Insects as food and feed:
Nutrient composition and environmental impact

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Abstract

Because of an increasing world population, with more demanding consumers, the demand for animal based protein is on the increase. To meet this increased demand, alternative sources of animal based protein are required. When compared to conventional production animals, insects are suggested to be an interesting protein source because they have a high reproductive capacity, high nutritional quality, and high feed conversion efficiency, they can use waste as feed and are suggested to be produced more sustainably.

This thesis starts with a historical perspective on insects as food and feed, and explains why insects are expected to be more suitable production animals.

In Chapter 2 the nutritional quality of insects is discussed based on a variety of insects that are commonly used as feed or food. Additionally, various environmental and dietary factors which are known to significantly affect insect chemical composition are reviewed.

In Chapter 3 the direct production of greenhouse gases (GHG) and ammonia, as well as carbon dioxide emission and average daily weight gain of five insect species is quantified. Differences regarding the production of GHGs (expressed as CO_2 equivalents) between insect species were mainly due to the production of methane. All species tested emitted lower amounts of GHG than cattle, had a higher growth rate than cattle or pigs, and emitted lower amounts of ammonia and either comparable or lower amounts of GHG than pigs.

Results from Chapter 3 were used in a Life Cycle Assessment conducted in Chapter 4. For a mealworm production system total GHG production, energy use, and land use were quantified and compared to conventional sources of animal protein. This chapter shows that mealworms should be considered a more sustainable source of edible protein, and that a large part of their environmental impact is due to the feed they consume.

In Chapter 5 four diets composed from industrial organic by-products were formulated such that they varied in protein and fat content. These diets were offered to four insect species and their feed conversion efficiency was determined. Diets used in large scale production systems were included and served as controls. Diet composition affected feed conversion efficiency in all species. It is concluded that the four investigated species are efficient production animals and can therefore be considered interesting for the production of feed or food. Furthermore, diet composition affected insect composition, indicating possibilities to tailor the composition of these insects to best meet consumers’ needs.

In Chapter 6 the suitability of chicken, pig, and cow manure was compared as feed for larvae of the Black Soldier Fly, which in turn could be used as feed for conventional production animals. Newly hatched larvae were directly inoculated on moistened manure. Whereas survival was high on all three tested substrates, the development time was greatly prolonged compared to the control diet. On pig manure, more nitrogen was utilised than on chicken and cow manure, while P utilisation was highest on cow manure. A large proportion of manure nitrogen was lost in all treatments, indicating that the production system would require a way to prevent this in order to make it ecologically sound. Furthermore, to improve economic viability, shorter development times would be required.

In Chapter 7 the suggestions why insects would make suitable production animals are evaluated based on both literature data, and data gathered in this thesis. Furthermore, prospects for insects as food or feed are put forward, with emphasis on the knowledge and legislation requirements for the further development of the insect production sector.

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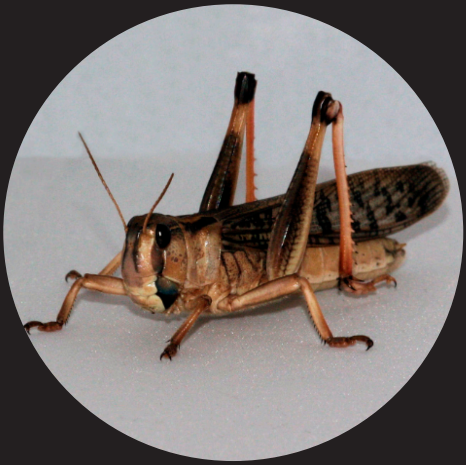
Chapter 1

General introduction



D.G.A.B. Oonincx

Introduction

The Class Insecta comprises most species of all taxa in the Animal Kingdom. The total number of species is estimated to be around 5 million, whereas the total number of described species is about one million (Ødegaard 2000; Finlay, Thomas et al. 2006). From those, at least 1900 insect species eaten by humans have been documented in literature, and the total number can be expected to be well over 2000 (Ramos-Elorduy 1997b; DeFoliart 2005; Jongema 2013). Besides insect species, other arthropods, in particular spiders and scorpions, are also consumed (Menzel and D'Aluisio 1998; Meyer-Rochow 2005). However, these non-insect arthropods are beyond the scope of this dissertation.

The practice of eating insects, also known as entomophagy, is widespread across the globe. In most temperate zones like Europe, North America and the former Soviet Union, the consumption of arthropods is virtually non-existent. However, countries in the temperate zone like Japan and China are notable exceptions (Bodenheimer 1951). In tropical countries, insects are often considered a relevant food source (Bodenheimer 1951). Their importance for human nutrition differs greatly between geographic areas; in some cases insects are merely a delicacy, eaten by a selected few at certain times, whereas in other cases insects provide the bulk of animal protein in the diet (Kitsa 1989). Edible insect species are predominantly collected from nature. Therefore, their availability is in many cases seasonal (Gomez, Halut et al. 1961; Ramos-Elorduy 1990; Agea, Biryomumaisho et al. 2008; Nonaka 2009). For example, the Mopane caterpillar (*Imbrasia belina* (Westwood); Lepidoptera: Saturniidae) is available twice a year (Illgner and Nel 2000).

In addition to insects used as food items themselves, several insect-derived products are used by humans. The best known, and best accepted product on all continents, is bee honey, with or without the larval brood (Finke 2005). The domestication of bees started at least 7000 years ago (Bloch, Francoy et al. 2010). Other insect products that are eaten are mushrooms (*Termitomyces* spp.) emerging from the fungi garden of termites (van Huis 1996). Also, the soil of termite mounds is eaten, which is termed geophagy (Luoba, Geissler et al. 2004). A positive correlation between geophagy and low iron status has been found. This might indicate that the consumption of termite soil is a way to provide dietary iron (Dominy, Davoust et al. 2004). However, it has also been suggested that geophagy might actually impair iron absorption (Geissler, Shulman et al. 1998; Nchito, Wenzel Geissler et al. 2004).

In most cases insects are eaten intentionally. However, in certain cases, people are unaware of eating insects. An example is the red dye, known in Europe as E120. It is made from cochineal (*Dactylopius coccus* (Costa); Homoptera:

Coccidae), which has been used for centuries in many different products such as cosmetics and food (De Jesús Méndez-Gallegos, Panzavolta et al. 2003). This product was first imported from America in the sixteenth century (Baskes 2005). Furthermore, in some products, insects are regularly present as contamination, such as eggs and larvae of fruit flies in tomato paste and puree (Hayes, Smith et al. 1998).

History

The history of entomophagy by hominids starts before the existence of *Homo sapiens* (Sutton 1990b). A diet with a high energy density, containing large amounts of polyunsaturated fatty acids (PUFAs), was needed to facilitate the expansion of the hominid brain (Tommaseo-Ponzetta 2005). Both the consumption of aquatic organisms, as well as the consumption of invertebrates, have been suggested to have aided this development. While little hard evidence is available to prove the latter hypothesis, there is circumstantial evidence. For instance, bones were made suitable by the early hominoid *Australopithecus robustus t*o dig into termite mounds, and it is known that termite species often contain large amounts of PUFAs (Backwell and d'Errico 2001)**.**

Based on the analysis of coprolites, insects were eaten in North and Middle America as early as 7500 BC and this practice has survived in certain parts up until the present day (Elias 2010). The first recorded evidence for the consumption of insects stems from the Paleolithic area (approximately 6000 BC), when *Homo habilis* evolved to *H. sapiens*. In the Araña Caves in Valencia, Spain, depictions of people collecting honey comb were found. This likely means that not only honey, but also bee larvae and pupae were collected and consumed (Ramos-Elorduy 1996), as is still the case in countries in southeast Asia (Yhoung-Aree and Viwatpanich 2005).

In the great Basin area in North America, the remains of large amounts of Mormon crickets (*Anabrus simplex* (Haldeman); Orthoptera: Tettigoniidae), together with the remains of other ingredients of "Desert fruitcake" were found in a cave dating back to approximately 2200 BC (Madsen and Kirkman 1988). Similarly, remains of chopped or ground locusts were found in a storage cist near the Utah-Colorado border. The latter site was dated between 1000 BC and 1000 AD (Madsen et al. 1988).

In approximately 700 BC, a bas-relief was made in Iraq (formerly Assyria), which depicts servants carrying locusts on barbecue sticks in preparation of a feast for their king (Berenbaum 1995). Roman aristocrats ate their locusts honeyed (Anonymous 2002), while the Greek ate both locusts and cicadas (Bequaert 1921; Berenbaum 1995). One of the earliest written pieces of evidence of consumption of insects comes from a play of Aristophanes (425 BC), who writes that locusts were offered at the market and that locusts are preferred to thrushes (Starkie 1909). Some years later, in his *Historia animalium* (350 BC), Aristotle provides information on when in their lifecycle the taste of cicadas was the sweetest (Thompson 1910).

Furthermore, the consumption of insects is mentioned several times in the Bible. For instance in the Old Testament food laws: "... you may eat any kind of locust, katydid, cricket or grasshopper." (Leviticus 11:20-23), and in the book of Matthew (3:4) where it is stated that John the Baptist lived on locusts and wild honey. Around 23 AD, Strabo describes how African people caught locusts: "The inhabitants catch them by throwing into the ravines materials which cause a great deal of smoke, and light them gently. The locusts, as they fly across the smoke, are blinded and fall down." (Hamilton and Falconer 1892). Similarly, there are accounts by Dioscorides that the Libyans consumed locusts (Osbaldeston and Wood 2000). Pliny the Elder writes in his encyclopedia on natural history (approximately 78 AD) about Ethiopians that eat only locusts, and preserve them by smoking and salting them. Furthermore, he writes that the Epicures considered certain beetle larvae, which lived in oak trees, as delicacies and that the wealthy Parthians ate both crickets and locusts (Bostock and Riley 1855). Some centuries later St. Jerome (347-420 AD) writes about the Turkish paying a good price for "fat white worms", which possibly are the larvae or pupae of *Xylophagus* (Diptera: Xylophagidae) and of people in the East and Libya eating locusts (Schaff and Wace 1890). Interestingly, he also points out the difference in culinary culture: "Compel a Phrygian or a native of Pontus to eat a locust, and he will think it scandalous. Force a Syrian, an African, or Arabian to swallow worms, he will have the same contempt for them". Several caches of stored insects were found in Colorado stemming from 650 AD (Sutton 1995).

According to Bodenheimer (1951), little is written on the practice of entomophagy during the middle ages. An exception is a discussion on the consumption of locusts according to Islamic tradition by the Muslim zoologist ad Damiri, published at the dawn of the 15th century. Around 1600, Leo Africanus confirms the consumption of locusts in Ethiopia, Arabia and northern India (Africanus 1896).

A renewed interest in entomology starts in the late Renaissance (Bodenheimer 1951). The works of Mouffet (1634) and Aldrovandi, Ferroni et al. (1638) are worth mentioning in this context. The latter mentions the consumption of several insect species, such as the consumption of fried silkworms by German soldiers residing in Italy, and the consumption of ants in India (Bodenheimer 1951). In the following centuries, many accounts of entomophagy in non-Western cultures exist which have been mentioned and summarized in various publications (Wallace 1854; Quin 1959; Sturtevant 1978; Sutton 1990a; Sutton 1990b; Berenbaum 1995).

In the 18th century, caterpillars were ravaging the fields in France. Since these were available in large quantities, it was suggested that the peasants could consume these caterpillars. This was seen as similar to the consumption of frogs and oysters, which also had seemed disgusting at first. However, the misconception that this species was poisonous prevented the people from consuming it (de Réaumur 1736).

In different parts of the world such as China, the East- and West Indies and Jamaica, several insect species were seen as luxurious food at that time. Amongst these were the larvae of the dung beetle (*Copris* (*Catharsius*) *molossus* (Fabricius); Coleoptera: Scarabaeidea) and the pupae of Domesticated silkworms (*Bombyx mori* (L.); Lepidoptera: Bombycidae) (Donovan and Westwood 1842). There are several records of Europeans visiting these countries also consuming these insects, for instance Palm weevils in the west Indies were also eaten by the French (Winterbottom 1803, as cited in Cowan 1865).

At the end of the 19th century, Holt (1895) published his book “Why not eat insects”, in which he, although fully aware of the deep-rooted prejudice against entomophagy, advocates eating insects in the Western world. His main arguments are the taste and nutritional quality of insects, and he compares insects as food with other “odd” food types which are considered delicacies, such as eel, sea slugs, and bird’s nest soup. A few years later an American scientist urged his colleagues to experiment with the consumption of locusts and beetle larvae (Howard 1915; Howard 1916). He felt certain that they would prove nutritious and that the prejudice against insects as food was “perfectly unreasonable”. Both Holt and Howard refer to the consumption of insect species in various non-Western countries as an argument for entomophagy in the Western world (Howard 1916; Holt 1995). The last centuries there seems to be a general aversion against the consumption of insects in the Western world. A noticeable exception is the consumption of the larvae of the cheese fly (*Piophila casei* (L.); Diptera: Piophilidae), which are consumed as part of a cheese in Croatia and Italy (Russo, Cocuzza et al. 2006). While the production of this cheese is forbidden in Italy, a black market has evolved in Sardinia (Trofimov 2000).

Entomophagy in non-Western cultures

It is beyond the scope of this dissertation to go into detail regarding entomophagy in non-Western cultures. Several reviews have been published on entomophagy around the world (Bodenheimer 1951; Quin 1959; DeFoliart 2002). Different regions of the world are covered in numerous other publications, for instance Asia (Pemberton 1994; Pemberton 1995; Hill 1997; Yhoung-Aree, Puwastien et al.

