Insects as food: Assessing the food conversion efficiency of the mealworm (Tenebrio molitor)

by

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ABSTRACT

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The use of insects as a source of food for human populations is gaining interest among small groups of researchers and entrepreneurs. One obstacle currently faced by this movement is the need to identify specific insects that can be raised and processed in a manner that is economically and environmentally sustainable. This thesis looks specifically at the Darkling beetle (Tenebrio molitor), and investigates the conversion efficiency of ingested food of the larva over the course of its development. Efficiency of conversion of ingested food (ECI) is presented here at a daily resolution. Data for this study was collected from ten cultures of mealworms reared over a period of four weeks. Measured values of the final dry masses of the mealworms was referenced with the expected initial dry masses and averaged for the ECI of each day. ECI is a valuable metric in the context of food system science, as it relates the amount of feed consumed by the insect to the amount of biomass (e.g., protein) gained by the insect. Such a measurement is important because it can be used to demonstrate both the economic feasibility of rearing a particular insect as well as the environmental impact the production process has on available resources. The results of this study are compared to similar metrics available for livestock. Implications of these results in terms of large-scale production are also discussed. Mealworm larvae reared had a final mean ECI of 0.3357 and average mass of 0.0491 g over the course of the 25-day observation period.

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CHAPTER 1: INTRODUCTION

The use of insects as a source of food for human populations is gaining interest, albeit at a slow pace and only by a select few researchers and entrepreneurs. One obstacle currently faced by this movement is the need to identify specific insects that can be raised and processed in a manner that is economically and environmentally sustainable. This thesis looks specifically at larvae of the Darkling beetle (Tenebrio molitor), and investigates the food conversion efficiency of the insect over the course of its development. The potential of various other insects as a food source will briefly be presented as will current trends of conventional livestock production. Food conversion efficiency of conventional livestock will be presented alongside that of T. molitor. It is intended for this comparison to demonstrate the advantage T. molitor has over conventional livestock in terms of resource demand (specifically, consumption of feed).

T. molitor (hereafter mealworm), of the insect order Coleoptera, is regarded by global food system specialists as a potentially valuable source of food for humans (Durst and Shono, 2010; Martin et al., 1976; Vantomme et al., 2012). In terms of value as a food source, mealworms, and indeed insects in general, have yet to receive significant attention beyond niche social groups. They do, however, represent a novel source of nourishment with obvious advantages over conventional food types. As is the case with many insect species, mealworms are quick to reproduce, require little care, can be raised in small spaces, and produce nutritional values that rival beef, pork, and poultry. Despite these general characteristics, the literature available on the

rearing and physiology of mealworms falls short of addressing the food conversion efficiency of the larvae. Furthermore, of the literature documenting food conversion efficiency of other insects, no published studies of food conversion efficiency at a daily resolution have been identified.

Fraenkel (1950) provides a thorough report on the nutritional needs of T. molitor larvae, citing development under varying conditions of temperature and humidity, and variations in the diet provided. This study pays close attention to the weight gain of the larvae over the study period but neglects to analyze the amount of food consumed or excrement produced and thus lacks a manner of reporting on efficiency of conversion of food.

Efficiency of food conversion is a valuable metric in the context of food system science, as it relates the amount of feed consumed by the insect to the amount of biomass (e.g., protein) the insect produces. Such a measurement is important because it can be used to demonstrate both the economic feasibility of rearing a particular insect as well as the environmental impact the production process might have on available resources.

Presently, the artificial rearing of insects in the United States serves to fulfill the demands of three primary markets: exotic pet feed; livestock/aquaculture feed; and laboratory research. These markets are interested in ease and cost-effectiveness of production, nutritional value of insects, and the physiological and behavioral characteristics associated with pest management. This thesis aims to provide greater insight into the feasibility of raising insects as a food source. As such, it focuses specifically on the capacity of T. molitor to produce a sustainable alternative to

conventional meat products. Furthermore, a secondary aim of this thesis is to increase our understanding of the methodology employed to quantify the food conversion efficiency of insects. There is currently little data available on this metric and that which is published remains questionable in terms of consistency. Methods for this calculation come in the form of both arithmetic and geometric formulas and may pertain to both short term and long term observations. Still other formulas are unique in that they differentiate between feed ingested and feed digested. As such, the results presented here will help to clarify proper use and representation of efficiency of conversion of ingested food (ECI) and aid in establishing consistency for future research.

BACKGROUND

The current availability of resources to those interested in studying, raising, or consuming insects as part of the human diet is relatively limited. At the same time entomophagy (the practice of eating insects) has been identified as a potential practice capable of alleviating food shortages, decreasing impacts of conventional livestock, and improving nutrition in the human diet (Dusrt and Shono, 2010; Vantomme et al., 2012; Gracer, 2010; Steinfeld et al., 2006; Mitushashi, 2010). The Food and Agricultural Organization of the United Nations has identified edible insects as a subject of priority for investigation and encourages the exploration of the many facets entomophagy has to offer (Durst et al., 2010; Johnson, 2010). Much of the existing literature on the subject is geographically centered in the South Pacific region but numerous sources cite a growing interest in the applicability of entomophagy to the

food systems of the United States and other industrialized cultures (Gracer, 2010; Vantomme et al., 2012; Durst et al., 2010).

Entomophagy may be a solution to current and future impediments to food security (Thrupp, 2000; Daily et al, 1998) by reducing the dependency of societies on large-scale mono-cultural food products. The raising of livestock, for example, is focused on three primary animals (cattle, chicken, pigs), which make up a majority of the meat consumed in the United States (Steinfeld et al., 2006). At the same time, these livestock are increasingly raised under industrialized settings with a decreasing degree of diversity in their diet and diversity among species and varieties. The result is that there exists a vulnerability of society's food system to such factors as population growth, terrorism, and climate change (Watson et al., 1997; Vantomme et al, 2012; Gahuker 2011). In other words, this type of food production is experiencing an increasing reliance on a decreasing set of variables, and a disruption in any one of those variables could favor the odds of food shortage.

Entomophagy also exhibits the potential to resolve issues of environmental degradation arising from conventional methods of food production. As stated, conventional food systems, such as that of the United States, are highly invested in the large-scale industrialized production of relatively few products. As such, there exists unfavorable byproducts that tend to be generated at comparable scales (Steinfeld et al, 2006). These byproducts range from air, water, and soil pollution to the absorption of harmful chemicals into food products and the excessive allocation of energy resources (Steinfeld et al., 2006; Watson et al., 2000). As indicated above, conventional food production has a set of inputs and outputs associated with various

types of livestock. These include methane gas emitted from cattle and hormones/steroids added to their diet.

Insects have been identified as a solution to these problems for a number of reasons. First, they require relatively little input in terms of energy, feed, space, and time. Second, they create relatively few outputs in terms of environmental wastes. And third, based on existing data, they are a very concentrated source of protein (Verkerk et al., 2007) along with containing high levels of essential fatty acids and other micro-nutrients (vitamins/minerals) (Banjo et al., 2006; Bukkens, 1997; Fast, 1966; Xiaoming et al., 2010). Some research has been aimed at studying the economic value of insects as a commercial product (primarily as a source of protein) (Mercer, 1997; Watanabe and Satrawaha, 1997; Boulidam, 2010). This thesis has been unable to identify documentation that compares the nutritional value or the economic value of insect fatty acids to other sources.

In order for entomophagy to make an impact on our current food system several issues need to be addressed. At present, there is a significant disparity between the potential that entomophagy can offer in helping to secure the safety and sustainability of our food system and the cultural biases and dietary preferences exhibited by most people of the United States and other industrialized nations (Gracer, 2010; Schonwald, 2012; Durst and Shono, 2010; Mitsuhashi, 2010)). In addition to the cultural hurdles, the advancement of entomophagy is also obstructed by a lack of an integrated network of researchers and producers specializing in the field (Johnson, 2010). This has the affect of making it more difficult to disseminate knowledge and discoveries amongst researchers. And considering that entomophagy

is regarded to be very much an interdisciplinary field, the development of a reliable network to share research is that much more valuable. Beyond these social and organizational barriers (which could possibly be successfully addressed through educational outreach (Durst et al., 2010)), there is also a lack of knowledge in general on the subject. Such areas of knowledge that need to be explored include: (1) the ecology and biology of important edible insect species; (2) a clearer understanding of the role insects would play in human nutrition; (3) the measurable benefits entomophagy could have over those of current food production; and (4) methods of commercial and personal production (Johnson, 2010; Dusrt et al, 2010; Meyer-Rochow, 2010).

In all cases encountered in the course of this research, insects produced by means of foraging have been used as: (1) a food; (2) as a bait or feed for the acquisition of higher trophic level food; or (3) as a preserved specimen (Mercer, 1997; Sutton, 1988,). This is in contrast to the farming of insects, in which the insects appear to be raised: (1) as feed for captive, non-food animals; (2) for scientific experimentation; or (3) for a byproduct (e.g., silk from the silk moth or honey from the bee) produced by the insect (Grisdale, 1973; Singh and Moore, 1985; Hardouin, 1997; Beets, 1997). Katayama et al. (2005, 2006, 2008) are in a unique position as they appear to be leaders in entomophagy research with the direction being extra-terrestrial agriculture. They are investigating various methods in the lab that they intend to apply to space agriculture models. The ranching of insects also has specific uses that can be anticipated. Insects that are managed in natural settings tend to be: consumed by livestock (free-range chickens); utilized for an insect byproduct (e.g.,

honey from bees); or for other purposes similar to those of foraging (Shinn, 1980; Sutton, 1988; Defoliart, 1995, Hardouin, 1997; Boongird, 2010).

At present, research on the use of edible insects within society is taking place in areas such as Africa, Indonesia, China, Mexico, and Australia (Banjo, 2006; Mercer, 1997; Ramos-Elorduya, 1997; Yen, 2010; Ramandy and Mastrigt, 2010; Onore, 1997; Zhi-Yi,1997), with very little attention addressing the role or potential role of these insects in industrialized western nations. As pointed out, several of the arguments for the increase use of insects as food comes from a collection of problems stemming from and facing industrialized western nations. For this reason, it makes sense to pursue an investigation of the incorporation of entomophagy into these societies. In a recent publication released by the Food and Agricultural Organization of the United Nations (Dusrt et al., 2010), it has been recommended that these investigations, in regards to entomophagy across global societies and cultures, address a number of key issues, among them:

- Conduct research on the ecology and life cycle of edible insects.
- Conduct research on the management potential of wild edible insects to enhance harvests, to ensure sustainability in nature and to assess potential for rearing of promising species.
- Assess the economic feasibility of rearing manageable insects, examining its potential to contribute to rural food stocks and development.

