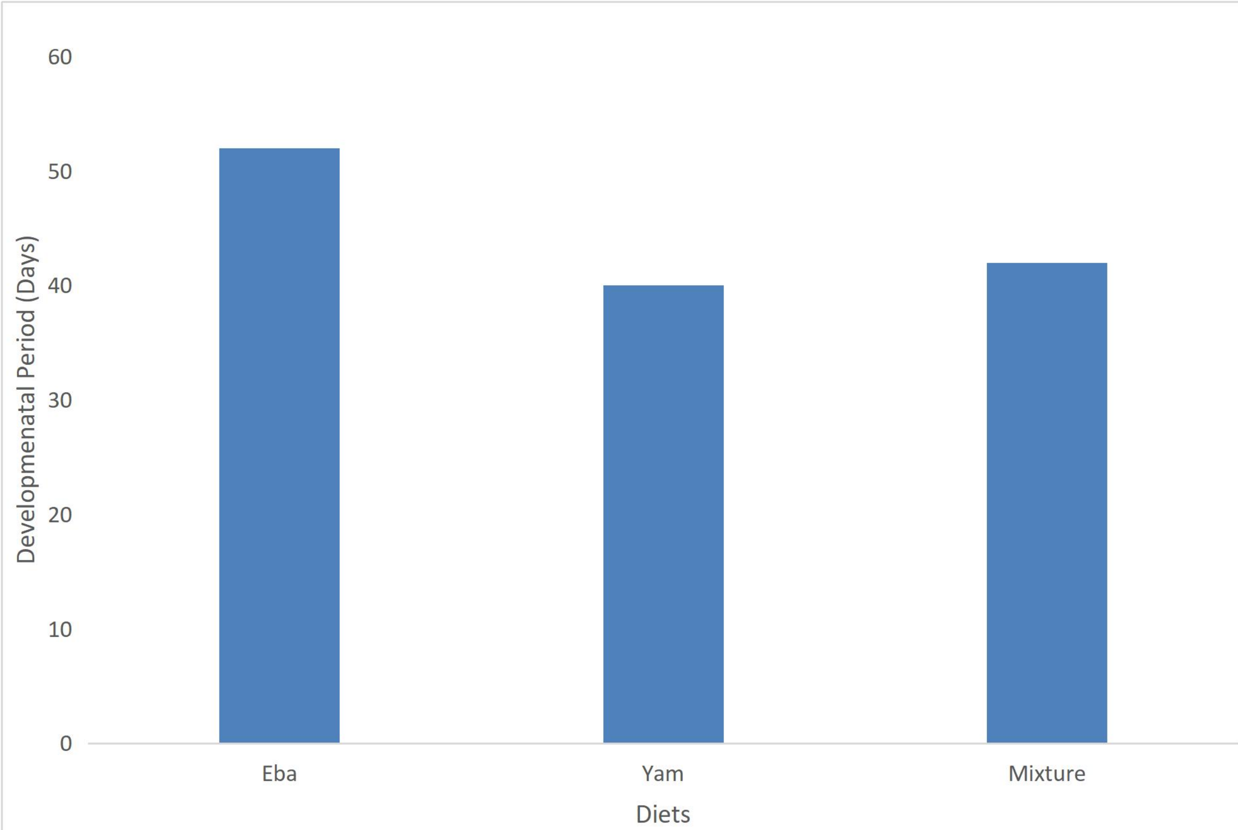

Figure 4.0: Developmental period (Days) of *Hermetia illucens* fed with different diets