1997; Zhi-Yi 1997), Africa (Oladipo 1996; Allotey and Mpuchane 2003; Huis 2003; Kruse and Kwon 2004; van Huis 2005), and South and Central America (Ruddle 1973; Ramos-Elorduy de Conconi and M. 1979; Dufour 1987; Ramos-Elorduy 1989; Paoletti and Dufour 2005) . Entomophagy was and is common in many countries around the world. The Western-world view on entomophagy, however, has had a negative influence on this practice in non-Western cultures (DeFoliart 1990; Ramos-Elorduy 1990; Paoletti et al. 2005).

Some 500 years ago, the Europeans came into contact with the indigenous people of Mexico. This impacted the diet of these indigenous people, which shifted away from the consumption of insects. While upon arrival the inhabitants had a diet both quantitatively and qualitatively sufficient, nowadays a large part of the country is poorly nourished and nutritional deficiencies have arisen. These dietary and nutritional problems can partially be explained by the shift in dietary choices first imposed by the European conquerors (Ramos-Elorduy 2006). In Mexico this resulted in lower energy intakes, decreased food diversity and a decrease in quality of the diet as a whole (Ramos-Elorduy 1990). Ironically, in rural areas where the harvesting of insects did continue, this harvesting led to a decrease in natural populations of certain edible insect species. An estimated 40 species are now considered threatened because of overexploitation (Ramos-Elorduy 2006). Similar accounts of the negative influence of the Western view on entomophagy, and consequently on nutrient provision have been described for several parts of the tropical world (Quin 1959; Meyer-Rochow 1973; Looy, Dunkel et al. 2013).

The insect production sector

In the 1980s, a company harvesting larvae of *Oryctes* spp. (Coleoptera: Scarabaeidae) was founded in Nigeria. This lasted until 1997 when insufficient larvae were available for harvesting, illustrating an interesting market for insects as a food source (Tchibozo, van Huis et al. 2005). In West Kasai, in the Democratic Republic of Congo, a survey indicated that during the main season 2.36 kg of insects was consumed per capita, per month. This amounts to 19.6 grams of protein per day, which is half of the total protein consumption (Kitsa 1989). Two surveys conducted at Amazonian tribes reported that 2.6% and ~6% of the protein consumed consisted of insects (Lizot 1977; Dufour 1987). However, reliable quantitative data for entomophagy are difficult to obtain, because interviewees are reluctant to provide information as disproval of the practice by Western investigators is assumed (Ruddle 1973). Furthermore, seasonality can strongly influence results (Dufour 1987).

The Mopane caterpillar, one of the most commonly consumed insects in southern Africa, has an estimated turnover of 1600 tons of dried product per year (Dreyer and Wehmeyer 1982). This species is sold by street vendors in Botswana as processed food and in restaurants (Allotey et al. 2003), but it is also exported to the USA and Korea (Mpuchane, Gashe et al. 2000). A similar example of insects being exported as food to a Western country are bee pupae from Japan sent to Canada, which have an estimated 100 kg annual turnover (Hocking and Matsumura 1960). Also, the Thai Giant waterbug (*Lethocerus indicus* (Lepeletier & Serville); Hemiptera: Belostomatidae) is exported to the USA as part of a paste and as an alcohol extract. The fact that it is not mentioned on the English ingredient label could be seen as an indication of the expected cultural aversion against insects as part of a food product (Pemberton 1988).

A common Western misconception is that insects are consumed in tropical countries because no alternatives are available. This, however, does not seem to be the case. Certain traditional insect foods are preferred to fresh meat by the Yukpa in South-America and the Pedi in South Africa (Quin 1959; Ruddle 1973). Van Huis (2003) reports that his African interviewees eat insects, particularly termites, simply because they are delicious. Prices for caterpillars in the Democratic republic of Congo are four times as high as those for beef (Huis 2003). In Mexico, currently high prices are asked for ant larvae and pupae, (*Liometopum apiculatum* (Wheeler); Hymenoptera: Formicidae) (200 $/kg), white agave worms (*Aegiale hesperiaris* (Walker); Lepidoptera: Hesperiidae*)* (250 $/kg), and larvae of the red agave worm (*Xyleutes redtembacheri* (Hamm); Lepidoptera: Cossidae) (200 $/kg) used in the traditional “Mezcal” (Ramos-Elorduy 2006). Comparing these prices with the price of beef (14 $/kg) illustrates the preference for insects (Ramos-Elorduy 1990; Ramos-Elorduy 1997a). The price for these collected insects has increased greatly in the last decades; supposedly the price for “escamoles” was 35 $/kg and the price for red agave worms 22 $/kg in 1988 (Ramos-Elorduy 1990). The reason for this price increase is suggested to be the increased wealth of Mexicans that have moved from rural areas to the USA, and who wish to retain their culinary habits.

Production of insects

Certain species of insects are seen as acceptable food; commodity goods. For the production of these and other non-conventional production animals, the term mini-livestock was introduced in the 1990s (DeFoliart 1995; Hardouin 1995). Two main forms of insect production systems can be distinguished; extensive and intensive. Extensive systems are mostly used in tropical settings where most of the requirements of the insect regarding temperature, humidity and feed are already met. The yield is then optimized by managerial adaptations such as protection from predators and enhancement of the insect's food source (Van Itterbeeck and van Huis 2012). One of the first steps from gathering to producing is the protection of the insect in natural situations. An example is the practice of early burning of forested areas in Zambia. This enables the edible caterpillars, locally called 'mumpa' (*Gonimbrasia zambesina*; Lepidoptera: Saturniidae), to feed on the regenerated woodlands when they are in season, instead of being wasted by fires (DeFoliart 2005).

The rearing of insects for human consumption is not new. Insects, for instance "Axayacatl" (Hemiptera: Corixidae), have been cultured in Mexico before the Hispanic period (1502 AD) (Ramos-Elorduy 2006). The eggs of these aquatic bugs were cultured by means of placing grass bundles on the bottom of lakes and ponds. After the bugs have deposited their eggs, these are harvested (Ramos-Elorduy 1997a). This practice has become threatened by drying up of water bodies and by pollution (Ramos-Elorduy 2006).

In the last decades, several studies have been conducted to estimate the productivity and feasibility of commercial insect rearing. A study on *Anaphe infracta* Walsingham (Lepidoptera: Notodontidae), reared for human consumption, quantified a production of 9.32 kg of caterpillars in a period of 56 days using a 50x50x50 cm cage. The authors concluded that the rearing of this species would be commercially interesting (Munyuli Bin Mushambanyi 2000). In eastern Africa, semi-captive rearing by means of net sleeves placed on tree branches to protect the larvae of *Gonometa postica* Walker (Lepidoptera: Lasiocampidae) was investigated. This method of semi-rearing reduced losses due to parasitism and predation by 35 to 55%, resulting in an effective farming technique (Ngoka, Kioko et al. 2008).

In western Africa, larvae of the palm weevil (*Rhychophorous* spp.; Coleoptera: Curculionidae) are accepted as food by many of the different cultures present there. The beetles oviposit in decaying palm trees from which the larvae are taken, and subsequently raised by gatherers until they reach their full size. Until recently little was known regarding the rearing of this species (Oladipo 1996; Cerda, Martinez et al. 2001). However, captive commercial rearing in Thailand of *Rhychophorous ferrugineus* has recently been described (Hanboonsong, Jamjanya et al. 2013).

If sustainable insect rearing systems are established, these aid to the provision of important nutrients (mainly proteins and fat) and at the same time aid in the sustainable economic development of a region (Illgner et al. 2000). Another advantage of shifting from gathering to production of insects as a food source is a relief of pressure on the wild population (Hardouin, Thys et al. 2003). As mentioned before, cochineal is used as a red dye. It is cultured in several countries, amongst others Peru, Argentina, Mexico, South-Africa and the Canary islands (De Jesús

Méndez-Gallegos et al. 2003). Research efforts are made to improve production through protection of the host plant (*Opuntia* spp.; Cactaceae: Caryophyllales) (Aldama-Aguilera, Lianderal-Cazares et al. 2005).

Besides producing insects for food, they can also be used as a feed source for livestock, for instance poultry (Munyuli Bin Mushambanyi and Balezi 2002). An extensive way of producing termites for feed is known from Burkina Faso; calabashes with organic matter are buried into the soil and the termites are harvested a few days later (Farina, Demey et al. 1991). One important benefit of certain insect cultures is the ability to convert biomaterials that would otherwise not be used. A termite culture, for instance, can convert leftovers of different husks and straws and thereby enhance the nutritional value of these plant residues. This mix of converted roughage and termites can then be used as a nutrient-rich feed for cattle (Abasiekong 1997), or poultry (Munyuli Bin Mushambanyi et al. 2002).

In the Western world, mostly intensive production systems are used, in which the requirements of the insect are met in a closed system. The advantage of such a system is that it allows for a more controlled product regarding both quantity and quality. These systems currently focus on the production of feed for birds, lizards or amphibians, which is sold through the pet trade. This type of business has been around for a few decades and it seems likely that further process innovations will be implemented in the coming decades. Sharing or publishing information regarding production methods is uncommon, because these cannot be patented. This makes it difficult to compare insect production systems with conventional animal production systems.

Although detailed information is lacking, the basic production steps are known: 1) The setting up of cages with adults 2) Extraction of the eggs or young, which are then placed in a separate cage according to age or stage of development 3) Extraction of the product (reared insects) from the cage. These are then prepared for sale or used for the production of the next generation.

Insect rearing, when compared to insect gathering, has the disadvantage that all requirements of the insect species need to be met in an artificial setting. This includes the provision of a suitable diet and the creation of a suitable environment. Because insects depend on environmental temperature for growth and developmental, and because most species reared in captivity prefer tropical temperatures, in temperate zones heat often needs to be provided. It seems likely that this, together with costs for feed and labour, contributes most to production costs. However, such a controlled system of production does allow for a uniform product, which can prove useful when dealing with quality, and food safety issues.

Food safety

Generally speaking, three risk areas can be distinguished regarding food safety: chemical, physical and microbiological contaminations (Mollenhorst, Berentsen et al. 2006).

Chemical contamination in insects can be caused by the use of pesticides when insects are collected from nature, for instance with the collection of locusts (Looy et al. 2013). Furthermore, certain species of insects naturally contain toxins, used as a defence against predators. This, however, need not be a reason to discard them as food. For instance, the larvae of *Cirina forda* Westwood (Lepidoptera: Saturniidae) are known to be toxic but can be detoxified by processing (Akinnawo, Abatan et al. 2002). In the Western world, where insects are normally reared rather than collected, the primary sources for chemical contamination are medication, the feed, and possibly cleaning agents used in the production process. These risks are similar to those found in conventional animal production systems (Mollenhorst and de Boer 2004). Therefore, feed should be certified, i.e. guaranteed free from contamination. Chemicals impairing food safety should be identified and specific tests are required to quantify the levels of these chemicals in the final product.

Physical contamination can come in many different forms and often originates from processing steps, e.g. contamination with pieces of metal in a hammer mill. In a controlled environment, procedures can be developed to safeguard against physical contamination. Special attention should be paid to physical contamination in the feed, especially when the insect is consumed as a whole, and could therefore contain the contamination in the gastrointestinal tract.

Microbiological contamination in animal products can come in the form of zoonosis, i.e. a disease that can be passed from animals to humans. Foodstuffs of animal origin are the most important source of zoonosis, and *Salmonella* spp. and *Campylobacter* spp. are the primary bacterial agents of food-borne zoonosis (Valkenburgh, R. van Oosterom et al. 2007). Since insects are taxonomically and biologically distinct from mammals, they might not be suitable hosts for many human pathogens. However, certain pathogenic bacteria are potential threats. For instance Enterobacteriaceae (which include *Escherichia coli* and *Salmonella* ssp.), and spore-forming bacteria can be present in insects and insect-derived products in similar levels as found in unprocessed plant based food stemming from agricultural fields. Enterobacteriaceae can be eliminated by a short heating step like blanching, or by acidification. However, spore forming bacteria are decreased , but not fully eliminated by these processing methods (Klunder, Wolkers-Rooijackers et al. 2012). Research performed on the lesser mealworm (*Alphitobius diaperinus* (Panzer); Coleoptera: Tenebrionidae), a pest species in broiler chicken production, indicated that both larvae and adults are capable of harbouring *Escherichia coli* for at least

10 days (McAllister, Steelman et al. 1996). An earlier study reported the ability of *A. diaperinus* to harbour *Salmonella enteriditis* for at least 28 days (Davies and Wray 1995). In both these studies the animals were inoculated with the pathogens, and the pathogen count decreased in time, suggesting that they might not be an apt host for the pathogen. A similar study on turkey broilers also indicated the possibility of *Salmonella* and *Escherichia* transmission by *A. diaperinus* (Harein 1972).

Although cockroaches are generally seen as vectors of disease, it has been suggested that disease transmission mainly occurs through mechanical distribution (Bennett, Selby et al. 1993). A study on bacterial presence on American and German cockroaches (*Periplaneta americana* (L.), and *Blatta germanica* (L.); Dictyoptera; Blattidae) in Iran indicated that most bacteria were found on external parts, however, there were also bacteria present in the alimentary canal (Vahabi, Rafinejad et al. 2007). In a study on *P. americana* in Morocco, eleven species of pathogenic bacteria were identified (Lamiaa, Mariam et al. 2007), which concurs with earlier studies in France (Rivault, Cloarec et al. 1993). For *P. americana*, it was shown that lectins play a role in the recognition of bacteria by the immune system, which could inhibit biological transmission of pathogens (Basseri, Emmami et al. 2008). A study on the presence of protozoa and helminths in cockroaches indicates that cockroaches can play a role in the distribution of human intestinal parasites (Kinfu and Erko 2008).