- Promote the adoption of local insects as an element of government strategy for rural development and agricultural diversification where applicable.

(Durst et al., 2010)

The content of this thesis will be most applicable to the first and third bullet points listed above. Through the analysis of the ECI, the research presented herein can be directly related to and incorporated into the existing knowledge of the mealworm's physiology and its growth cycle. This thesis will also support the advancement of our understanding of economic feasibility of large-scale production by assessing how the daily ECI of mealworms compares to that of other food sources. ECI can be used as a metric in food production science for the purpose of identifying unnecessary financial investments in regards to animal production.

CHAPTER 2: REVIEW OF THE LITERATURE

As indicated in the introductory chapter, there is much to consider in terms of the applicability of the mealworm to the future of the human food system. In this chapter a review of the existing literature will provide greater insight into specific aspects of the future of entomophagy, summarize those areas of the field that are currently lacking, and establish a rationale for the focus of the remainder of this study. Key points that will be addressed include: the biology and physiological development of mealworms, nutritional content of mealworms, current rearing technology, impediments to production and markets, and comparisons with the production of conventional livestock. Of particular interest is research-to-date that relates food utilization efficiencies of mealworms to that of cattle and other livestock, and how a more thorough understanding of this concept can aid in establishing reliable mass-rearing endeavors.

The field of research specializing in entomophagy is a relatively new academic discipline. As such, there are few resources available to those interested in pursuing published information. One of the most recent additions to the modest body of literature was released in 2010 by the Food and Agricultural Organization (FAO) of the United Nations. That document, titled "Forest insects as food: Humans bite back," is a volume of 21 essays that specifically addresses the potential role of insects in today's changing world. Along with "Forest insects as food," the FAO is responsible for several other publications aimed at encouraging the advancement of entomophagy in a systematic manner.

Preceding "Forest insects as food" was the periodical "The Food Insects Newsletter," which was published from 1988 to 2000. This newsletter, spearheaded by Gene DeFoliart of the University of Wisconsin, took a grassroots approach to the sharing of knowledge about anything and everything related to entomophagy. With the inclusion of content from both professional researchers and casual practitioners it is a valuable resource for those interested in understanding the history of entomophagy over the past 25 years.

In addition to the two works highlighted above, the journal "Ecology of Food and Nutrition" published a special issue in 1997 that focuses on various aspects of insects as food. A final source worth noting is not in the form of a specific piece of literature but rather an academic department at a research university in the Netherlands. During the last few years, Wageningen University has begun to emerge as a leader in entomopahgy research. One of the more recent articles put out by Wageningen assesses the environmental impact of mealworms as a food for humans (Oonincx and de Boer, 2012). These four sources appear to encapsulate the most thorough content of literature on the subject of entomophagy to date.

Aside from the sources mentioned above, little else was identified as specifically addressing entomophagy. Much of the remaining literature pertinent to this study has been published in either entomological or zoological journals, and in some cases animal husbandry and agricultural journals.

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TENEBRIO MOLITOR – BASIC BIOLOGY

The mealworm beetle (Tenebrio molitor) is a member of the Tenebrionidae family of the insect order Coleoptera. It is relatively well known as both a pest of stored food products (e.g., cereals and grains) and as a feeder insect for captive insectivorous animals (e.g., birds and reptiles). The mealworm is cosmopolitan in distribution and, as it has trouble breeding in the tropics, will be found primarily in temperate regions at northern latitudes (Hill, 2003; Bousquet, 1990). Like all insects of the superorder Endopterygota, the mealworm beetle exhibits holometabolism, or complete metamorphosis, in which the insect develops through a series of four stages in its life cycle – embryo (egg), larva, pupa, and imago (adult). Development within the larval stage may be further distinguished by the number of instars (molts) the insect experiences as it grows prior to pupating. As many as 22 instars have been reported by Ludwig (1956). It is at the larval stage in which this insect is regarded as

a suitable food. The entire life cycle will take place in the same ecosystem, that being the foodstuff occupied and eaten by the insect.

Figure 2.1 – Tenebrio molitor adult (left) (USDA,a) and larvae (right).

Hill (2003) provides a brief description of the life stages of the mealworm and several related species. Upon mating the female will lay up to 500 eggs over. The egg stage is the shortest period of the cycle. The eggs present as tiny, white, spherical, sticky masses that will hatch in 10 to 12 days at temperatures of approximately 20° C. During the larval stage, which from reported estimates can vary from about one month to over a year, the mealworm will go through 9-20 molts, or instars. These molting periods are characterized by larval growth and the shedding of the exoskeleton, of which contains a relatively high amount indigestible chitin. The larvae appear as yellow-brown in color and are smooth and cylindrical, and can grow to 28 mm in length. The pupal stage is characterized by a final molt along with

a complete loss of the carapace which serves as a distinct protective plate above the head and thorax. The pupa will curl ventrally inward along its longitudinal axis and undergo a final metamorphosis of approximately 20 days. It was observed during this study that the pupae appeared to be relatively dormant. The only signs of movement was a twisting of the body in response to touch. No records were kept on those larvae that entered the pupal stage but in general it seems as though the pupal stage was shorter than the 20 days reported by Hill (2003).

The adult beetle emerges as an off-white color, gradually darkening to dark reddish-brown to black and is approximately 15 mm in length. Their life expectancy at this point is approximately 1-3 months. The total length of the life cycle is reported to be 280-630 days. It should be noted that the duration of the various stages of the life cycle can be shortened or lengthened depending upon environmental conditions (Martin et al, 1976; Connat et al., 1991; Hill, 2003). Although temperature and humidity may play primary roles in the onset of pupation it has also been reported that some commercial facilities will manipulate the population of larvae to such high densities that they will in fact not enter into the pupal state (Connat et al., 1991). Additionally, although the mealworm remains conspicuous, compared to other insects pests it is said to be a low threat to stored foodstuffs. This is due to its slow rate of reproduction and small populations and thus any damage incurred by the stored product will remain limited (Hill, 2003).

A very closely related species to the mealworm is the mini mealworm (Tenebrio obscurus), differing phenotypically by a smaller size (12-19 mm) and darker exoskeleton. T. obscurus is also known to develop faster, attributed to an

appreciably shorter larval stage (Hill, 2003). Other related species include those of the Tribolium genus, notably T. confusum (confused flour beetle) and T. castaneum (red flour beetle), and also the superworm, Zophobas morio. In addition to moderate variation in the physical appearance of the two species of Tribolium (adults will grow only to 3-4 mm long) from that of T. molitor, they differ also by exhibiting a relatively shorter anticipated life cycle and being more likely to cause considerable damage to stored food products. Both Tribolium species are likely to practice cannibalism, which is reported to be an important factor in controlling their populations. The major difference between the two is that, like T. molitor, T. cunfusum is found more often in cooler northern latitudes whereas T. castaneum thrives in warmer regions. Z. morio is notable for its slightly larger size and smaller amounts of chitin, and for this reason could be worth the attention of future entomophagy (entomophagical) studies. Similar to T. molitor, Z. morio is currently reared as a feed for reptiles, albeit on a considerably smaller scale. These four species are also regarded as common pests of stored food products (e.g., grains and cereals), and like the mealworm, the larvae are also reared for use as a live food for captive insectivorous animals (primarily birds and reptiles).

ENVIRONMENTAL INFLUENCES

A number of environmental (extrinsic) factors will invariably influence rate and extent of the development of T. molitor larvae (Martin et al., 1976; Connat et al., 1991; Wallach, 1972). Among these are temperature (Ludwig, 1956), humidity (Urs and Hopkins, 1973; Pielou and Gunn, 1940), diet/nutrition (Fraenkel, 1950)),

photoperiod (Tyschchenko and Sheyk Ba, 1986), stress (Weaver and McFarlane, 1990, Connat et al., 1991), and pathogens (Shea, 2005a,b). Although it is difficult to say which of these factors might induce the most profound effect on larval development, most of the studies indicating specific parameters of the rearing methods regularly cite temperature and relative humidity as meaningful variables.

Appropriate rearing temperature for T. molitor larvae is debatable but in general any temperature between 20º C and 30º C would be entirely sufficient for larval survival. Ludwig (1956) and Ludwig and Fiore (1960) have observed that not only will the duration of the larval period increase under colder temperatures but the number of instars will also increase – with up to 22 such molts being observed (Cotton, 1927). Results of these studies (Ludwig, 1956; Ludwig and Fiore, 1960) indicate that as rearing temperature increases from 25º C to 30º C so to do the speed of growth and the number of molts. Martin et al. (1976) present the only identified study with rearing temperatures as low as 20º C. They also report that temperatures above 35º C are "unstable," and that death is imminent at 40º C. Most studies referenced herein cite 25º-27º C as the recommended rearing temperature.

The range of acceptable relative humidity for the rearing of T. molitor larvae is between 13% and 70% (Martin et al, 1976). At the low end it is reported that no growth occurs although food intake remains constant (Fraenkel et al., 1950). Additionally, it has been reported that mealworm cultures reared at low humidity levels may result in cannibalism (Wallach, 1972). And humidity above 70% is likely to result in the growth of molds in the culture with the risk of high mortality rates. It is also reported that at a humidity above 70% food intake by the larvae will drop

appreciably, though they will continue to grow (Martin et al.,1976). The continued growth of the larvae coupled with the absence of increased food intake may be attributed to the insect's ability to assimilate atmospheric moisture through specialized glands in its rectum (Machin, 1976). Martin et al. (1976) report a difference in mass of 50 mg between larvae reared at 30% relative humidity and those reared at 70%. Similarly, Mellanby (1932) reports that at a rearing humidity between 0% and 90%, those larvae that are subjected to higher humidity will consistently retain more weight than those subjected to lower humidity.

In regards to those studies referenced above it is unclear as to whether or not growth and weight gain cited by the authors is in terms of either water weight or biomass. This point will be addressed in the discussion section as it could imply a relative humidity value that favors a more efficient conversion of food into biomass than either higher or lower values. Key points to understand regarding humidity levels are as follows: (1) At low enough levels biomass gain of the larvae will stagnate despite the continued ingestion of food. (2) As humidity levels increase so to does the risk that harmful molds may become established. (3) Further research may by advisable so as to better understand the effect of increased humidity on biomass gain.