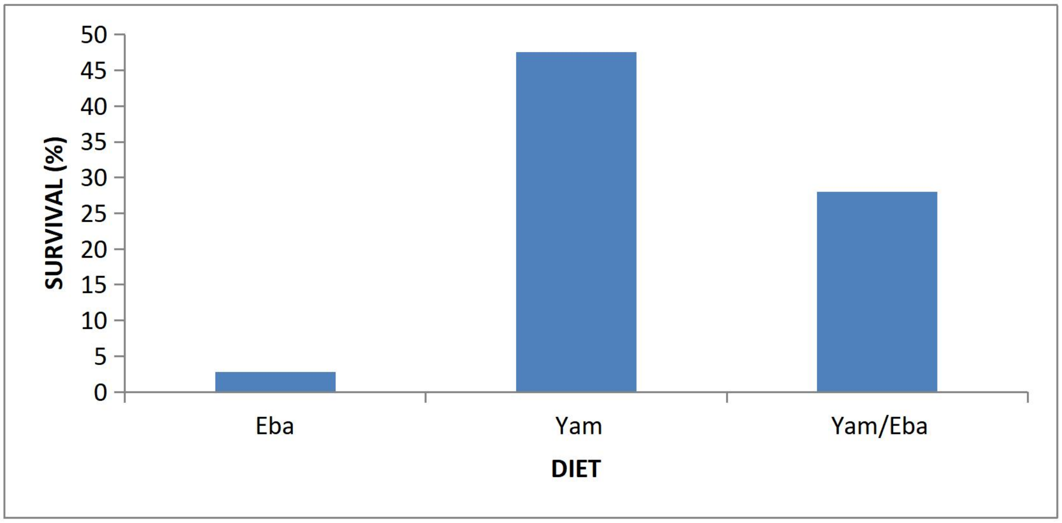

Figure 4.1: The percentage of Larvae of *Hermitia illucens* that survived in Eba, Yam and Yam/Eba

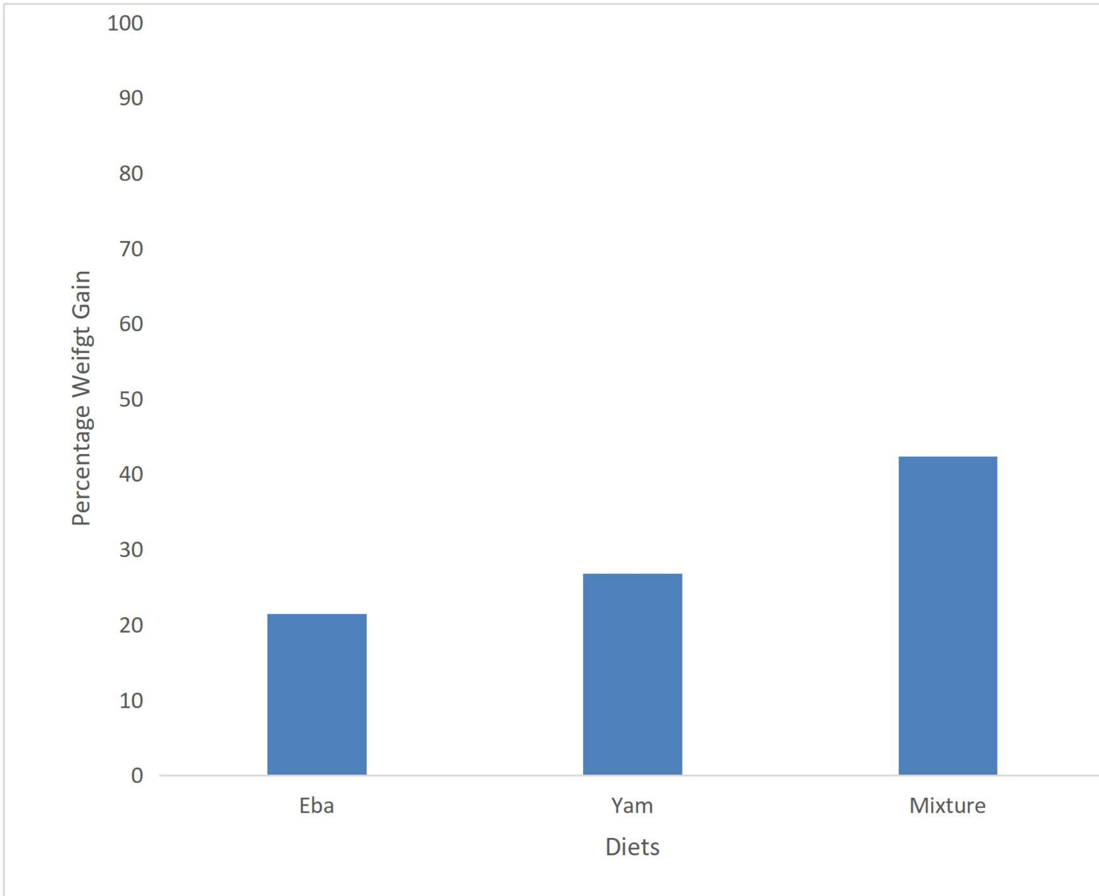

Figure 4.2: Percentage weight gain of *Hermitia illucens*

4.3 Sex Ratio

The percentage sex ratio of the adult; *Hermitia illucens* adults is shown in Figure 4.3.The percentage of the male and female raised from Eba, Yam and Mixture (Yam/Eba) were 31.82%, 68.18%, 47.78%, 52.22%, 54.77% and 45.23%. There was significant variation in Eba and Yam diet as the females were more than the males. However, in the Mixture (Yam/Eba) diet, the males produced were more than the females.

4.4 Proximate analysis

There was significant difference between the proximate analysis of Eba and Yam diet ($P<0.05$). Furthermore, the dry matter, crude fiber and carbohydrate of Yam was significantly higher than that of Eba in Table 4.0. Also, the moisture content, lipid, ash and protein of Eba was significantly higher than that of Yam.