Insect rearing facilities, producing for the human food market, should safeguard the health of the consumer by means of control measures. Validation could be based on guidelines for the validation of food safety control measures as suggested by the Codex Alimentarius Commission (CodexAlimentarius 2008). In summary, it can be assumed that certain pathogens can be distributed by certain insect species. Further studies are required to determine which pathogenic bacteria are present in edible insects, in order to develop hygienic rearing protocols for the prevention of bacterial contamination, and to determine optimal processing methods in order to guarantee food safety.

Reasons for entomophagy

In 2001, Cunningham posed the question why people eat insects (Cunningham 2001). However, from a global perspective, the question why the Western world has *not* generally accepted the consumption of insects is more logical. Especially since other exotic culinary food items, such as snails, lobsters, oysters, sushi and frog legs have been accepted (Kiuchi and Tamaki 1990; Illgner et al. 2000; Gullan and Cranston 2005). Food choice is determined by cultural, psychological, and lifestyle factors as well as food trends (Asp 1999). Food attitude is formed in early childhood and is difficult to change in later life (Cervellon and Dubé 2005). Fear of new food items is greater for products of animal origin than for other food sources (Pliner and Pelchat 1991). The association of entomophagy with having a low social status might be an important restraint for Westerners (Nielsen 2000). It has been suggested that in early life, children have not yet acquired a cultural bias against eating insects. If Westerners are informed about the positive aspects of entomophagy, they are more inclined to surmount their aversion (Mignon 2002; Verbeke 2015). What are those positive aspects?

Nutritional quality

Some decades ago it was suggested that "in-depth studies are needed on the composition and dietary quality of… selected insects" (DeFoliart 1989). Since then, a large amount of data on the chemical composition of insects has been gathered. Several feeding trials in which insects were offered to animals have been conducted. Most studies report similar or increased production when insect protein is used to replace other protein sources (such as fishmeal or soymeal), indicating a good nutritional value (Farina et al. 1991; Ekoue and Hadzi 2000; Munyuli Bin Mushambanyi et al. 2002).

Currently, little is known on the digestibility, and thereby nutrient availability of insects as food for humans. However, insects are suggested to be good food; nutritionally superior to vegetables, and similar to meat (Berenbaum 1995; Looy et al. 2013). It is suggested that insects can contribute to healthy ageing and therefore could become a normal part of the diet for Westerners by the end of this century (Heinrich and Prieto 2008). Possibilities for the optimization of the nutritional composition of insects, especially regarding nutrients with an added health value in the Western world, should be explored.

Reproductive capacity

Insects in general are both prolific breeders and have short production cycles. This high reproductive capacity (DeFoliart 1999; Collavo, Glew et al. 2005) can be seen as an advantage in comparison with the conventional production animals (Premalatha, Abbasi et al. 2011). A female House cricket (*Acheta domesticus* (Fabricius); Orthoptera: Grillidae), can produce 200-300 offspring in her lifetime of 12 weeks, a Black soldier fly (*Hermetia illucens* (L.); Diptera: Stratiomyidae) female can lay a clutch of more than 1000 eggs within one week of eclosion, and a female of the super worm (*Zophobas morio* (Fabricius) Coleoptera; Tenebrionidae) can lay 1500 eggs in her one year lifetime (May 1961; Friederich and Volland 2004). These numbers are much larger than reproductive numbers for cows, pigs, or even chickens.

Feed conversion efficiency

Animal protein production through insects has been reported to be more efficient than through conventional production animals (Nakagaki and deFoliart 1991; Berenbaum 1995; Gullan et al. 2005; Ramos-Elorduy 2008; Premalatha et al. 2011; Looy et al. 2013). This seems plausible because insects, unlike endotherms, do not use energy obtained from feed to maintain a constant body temperature. Therefore, a greater proportion of the ingested feed can be allocated to growth.

Several studies have calculated conversion efficiencies in different insect species. In entomological studies this efficiency is normally expressed as the ECI (the efficiency of conversion of ingested feed to body substance (Waldbauer 1968)). ECI's from 8 to 87% have been reported for a range of insect species, which illustrates the large differences between species (Slansky and Scriber 1985).

House crickets have been reported to have a fresh weight ECI of 95% on a chicken diet, which is practically double that of broilers (Nakagaki et al. 1991). This means that for each kg of feed consumed a little under one kg of live crickets is produced. Another study on House crickets reports an ECI of 59%, with an optimum of 64-65% at an age of eight weeks (Collavo et al. 2005).

These values are on a fresh weight basis, in which the moisture content of chicken feed is likely to be much lower than for the cricket (≈70%). For last instar House crickets a dry weight ECI of 27% was reported (Woodring, Roe et al. 1977; Woodring, Clifford et al. 1979), which approximates the ECI from the study of Collavo et al. (2005) when assuming a moisture content of the feed after grinding of 10%. Ramos- Elorduy et al. (2008) report dry weight ECIs for House crickets ranging from 35 to 44% and between 53 and 73% for mealworms (*Tenebrio molitor* (L.); Coleoptera: Tenebrionidae) when these species are reared on organic wastes of vegetable origin.

While ECI is a relevant indicator of the feed conversion efficiency of a certain species, other parameters, such as the chemical composition, and the form in which a diet is offered have a large effect. High feed conversion efficiencies are expected if diet composition is optimal, whereas low ECIs are expected on less suitable diets. ECI, however, is a crude measure that does not discriminate between the different nutrients. Some nutrients are well assimilated by insects, when provided in appropriate concentrations and form. For instance, a nutrient ECI above 90% has been described for copper and zinc in the bruchid beetle (*Bruchidius sahlbergi* (Schilsky); Coleoptera: Chrysomelidae) (Ernst 1992). However, in the same study the nutrient ECI for calcium and magnesium was below 20%. These differences could be due to the availability, or concentration of these minerals, but also due to the requirements of the beetle.

Insects have three ways of optimizing their nutrient intake: by regulation of total food intake, by selective absorption of nutrients and by selection of different diet constituents (Simpson and Abisgold 1985; Simpson, Raubenheimer et al. 2002; Raubenheimer and Simpson 2003; Fielding and Defoliart 2008). Self-selection behaviour is a strategy insects employ to optimize their nutrient intake. Studies have shown similar diets being chosen when comparing optimal diets and self-selected diets (Nation 2008). These optimal diets can be artificial diets, or composed of natural ingredients. If the digestibility of nutrients and the requirements of a certain species are known, this will allow the production of this species with a minimal use of resources.

Sustainability

The production of animal protein is associated with several environmental problems (Steinfeld, Gerber et al. 2006; Pelletier and Tyedmers 2010; Steinfeld 2012). Environmental pollution can be divided into different aspects such as acidification, eutrophication, global warming, aquatic and terrestrial ecotoxicity, and land use (Mollenhorst et al. 2004). The livestock sector is an important source of environmental pollution; 15% of the global anthropogenic production of greenhouse gases, and the use of 70% of all agricultural land is attributed to this sector (Steinfeld et al. 2006; Foley, Ramankutty et al. 2011; Steinfeld 2012). An absolute increase in population size, accompanied by a relative increase in demand per individual in developing countries, is estimated to result in an increased animal protein demand of 70-80% between 2012 and 2050 (Steinfeld et al. 2006; Pelletier et al. 2010; Steinfeld 2012). This increase in demand goes hand in hand with an increase in carbon emissions and as such with environmental problems on a global scale (Sebek, Kuikman et al. 2008). Furthermore, the growth in human population size results in an increased demand for fuel. Alternative fuels like so-called bio-fuels compete for agricultural land with food production (Lewandowski and Faaij 2006; Martines-Filho, Burnquist et al. 2006). More sustainable animal production systems are therefore required. Several authors have suggested insects to be a sustainable food source (Hardouin et al. 2003; Barwa ; Das, Ganguly et al. 2009; Premalatha et al. 2011; Looy et al. 2013).

Limited reliable data on the sustainability of insect production are available. While for instance the direct use of land for insect production can be limited, and it has been suggested that the production system as a whole requires less land, quantitative data is limited or absent (Das et al. 2009; Premalatha et al. 2011). Regarding greenhouse gas production it is known that, similar to ruminants, certain insect species (within the Cetonidae and Blattidae families) produce methane originating from gut-associated methanogenic bacteria (Hackstein and Stumm 1994; Cazemier, Hackstein et al. 1997; Kammann, Hepp et al. 2009). The quantitative relevance of this enteric methane production in comparison with conventional production animals deserves further attention. Moreover, a method to evaluate the environmental impact of entire insect production systems, for instance by input–output accounting, ecological footprint analysis, or life cycle assessment (LCA), should be implemented.

"Waste" as a substrate

According to Kollikkathara et al. (2009) "Waste is simply resources out of place". In small, closed systems, where resources are limited, the urgency to avoid waste is most apparent. Several authors have suggested to use insects to convert waste to food in order to facilitate space travel or extra-terrestrial settlements (Kok 1983; Katayama, Yamashita et al. 2005; Katayama, Ishikawa et al. 2008; Katayama, Yamashita et al. 2008; Yu, Liu et al. 2008). However, more down-to- earth applications to use waste as feed for insects have also been described. For instance, mealworm larvae can be reared on a diet consisting of organic waste, supplemented with yeast (Ramos-Elorduy, Gonzalez et al. 2002). Another example is the Black soldier fly, which can convert several organic waste types, for instance compost, poultry manure, or coffee pulp (May 1961; Larde 1990; Sheppard, Larry Newton et al. 1994). Similarly, poultry manure can be used as feed for the house fly (*Musca domestica* (L.); Diptera: Muscidae) (El Boushy 1991; El Boushy and van der Poel 2000).

Silk production entails the production of Domesticated silk worm pupae, which are used both as food for people and as feed for poultry (Pereira, Ferrarese Filho et al. 2003). This species can be produced on mulberry leaves and the frass produced by the silk worms can subsequently be used as a fertilizer for the mulberry trees (DeFoliart 1989). The examples above indicate that insects can be used in closing loops in production systems in which waste is used to create an ecologically, and economically, sound product.

Outline of this thesis

The current thesis examines the arguments for entomophagy identified above, and, for a variety of insect species, adds experimental data regarding their chemical composition, their production of greenhouse gases, the environmental impact associated with their production, and the efficiency with which they convert their feed to body mass, in order to determine the validity of these arguments. Chapter 2 reviews the literature on the chemical composition of insects, gives an indication of the nutritional quality of insects, and details ways in which their composition can be altered.

In Chapter 3, the greenhouse gas production of a selection of insect species (*T. molitor*, *A. domesticus*, *Locusta migratoria* L. (Orthoptera: Acrididae), *Pachnoda marginata* Drury (Coleoptera; Scarabaeidae), and *Blaptica dubia* Serville (Dictyoptera: Blaberidae)), is quantified. This direct production of greenhouse gases quantifies the direct contribution of the animals themselves and their substrate to an insect production system. These values are subsequently compared to values published on conventional production animals and provide the first indications of the global warming potential associated with the production of insects.

In Chapter 4, the environmental impact of a production system of two species of mealworms (*T. molitor* and *Z. morio*) is examined. This is done by means of a Life Cycle Assessment, in which three indicators are quantified; global warming potential, depletion of fossil energy, and land use. These indicators include all flows entering the production system and are calculated up till the end product (live mealworms ready for further processing). Presented data are expressed as kg of edible protein, and a comparison is made with conventional production animals.

In Chapter 5, a selection of side streams from the Dutch food industry were combined to formulate four diets, varying in protein and fat content. These diets are tested on four insect species (*T. molitor*, *A. domesticus*, *H. illucens* and *B. dubia*), and the resulting chemical composition of these species in terms of protein and fat is determined. Production parameters, such as development time and mortality are reported. The goal of this experiment was to determine the suitability of these substrates as feed for insects and to obtain an idea on the effect of diet on the chemical composition of these insect species. In Chapter 6, manure of chicken, pigs and cows is used as a substrate to rear larvae of *H. illucens*. Manure is a true waste product, which has a negative economic value. The environmental impact of manure used as feed, when seen from the perspective of an attributional Life Cycle Assessment using economic allocation, is considered zero. This experiment had three goals: (1) to determine the suitability of these types of manure as a substrate for *H. illucens* larvae, (2) to determine their chemical composition, which is an indication of their suitability as animal feed; (3) to determine the effects of this processing method on the N- and P- content of the manure.

Finally, in Chapter 7, the findings of this thesis are discussed, and put in a wider perspective. Here the focus is on the arguments put forward in favour of entomophagy. Furthermore, a selection of insect species is compared to conventional production animals regarding their suitability as production animals. Lastly, I discuss future perspectives on the use of insects as a source of food and feed.

Chapter 2

Insects as food for insectivores

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Abstract

A variety of insects are commonly fed to captive insectivores. We review the nutrient content of a variety of commercially raised insects and compare those values to the data available for wild insects. These data are discussed in light of the nutrient requirements for domestic animals to identify nutrients of concern for captive insectivores. Additionally, various environmental and dietary factors which can significantly affect insect nutrient composition are reviewed. We then evaluate the various techniques that are currently used to enhance the nutrient content of commercially bred insects including gut loading and dusting. Lastly possible negative considerations, including pathogens, pathogenic microorganisms, toxins, and anti-nutritional factors, that might be important factors when feeding captive insectivores, are discussed.