Although the influence of stress in the onset of metamorphosis of T. molitor larvae has at times been overlooked (Connat et al., 1991), it remains an important factor in assessing overall development. As reported by Weaver and McFarlane (1990) and Connat et al (1991), a positive correlation exists between an increase in stress and a decrease in developmental progression. Weaver and McFarlane highlight

the role that high larval densities have on stress. Their results show that at high larval densities (20 individuals per 4.5 L jar as opposed to 1 individual per 4.5 L jar) the percent survival of larvae at pupation decreases by close to 50%. These findings may be closely related to the available oxygen in the rearing chamber, whereby a greater number of larvae developing as a group in a finite space might use up the available oxygen thus creating a hypoxic environment and ultimately death (Greenberg and Ar, 1996). Connat et al. (1991) also note that high larval density has been observed to lead to some cases of cannibalism amongst the larvae.

NUTRITIONAL VALUE OF MEALWORMS

According to Martin et al. (1976), there is little information available on the nutritional value of mealworms. They also note that their own efforts at documenting such data "have raised more questions than they have answered" (Martin et al., 1976). Despite this supposed lack of reliable records of nutritional values for mealworms, several publication report information such as protein, fat, vitamins and minerals. These reports will be used as a reference for establishing general expectations of mealworm protein values and for comparison of the results presented in this study, as well as understanding differences in how protein values can be measured and presented.

The following three studies report protein content for mealworms. In each of the studies, the protein content of the larvae is expressed as a percent and is based on the insect's dry weight. The values are calculated by multiplying the analyzed nitrogen content by 6.25. Jones et al. (1972) also provide a value for protein content

(22.32%) obtained from fresh (wet) larvae samples as opposed to dried. Jones et al. (1972) report protein at 52.8%, Redford and Dorea (1984) at 54.5%, and Davis and Sosulski (1974) at 47.2% to 69.0%. Davis and Sosulski provide a range due to the fact that they were interested in the change of protein content over the course of larval stage. In this case the lower percentage (47.2%) is obtained from the larvae which have gone through a longer period of development and the higher percentage (69.0%) is obtained from those that experienced a comparatively shorter period of growth. Clearly, these figures demonstrate that over the course of development the larvae had diminishing protein content despite overall positive mass increase.

Table 2.1 provides an overview of various animal and insect meat types along with their corresponding protein content. Unless otherwise noted the values for the insects were obtained from dried, uncooked samples and represent only the portion of the insect that is customarily consumed. For example, the legs of crickets are typically removed prior to eating. The protein values of the animal meat were obtained from raw, fresh (not dried) samples and, again, have been cleaned of inedible tissue (e.g., hide, bones, etc.). Although there does exist a discrepancy between percent protein obtained from dry versus wet samples, a good reference point can be established with the dried sample of cattle.

The data presented in Table 2.1 have been calculated using the Kjeldahl technique of crude protein estimation. This technique utilizes a set of established ratios for the conversion of nitrogen content of various types of food/protein tissue

	% Protein	Notes	Source
Insects			
Mealworm	54.5		Redford and Dorea, 1984
	52.7		Bernard and Allen, 1997
	22.3, 52.8	Fresh, dried	Jones et al., 1972
	18.7, 67.9	Fresh, dried	Tjhuis et al., 2011
	22.4, 57.2	Fresh, dried	Martin et al, 1976
	23.7	Frozen	Finke, 2002
Cricket	15.0	*A. domesticus*	Woodring et al., 1977
	13.7	*B. membranaceus*	Wu Leung et al., 1968
	12.9	*G. bimarculatus*, prepared	Yhoung-Aree et al., 1997
	58.0	*A. simplex*	DeFoliart et al., 1982
Caterpillar	56.8	*C. belina*	Wu Leung et al., 1968
	55.3	Mean of five *Notodontidae* species	Malaisse and Parent, 1980
Beetle	29.6	*A. trifasciata, larvae*	Banjo et al., 2006
Fly	17.5	*H. illucens, larvae*	Finke, 2012
Ant	6.5	*Carebara sp.*	Wu Leung et al., 1968
Livestock			
Cattle	17.0		Ensminger, 1980
	18.2		Meyer and Nelson, 1963
	17.1		Berg and Butterfield, 1976
	17.5		USDA, 2013b
	31.1	Cured, dried	USDA, 2013b
Pig	13.0		Ensminger, 1980
	12.7		Meyer and Nelson, 1963
	14.7		Whittemore and Elsey, 1976
	13.9		USDA, 2013b
Chicken	18.8		Ensminger, 1980
	28.6		Meyer and Nelson, 1963
	21.4		USDA, 2013b
Egg	12.6	Presumably chicken	USDA, 2013b
	48.4	Dried, presumably chicken	USDA, 2013b

Table 2.1 – Mealworm data is from larvae of the T. molitor species. All values of protein presented in the table were determined via the Kjeldahl technique (nitrogen × 6.25). See text for further explanation concerning Kjeldahl technique.

into a crude protein equivalent (Jones, 1941). In the case of animal meat and insect tissue the conversion is percent nitrogen multiplied by 6.25.

The problems associated with a reliable quantification of nutrient values of a foodstuff (Stewart, 1997) is threefold: 1) variation of nutrient content among samples analyzed, 2) the degree to which that variation may be affected by different rearing methods, and 3) how the physiology of the consumer (i.e., humans) responds to the nutrients (Martin et al., 1976). This last point can be looked at as the percentage of crude protein of a food versus the quality of that protein. It has discussed by Jones (1931) that amount of measured protein in a food is not in itself an accurate assessment of the usable protein. Because crude protein is merely extrapolated from the measured nitrogen content it falls short of quantifying the presence, type, and amount of amino acids (i.e., the "quality" proteins) that are actually processed by the human body (Jones, 1931).

It is useful to note here that chitin $(C_8H_{13}O_5N)_n$, is a long-chain polymer sharing similarities to both cellulose and keratin. It is a primary constituent in insect exoskeletons, be that adult shells or larval molts, and has for the most part been considered to be indigestible by humans (Bukkens, 1997; Paoletti et al., 2007). Due to this structural similarity between chitin and cellulose, it has also been widely assumed that the fiber in insects represents chitin (Finke, 2007). Finke points out, however, that a customary approach to estimating the chitin content of insects likely results in overestimates of that chitin which is actually present. Due to the relatively small amount of chitin, and thus nitrogen in the form of chitin, Finke goes on to justify the rational of regarding crude protein content of insects as a reasonable estimate of the true protein. The premise of Finke is that the less nitrogen in the form

of chitin (i.e., indigestible protein) then the more nitrogen must be present in the form of true protein (metabolically digestible).

Paoletti et al. (2007) seek to provide a greater depth of understanding of the role chitin plays in the digestion of insect-consuming peoples. They attribute the presence of enzymes capable of digesting chitin to the combination of a person's lower socio-economic status (and thus the ensuing cultural norm diet) and genetic ancestry. Their discussion of findings credits a person's potential to assimilate chitin with that person's history of insect consumption.

Martin et al. (1976) provide an analysis of three treatments used for sampling mealworms: one group was reared from egg to adult in the lab, one group was reared acquired as larvae from a supplier and subsequently reared in the lab, and the third group was used as is from a commercial supplier. Methods for the first group (sample A) consisted of setting up a colony of 200 commercially supplied mealworms in a 19 L enclosure. The rearing medium consisted of wheat bran, oats, and apple. It was maintained at 12 hours light and 12 dark each day with average temperature and relative humidity of 20-23°C and 35-70%, respectively. The larvae were harvested after two weeks. The second treatment group (sample B) was reared in 800 cm2 containers with a mixture of flour, bran, yeastamin, and vionate. This sceond group of cultures was maintained in such a manner to facilitate the entire life cycle of the insect. The third group (sample C) was acquired at the larval stage from a supplier upon which they were immediately prepared for nutritional analysis. The primary objective of their study was to determine differences in nutritional value between commercially obtained mealworms and those that were homegrown.

Martin et al. report the results of this study along with a discussion of the complications associated with the interpretation of the available data. Despite the issues in securing reliable data, a moderate collection of published data has been identified for the establishment of reference metrics for use in this study. These sources (cited below), along with the analysis provided by Martin et al. (1976), serve as a reference for establishing estimates of protein, fat, and vitamins/minerals of T. molitor larvae. Results of the Martin et al (1976) analysis indicate that there was little difference in nutrient content amongst the three experimental groups. The results shown in Table 2.2 below are for protein and represent final larvae weight at time of collection.

Sample	A	B	C
Fresh Weight	21.2	24.5	21.6
Dried Weight	56.4	60.3	55.0

Table 2.2 – Percent crude protein of mealworm samples A, B, and C (Martin et al., 1976).

The protein values presented by Martin et al. (1976) are largely consistent with those provided by similar studies. Furthermore, there was only slight variation of protein values within the three experimental groups surveyed, with a difference of only 5.3% (55.0%, 56.4%, and 60.3%). Similarly, total fat content varied by only 5.6 percent between the three groups (31.3%, 35.5%, and 36.7%). Percent protein, again as measured by dry weight, is reported by Jones et al. (1972) and Allen and Oftedal (1994) to be 52.82% and 48%, respectively. Although beyond the scope of this paper, an extensive survey of the biochemistry of various feeds and feed additives on

the protein production capacity of T. molitor larvae is presented by Davis and Sosulski (1972).

CURRENT STATUS OF PRODUCTION

Practically speaking, no established methods for the large-scale, industrial production of mealworms currently exists. That is to say, the methods that are currently being used are at a scale inconsistent with anticipated future demand (Gracer, 2010). Those methods are primarily aimed at producing mealworms to supply the demand of the pet food industry and, to a lesser degree, scientific research and as chicken feed. Additionally, there does exist a fringe sector of private corporate interests that are developing methods of large-scale production, however these methods are being protected as proprietary and therefore inaccessible to the public (Kok, personal communication, 2013).

Although not at a point in its development where one can expect to see implementation in the immediate future, the mass rearing of insects has become a subject of formal study. As an example, Kok et al. (1988) have published the results of their trials with what they refer to as an "insect farm." Their purpose aims to develop reliable methods for the mass production of the larvae of Tenebrio confusum, confused flour beetle (a close relative of T. molitor) as a human food product. Although they indicate moderate success in rearing, there remain aspects of the process that have prevented its immediate implementation. Notably, there is a need to increase the output of the system, incorporate the use of an inexpensive, cellulose

feed (e.g., agwaste), analyze the economics of the process, and identify a consumer for the waste generated.