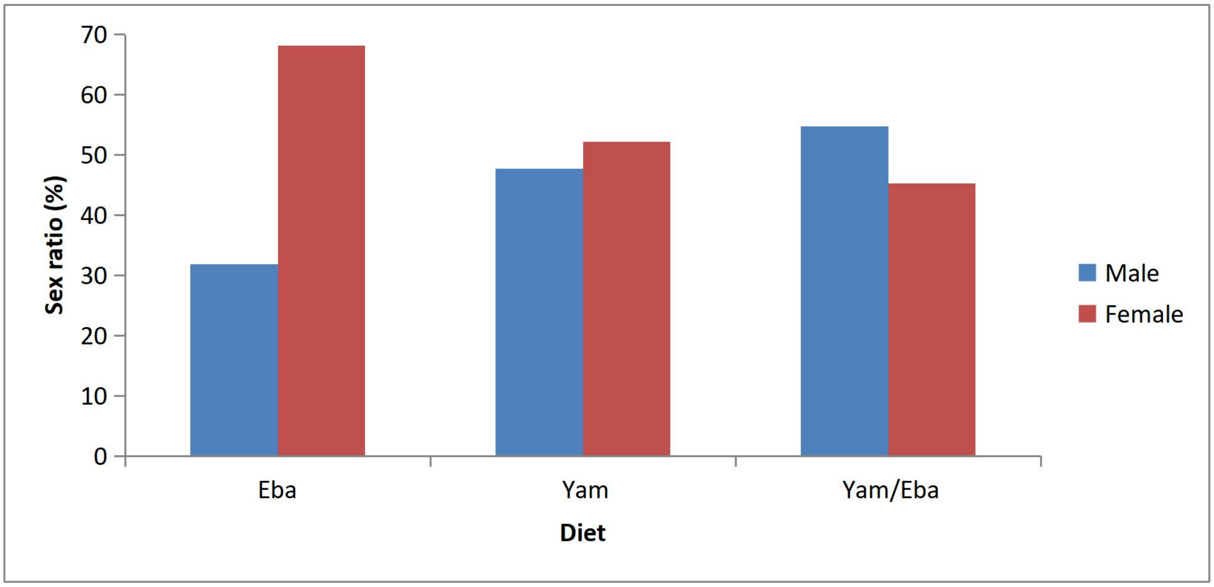

Figure 4.3: Sex ratio of *Hermitia illucens* adult raised from various diet

Table 4.0: Proximate analysis of diets

	Moisture	Dry matter	Lipid	Ash	Protein	Crude fiber	Car. (NFE)
Yam	68.44	31.56	0.18	1.28	0.33	1.94	27.83
Eba	67.22	32.78	0.07	0.97	0.25	2.58	28.91

CHAPTER FIVE

5.0 Discussion

Nutritional strategies used by an individual or group of insects are shaped by their physiology, behaviour, and performance (Lihoreau *et al.,* 2015). The processes of seeking, obtaining, utilising, and allocating nutrients are influenced by several biotic and abiotic factors, such as insect density and nutrient concentrations. Studying the effect of these factors on nutrition and development of insects is particularly important for mass-rearing insects for feed and food in order to optimise productive performance and nutritional quality (karol *et al.,* 2018).

Eba and yam fed larvae developing at the same rate could be due to low protein content. Several authors observed that when BSF larvae were fed with diet high in protein and fat they had higher growth rate than those fed with low protein and fat diets (Nguyen *et al.,* 2013).

The highest survival rate of Yam fed larvae may be attributed to the higher lipid content. Biologically, lipid is for storage, indicating more energy reserve for survival.

The absence of disparity in weight gain among the output of diet indicate that the Mixture of these diets may be productive in rearing *Hermetia illucens.* This research findings in the line with (Nguyen *et al.,* 2013) who reported that the type of diet significantly affected black soldier fly ability to develop with respect to developmental period, size of larvae and mortality and number of larvae successfully reaching each stage of development.

Furthermore, the results of the frequency of adults emerging from pupae on the three diets showed that the Eba produced more female compared to Yam and Mixture (yam/eba) diet. This implies that Eba may have innate fertility quality as research has shown that more female are produced by suitable diets.

Sex ratio seems to be determined by nutrition (diets) of each diet. Simon et al., (2011) suggested that diets with a higher proportion of protein increase developmental time and survival rate of some predatory fly species. This study indicates that larval diets affect the population of *Hermitia illucens*.

5.1 CONCLUSION

In conclusion, the experiments demonstrated that waste diet (Eba and Yam) had effect on *Hermetia illucens* developmental period, survival rate. weight gain and sex ratio.

Their mixture is advised.