Keywords: Insects, Nutrients, Minerals, Vitamins, Amino Acids, Fatty Acids, Gut Loading, Dusting, Insectivores

Introduction

Insects are generally considered a good source of most nutrients (DeFoliart 1992) and many insect species have been analysed for their nutrient composition. These analyses include insects taken from the field (Bukkens 1997; Punzo 2003; Gullan et al. 2005) and those commercially reared (Simpson and Raubenheimer 2001; Finke 2002; Ramos-Elorduy et al. 2002; Dierenfeld and King 2008; Oonincx, Stevens et al. 2010a; Oonincx and Dierenfeld 2012b; Finke 2013). In nature, most insectivores will feed on a variety of arthropod prey species. As a general rule, it is better for insectivores to be offered a varied diet rather than a single insect species (Bernard and Allen 1997). When the insectivore is able to select between different insects offered, this allows self-selection of nutrients (Oonincx et al. 2010a; Senar, Møller et al. 2010).

A number of variables can influence the chemical composition of insects, such as gender (Hoffmann 1973; Ali and Ewiess 1977; Sönmez and Gülel 2008), stage of development (McClements, Lintzenich et al. 2003), diet (Calvez 1975; Simpson et al. 2001; Ramos-Elorduy et al. 2002; Oonincx and van der Poel 2011) and environmental factors such as temperature (Hoffmann 1973; Sönmez et al. 2008), day length (Ali et al. 1977; Shearer and Jones 1996; Koc and Gulel 2008) and humidity (Han, Parajulee et al. 2008; Ali, Mashaly et al. 2011; Nedvěd and Kalushkov 2012). Additionally, other factors such as light intensity and spectral composition might also affect the chemical composition of insects.

Nutrient densities can be expressed on a fresh matter basis. However, although insectivores consume live prey, in most publications data are expressed on a dry matter basis. Variation in moisture content can strongly influence the reported amount of nutrients when these are expressed as fresh material. While water is a critical nutrient, in most cases providing it is not the primary function of offering insects as food. Therefore, in this chapter nutrient densities are expressed on a dry matter basis to be consistent with the existing literature. Large variations in dry matter content exist between species and developmental stages. As a rule of thumb, the dry matter content typically ranges from 15 to 40% of the fresh weight (Bernard et al. 1997; Barker, Fitzpatrick et al. 1998; Finke 2002; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013).

Nutrient content of insects

Protein and amino acids

The protein content of insects is highly variable and ranges between 7.5% and 91% with many species containing around 60% protein on a dry matter basis (Bernard et al. 1997; Bukkens 1997; Barker et al. 1998; Finke 2002; Ramos-Elorduy et al. 2002; Punzo 2003; Oonincx et al. 2010a; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013). Protein content is commonly determined by multiplying the amount of nitrogen times 6.25, known as the crude protein content. While convenient, this method assumes an even distribution of amino acids and may lead to a slight overestimation of true protein content due to the presence of other nitrogen containing compounds (such as chitin). In most papers where all amino acids are properly quantified, protein recovery (sum of the amino acids divided by crude protein content) is generally quite good, suggesting crude protein is a reasonable estimate of true protein in most species of insects (Finke 2007; Finke 2013).

Amino acids are the building blocks of proteins. Certain amino acids are known as essential amino acids, since they cannot be synthesized from simpler molecules by most animal species (Bender 2002). It seems plausible that amino acid composition is fairly constant in a given species, in a given developmental stage relative to the total amount of protein (Sealey, Gaylord et al. 2011), although plasticity has been suggested (Ramos-Elorduy et al. 2002).

Prediction of an insect's amino acid profile based on species or diet provided is difficult at best. In addition to these factors various analytical techniques are used, which increase variation (Bukkens 1997; Finke 2002; Finke 2013). However, certain trends regarding protein composition can be distinguished. Table 1 (Finke 2002; Finke 2007; Finke 2013) provides expected ranges for specific amino acids. The amino acids in table 1 together typically account for over 90% of the crude protein in most insect species. The small amount of nitrogen that is not accounted for by these amino acids is likely due to other nitrogen containing compounds such as other amino acids, chitin and uric acid plus the nitrogen that is lost in the conversion of glutamine to glutamic acid or asparagine to aspartic acid, during protein hydrolysis.

The nutritional quality of insect protein has generally been described as good, but the quality depends on the digestibility of the amino acids and the match of the amino acid profile to the requirements of the insectivore in question (Finke, DeFoliart et al. 1987; Finke, DeFoliart et al. 1989; Ramos-Elorduy 1997b). Unfortunately the amino acid requirements of most insectivores are unknown so most comparisons have been made using more common laboratory animals, such

as rats or chickens (Finke 2002; Finke 2013).
The crude protein digestibility of Housefly (*Musca domestica* L.) larvae and pupae is between 70 and 80% in chickens, and the nine amino acids tested had a digestibility between 86 and 100% (Pretorius 2011). Crude protein digestibility of the larvae of the Black soldier fly (*Hermetia illucens* L.) fed to mountain chicken frogs (*Leptodactylus fallax* (Müller)) was quite low (44%) when the larvae were offered alive, but when the larvae were mashed, the digestibility increased to 77% (Dierenfeld et al. 2008). The crude protein digestibility of intact House crickets (*Acheta domesticus* L.) in these frogs was 95% (Dierenfeld et al. 2008). The lower protein digestibility of intact larvae versus mashed larvae could be due to the protein in the exoskeleton, which might need to be mechanically or enzymatically treated before all of the digestible nutrients can be accessed. The apparent protein digestibility of dried Yellow mealworm larvae (*Tenebrio molitor* L.) was 75% when fed to rats which was slightly lower than that for both soy protein concentrate (84%) and casein (88%) (Goulet, Mullier et al. 1978). It is likely that the digestibility of protein from insects is highly variable. Insects that have a larger proportion of their amino acids in cuticular protein complexed with chitin might be expected to have a lower protein digestibility than those that do not (Finke 2007).

The first limiting amino acid depends on both the insect species being consumed and the species consuming the prey. Since different insectivore species might require different proportions of amino acids, the first limiting amino acids could differ between them. In chickens fed purified diets where Mormon crickets (*Anabrus simplex* Haldeman) or House crickets were the sole source of protein, the first limiting amino acids were methionine and arginine (Finke, Sunde et al. 1985; Nakagaki, Sunde et al. 1987). When fed to growing rats, the first limiting amino acids in insect protein from Yellow mealworm larvae, Housefly larvae and adult Mormon crickets was methionine (Goulet et al. 1978; Finke et al. 1987; Finke et al. 1989; Onifade, Oduguwa et al. 2001). Similarly, methionine and cysteine were calculated to be the first limiting amino acids for rats in Super worm larvae (*Zophobas morio* F.), larvae of the Greater wax moth (*Galleria mellonella* L.), Domesticated silkworm larvae (*Bombyx mori* L.), honey bee larvae and pupae (*Apis mellifera* L.), adult Houseflies, Black soldier fly larvae, Rusty red roaches (*Blatta lateralis* Walker) and Butter worm larvae (*Chilecomadia moorei* Silva) (Finke 2002; Finke 2013). As such it seems likely that for most mammalian insectivores the sulphur amino acids are likely to be first-limiting. For fish, birds, and other species, especially those without a functioning urea cycle, other amino acids such as arginine may be important. It has been proposed that adult passerines have a preference for spiders, driven by a need for enhanced levels of methionine, cysteine or perhaps taurine by their growing chicks (Ramsay and Houston 2003).

Fats and fatty acids

The most common way to estimate insect fat content is by determining the total weight of all fat-soluble molecules (mostly lipids but also waxes and some other compounds). Fat tissue is used for energy storage in the body. It is either obtained and stored directly from a dietary source or synthesized from carbohydrates (Fast 1970; Hanson, Cummins et al. 1983; Bender 2002). The main storage site for insect lipids is the fat body (Beenakkers, Van der Horst et al. 1985). As a dietary component, fat is not only an important energy source, but may also play a role in the palatability of the insect when fed to insectivores. Large variations in the lipid content (4.6-64% dry matter) of insects have been reported (Bukkens 1997; Yang, Siriamornpun et al. 2006; Finke 2013). As a rule of thumb, most adult, wild insects contain less than 10% fat on a fresh weight basis (Fast 1970; Lease and Wolf 2011).

There are indications that a certain amount of protein is needed to reach adulthood in migratory locusts (*Locusta migratoria* L.). The adult weight in this species varies, but this is mainly due to differences in fat content (Simpson et al. 2001; Oonincx et al. 2011). This could indicate that differences in weight upon reaching adulthood are a crude indication of differences in fat content.

The behavioural ecology of a species likely influences the amount of fat stored (Thompson 1973). In certain species, the males have a higher fat content than the females (Nakasone and Ito 1967; Fast 1970). For species where male combat is customary (for instance Odonata), this can be explained by a need for greater energy reserves. In several families within the order Lepidoptera, males contain greater fat reserves than females (Beenakkers et al. 1985). In most other species, females have greater fat reserves than males (Zhou, Honek et al. 1995; Nestel, Papadopoulos et al. 2005; Lease et al. 2011). In species that use accumulated energy reserves obtained during their larval stages for reproduction, greater reserves would have a positive effect on fecundity as eggs have a high lipid content and therefore provide fitness advantages (Downer and Matthews 1976; Beenakkers et al. 1985; Lease et al. 2011). Before oviposition, these females would have a higher fat content than after oviposition (Nestel et al. 2005). Insects collected from the wild seem to have a lower fat content than insects which are commercially produced (Finke 2002; Yang et al. 2006; Oonincx et al. 2012b; Finke 2013). This might be a result of decreased movement in captivity, access to high energy diets or a combination of the two.

Fatty acids are the building blocks of fat. Two or three fatty acids are coupled to glycerol to form diglycerides and triglycerides respectively. These fatty acids are stored in the insect fat body, making up over 90% of the total fat body lipid (Downer et al. 1976; Beenakkers et al. 1985; Bender 2002). Based on the degree of saturation, fatty acids can be subdivided into saturated fatty acids (those with no double bonds) and unsaturated fatty acids (those with one or more double bonds). Unsaturated fatty acids can be divided in mono-unsaturated fatty acids and poly-unsaturated fatty acids. Poly-unsaturated fatty acids can be further subdivided into omega 3, 6 or 9 unsaturated fatty acids based on the relative position of the first double bond. Both the absolute occurrence of unsaturated fatty acids (Haglund, Wallin et al. 1998) and the relative occurrence of specific unsaturated fatty acids (Schmitz and Ecker 2008) is associated with health in humans, and these proportions might also play a role in the health of some species of insectivores. For most insect species, more than half of the fatty acids are unsaturated, a notable exception being the Hemiptera (Thompson 1973). The main saturated fatty acids found in insects are C16:0 and C18:0, and, as observed with most other land animals, C16:0 is normally present in larger quantities than C18:0 (Fast 1970; Thompson 1973; Majumder and Sengupta 1979; Yang et al. 2006). The most prevalent unsaturated fatty acids found in insects are C16:1, C18:1, C18:2 and C18:3 (Fast 1970; Majumder et al. 1979; Cookman, Angelo et al. 1984; Beenakkers et al. 1985; Bukkens 1997; Yang et al. 2006; Ekpo, Onigbinde et al. 2009). Poly-unsaturated fatty acid content has been reported to vary between 0.4% and 52.4%, but is usually a large proportion of the unsaturated fatty acids (Bukkens 1997; Yang et al. 2006). For instance, the fat of the Chinese grasshopper (*Acrida cinerea* (Thunberg)) contains 41% C18:3 and 12% C18:2 (Yang et al. 2006). In Yellow mealworm larvae, C18:1 seems to be the most prevalent fatty acid, but C18:2 and C18:3 constituted 25.5% and 0.3% of the total fat, respectively (Aguilar-Miranda, Lopez et al. 2002). A similar trend was reported for commercially raised feeder insects (Finke 2002).

The fatty acid composition of insects is affected by four main variables: 1) species, 2) developmental phase, 3) diet, and 4) environmental factors such as temperature, light and humidity.

Phylogeny is not the main determining factor for fatty acid composition, although some general distinctions can be made (Fast 1970; Fontaneto, Tommaseo-Ponzetta et al. 2011). For instance, aphids and other Hemiptera tend to contain large amounts of the short-chained fatty acids C14:0, reaching up to 80% of total fatty acids (Thompson 1973). Coccids contain large amounts of C10:0 and C12:0 (Fast 1970). Most fatty acids found in Diptera are shorter than 18 carbons with C16:1 being especially abundant (Fast 1970; Thompson 1973). In adult houseflies less than half of the fatty acids were shorter than 18 carbons while C18:1 and C18:2 made up about half of the fatty acids (Finke 2013). Black soldier fly larvae seem to contain high levels of C12:0 although the exact amounts are affected by the insect’s diet (St-Hilaire, Sheppard et al. 2007b). Lepidopterans tend to have a higher C18:3 content than other insect species (Fast 1970; Majumder et al. 1979; Fontaneto et al.

2011), while Dictyoptera, such as cockroaches, contain little or no C18:3 (Thompson 1973; Finke 2013).