As discussion of production methods continues among scholars, it should be noted that there exists reports of allergic reaction upon exposure to T. molitor (Schroekenstein et al., 1990). Persons suffering from this allergic reaction present with symptoms of rhinoconjuctivitis and include the following: nasal congestion, runny nose, post-nasal drip, sneezing, red eyes (conjunctivitis), and itching of the nose or eyes. During the winter and spring of 2013, the principle investigator of this thesis exhibited similar symptoms, notably nasal congestion, runny nose, post-nasal drip, sneezing. The pathway of infection was not apparent. Initial attempts of reducing airborne exposure to allergens by use of an N95 respirator was unsuccessful. During a period of five days away from the cultures symptoms abated and general health improved. Upon returning to the lab for data collection of the cultures, disposable nitrile gloves were worn along with an N95 respirator. Gloves and mask were utilized throughout the remainder of the study and no further signs or symptoms of allergic reaction were observed. Ironically, despite that personal protective barriers were not utilized during the fall of 2013, no signs or symptoms of allergic reaction were experienced. The potential of T. molitor to induce allergic reaction or other adverse health effects remains questionable and should be more closely scrutinized as entomophagy studies move forward.

THE NATURE OF LIVESTOCK AND ITS SUSTAINABILITY

As is the case with cattle livestock, it is hypothesized that there exists a peak or plateau of food conversion efficiency in insects in which resources invested in the insect's growth will gradually begin to result in diminishing returns. The intent of this study is to investigate the sustainability and supposed low environmental impact of the production of T. molitor larvae. For this purpose, sustainable production is considered to be that which requires a minimum of environmental inputs in the form of mealworm feed. The sustainable production of livestock, specifically cattle (beef), will be used here to demonstrate the potential of mealworms to meet the protein requirements of human consumers. Cattle production is of interest because beef is currently one of the most highly consumed sources of protein in developed countries at approximately 80 kg per person per year. And over the next 15 to 40 years consumption is projected to rise dramatically in developing and transition countries from a current rate of approximately 30 kg per person per year (Steinfeld, 2006). At the same time, cattle production requires more resources in the form of land, water, and feed than any other agricultural product.

Livestock production has been reported to be the “single largest user of anthropogenic land (Steinfeld, 2006),” accounting for 70 percent of all agricultural land and 30 percent of the Earth's land surface. These resource consumption patterns ultimately translate into a complex suite of environmental problems in the from of air, water, and land pollution (Steinfeld, 2006).

In order to make livestock production more efficient (both economically and environmentally) certain parameters have been closely studied, the findings of which

have been implemented into current practices. One of these parameters is the efficiency with which cattle will convert feed into biomass (Kilpatrick and Steen, 1999). Simply put, a livestock animal will reach a maximum size in which the feed that continues to be consumed will have little impact on growth. To feed an animal beyond its point of significant growth will result in a loss of profits due to excessive feeding costs along with a loss of investment in natural resource to the animal's metabolic activity.

Kilpatrick and Steen (1999) offer a two-component model for the process of beef cattle production. They indicate that the proposed model is necessary for effectively managing for the effects that the provided feed has on growth rate and carcass composition (i.e. marketable meat). First, an accurate assessment of the amount of food consumed "ad libitum" by the cattle must be maintained. And second, there must be an accurate assessment of the resulting diet intake on growth rate and carcass composition. They cite a study published by the Department of Agriculture of Northern Ireland (1987) that indicates a lack of accuracy in previous equations used to predict growth rate and carcass weight (Agricultural Research Council, 1980).

In their attempt at producing a model for the reliable prediction of cattle growth rate and carcass weight Kilpatrick and Steen (1999) identified two potential approaches: (1) an empirical approach in which relationships are derived from experimental data and (2) a mechanistic approach in which attempts are made to simulate energy metabolism of biological processes (France et al., 1987). Their rejection of the mechanistic approach and acceptance of the empirical approach as a

framework for their model parallels methods utilized by Slansky (1985) and Farrar et al., (1989) in their assessments of similar models used for quantification of growth rates and biomass gains in insects.

UNDERSTANDING FOOD UTILIZATION OF INSECTS

Food utilization efficiency is the standard by which insects can be assessed for physiological responses to food consumption (Waldbauer, 1968; Slansky, 1985; Farrar et al., 1989). According to Slansky, "[...when coupled with measures of consumption rate, food utilization efficiency helps identify the reasons for changes in consumer growth rate and developmental time, such as those related to changes in nutritional quality of food (Slansky, 1985).]" There exists a variety of metrics for the quantification of insect feeding behavior. A thorough analysis of those metrics are provided by both Slansky (1985) and Farrar et al. (1989). Each of their individual purposes is to clarify the strengths and weaknesses of the various methods used within the literature and to offer solutions to the inconsistencies that are identified.

Slansky (1985) reviews the available literature on insect consumption data and corresponding food utilization efficiencies for the purpose of assessing: 1) possible methodological sources error, and 2) discrepancies between calculations of dry mass values and energy units values. Three food utilization efficiencies are noted by Slansky as the most commonly referenced: approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI). ECI is a direct measure of body mass gained relative to the amount of food ingested (total consumption), whereas ECD ignores food that passes through the

insect undigested. ECI is also presented by Farrar et al. (1989) in a critical evaluation of the various methods used in calculating insect consumption rates and growth rates. Because the goal of this study is to identify growth patterns of mealworms (in the form of harvestable biomass) relative to the investment in primary resources (e.g., food), it is ECI that is of primary interest.

Results of Slansky's (1985) study identify the following factors as potential sources of error in final efficiency calculations. Implications of these errors along with the associated discussion encourage and reaffirm the use of ECI based on dry weight values as the metric of choice for this study.

1) Conversion of dry weight values to energy values.
2) Inconsistencies between the environments of experimental samples.
3) Evaporation from the feed being mistaken as consumed food.
4) Incomplete assessment of biomass produced (i.e., ignoring shed exoskeletons).
5) Energy values based on dry weight as opposed to ash-free dry weight .
6) Improper/inadequate sample preparation.
7) Improper manipulation of equipment (i.e., failing to calibrate and/or replicate/verify findings).

Farrar et al. (1989) focus on a slightly different aspect of the quantification of food utilization in insects. They are primarily interested in determining: 1) the merits of an arithmetic versus geometric approach to calculations of food consumption and growth, and 2) the appropriateness of initial insect weights versus mean insect weight

in regards to the length of the experiment (e.g., period of growth). The arithmetic approach calculates the mean insect weight by adding the initial and final masses and then dividing the sum by 2. The geometric approach multiplies the initial and final weights and then takes the square root of the product. Farrar et al. (1989) cite studies by Gordon (1968), Klein & Kogan (1974), and Kogan (1986) in which arguments are made for geometric as opposed to arithmetic calculations because of the tendency of insects to exhibit exponential rather than linear trends in growth. Farrar et al. (1989) point out that this is likely to be true only in cases where the period of observation spans more than one instar of the insect's larval growth. Such metrics would be of interest for the purpose of predicting the loss of stored grains from insect pest consumption. This study adopts the arithmetic rather geometric approach as it seems to be the most universal for purposes of data comparison.

CHAPTER 3: METHODS

SETUP

This study was conducted over the course of two distinct periods during the spring of 2013 (February – May) and fall of 2013 (October – November). The spring period will be referred to as Trial 1 and the fall period will be referred to as Trial 2. Both periods took place in laboratory facilities located in campus buildings of the Evergreen State College, Olympia, Washington (47.03° N, 122.9° W). Unless otherwise noted these methods and ensuing analysis/results are in reference specifically to the period of October to November, 2013 (Trial 2). Due to circumstances beyond the control of this researcher, Trial 2 was relocated from from one lab building to a second lab building. This relocation occurred on Day 20 of the study. Other than a change in environmental conditions (i.e., temperature and humidity – recorded with a data logger) there was no other variation in the lab setup. The temperature of the lab varied from approximately 20°C to 23°C (68-74°F) and the relative humidity averaged approximately 25-30% (see Figure 3.1). Temperature and relative humidity were recorded via a Vernier LabQuest data logger. The logger was programed to record six measurements per hour. The photo regime in the room was influenced most strongly by daylight and consequently reflected the pattern of sunrise and sunset. No attempt was made to maintain a strict or consistent photoregime or to control for the influence of artificial lighting within the room.

Several factors contributed to choosing Rainbow Mealworms as the source to supply the needed larvae. First, the company offers several sizes of mealworms. The

choice of mealworm size is helpful because it provides the opportunity to focus the data collection on a specific stage of the larvae development. In this case, due to the relatively short time period available to conduct the study, the medium larvae were selected. The mini mealworms were selected for the first trial of this study because of the relatively young age associated with this size. The young age of the mealworms thus provides a more representative record of the larvae development to pupation. Second, whereas many other suppliers ship their mealworms directly in the mealworm's substrate, Rainbow Mealworms ships their larvae in a newspaper medium.

There are two benefits of this type of packaging. The mealworms will have a relatively empty gut upon arrival, thus establishing a more reliable baseline for beginning this study. Also, the absence of a substrate means the larvae will not have to be subjected to the arduous process of separation from a substrate prior to being placed in the bins prepared for this study. A final reason for selecting this source is because of the company's specialization in mealworms. This in turn provides reliable assurance of the quality of their product. Drawbacks to this method of acquisition include uncertainties resulting from the long transit. The mealworms were in transit for approximately 48 hours spanning a distance of roughly 1,000 miles. During this time, the temperature, humidity, and other potential environmental stresses remain unknown. Upon arrival, the mealworms were observed for signs of mortality and with the exception of a few dead larvae (less than 0.01%) appeared to be in good health.