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APPENDIX I

THE MEAN DEVELOPMENTAL PERIOD OF *Hermetia illucens* FED WITH EBA AND YAM DIET

Mean Development Period

	Diet		
No. of Larvae	**Eba**	**Yam**	**Mixture**
50L	13.2	9.0	10.3
100L	11.6	10.0	11.2
200L	14.5	11.0	10.0
400L	12.6	10.0	10.7
Total	51.9	39.76	42.2
Days	52	40	42

APPENDIX II

EFFECT OF VARIOUS DENSITY OF DIET ON SEX RATIO OF MALE AND FEMALE

	DIET								
	Eba			Yam			Mixture		
Nos.of Larva	M	F	M:F	M	F	M:F	M	F	M:F
50	1	3	1:3	13	14	13:14	49	10	49:10
100	1	3	1:3	30	37	30:37	13	15	13:15
200	2	3	2:3	60	67	60:67	32	40	32:40
400	3	6	3:6	69	70	69:70	38	44	38:44
Total	**7**	**15**	**1.2**	**172**	**188**	**1.1**	**132**	**109**	**1.1**
%	**31.82**	**68.18**		**47.78**	**52.22**		**54.77**	**45.23**	

APPENDIX III

WEIGHT GAIN OF *Hermitia illucens* LARVA FED WITH EBA AND YAM AFTER 15 DAYS

DIET

Initial density NOS.	Eba				Yam				Mixture			
	Initial density WT.	Final density NO.	Final density WT.	Final Weight	Initial density WT.	Final density NO.	Final density WT.	Final Weight	Initial density WT.	Final density NO.	Final density WT.	Final Weight
50	1	11	0.22	1	1	33	0.66	2	1	29	0.58	2
100	2	5	0.10	1	2	74	1.48	3	2	37	0.74	4
200	3.5	12	0.21	1	3.5	141	2.47	4	3.5	100	1.75	5
400	5	12	0.15	1	5	146	1.83	4	5	103	1.29	5
Total	11.5	40	0.68	4	11.5	394	6.44	13	11.5	269	4.36	16

APPENDIX IV

SURVIVAL RATE OF *Hermitia illucens* FED WITH EBA AND YAM DIET

	DIET								
	Eba			Yam			Mixture		
Nos. of larva	Pre-pupa	Pupa	Adult	Pre-pupa	Pupa	Adult	Pre-pupa	Pupa	Adult
50	5	4	3	30	29	27	28	26	25
100	5	4	4	68	67	67	31	30	30
200	6	5	5	134	129	125	87	87	73
400	9	9	9	143	142	138	88	88	82
Total	25	18	21	375	367	357	234	231	210
%			2.8			47.6			28

APPENDIX V

KOLMOGOROV-SMIRNOV GOODNESS OF FIT TEST SUMMARY TABLE FOR THE MEAN DEVELOPMENTAL PERIOD

MEAN DEVELOPMENTAL PERIOD OF *Hemitia illucens*

	1	2	3	N
Observed	52	40	42	134
Expected	44.7	44.7	44.7	
Cum observed	52	92	134	
Cum expected	44.7	89.4	134	
D	7.3	2,6	0	

Calculated = 7.3/134 = 0.05

Tabulated = 1.34/ $\sqrt{N}$ = 1.36/11 .57 = 0.12

Not Significant different (P>0.05)

APPENDIX VI

KOLMOGOROV-SMIRNOV GOODNESS OF FIT TEST SUMMARY TABLE FOR THE MEAN DEVELOPMENTAL PERIOD WEIGHT GAIN OF *Hemitia illucens* FED ON EBA AND YAM DIET

	1	2	3	N
Observed	21.42	26.78	42.33	90.53
Expected	30.18	30.14	30.18	
Cum observed	21.42	48.20	90.53	
Cum expected	30.18	60.36	90.53	
D	8.76	12.16	0	

Calculated = 12.16/90.53 = 0.13

Tabulated = 1.34/ $\sqrt{N}$ = 1.34/9.515= 0.14

Not Significant different (P>0.05)

APPENDIX VII

KOLMOGOROV-SMIRNOV GOODNESS OF FIT TEST SUMMARY TABLE FOR THE MEAN DEVELOPMENTAL PERIOD SURVIVAL RATE OF *Hemitia illucens* FED ON EBA AND YAM DIET

	1	2	3	N
Observed	2.8	47.6	28	78.4
Expected	26.13	26.13	26.13	
Cum observed	2.8	50.4	78.4	
Cum expected	26.13	52.26	78.4	
D	23.33	1.86	0	

Calculated = 23.33/78.4 = 0.28

Tabulated = 1.34/ $\sqrt{N}$ = 1.34/8.84= 0.15

Significant difference ($P<0.05$)