There also seems to be a difference between the fats found in aquatic and terrestrial insects, presumably as a result of their diet. Aquatic insects have a higher mono-unsaturated fatty acid content than terrestrial insects, while terrestrial insects tend to have a higher poly-unsaturated fatty acid content, especially the omega-6 fatty acids, C20:3 and C20:4 (Fontaneto et al. 2011).

Insects contain higher amounts of C18:3 than most mammals. Similar to vertebrates, *de novo* synthesis of saturated fatty acids and poly-unsaturated fatty acids by elongation and desaturation occurs in insects (Tietz and Stern 1969; Beenakkers et al. 1985). Certain poly-unsaturated fatty acids such as C18:2 and C18:3 are considered essential because most animal species are unable to synthesize them (Bender 2002). This also appears to be true for most insect species, which makes these essential fatty acids a dietary requirement (Fast 1970; Thompson 1973; Cookman et al. 1984; Beenakkers et al. 1985). A few noticeable exceptions have been identified, for example the American cockroach (*Periplaneta americana* L.) and the House cricket (Beenakkers et al. 1985; Blomquist, Borgeson et al. 1991). It was originally assumed that bacterial fermentation was the source for C18:2 in these insect species, but more recent studies have shown that it can be synthesized *de novo* in these and other insect species distributed over four different orders (de Renobales, Cripps et al. 1987; Blomquist et al. 1991; Borgeson, Kurtti et al. 1991; Borgeson and Blomquist 1993).

Carbohydrates

Few publications have focused on the carbohydrate content of insects. In general, carbohydrates are present in relatively small amounts in insects (Finke 2002; Finke 2013). In the field cricket (*Gryllus bimaculatus* De Geer), polysaccharide and carbohydrate content are approximately 0.3% of the fresh weight (Hoffmann 1973) and free carbohydrate content of the fat body in females of this species is less than 0.5% dry matter (Lorenz and Anand 2004). In Yellow mealworm larvae, the carbohydrate content can vary between 1 and 7% dry matter, depending on the diet provided (Ramos-Elorduy et al. 2002) although some of these differences may be the result of the food remaining in the gastro-intestinal tract.

Fiber & chitin

Insects contain significant amounts of fiber as measured by crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Finke 1984; Pennino, Dierenfeld et al. 1991; Barker et al. 1998; Finke 2002; Punzo 2003; Finke 2007; Lease and Wolf 2010; Oonincx et al. 2012b; Finke 2013). While insects contain significant amounts of fiber, the components that make up these fibers are unknown. While it has been suggested that the fiber in insects represents chitin, since chitin (linear polymer of β-(1→4) N-acetyl-D-glucosamine units) is structurally similar to cellulose (linear polymer of β-(1→4)-D-glucopyranose units) found in plant material. In reality the fiber in whole insects likely represents a variety of different compounds including chitin, sclerotized proteins and other substances that are bound to chitin (Finke 2007). Chitinase activity has been reported in certain species of frogs, lizards, tortoises, fish, and bats, suggesting that certain insectivores might be able to digest chitin (Fujimoto, Suzuki et al. 2002; Whitaker Jr, Dannelly et al. 2004; Donoghue 2006; German, Nagle et al. 2010).

In insects chitin exists in a matrix with proteins, lipids, and other compounds, which together comprise the cuticle (Kramer, Hopkins et al. 1995). Since chitin is present only in the insect's exocuticle, the chitin content of insects is likely not high. Little quantitative data exists concerning the chitin content of whole insects but using an enzymatic assay aquatic insect larvae contained between 2.9 and 10.1% chitin on a dry weight basis (Cauchie 2002). In contrast, in most insects studied, protein, not chitin is the predominant compound in the cuticle (Kramer et al. 1995). The amino acid patterns of whole insects and the proteins in the ADF fractions were different, and the amino acid patterns of the ADF fraction also differed between insect species (Finke 2007). These differences likely reflect specificity in cuticular proteins present in insects which contribute to their unique properties.

While "harder bodied" insects like adult beetles contained higher levels of ADF than softer bodied insects like Yellow mealworm larvae, silkworm larvae or cricket nymphs, those fiber levels were a result of higher levels of amino acids in the ADF fraction (Finke 2007). This suggests that insects with "harder" cuticles do not necessarily contain more chitin than softer bodied insects but rather contain higher levels of cross-linking proteins that are essential for sclerotization.

Minerals

Minerals can be broadly classified as macro-minerals and micro- or trace minerals based on the amounts needed to meet requirements. The essential macro-minerals include calcium, phosphorus, magnesium, sodium, potassium and chloride. The essential micro minerals include iron, zinc, copper, manganese, iodine and selenium. The macro-minerals calcium, phosphorus and magnesium play a primary role in helping maintain the skeletal structure in vertebrates while sodium, potassium and chloride function as electrolytes and help maintain acid-base balance. The trace minerals play wide ranging roles ranging from oxygen transport to functioning as cofactors in a variety of enzyme systems.

Most species of insects contain little calcium since insects as invertebrates do not have a mineralized skeleton. Calcium levels are typically less than 0.3% dry matter (Jones, Cooper et al. 1972; Martin, Rivers et al. 1976; Frye and Calvert 1989; Barker et al. 1998; Finke 2002; Hatt, Hung et al. 2003; Punzo 2003; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013). The higher levels of calcium occasionally reported for feeder crickets likely reflect calcium in the gut contents (Frye et al. 1989; Barker et al. 1998; Finke 2002; Hatt et al. 2003; Punzo 2003; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013). The exoskeleton of most insects is primarily composed of protein and chitin, although some insects have a mineralized exoskeleton in which calcium and other minerals are incorporated into the cuticle (Dashefsky, Anderson et al. 1976). Examples include larvae of the face fly (*Musca autumnalis* De Geer), and the Black soldier fly (Dierenfeld et al. 2008; Finke 2013). Black soldier fly larvae contain a high level of calcium and are now commonly sold commercially as food for captive insectivores. Concerns have been raised concerning the availability of the calcium in Black soldier fly larvae especially for insectivores that swallow their prey whole. When fed to mountain chicken frogs, the calcium digestibility of whole Black soldier fly larvae was only 44% compared to 88% for larvae that had been “mashed” (Dierenfeld et al. 2008). Even though calcium availability was relatively low, given the high level of calcium in this species, they should provide sufficient calcium for most insectivores.

Other invertebrates such as millipedes and isopods also have a mineralized exoskeleton and likely serve as a source of calcium for wild insectivores (Reichle, Shanks et al. 1969; Gist and Crossley 1975; Graveland and van Gijzen 1994; Oonincx et al. 2012b). However, one effective way of supplying this mineral might be offering calcium grit, powder, or other calcium rich materials separately to insectivores (Tordoff 2001; Rich and Talent 2008). Wild birds seek out calcium rich invertebrates when calcium requirements are high such as during egg laying and nestling growth (Graveland et al. 1994; Bureš and Weidinger 2003).

The phosphorus content of feeder insects is much higher than calcium levels in most insect species, except for Black soldier fly larvae (Jones et al. 1972; Martin et al. 1976; Barker et al. 1998; Finke 2002; Hatt et al. 2003; Punzo 2003; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013). Most insects would likely contain adequate levels of phosphorus to meet the requirements of insectivores especially since the phosphorus in insects is likely to be readily available as was shown for face fly pupa (Dashefsky et al. 1976).

Most species of feeder insects contain levels of magnesium ranging from 0.08 to 0.30% dry matter. These levels would likely be sufficient to meet the dietary requirements of most species of insectivores. Like calcium, the magnesium content of Black soldier fly larvae was 3 – 10 times higher than that of other feeder insects. It seems likely that both calcium and magnesium form a complex with chitin in the larval cuticle in this species.

There are few reports on the sodium and potassium content of captive bred insects (Finke 2002; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013) but these data are similar and comparable to the values obtained for wild-caught insects (Reichle et al. 1969; Levy and Cromroy 1973; Studier, Keeler et al. 1991; Studier and Sevick 1992; Oyarzun, Crawshaw et al. 1996). Levels of potassium generally range from 0.6 – 2.0% dry matter while sodium levels are somewhat lower ranging from 0.1 – 0.6% dry matter. There are limited data concerning the chloride content of feeder insects with values ranging from 0.16 – 0.97% dry matter (Finke 2002; Finke 2013). These data suggest that most insects likely contain adequate amounts of these three minerals to meet the needs of most species of insectivores (Finke 2002; Oonincx et al. 2012b; Finke 2013).

Most insects appear to contain relatively high levels of the trace minerals iron, zinc, copper and manganese. While the high fat larval stage of some species of feeder insects like Greater wax moth larvae, Yellow mealworm larvae and butter worm larvae might be marginally low in iron, relative to energy content most insects likely supply adequate amounts for the typical insectivore (Barker et al. 1998; Finke 2002; Hatt et al. 2003; Punzo 2003; Oonincx et al. 2011; Oonincx et al. 2012b; Finke 2013). For unknown reasons both adult houseflies and fruit flies contain relatively high levels of iron (Barker et al. 1998; Oonincx et al. 2012b; Finke 2013). House crickets can contain up to nearly 200 mg/kg dry matter although other reports documented iron content to be around 60 mg/kg dry matter (Bernard et al. 1997; Finke 2002). These variations again are likely to be due to food remaining in the gastro-intestinal tract when the insects were analysed. Wild caught insects also appear to contain significant amounts of iron (Studier et al. 1992; Punzo 2003).

Insects are generally a good source of zinc with values for commercially raised insects ranging from 61.6 to 340.5 mg/kg dry matter (Barker et al. 1998; Finke 2002; Punzo 2003; Oonincx et al. 2012b; Finke 2013) . These values are similar to those obtained for wild caught species (Levy et al. 1973; Punzo 2003). Copper in commercially raised feeder insects ranged from 3.1 to 51.2 mg/kg dry matter (Barker et al. 1998; Finke 2002; Punzo 2003; Oonincx et al. 2012b; Finke 2013). The lowest value seen in captive raised insects was 3.1 mg/kg dry matter for Greater wax moth larvae reported by Barker et al. (1998), while Finke (2002) found a much higher level for this species (9.2 mg/kg dry matter). All other species analysed had values greater than 7 mg/kg dry matter suggesting insects are typically good sources of copper. Wild caught insects also appear to contain significant amounts of copper (Levy et al. 1973; Punzo 2003).

Levels of manganese in feeder insects range from 1.5 to 364 mg/kg dry matter (Bernard et al. 1997; Barker et al. 1998; Finke 2002; Punzo 2003; Dierenfeld et al. 2008; Oonincx et al. 2012b; Finke 2013). Like calcium and magnesium, the highest levels of manganese observed were seen in Black soldier fly larvae (Dierenfeld et al. 2008) although the reasons for elevated levels of manganese in this species are unclear. Some species of stored product insects contain elevated levels of zinc and manganese in their mandibles presumably to harden them in order to better penetrate whole seeds (Morgan, Baker et al. 2003). Wild caught insects also appear to contain significant amounts of manganese (Levy et al. 1973; Punzo 2003).

There are very little data regarding the iodine content of insects. Of the twelve species of feeder insects analyzed, only six had any detectable iodine with levels ranging from 0.45 to 1.22 mg/kg dry matter (Finke 2002; Finke 2013). Bee brood (pupae and larvae) did not contain detectable levels of iodine (Finke 2005). No other data are available concerning the iodine content of insects.

As is the case for iodine there are only limited data on the selenium content of feeder insects. While Butterworm larvae did not contain any selenium the other eleven species of feeder insects contain selenium at levels ranging from 0.27 to 0.97 mg/kg dry matter (Finke 2002; Finke 2013).

Since the contents of the gastrointestinal tract can represent a significant percentage of the total weight of the insect (Finke 2003), it can have a significant effect on the mineral content of the insect if it is analysed when fully fed. Additionally studies of wild insects show both seasonal variation as well as variation between different populations of the same species living in the same general area (Finke 1984; Studier et al. 1991).

Vitamins and carotenoids

Vitamin A

Vitamin A plays a role in a wide variety of physiological processes including vision, cell differentiation, immune response, reproduction and growth. There are limited data regarding the vitamin A content of wild insects and most species of captive bred insects contain relatively low levels of vitamin A/retinol (typically less than 300 µg retinol/kg dry matter) (Pennino et al. 1991; Barker et al. 1998; Finke 2002; Hatt et al. 2003; Punzo 2003; Oonincx et al. 2011; Finke 2013). While migratory locusts fed a grass diet supplemented with wheat bran and fresh carrots contained significantly more retinol than those fed only a grass diet, the retinol levels for all locusts were well below the requirements of the rat (Oonincx et al. 2011).