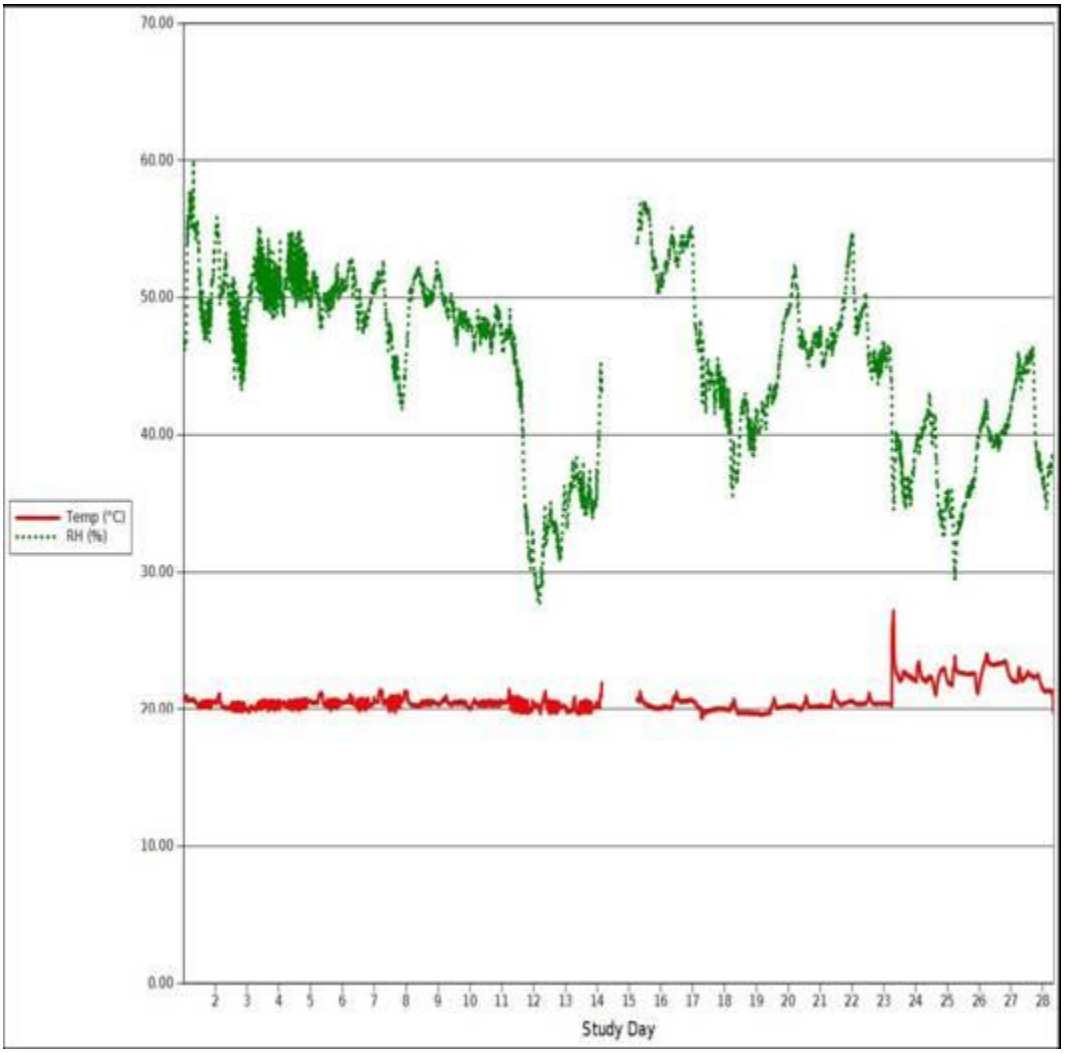

Figure 3.1 – Graph showing the temperature (°C, red line) and the relative humidity (%, green dotted-line) of the lab environment. Temperature and humidity are nearer the low end of the conditions reported in the literature as acceptable. Note the missing data on Days 14 and 15 when the data logger was mistakenly unplugged. Data collected by Vernier LabQuest and processed using Gnumeric 1.10.16.

A total of 5,000 mealworms were used in this study and were acquired from Rainbow Mealworms of Compton, CA on October 16, 2013. They were marketed as "medium" larvae. Unfortunately, this supplier was unable to provide an estimate the

age of the larvae. The inability of the supplier to confirm an age was unexpected as the larvae used during the spring study period (same supplier) were reported to be one week old. Rainbow Mealworms claim that there is a greater degree of variability in growth as the larvae get older. This, apparently, was due to fluctuations of temperature and humidity at the rearing facility (personal correspondence). Similarly, no indication was available as to the number of instars of the larvae, which is commonly used to identify the stage of larval development, or number of molts. The larvae were reared by Rainbow Mealworms on a diet of oat and carrot. Upon arrival to the laboratory their digestive tracks were regarded to be relatively empty, given the couple days they were in transit without feed. This assumption was supported by the amount of frass that had accumulated in the packaging along with procedures reported in other studies. 2.0070 grams of frass was recovered from 555.8043 g of mealworms, slightly more than the average frass egested by the larvae over the course of this study.

Cultures were comprised of approximately 55.6 g (± 1.0 g) of larvae, which is estimated to be 500 mealworms each. At ten larvae per sample per day, this amount would provide enough mealworms for a study period of 50 days. Based on observations recorded during the first trial period of spring 2013, and given the older larvae used for this second trial, it was anticipated that 50 days would be adequate to collect the necessary data before the larvae began pupation. Rolled oats (~800 mL per culture) were used as feed and carrots were provided as a source of water.

Each of the ten larvae cultures used in this study was reared in a plastic storage container measuring of 12 inches by 6 inches (Figure 3.2). The containers

were clear with opaque lids. Although no aspect of the lids were measured or controlled for within the scope of this study it was documented that cultures 1 – 5 had white lids and cultures 6 – 10 had pink lids. Each lid was drilled multiple times to allow for air circulation. The containers were stored on the lab counter in a cluster of five groups stacked two high.

Figure 3.2 – Mealworm cultures on lab counter. A single layer of oats lines the bottom of the plastic bins. Holes in the lids facilitate air movement.

DATA COLLECTION

On a daily basis, ten larvae were selected at random and removed from each of the cultures. They were passed through a series of four stages over the course of four days before being discarded. Larvae were selected one each from 10 sectors laid out over the culture and removed with the use of forceps. The only discretion used in the selection process was that the larvae showed signs of movement, thus assuring no possibility of selecting a recently deceased larva. The larvae were collected on a watch glass prior to all measurements and subsequently weighed using an analytical balance with a precision of 0.0001 g (0.1 mg) (Figure 3.3). Prior to beginning the daily collection of data, the analytical balance was adjusted to zero level and calibrated. The watch glass was then weighed and the mass recorded.

Figure 3.3 – Fresh mealworms on watch glass ready to be weighed. The mealworms in this photo had been stored in the refrigerator, which explains their curled position. These mealworms were not sampled as part of the data.

The first stage of sampling was designed to provide an opportunity for the larvae to expel any contents contained in their gut, thus providing for the establishment of a consistent starting point for mass measurements. During this stage the larvae were deposited into 7 oz. paper cups (Figure 3.4). The larvae were restricted from feed but were provided with water crystals so as to maintain hydration. The continued use of carrots at this point in the study was avoided due to their relatively high fiber and caloric value. The cups were covered with aluminum foil over the top so as to prevent possible escape and punctured with a pencil-sized hole to allow for air circulation. The larvae were kept in these cups for approximately 24 hrs before being removed and weighed.

Figure 3.4 – Example of the sample cups used in this study. The image on the left shows the cup sealed with foil. This method of securing the sample larvae proved to be effective as no larvae escaped the cups during the study. The image on the right shows an aerial view of a cup after being taken out of the drying oven (foil removed).

After being removed from the first set of cups and weighed, the larvae were moved to a second set of cups which contained approximately 46 to 47 grams of oat

feed each. Again, the cups were sealed with foil and the larvae remained there for a 24 hour period before being removed and weighed. The oats were also weighed so as to determine how much feed was consumed by the larvae. It should be noted thatfrass accumulation in the oats was present in very small quantities, an observation that receives additional attention in the Discussion section. The oats were replaced with fresh oats every couple days. Because the water crystals used to provide hydration tend to have a sticky consistency they were omitted from these cups so as not to interfere with the oat feed.

The third stage in the sampling procedure consisted of frass collection (Figure 3.5). For this step, the larvae were moved from the feed cups to a third set of empty cups. Again, similar to the cups containing feed, there were no water crystals in these cups due to the likelihood that the frass would adhere to them. The larvae remained in this cup sealed with a piece of punctured foil overnight and were measured again the following day. Frass that had been deposited in the cup was dumped out onto the watch glass and measured on the balance.

The fourth stage of the procedure was designed to facilitate the collection of dry mass measurements of the larvae (Figure 3.6). Samples were removed from the drying oven and allowed to reach room temperature before weighing. This assured that there would be no temperature gradient between the sample and the balance (Mettler Toledo, 2012). The samples were weighed in the same manner as previous samples and the mass recorded.

Figure 3.5 – A sample of the frass collected during the study. The frass had a sand-like consistency and varied in color from light yellow to dark brown. Under magnification the frass appeared to display translucent and crystalline properties. Note forceps for scale.

Figure 3.6 – Mealworms after having been removed from the drying oven. The mealworms were rigid and brittle their color presented as dark brown to black.

ANALYSIS

It was important to establish consistency in regards to the manner in which measurements were taken with the balance. As an example, observations showed that the reading of the balance would occasionally "drift" to either higher or lower values. This means that a reading of 5.1000 g might, over the duration of a minute or so, slowly drift up to 5.1500 or down to 5.0500. In this study the drift was observed to be at most 0.0010 g. It was also observed that the drift tended to occur when the samples supposedly had either relatively high water content or relatively low water content. This would be the case for samples taken from the first stage cup (with

water crystals) or from the fourth stage cup (oven dried). For this reason, the mass value was immediately recorded upon stabilization and any subsequent drift was ignored.

Because this study aims to quantify the ECI of dry larvae, it was necessary to establish a baseline for comparison of the stage 4 (oven-dried) larvae with their initial weight during stage 1. To achieve this, representative samples were taken during the onset of the study and prepared as per stage 1. They were then moved directly to the oven and dried as per stage 4. The difference between wet and dry mass was determined and a percent water loss value was established. Of seven samples taken over the course of the study an average of 62.2% water loss was determined. This value is comparable to wet and dry larvae masses as reported by Martin et al. (1976), in which the wet and dry masses yielded a water loss of 62.9%. Likewise, Jones et al. (1972) report a moisture content of 57.8% and Redford and Dorea (1984) report 66.4%. The percent water loss value obtained in this study was applied to the initial masses of all the larvae samples so as to make meaningful comparisons at the outset of the study. For example, a sample that is weighed upon being removed from the first stage of the sampling procedure might have an initial wet mass of 1.2088 g. Adjusting this wet weight for expected water loss will yield an initial dry mass of 0.4570 g (1.2088 x 0.6220 = 0.4570). Oat feed was also analyzed in a similar procedure so as to base calculations of ingested feed on dry rather than wet values.

CHPATER 4: RESULTS

EFFICIENCY OF CONVERSION OF INGESTED FOOD

The primary analysis of this study aimed to quantify the growth of larvae relative to the amount of food they were consuming over daily time intervals. ECI is the standard metric used for such purposes and is calculated by dividing the change in weight of the larvae (grams) by the amount of food they ingest (grams) ($ECI = \Delta B/I$). ΔB was calculated using the difference in weight between subsequent measurements. For example, the final dry mass of larvae (stage 4) of Day 5 would be subtracted from the initial mass of larvae (stage 2) of day 3. Ingested feed (I) values were obtained from the amount of food consumed over the same periods as ΔB. ECI results are shown in Table 4.1. ECI of the entire 25-day study period is 0.3357 (see Table 5.1). The final ECI obtained of 33.6%, can be interpreted as the larvae converting 33.6% of the feed ingested into biomass between Day 1 and Day 28 of the study.

Excluded from the final ECI measurement are two extreme outlier values recorded on Days 16 and 21. The sample taken from culture 2 on day 16 was excluded as was the sample taken from culture 8 on day 21. These two cultures represent one each of ten samples collected on those days. In other words, of 250 total ECI measurements over the course of the study, 248 measurements were incorporated into the results and analysis. Figure 4.1 shows how extreme these values are in relation to the rest of the data. (Refer to Figure 4.4 for a comparison to the data after being cleaned.) In both Days 16 and 21, the extreme outliers can be traced back to the mass of feed ingested. The values for I (feed ingested) are

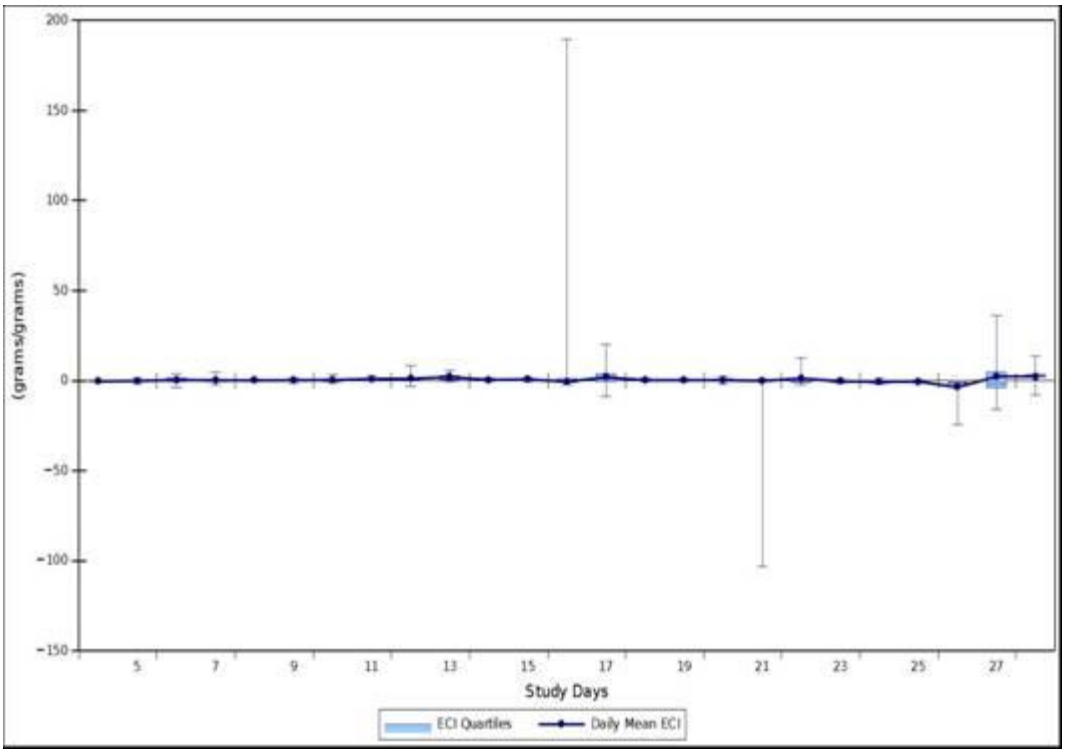

Figure 4.1 – Box plots of data prior to being cleaned. Note maximum outlier on day 16 and the minimum outlier on day 21. Sample from culture 2 was omitted from ECI of day 16, as was sample from culture 8 on day 21. See Figure 4.4 for comparison.

extremely low in comparison to those recorded for the rest of the study period, thus resulting in a samll denominator and a large quotient (i.e., ECI). It is unclear why the values are so small but it is suspected that a majority of the larvae may have molted on these days and thus deposited there exoskeletal remains into the feed. The extra mass of the molted tissue may have been substantial enough to make up the difference of the feed that was consumed. Another explanation for the extremes could be that there was a very small accumulation of frass in the feeding cups. Given that previous studies report 24 hours as being a sufficient length of time for larvae to empty their guts of food/waste matter, the following two points are suspected as contributing to frass accumulation. First, larvae of stage 1 could be consuming the

exoskeletal remains as they are molted, leading to the elimination of the ingested matter during stage 2. Second, mealworms may have a quicker digestion time than previously anticipated and so are actually eliminating that feed which they are ingesting during stage 2.

Figure 4.2 depicts the average ECI (as a percentage) of the sample larvae during the study period. The average mass of the larvae is included for comparison. Note that there is an upward trend in larvae size (mass) during the first half of observation. This is followed by a generally slight decrease in size. The ECI of the larvae increase slightly during the first half of the study and then tend to decrease considerably. It is believed that the decrease in ECI corresponds to a point in development where the larvae are approaching the pupation phase (refer to Table 4.1 and Figure 4.3). The average larvae mass supports this due to the fact that there are no longer signs of mass gain.

Figure 4.4 displays the spread of the corrected ECI data (as a ratio) on each day of the study. The spread of the data is broken down into quartiles (25%, 50%, and 75%) with the whisker ends representing minimum and maximum values of each day. Note that after being cleaning the data, the median (50% mark) on Day 16 is actually slightly in the negative, with the extreme outlier having previously had a very strong influence on the ECI. A similar example can be seen on Day 21, although in this case the outlier and median are both in the negative. Days 22 and 26 should also be noted as having strongly influential outliers as can be seen in the significance of the offset between median and mean. Day 27 is unique in that is has a relatively large spread between the 25th and 75th percentiles, spanning over 8 %. The next largest

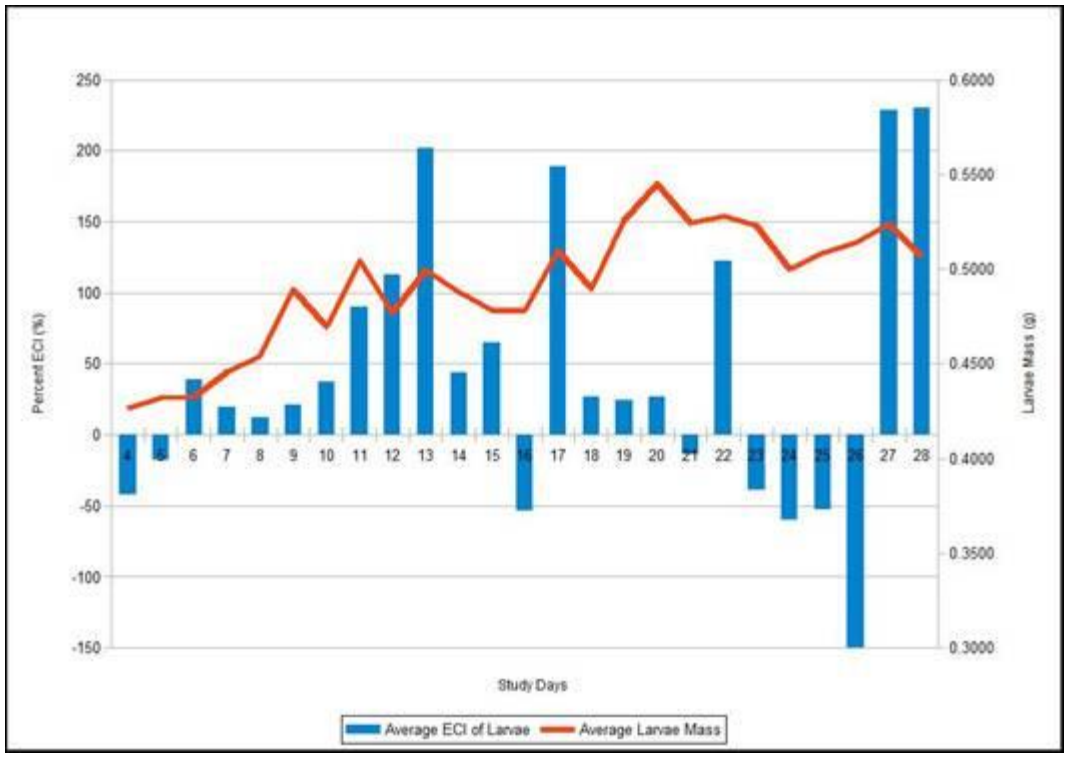

Figure 4.2 – Graph showing the change in ECI of larvae from day to day (blue bars) and the average mass of larvae (orange line). ECI is expressed as a percent (e.g., ingesting 0.05 g of feed and gaining 0.025 g in mass yields a 50% ECI). Note that the data begins at day four of the study. This is because day four is the first day that dried larvae samples were removed from the oven.

spread is only 4% (apx) (Day 17), with most days having a spread of < 1% to 2%. As a final point, it should be recognized that in most cases the mean daily ECI very closely falls in line with the corresponding daily median value.

Study Day	Date	Daily Mean ECI	Average Mass	Average Food Ingested	Pupae
4	10/26/13	-0.4239	0.4264	0.0348	0
5	10/27/13	-0.1805	0.4320	0.0217	0
6	10/28/13	0.3878	0.4328	0.0169	0
7	10/29/13	0.1927	0.4457	0.0201	0
8	10/30/13	0.1186	0.4542	0.0346	3
9	10/31/13	0.2093	0.4891	0.0194	2
10	11/01/13	0.3744	0.4696	0.0040	0
11	11/02/13	0.8974	0.5046	0.0211	0
12	11/03/13	1.1238	0.4771	0.0120	1
13	11/04/13	2.0208	0.4995	0.0089	3
14	11/05/13	0.4365	0.4883	0.0240	12
15	11/06/13	0.6486	0.4781	0.0124	27
16	11/07/13	-0.5340	0.4819	-0.0086	36
17	11/08/13	1.8916	0.5097	0.0417	32
18	11/09/13	0.2681	0.4896	-0.0219	32
19	11/10/13	0.2437	0.5259	0.0502	5
20	11/12/13	0.2669	0.5451	0.0303	2
21	11/13/13	-0.1409	0.5227	0.0217	9
22	11/14/13	1.2273	0.5284	0.0065	10
23	11/15/13	-0.3889	0.5233	0.0290	2
24	11/16/13	-0.5959	0.5004	0.0298	6
25	11/17/13	-0.5289	0.5088	0.0235	7
26	11/18/13	-3.7101	0.5141	0.0150	15
27	11/19/13	2.2844	0.5236	0.0022	36
28	11/20/13	2.3039	0.5065	-0.0049	27

Table 4.1 – Daily mean ECI. Data used to generate the graph in Figures 4.2, 4.3, and 4.4. For consistency, “Average Mass” and “Average Food Ingested” were also cleaned of culture 2 on day 16 and culture 8 on day 21.