In fruit flies only the eyes contained measurable quantities of retinoids and the amount detected was a function of the carotenoid content of the larval diet (Goldsmith and Warner 1964; Seki, Isono et al. 1998; von Lintig 2012). The low values reported in the literature for the vitamin A content of captive bred insects may be a result of a several factors. First, one analytical method used for vitamin A analysis of insects is specific for retinol and may not detect the other retinoids (retinal and 3-hydroxyretinal) found in insect eyes (Goldsmith et al. 1964; Smith and Goldsmith 1990; Seki et al. 1998). It is unclear if 3-hydroxyretinal can serve as a source of vitamin A since it is unknown if 3-hydroxyretinal can be converted into retinal by insectivores. Honey buzzards (*Pernis apivorus* (L.)), a primarily insectivorous bird were shown to contain high levels of 3-4 didehydroretinol in their plasma suggesting insectivorous birds might be able to utilize some of the atypical retinoids found in adult insects (Müller, Raila et al. 2012). Second, since the retinoid levels in fruit fly eyes was a function of dietary carotenoid content it may be that diets fed to commercially raised insects do not contain sufficient levels or types of carotenoids to optimize the retinol, retinal and 3-hydroxyretinal content of insects. Third, many insect species raised for food are fed to insectivores as larva. Since insect larvae do not possess compound eyes, where retinoids are synthesized from carotenoids, the larval stage of these insects would not be expected to contain large quantities of retinoids (von Lintig 2012). In addition to retinol, certain carotenoids can be converted into vitamin A in many species of animals although it is unclear if all insectivores have the ability to convert beta-carotene to retinol (Olson 1989; Bender 2002; McComb 2010; Levi, Ziv et al. 2012). Carotenoids are found at high levels in many species of wild insects while captive bred insects contain significantly lower levels (Isaksson and Andersson 2007; Eeva, Helle et al. 2010; Oonincx et al. 2011; Finke 2013). The reason for this discrepancy is unclear but is likely a function of dietary carotenoid content. It may be that wild insectivores use a combination of retinoids (retinol, retinal and 3-hydroxyretinal) as well as carotenoids to meet their vitamin A requirements. A better understanding of the retinol, retinal and 3-hydroxyretinal content of insects and the utilization of the retinoids and carotenoids found in insects as a source of vitamin A in insectivores is important, as vitamin A deficiency has been reported in several species of captive insectivores (Ferguson, Jones et al. 1996; Miller, Green et al. 2001; Pessier, Linn et al. 2005; Hoby, Wenker et al. 2010).

Vitamin D

Vitamin D can be considered conditionally essential for many species of insectivores as it can be synthesized by most animal species provided the proper environmental conditions. An analysis of the vitamin D content of a variety of species of commercially raised insects detected no vitamin D although the threshold for detection was 250 IU/kg (as is) or roughly 595 – 1445 IU/kg dry matter (Finke 2002). Using a more sensitive technique, Black soldier fly larvae, Butter worm larvae and Rusty red roaches were shown to contain 388 - 633 IU vitamin D_3/kg dry matter while house flies contained no detectable vitamin D_3 (Finke 2013) . These values are similar to those obtained for yellow mealworms (150 IU vitamin D/kg dry matter) and House crickets (934 IU vitamin D/kg dry matter) (Oonincx et al. 2010a). There is no analysis of the vitamin D content of wild insects available for comparison. Further studies on the vitamin D content of both captive raised and wild caught insects should be conducted to provide a better understanding of insect vitamin D content. However, it seems likely that exposure to ultraviolet light is the appropriate way to ensure an adequate vitamin D status in most insectivores (Ferguson et al. 1996; Oonincx et al. 2010a).

Vitamin E

Vitamin E serves as an anti-oxidant and therefore helps maintain the functionality of a variety of lipid soluble compounds in the body. Insects contain varying amounts of vitamin E. Both House crickets and yellow mealworms have been shown to contain widely varying levels of vitamin E. Values for House crickets ranged from 5-79 mg/kg dry matter (Pennino et al. 1991; Hatt et al. 2003) while yellow mealworm larvae ranged from less than 15 mg/kg to 50 mg/kg dry matter (Pennino et al. 1991; Finke 2002). The large variations found could well be due to the diet provided to the insect, both the vitamin E incorporated into the body tissue as well as the vitamin from any diet remaining in the insect's gastrointestinal tract. Vitamin E content of other commercially raised insects was relatively low (typically less than 15 mg/kg dry matter) (Barker et al. 1998; Finke 2002; Oonincx et al. 2012b; Finke 2013). Several species, including Butterworm larvae and silkworm larvae had somewhat higher levels (33-35 mg/kg dry matter) while much higher levels (110-120 mg/kg dry matter) were reported for fruit flies, house flies and false katydids (*Microcentrum rhombifolium* (Saussure)) (Finke 2002; Oonincx et al. 2012b; Finke 2013). There are few data on the vitamin E content of wild insects, although they appear to contain vitamin E at levels on the higher end of the range or exceeding that seen in captive bred insects (Pennino et al. 1991; Punzo 2003).

B-Vitamins

There is limited comprehensive information regarding the B-vitamin content of most insects. Recent research has focused on the B-vitamin content of the most common commercially raised feeder insects (Finke 2002; Finke 2013). There is much less information regarding the B-vitamin content of unprocessed wild-caught insects for comparison. Complicating a comparison between the data for captive bred insects and that for various dried insects are differences in the analytical methods used (microbiological versus chemical techniques) and the method used for sample preparation. Some B-vitamins are relatively unstable when exposed to heat, light or oxygen. Therefore values obtained by analysing dried insects that have been processed for human consumption may not be representative of the values found in live whole insects. This also means that commercially available dried whole insects may contain lower levels of some B-vitamins due to processing, drying and storage. Thiamine (vitamin B_1) is needed for the production of several important enzymes associated with energy metabolism (Thurnham, Bender et al. 2000). A number of species of feeder insects including House crickets, adult Yellow mealworms, Superworms, Butterworms, and Rusty red roaches contained relatively low levels of thiamine (0.8 to 2.9 mg/kg dry matter) while other species of feeder insects (Black soldier fly larvae, adult house flies, silkworms, Yellow mealworm larvae and waxworms) contained much higher levels (5.6 to 44.8 mg/kg) (Finke 2002; Finke 2013). Similarly, for a selection of collected Nigerian insects comprising five orders, large variations were reported (0.3-32.4 mg/kg dry matter) (Banjo, Lawal et al. 2006). When analysed using a microbiological method, high levels of thiamine were reported for the African palm weevil larva (*Rhynchophorus phoenicis* (F.)) and the larvae of the Cavorting emperor moth (*Usta terpsichore* (Maassen and Weymer)) (30.2 and 36.7 mg thiamine/kg dry matter) while the values for termites (*Macrotermes subhyalinus* (Rambur)) were very low (1.3 mg/kg dry matter) (Santos Oliveira, Passos De Carvalho et al. 1976). Dried smoked Attacidae caterpillars from Zaire analysed using a microbiological method also contained low levels of thiamine (1.5-2.7 mg/kg dry product) (Kodondi, Leclercq et al. 1987). Since thiamine is relatively unstable and the values reported are for dried insects processed using a variety of traditional methods (drying, smoking, and frying) it is unclear how representative these values are of raw whole insects (Santos Oliveira et al. 1976;

Kodondi et al. 1987; Banjo et al. 2006).

Riboflavin (vitamin B_2) functions as a co-enzyme required in the metabolism of a variety of other nutrients (Thurnham et al. 2000). Most species of commercial feeder insects contain relatively high levels of riboflavin ranging from 17.6 to 306.3 mg/kg dry matter (Jones et al. 1972; Finke 2002; Finke 2013). A similar concentration 32.4 mg/kg dry matter was found for honeybee brood (larvae and pupae) (Finke 2005; Banjo et al. 2006). Dried smoked Attacidae caterpillars also contained high levels of riboflavin (32-51 mg/kg dry product) (Kodondi et al. 1987). These values are slightly higher than those for termites, palm weevil larvae and a species of Saturniid larvae processed for human consumption (11.4 – 22.4 mg riboflavin/kg dry matter) (Santos Oliveira et al. 1976). Banjo reported highly variable levels of riboflavin in 14 species of insects from Nigeria ranging from 0.9 to 32.4 mg/kg dry matter (Banjo et al. 2006). It should be noted that after collection these insects were kept dry (adults) or stored in 70% alcohol (larvae). Since riboflavin is degraded by light it is unclear whether these represent real differences or resulted from losses during storage prior to analysis.

Niacin (vitamin B_3) plays a role in metabolism and tissue respiration (Thurnham et al. 2000). It appears to be abundant in insects with commercially reared feeder insects containing levels ranging from 76.7 to 359.1 mg/kg dry matter (Finke 2002; Finke 2013). A variety of dried insect species typically consumed in Africa including termites, palm weevil larvae and several different species of lepidopteran larvae have been analysed for niacin. Even though these insects were processed using a variety of different methods (drying, smoking and frying) they contained high levels of niacin ranging from 52 to 110 mg/kg dry matter (Kodondi et al. 1987), while larvae of the Cavorting emperor moth contained only 3 mg niacin/kg dry matter (Santos Oliveira et al. 1976). Niacin is a relatively stable B-vitamin and therefore the values in these studies are likely more representative for live insects when compared to other B-vitamins.

Pantothenic acid (vitamin B_5) functions as a component of coenzyme A and as such plays a role in the citric acid cycle, fatty acid synthesis and oxidation reactions. It is widely distributed in most foodstuffs and commercially raised insects appear to be no exception, with levels ranging from 46.1 to 179.8 mg/kg dry matter (Finke 2002; Finke 2013). Bee brood also appears to be a good source of pantothenic acid containing 51.3 mg/kg dry matter (Finke 2005). There are little additional data available on the pantothenic acid content of insects, although using a microbiological assay dried smoked Attacidae caterpillars were found to contain very low levels of pantothenic acid (0.073-0.102 mg/kg dry product) (Kodondi et al. 1987).

Pyridoxine (vitamin B_6) plays an important role in a variety of metabolic reactions most notably those involved in amino acid metabolism. Commercially raised insects appear to be good sources of pyridoxine with values ranging from 3.1 mg/kg dry matter for Greater waxworm larvae to 22.3 mg/kg dry matter for Yellow mealworm beetles (Jones et al. 1972; Finke 2002; Finke 2013). Most insect species, however, fall in a fairly narrow range between 6 and 10 mg pyridoxine/kg dry matter. Using a microbiological technique very low levels of pyridoxine (0.37 to 0.63 mg/kg) were detected in three species of dried smoked Attacidae caterpillars (Kodondi et al. 1987). Since pyridoxine is unstable it is likely that the low values obtained are a result of the smoking and drying process used to preserve the dried insect product.

Biotin (vitamin B_8) is a carrier of carboxyl groups in reactions involving ATP. The biotin content of commercially raised insects is highly variable ranging from 0.23 to 2.69 mg/kg dry matter, however, the values for most species ranged from 0.7 to 1.4 mg/kg dry matter (Finke 2002; Finke 2013). Dried smoked meal from Attacidae caterpillars have been reported to contain 0.23-0.45 mg/kg (Kodondi et al. 1987). It is unclear if these lower values reflect biological differences, biotin loss during processing (drying and smoking) or analytical techniques used.

Folic acid (vitamin B_9) plays an essential role in DNA synthesis and one carbon metabolism (Thurnham et al. 2000). Using a chemical method commercially raised insects were shown to contain high levels of folic acid ranging from a low of 1.57 mg/kg for silkworm larvae to 7.22 mg/kg for house flies (Finke 2002; Finke 2013). In contrast using a microbiological method very low folic acid values (0.20-0.63 mg/kg) for smoked and dried product from three species of Attacidae caterpillars were found (Kodondi et al. 1987). Since folic acid is susceptible to degradation both by light and oxidizing agents the low values seem likely a result of the smoking and drying process used to preserve the dried insect product.

Cobalamin (vitamin B_{12}) is found exclusively in products of animal origin and plays a key role in reactions involving methyl donors. While silkworm and Greater waxworm larvae contain no detectable vitamin B_{12} (less than 3-4 µg/kg dry matter), most other species are good sources of vitamin B_{12} (Finke 2002; Finke 2013). In particular House crickets, Black soldier fly larvae, and Rusty red roaches contained extremely high levels ranging from 143 to 767 µg/kg dry matter. Vitamin B_{12} levels of 140-250 µg/kg dry matter were reported for dried and smoked products from three species of Attacidae caterpillars (Kodondi et al. 1987), levels similar to those obtained for commercially raised species.

Vitamin C

Vitamin C is needed to form connective tissue and functions as an antioxidant. Insects contain some vitamin C and it has been suggested that certain insects, for instance the grasshopper *Melanoplus sanguinipes* (F.), use enzymes to reuse antioxidants such as vitamin C and E (Barbehenn 2003). Honey bees contain relatively high amounts of vitamin C (102.5 to 163.8 mg/kg dry matter) (Banjo et al. 2006). Most other species in which vitamin C was determined contained low levels from 0 to about 50 mg/kg dry matter, although both adult House crickets and adult Yellow mealworms contained levels similar to those reported for honey bees (Finke 2002; Banjo et al. 2006; Finke 2013).

Effects of insect size/life stage on nutrient composition

In general, the lipid content of wild insects is approximately 30% for larvae and 20% for adults (Fast 1970). Fat stores are usually largest in the final larval stage prior to metamorphosis (Fast 1970). This depends to some degree on whether species undergo a complete metamorphosis (holometabolous species; for instance Yellow mealworms) or an incomplete metamorphosis (hemimetabolous species; for instance House crickets).