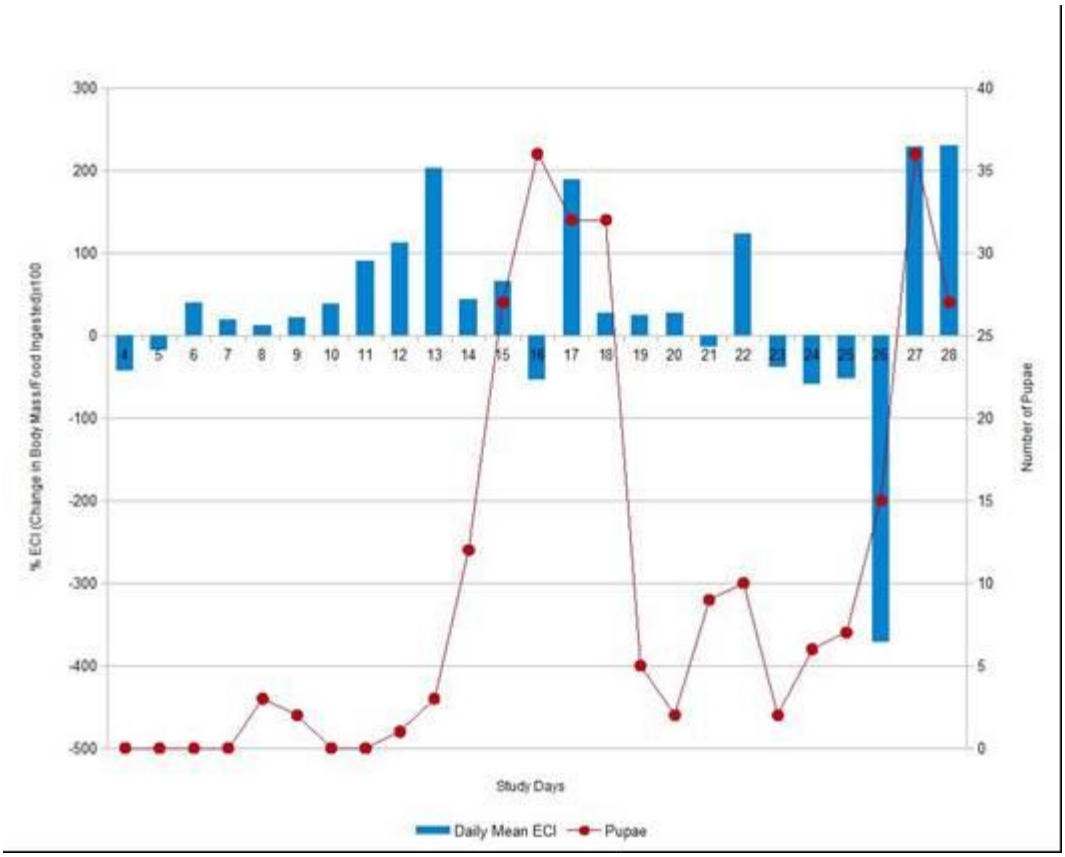

Figure 4.3 – Graph depicting pupation in comparison to ECI. Note that ECI tends to decrease during those periods when more larvae are pupating.

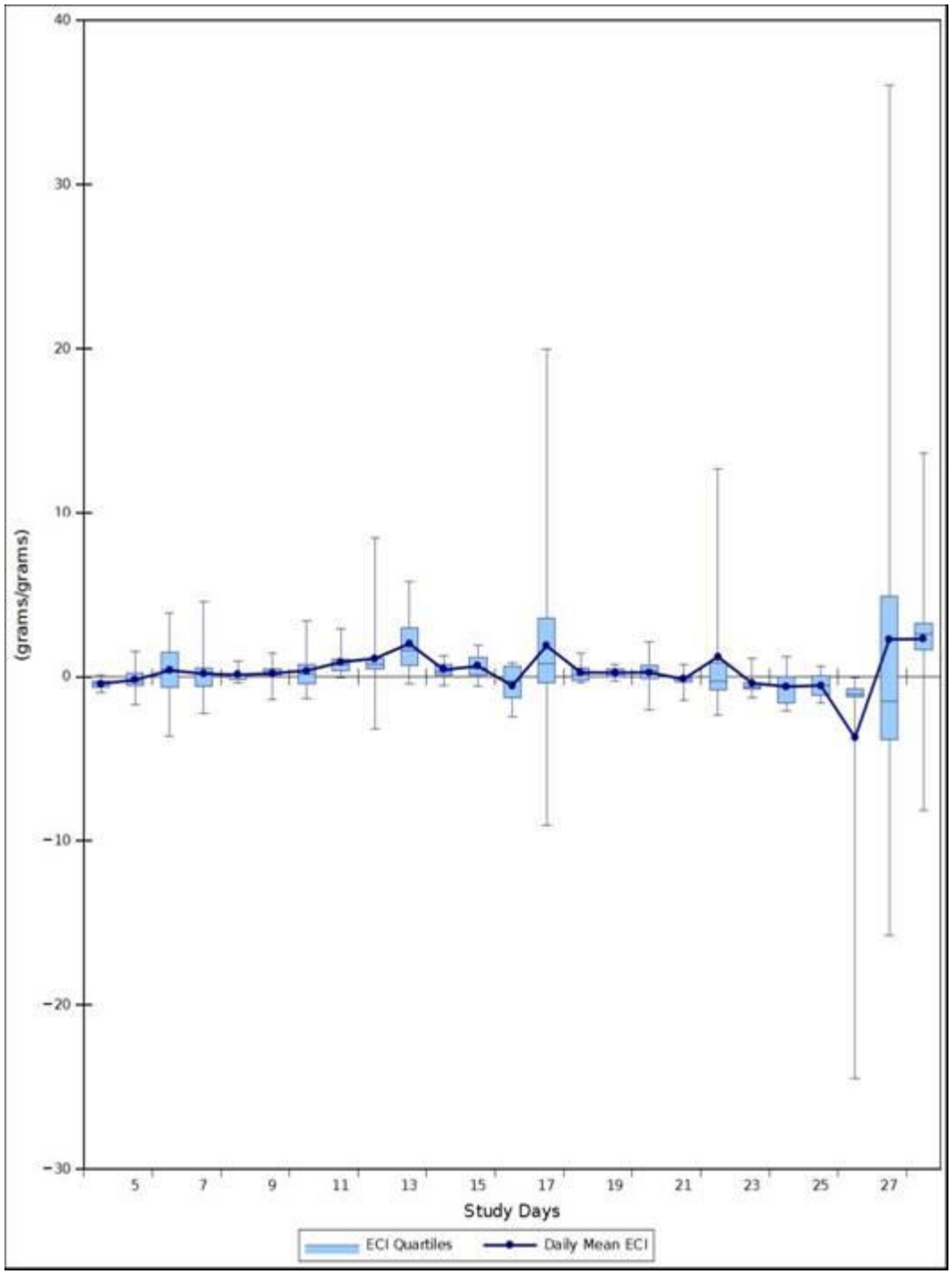

Figure 4.4 – Cleaned data. Box plots showing spread of ECI values for each of the ten cultures on each day of the study. Daily mean ECI is included for reference and demonstrates the overall reliability of the data. The graph represents the data presented in Table 4.1.

CHPATER 5: DISCUSSION

MEALWORMS COMPARED TO CONVENTIONAL LIVESTOCK

Figure 5.1 has been adapted from data presented by Thiessen et al. (1985) on the efficiency of food conversion of cattle. Their data was presented as grams of cattle mass gained per kg of food consumed. As the graph demonstrates, the data was collected once every 12 weeks over a duration of 60 weeks. (No data was reported for the period 0-12 weeks.) This data represents the live weight of the whole cattle. A total of 292 female animals representing 25 breeds were used for the study. The ECI's would be expected to decrease if slaughter weight was to be taken into consideration (i.e., exclusion of hide, bones, etc.). The combined ECI over the five

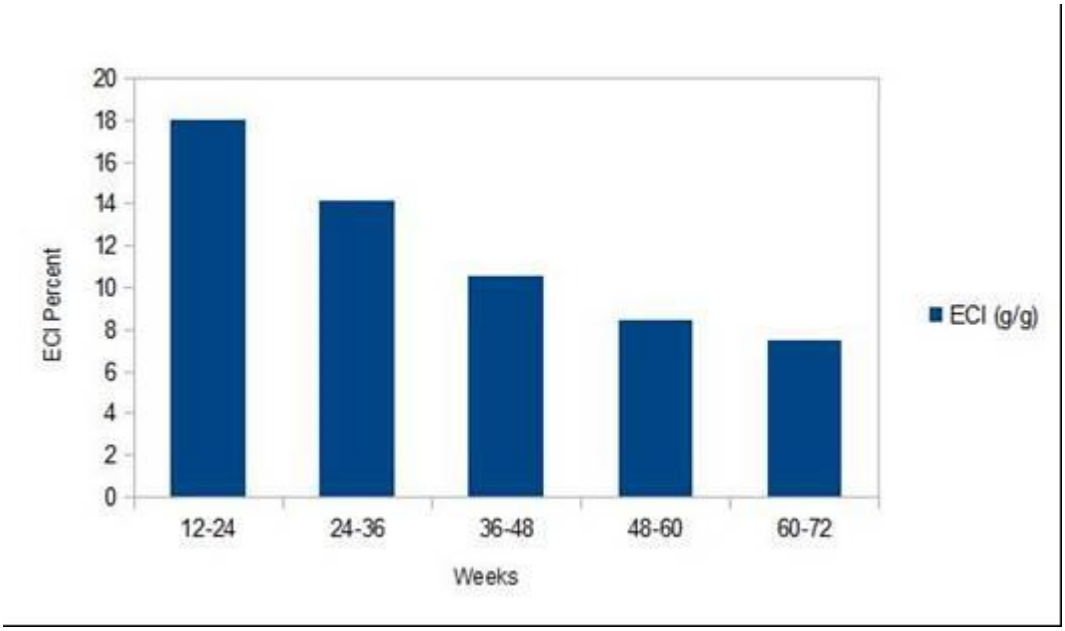

Figure 5.1 – Graph of ECI for cattle over a 72-week period. Data adapted from Thiessen et al. (1985). Average ECI from week 12 to week 72 is 11.7%.

data periods recorded equals 11.7%, which is comparable to those ECI values for cattle presented in Table 5.1. The downward trend of the ECI values is similar to that presented in Figure 4.2 for the mealworms with the exception that the study period ends (i.e., cattle are harvested) prior to the occurrence of negative ECI values.

	% Wet Weight ECI (whole body)	% Dry Weight ECI (dressed meat)	Source
Insects			
Mealworm (larvae)	-	33.6	This paper.
Cricket	92.0	21.1	Nakagaki and DeFoliart, 1991
Livestock			
Cattle	16.0	-	Meyer and Nelson, 1963
	13.0	-	Lovell, 1979
	11.7	-	Thiessen et al., 1985
	-	3.6	Nakagaki and DeFoliart, 1991
Pig	28.0	-	Meyer and Nelson, 1963
	31.0	-	Lovell, 1979
	-	8.5	Nakagaki and DeFoliart, 1991
Chicken	35.0	-	Meyer and Nelson, 1963
	48.0	-	Lovell, 1979
	-	9.9	Nakagaki and DeFoliart, 1991

Table 5.1 – "Whole body" has not been processed in any way. "Dressed meat" refers to animal/insect meat that has been cleaned of inedible tissue (e.g., bone) and prepared as it would be for market. % dry weight ECI for mealworms was calculated from the data generated by this study. The daily ECI values (Table 4.1) were averaged across the length of the study to produce a final ECI. Cricket species is Acheta domesticus.

INTERPRETATION OF RESULTS

A decrease in the ECI of the larvae demonstrates that they are becoming less efficient at processing their feed into biomass. Although the ECI can be closely correlated to larvae mass, a change in efficiency can occur under various

circumstances. As Figure 4.2 illustrates, as the study period approaches day 28, there is a small increase in larvae mass (days 24 – 27). However, this increase in mass corresponds to a drop in ECI, indicating that the larvae are ingesting more food for smaller gains. From a food production point of view, any indication of an increase in larvae mass would tend to be favorable so long as the corresponding ECI is constant or increasing.

Under circumstances favorable to a longer period of observation and data collection, it would be beneficial for this study to be repeated with very young larvae so as to create a longer history of their development. The study as presented here is satisfactory in that there is justification for a shorter rearing/observation period due to logistical limits and the fact that a developmental history had been, at least in part, established during the spring 2013 trial. The results successfully accomplished the purpose of this study which was to identify a trend in the ECI of developing larvae and to establish a stronger comparison between mealworm production and that of cattle. As can be seen in the data of Table 5.1, mealworms are approximately 30% more efficient at turning their feed into biomass than are cattle.

Figure 5.2 is a graph of data collected during the first trial period (spring 2013). It can be directly compared to Figure 4.2. Notable differences include the relatively lower ECI values and the longer study period. Overall, the trends are for the most part similar. The larvae used in trial one can be more accurately approximated to a specific age. They were reported to be approximately one and a half weeks old at the beginning of the study which makes them at least nine weeks old at the end of the study on day 55. Note that at day 39 there is less positive growth

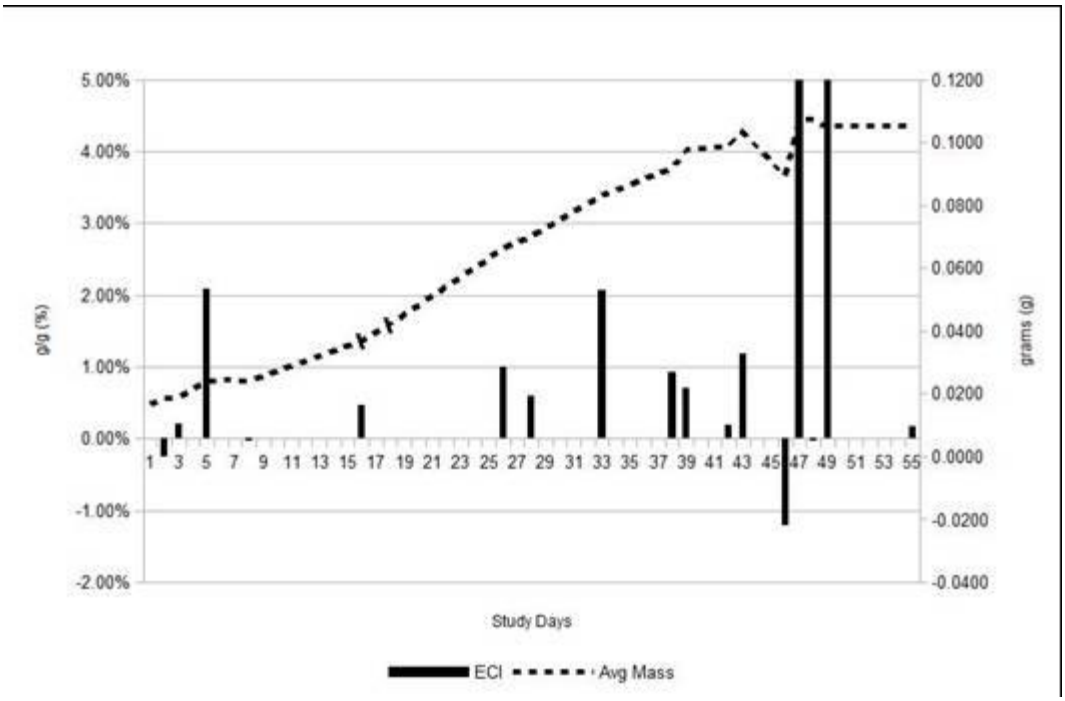

Figure 5.2 – Graph of daily ECI of larvae from Trial 1 (Spring 2013). The solid bars represent ECI and the dotted line represents the average mass of larvae on those study days. During Trial 1, samples were composed of 20 larvae, as opposed to ten larvae per sample during the Fall 2013 study period.

occurring and the average mass seems to top out for the remainder of the period. A similar trend occurs at day 20 during the trial 2. With this information, the approximate age of the larvae at day 20 can be estimated to be 7 weeks old.

ADDITIONAL OBSERVATIONS AND RECCOMENDATIONS

Although the second trial period in this study resulted in more reliable results than the first trial period, improvements can still be made. For example, during phase two of the fall trial, there was a very slight accumulation of frass in the feeding cups. This was to be anticipated. The accumulation of frass was partially controlled for by regularly replacing the oat feed with fresh oat. Nonetheless, the frass would have

skewed the feed measurements to some degree. Future studies might benefit from a more sophisticated mechanism to control for frass deposition.

Additionally, the presence of frass did not distort the measurements to the degree that was observed during the spring study period. Due to the physical properties of the frass it was not feasible to attempt the separation of frass from oats. Evans et al. (1939) provides discussion of methods utilized which reportedly accomplished the task of separating T. molitor frass from oats in which the larvae had been feeding. Their methods, unfortunately, were unable to be effectively reproduced for this study. In addition to their methods lacking the precision that the present study aims to achieve, it was also observed that the frass being produced during this study had a tendency towards irregular and oblong physical characteristics (see Figure 5.3). This is in contrast to that which was reported by Evans et al. (1939) in which the frass was round and spherical and easily rolled away from oat and oat flour particles. Figure 5.3 shows the variability in size and shape of the frass.

Observations show that the value of the mass of the watch glass fluctuated by ~2.0 mg (0.0020 g) over the duration of the study. It is understood that the analytical balance used for these mass measurements is sensitive to environmental conditions and will respond accordingly. Those conditions which are believed to have potentially had some influence during the sampling procedure are: relative humidity, temperature, and electrostatics. The fluctuations occurred on a day-to-day basis but did not appear to present obvious fluctuations during the ~2 hour sampling procedure. This was confirmed by periodically reassessing the mass value of the empty watch glass during the procedure. Part of the fluctuation in mass readings of the watch glass

Figure 5.3 – Frass collected during the second trial study period. Various sizes and shapes of frass are visible in the image. The piece of frass indicated in red is 1.4 mm long by 0.1 mm wide. Scale bar is 2.0 mm. Image captured with an automontage microscope under 2.5 magnification.

is due to a small chip having occurred during measurements on the 15th day of the study. The change in mass was taken into account at the time.

It was found in both trials 1 and 2 that screen proved to be an inadequate material for securing the larvae. Both poly and nylon screens were used intermittently over the course of both trial periods and it was found that the larvae effectively chewed through the screen material. Subsequently, it was found that the foil-enclosed paper cups were effective at securing the larvae. All of the data presented in this thesis came from samples reared in the foil-cup enclosures.

Agar was used early on in this study as a source of feed for the larvae samples. The purpose of agar as a feed was to (1) maintain greater control over the dispersal of feed fragments and (2) to provide additional moisture to the larvae during the sampling procedure. There was no indication that the larvae had suffered in any way from a lack of moisture during the sampling procedure, as would be evidenced by dark, rigid dead larva. In fact, the only larvae that were observed to die during the sampling procedure occurred early in the study before the switch was made from agar to oats, thus suggesting an adequate amount of water in the larval diet. All of the data presented here came from larvae samples reared entirely on oats.

CHAPTER 6: FINAL THOUGHTS

The food conversion efficiency of the mealworm is just a very small piece of the larger, more complex global food system. The ECI values presented in this thesis are a step towards addressing just a specific component of that system. There are, of course, additional steps being taken, some of which are also aimed at food conversion efficiency but with different insects. Others focus on nutritional needs of the global population. And some explore ways of tackling culture biases against insect-eating.

A study by Nakagaki and DeFoliart (1991), for example, quantifies the ECI of crickets. That study was both a valuable tool for the investigation of this thesis as well as now being a compliment to it. Other studies also deal with mealworms, but as opposed to focusing directly on the metabolic processes, they focus on measurable environmental impacts of production. A recent study from the Netherlands, for example, quantifies the global warming potential, land use, and energy use resultant from the production of food-grade mealworms (Oonincx and de Boer, 2012).

The most effective way to approach problems of land, air, and water degradation, to improve the health and nutrition of people, to more fully understand and respect the life of other organisms, and to secure a more sustainable future is by breaking the food system down into its most fundamental component parts. Critically evaluating those parts and addressing them individually is necessary in order to make meaningful progress towards food security and environmental sustainability. Growing and eating mealworms and other insects may be a novel approach, but also one that is beginning to emerge as a valuable technology for the coming future.

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