Larvae of holometabolous species have a higher fat content than adults (Punzo 2003; Lease et al. 2011). Yellow mealworms, for example, increase their fat reserves during larval growth (McClements et al. 2003). These fat reserves are used as an energy source during metamorphosis, resulting in a lower fat content of adults and a subsequent increase in the relative content of protein and ash (Downer et al. 1976; Bernard et al. 1997; Barker et al. 1998; Finke 2002; Lease et al. 2011; Oonincx et al. 2012b). A similar trend is seen in fruit flies. Furthermore, during metamorphosis the protein composition of Yellow mealworms changes; glycine, tryptophan and taurine content increase, while tyrosine content decreases (Finke 2002). The taurine content of both fruit flies as well as a species of noctuid moth (*Mamestra configurata* Walker) also increased after metamorphosis (Bodnaryk 1981; Massie, Williams et al. 1989). During pupation of the fly *Agria affinis* (Fallén), two thirds of their fat reserve is used. This coincides with a relative decrease in C16:0, C16:1 and C18:1, and a relative increase in C18:2 and C22:1, while the relative concentration of C18:0 is not affected (Barlow 1965). Different changes in fat composition occur in silkworms. During larval development, both fat content and composition change; the total fat content increases and relative increases of C16:0, C16:1 and C18:1 and decreases of C18:0 and C18:2 are observed. During pupation, C18:1 continues to increase while C16:0 continues to decrease. The levels of C18:3 in silkworm larvae differed between males and females (Nakasone et al. 1967). In the velvet bean caterpillar, total lipid content decreases during metamorphosis, as would be expected. However C16:0 and C18:1 levels increase, C18:3 levels decrease and C18:2 levels remain stable (Cookman et al. 1984). To which extent these changes are species-specific or constitute differences between Diptera and Lepidoptera needs further investigation. Similar to species preparing for pupation, preparation for diapause or hibernation results in increased energy (fat) reserves (Downer et al. 1976; Ali et al. 1977). In three species of aquatic insects the concentration of C18:3 increased while C20:4 and C20:5 decreased after the larval phase, when the insect left the aquatic environment (Hanson, Cummins et al. 1985).

Unlike holometabolous insects, hemimetabolous adults tend to have a higher fat content than nymphs (Lease et al. 2011). This holds true for certain studies conducted on Migratory locusts, but not all (Oonincx et al. 2010a; Oonincx et al. 2011). Probably the time of sampling, whether directly after adult emergence or later during adulthood, explains these differences since fat continues to accumulate after adult emergence (Beenakkers et al. 1985). Generally, flying and migratory insects have a tendency to accumulate more fat, which is used as an energy source for their flight muscles (Downer et al. 1976). In this locust species dry matter content increases and ash content decreases between the penultimate instar and adulthood, although copper and iron levels increase (Oonincx et al. 2011). Similar to these locusts, the dry matter content in House crickets significantly increases (from 23 to 30%) during the penultimate stage of development (Roe, Clifford et al. 1980). Furthermore, adults have a higher protein and a lower fat content than small and medium House crickets (Finke 2002; McClements et al. 2003). The amino acid composition seems similar between nymphs and adults of this species (Finke 2002). In a study on three cockroach species (Rusty red roaches, Six-spotted roaches (*Eublaberus distanti* (Kirby)) and Hissing roaches (*Gromphadorhina portentosa* (Schaum)), an increase in dry matter content and crude protein content, and a concomitant decrease in fat content, was noted between small and medium specimens. The opposite change was noted when Six-spotted roaches reached adulthood (Oonincx et al. 2012b).

Besides size differences, certain gender differences seem apparent in insects. Males often have a lower body weight than females, which might be caused by a lower fat content as explained previously (Hoffmann 1973; Ali et al. 1977; Sönmez et al. 2008).

Effects of insect diet on insect nutrient composition

Diet can have a significant effect on the nutrient composition of an insect. Since the entire insect is normally consumed, dietary effects described in the literature can partially be due to diet remaining in the gut as was mentioned previously. This is discussed in more detail later in this chapter. The body composition of the insect itself can also be altered to a certain extent through the diet. For instance, a higher water content of the bran (8.7 vs. 6.7%) provided to Yellow mealworm larvae increases their water content (64 vs. 59%) (Machin 1975).

For the macro nutrients, it seems that insect fat content is highly variable and that the content of protein and ash are subsequently affected. If an insect with a certain amount of protein increases its fat reserve, the percentage of protein thereby decreases (Simpson et al. 2001; St-Hilaire et al. 2007b; Oonincx et al. 2011). This might be the reason why the protein content stays constant during adulthood if a protein source is available and protein content decreases if only a carbohydrate source, which can be converted to fat, is available as was shown in Mediterranean fruit flies (*Ceratitis capitata* (Wiedemann)) (Nestel et al. 2005). Although the percentage of protein can be altered through the diet, it seems unlikely that the amino acid composition is affected. Within a specific life stage the relative abundance of amino acids is expected to be species-specific because specific parts of the insect body are made from certain amino acids. A study of the Black soldier fly found no effects of diet on amino acid composition (Sealey et al. 2011). However, a study of Yellow mealworms, reared on different diets, while not resulting in large differences in crude protein content, found differences in amino acid profiles (Ramos-Elorduy et al. 2002). It seems likely that these were not necessarily due to changes in the amino acid composition of the insect, but rather due to differences in the amino acid composition of the diet present in the gut of the larvae.

As stated before, the crude fat content of insects can be altered by the diet provided. Two independent studies on fruit flies using the same artificial diet showed a similar nutrient composition, especially regarding fat and iron. A third study on fruit flies, found a dissimilar composition, which could indicate a strong dietary influence (Bernard et al. 1997; Barker et al. 1998; Oonincx et al. 2012b). For House crickets, several studies are available on their chemical composition. Large differences in fat content have been reported ranging from 17 to 37% dry matter (Barker et al. 1998; Finke 2002; Hatt et al. 2003; Oonincx et al. 2010a). It seems likely that variation in fat content is a result of both the diet provided and the age of the cricket (Hatt et al. 2003). When sub-adult House crickets were provided with either water, lettuce or a commercial cricket diet, after three weeks the ones fed water or lettuce had higher moisture and protein contents and lower fat content than those fed the commercial cricket diet. During this period the fat content decreased for all three groups, which led to a concomitant rise in the other macro nutrients (Hatt et al. 2003). This may have been caused by a lower energy content in the three experimental diets than in the diet provided before the trial, especially since the crickets fed only water or lettuce showed the greatest decline. Additionally the sub-adult House crickets would be expected to become adults during those three weeks and as such their fat content would be expected to decrease (McClements et al. 2003).

Besides the plasticity of total fat content, large differences can be expected in the fatty acid composition when different diets are provided. In non-ruminant production animals, short term provision of poly-unsaturated fatty acids has been shown to increase the poly-unsaturated fatty acid content of the meat, indicating these fatty acids were incorporated in the body (Kouba and Mourot 2011). Studies on insects indicate that fatty acid composition of both larvae and adults tends to reflect the fatty acid composition of the diet provided (Cookman et al. 1984; St-Hilaire et al. 2007b). A study with Black soldier flies indicated that poly-unsaturated fatty acid content can be elevated by supplementing their diet with fish offal during the last month of development (St-Hilaire et al. 2007b). The fatty acid composition of the insect does not always directly match that of the diet suggesting selective accumulation or synthesis (Cookman et al. 1984). For instance, in migratory locusts the C18:1 and C18:3 content of the diet strongly relates to the fatty acid content of the locust fat body. However, for C18:2 this was not the case, possibly due to poor absorption or due to saturation of this fatty acid (Beenakkers and Scheres 1971). While diet composition influences the fatty acid composition directly, indirect effects have also been reported. For instance, a sufficient supply of C18:2 can be used to synthesize C20:4 and C20:5 (Hanson et al. 1983).

For many feeder insects, carrots are a well-accepted part of the diet or a means of providing moisture. In migratory locusts, the addition of carrots increased carotene content as well as retinol (vitamin A) (Oonincx et al. 2011). While the elevated carotene levels could be due to gut loading, it seems likely that the increased retinol content is due to conversion of carotene to retinol. Fruit flies are able to convert β-carotene to retinol, which might indicate that more insect species have this ability (von Lintig and Vogt 2000). Great tits (*Parus major* L.) have been shown to prefer carotenoid-enriched Yellow mealworms over non-enriched yellow mealworms (Senar et al. 2010).

Besides differences in required nutrients, undesirable effects can arise through long term provision of a certain diet. For instance accumulation of heavy metals, such as Cd and Pb, can occur in certain insect species (Zhang, Song et al. 2012).

Effects of environment on insect composition

A number of environmental factors, such as temperature, light and humidity, can affect growth, development and chemical composition of insects.

Temperature

Insects are poikilothermic, that is, cold-blooded; their body temperature depends to a large degree on the environmental temperature. Therefore, within a range of temperatures suitable for the specific species, their metabolic rate and growth rate should increase with higher temperatures (Ali et al. 1977; Hanson et al. 1983; Akman Gündüz and Gülel 2002; Angilletta Jr, Steury et al. 2004; Ali et al. 2011; Krengel, Stangl et al. 2012). A standard way of quantifying this is the relative difference when the temperature is increased by 10 degrees (Q10). For instance, in House crickets the development time is halved with a 10 degree increase in temperature (Roe et al. 1980). While growth rates are increased by higher temperatures, adult size generally seems to decrease; a colder environment results in larger animals (Akman Gündüz et al. 2002; Angilletta Jr et al. 2004; Krengel et al. 2012). Exceptions include both desert locusts (*Schistocerca gregaria* Forsskål) and migratory locusts where adult body weights are higher when reared at 30° and 31°C respectively, than at 25°C (Beenakkers, Meisen et al. 1971; Akman Gündüz et al. 2002). Temperatures between 32° and 43°C appear most suitable when appropriate humidity is provided (Hamilton 1936). Last instar female House crickets gain weight quicker, and more efficiently, at 35° than at 25° or 30°C (Roe et al. 1980; Roe, Clifford et al. 1985). At higher temperatures, lipid content decreases during the latter half of this instar, but at 25°C carbohydrates are still converted to lipids. It seems that optimal growth occurs at 35°C, which is similar to the 34°C for the field cricket. However, for the latter species, mortality is also highest at 34°C. Both growth rate and mortality are decreased at 27°C. The lowest adult fresh weight is achieved at ambient temperatures around 10°C, but the dry matter content is about 20% higher compared to crickets raised at 27°C. For field crickets temperature changes during rearing (alterations between 20°C and -1.5°C) versus constant temperatures (13°C) increase water content and decrease protein content (Hoffmann 1973). Alternating temperatures around the optimal growth temperatures appear to result in a higher protein and fat content in field crickets (Hoffmann 1974). At low temperatures (13° and 20°C vs 27°C), fat content is higher, as are the proportions of saturated fatty acids. This seems a likely adaptation to the thermal regime. In the fly *Pseudosarcophaga affinis* (Fallén), higher proportions of unsaturated fatty acids are known to increase heat tolerance (House, Riordan et al. 1958). A comparative study on the Seven-spotted lady beetle (*Coccinella septempuctata* L.) and the Asian lady beetle (*Harmonia axyridis* Pallas) indicated that the first species accumulates more fat in general and increases fat storage at elevated temperatures compared to normal temperatures (18° vs. 21°C). Fat accumulation remains low under both conditions in the latter species. Females of the Seven-spotted lady beetle have a higher fat content than males at normal temperatures, but a similar fat content at elevated temperatures. The authors suggested that temperatures before eclosion determine the fat content of adults in both species (Krengel et al. 2012). The carbohydrate and protein content of bean beetles (*Acanthoscelides obtectus* (Say)) is lower at 20°C than at 30°C, but lipid content is the same (Sönmez et al. 2008).

Humidity

Humidity is normally expressed as relative humidity (RH), which is the relative amount of water that can be stored in air of a certain temperature. Higher levels of humidity, within an appropriate range, seem to decrease development time (Han et al. 2008; Ali et al. 2011; Nedvěd et al. 2012). However, unlike in the case of increasing temperatures, this increase in growth rate does not seem to lead to a lower body mass in adults. For instance, the body mass of the pine caterpillar (*Dendrolimus tabulaeformis* Tsai et Liu) increases with higher humidity during their larval stages (20-100%) (Han et al. 2008).

A comparative study on the effects of humidity and temperature on locust species (*L. migratoria* and *S. gregaria*) indicated development is possible between 35 and 80% RH, and increasing temperatures require a higher RH (Hamilton 1936). Optimal RH is around 60-65%, while alternating the humidity to provide a day-night rhythm showed inconsistent effects on development. Similarly, pine caterpillars require a RH above 40% for development, while the optimal RH, resulting in increased body mass, seems to be around 80%. During diapause, this species can absorb water from the substrate, thereby increasing its fresh weight (Han et al. 2008). Fasting Yellow mealworms are capable of absorbing water vapor if relative humidity is above 88% (Fraenkel 1950; Machin 1975). Relative humidity of course has an indirect effect through the feed provided as well. If the feed provided to Yellow mealworms is in equilibrium with 70% RH, it allows rapid growth (Fraenkel 1950). Mealworms first exposed to high humidity, have a higher dry matter weight gain if feed is provided after exposure (Machin 1975). However long term exposure to high humidity (>85%) results in higher larval mortality, probably due to excessive hydration (Machin 1975). The observed increased dry matter weight gain could be due to the larvae compensating for the excessive hydration by an increased feed intake. Other problems are likely to occur at high

RH, for instance the development of fungi and/or mites (Machin 1975). The optimal growth rate for Yellow mealworms at 25°C is attained at 70% RH (Fraenkel 1950; Machin 1975).

As indicated before, the optimal RH is related to temperature, although other variables can play a role as well. The Asian lady beetle tends to grow larger at higher humidity levels (between 30 and 90%) if fed with the aphid *Acyrthosiphon pisum* (Harris) but not when fed on frozen eggs of *Ephestia kuehniella* (Zeller) (Nedvěd et al. 2012).

It seems that body weight and development rate of insects is higher at the top of their RH range. Also, the moisture content of insects seems to increase at a higher RH. However, little is known on how variables such as fat content and body composition are affected.

Photoperiod

The effect of photoperiod (daily exposure time to light) on insect composition has not been studied in detail; most studies have focused on behavioral effects, fecundity and body weight. For certain locust species, long photoperiods (up to 24 hours) increase their growth rate. Possibly, the food intake in this diurnal species is increased if a longer photoperiod is provided. This effect could, however, also be due to concomitantly higher temperatures. At constant temperature, Asian lady beetles tended to develop quicker with 16 hours of light compared to 12 hours of light, but adult weight was similar (Berkvens, Bonte et al. 2008). This seems likely for these diurnal species, but the green stink bug (*Nezara viridula* (L.)) that mainly feeds at night, has an increase in the rate of development with an increased photoperiod (10 vs. 14 hours) (Ali et al. 1977; Shearer et al. 1996). This increase in developmental rate coincided with an increase in body weight when reaching, and during adulthood, especially for females (Ali et al. 1977). Short photoperiods can induce diapause, while longer photoperiods are more likely to induce reproduction. It seems that for this species, more energy is accumulated when preparing for reproduction than for diapause, which would be likely to lead to a higher fat content. However, little is known on the influence of photoperiod on the chemical composition. For adults of the Giant wax moth, it has been suggested that the protein content of adults increases more rapidly if kept in constant light, compared to constant darkness (Koc et al. 2008). The effects of photoperiod on the nutrient profile of insects mostly seems to be indirect, acting through other processes, such as preparation for reproduction or diapause or concomitant changes in temperature.

Nutrient requirements of insectivores including diet availability

It is difficult to compare the nutritive value of insects as a group to the dietary requirements of insectivores because of the large number of insect species and the large differences in nutrient content between those species (Bukkens 1997; Barker et al. 1998; Finke 2002; Finke 2013). A proper evaluation of a food/insect requires it to be evaluated in the broader context of a complete diet, made up of a number of different foods/insects.

Availability and digestibility

It has been suggested that insects with sclerotized exoskeletons might have poor digestibility, which would be lowest in the last larval instar (Dufour 1987). This lower digestibility would be caused by the higher chitin content. For aquatic insects, this has been reported to be between 3 and 10% (Cauchie 2002). For the commonly reared House crickets, the larvae of the Giant wax moth and the giant mealworm (hormonally treated *T. molitor*), chitin contents were estimated to be between 1.6 and 2.0% (Finke 2007). Yellow mealworm adults contain about 7.4% chitin (Finke 2007), which concurs with the findings of Dufour (1987). A study of the protein digestibility of mopane caterpillars in rats showed a slightly lower digestibility when compared to other products of animal origin (Dreyer et al. 1982). When fed to rats the protein digestibility of freeze-dried Yellow mealworms was slightly lower than that of casein (Goulet et al. 1978), whereas the protein digestibility of dried caterpillars (*Clanis bilineata*) was shown to be similar to that for casein (Xia, Wu et al. 2012). The Chinese grasshopper *A. cinerea* showed a slightly higher true protein digestibility than fish meal when fed to poultry (Wang, W. Zhai et al. 2007). For grasshoppers, a protein digestibility of 62% has been reported in poultry (Ravindran and Blair 1993). For broilers, rendered beef meal can be replaced by a meal of the termite *Kalotermes flavicollis* F., or the cockroach *Blatta orientalis* L., with similar better growth rates of broilers (Munyuli Bin Mushambanyi et al. 2002). In general protein digestibility of insects seems relatively high and the variability reported in the literature is likely a result of differences in how the insect was prepared prior to being used and the proportion of amino acids that are used for sclerotization.

Enhancing the nutrient composition of insects as food for insectivores

While the exact nutrient requirements for most insectivores are unknown, certain nutrient deficiencies are known to occur regularly in captive insectivores. Probably the three most common in captive insectivores are calcium, vitamin A and D deficiencies. Nutrients which are expected to be present at low levels in insects can be enhanced in order to increase the concentration of these nutrients in the insectivore diet. There are two primary methods of providing these nutrients: gut loading and dusting. The goal of both gut loading and dusting is to increase the nutrient intake of selected nutrients by the insectivore. While this is valid for a certain range, one must be careful that this range is not surpassed, resulting in adverse effects. For instance, over-supplementation with calcium can decrease the absorption of other minerals, leading to secondary trace mineral deficiencies. Likewise over-supplementation of the fat-soluble vitamins, in particular vitamins A and D, can cause toxicity (Bender 2002). A difference in size (developmental stage) of the dusted or gut loaded insect leads to differences in surface:volume and gut size:volume ratios. Smaller insects have a relatively large surface area to which the dust can adhere. The same powder used for pinhead or adult House crickets could therefore have different effects on their chemical composition (Sullivan, Livingston et al. 2009). Similarly for gut loaded insects size differences could lead to differences in nutrient delivery of smaller versus larger insects (Finke 2003; Finke 2005).

Gut loading

Gut loading is the term used for the provision of a special diet to insects, shortly before the insects will be consumed. When this diet, which contains high levels of the desired nutrient(s), is consumed by the insect it will be present in the gut thereby increasing the insectivore’s nutrient intake when the insect is consumed. Due to the nature of gut loading, it is suitable for almost all nutrients as long as the diet is palatable to the insect and the diet can contain sufficient quantities of the desired nutrient(s) (Hunt-Coslik, Ward et al. 2009).

Most research on the effects of gut loading has focused on increasing the calcium content of insects. High calcium diets containing 4-9% calcium, typically from calcium carbonate, have proven effective in increasing the calcium content of wax moth larvae, House crickets, Yellow mealworm larvae and silkworm larvae (Strzelewicz, Ullrey et al. 1985; Allen and Oftedal 1989; Bernard et al. 1997; Anderson 2000; Klasing, Thacker et al. 2000; Finke 2003; Finke, Dunham et al.

2005). Chemical analysis of the diet provided might be necessary to verify the true calcium content of commercially available gut-loading diets (Finke et al. 2005). The calcium from gut loaded Yellow mealworms fed high calcium diets was shown to be readily available to growing chicks showing its usefulness in providing available calcium (Klasing et al. 2000). Different studies have found different results in optimal gut loading times and the optimal amount of time for properly gut loading insects seems to vary slightly. This is likely a result of the insect species being studied, the palatability of the gut loading diet and the environmental conditions (temperature, light and humidity). In general, however, gut loading for 24-72 hours appears to result in similar levels of nutrients in the intact insect. When gut-loading diets are fed for longer periods of time adverse effects on the viability of the insects have been observed (Klasing et al. 2000). In Yellow mealworms a gut loading period of 24 hours raised Ca:P ratios, and extending this period to 48 or 72 hours, resulted in slightly higher Ca:P (Anderson 2000; Klasing et al. 2000). For House crickets, a period of 48 hours seems sufficient to attain a significant increase in Ca:P ratios, while extending this to 72 hours does not affect this ratio (Anderson 2000). However, other studies report the highest calcium content after 1 day compared to 2, 3 or 7 days (Dikeman, Plesuk et al. 2007). It has been reported that offering certain gut loading diets longer than 2 days can reduce the initially increased calcium levels (Hunt-Coslik et al. 2009), which could be an effect of the palatability of the gut loading diet (McComb 2010).

In addition, the physical form of the diet and the presence of other nutrients such as amino acids and fatty acids, which affect diet palatability, is something to take into account when designing a gut loading diet (Anderson 2000). Addition of poly-unsaturated fatty acids to the gut loading diet has been suggested for insectivores stemming from temperate climates, which would be likely to encounter insects with relatively high poly-unsaturated fatty acids concentrations (Li, Vaughan et al. 2009).

A simple way of providing extra carotenoids to insectivores is the provision of carrots or certain other fruits or vegetables during the last 24 hours before feeding the insects to the insectivores. The amount of carotenoids that accumulates through gut loading differs per insect species. The field cricket, for instance, accumulates more carotenoids on a high carotenoid diet than the House cricket or the banded cricket (*Gryllodes sigillatus* (Walker)) (Ogilvy, Fidgett et al. 2012).

A study in which Yellow mealworms were gut loaded with chicken starter feed, increased their vitamin D content to 132 IU/kg dry matter while undetectable levels were present in mealworms provided a wheat bran diet (Klasing et al. 2000).

Dusting

Dusting is the term used for coating an insect with a fine powder containing the desired nutrients, such that the powder adheres to the outside of the insect. When the insect is eaten, the powder on the outside is also ingested. Little data exist on the nutritional effects of this method. One study in which House crickets were dusted with a fine calcium powder, the Ca:P ratio was raised from 1:5.7 to 5.3:1 and the digestibility of these minerals was high (84 and 94%, respectively) (Dierenfeld et al. 2008). An important factor in the effectiveness of this method is the time between dusting and consumption (Trusk and Crissey 1987). House crickets can groom off up to half of the amount of adhering powder in 90 seconds (Li et al. 2009). For animals which immediately consume their prey, dusting can be an acceptable method. A downside of dusting is that it is difficult to estimate the amount of dust adhering to the insect. Using two different calcium carbonate dusts, weight increases of 0.8 to 6.3% for Giant wax moth larvae, Yellow mealworm larvae and House crickets when dusted with two different types of calcium carbonate dusts have been reported (Winn, Dunham et al. 2003). The physical characteristics of the dust, the relative surface area of the insect and the physical characteristics of the surface of the insect exoskeleton can all affect the amount of dust adhering to the feeder insect. For aquatic insectivores, this way of enhancing the nutrient content is obviously unsuitable. A study with the Wyoming toad (*Bufo baxteri* Porter) indicated that both the composition and the method of providing the powder or gut load diet can lead to differences in the weight gain of the insectivore (Li et al. 2009). This might be due to a decreased consumption rate, which in turn might be caused by decreased palatability due to the vitamin powder (Li et al. 2009). A study of Puerto Rican crested toads (*Peltophryne lemur* Cope), compared the effects of direct oral application of vitamin A with gut loading and dusting in House crickets. Retinol blood values were significantly higher in toads offered dusted crickets (McComb 2010). This indicates the effectiveness of this method. Why the other methods were not effective is not clear although it may be that retinol absorption was enhanced when ingested with food. Dietary fat enhances the absorption of fat soluble nutrients like retinol and so the fat in the crickets may have increased retinol absorption compared to direct oral supplementation.

Other considerations

Pathogens/parasites

There is little information regarding commercial feeder insects as a source of pathogens. House crickets from five commercial suppliers in the United States were shown to be free of Oxyurids/pinworms but little other information is available regarding insects as a source of parasites for insectivores (Klarsfeld and Mitchell 2005). The Lesser mealworm *Alphitobius diaperinus* (Panzer) has been shown to be a potential vector for *Salmonella* spp. with both larvae and adults being able to carry *Salmonella* spp. both externally and internally (Crippen, Sheffield et al. 2009). Additionally *Salmonella* could be detected in newly emerged adult beetles from infected larvae suggesting some bacteria are carried through metamorphosis (Crippen, Zheng et al. 2012). Since several species of grain beetles (*Tenebrio* sp. and *Z. morio*) are commonly used as feeder insects it seems likely that these species could serve as a vector of *Salmonella* spp. and other pathogenic microorganisms. To minimize this risk, feeder insects should be obtained from qualified suppliers, fed an appropriate diet and maintained under hygienic conditions to minimize the risk of transmitting pathogenic bacteria.

Toxins

It is well known that many species of insects sequester toxic compounds from their diet making them unpalatable or even toxic to certain insectivores. In the wild these species are generally brightly colored (aposomatic) to warn potential predators of the consequences of feeding on these species. Monarch butterflies (*Danaus plexippus* L.) and milkweed bugs (*Oncopeltus fasciatus* Dallas) are but a few of the many species that sequester toxins from their food (Brower 1969; Berenbaum and Miliczky 1984). Since most captive raised feeder insects are fed controlled diets containing commercial feed ingredients that are used to feed domestic animals, it seems unlikely that they would accumulate few if any toxins from their diet as long as the diet was properly made and stored.

Ingredients commonly used in commercial insect diets can become contaminated with various species of molds that can produce mycotoxins including aflatoxin, fumonisins, and zearalenone. Little is known regarding the effects of these mycotoxins on both insects and insectivores. However, at least some insect species seem to be relatively resistant to moderate levels of aflatoxin (McMillian, Widstrom et al. 1981). None of the insect species commonly used to feed captive insectivores have been studied with respect to mycotoxins but feeder insects should be sourced from qualified suppliers. Additionally the diet fed the insects prior to use should be stored properly and periodically checked to insure they are free of molds which might produce mycotoxins.

Almost nothing is known regarding the potential anti-nutritional properties of insects with the exception of thiaminase. Thiaminase is an enzyme that when ingested splits thiamin, effectively destroying its vitamin properties. While thiaminase is typically associated with certain species of fish it has been reported in both Domesticated silkworm larvae and African silkworm pupae (*Anaphe* spp.), although the levels found in Domesticated silkworm larvae were only one third of those found in African silkworm pupae. These authors noted that in addition to thiamine, pyridoxine, taurine, and other nutrients could also serve as a substrate for this enzyme. The consumption of *Anaphe* pupae has been associated with a seasonal ataxia in local populations in Nigeria which is presumably due to thiamine deficiency (Adamolekun 1993; Adamolekun, McCandless et al. 1997; Nishimune, Watanabe et al. 2000). The extent to which thiaminases are found in other species of insects and their potential effect on insectivores is currently unknown.

Conclusions

Insects are a good source of many nutrients although for most nutrients the values vary widely depending on the insect species, the life stage and the conditions in which they are raised. In general most species appear to be good sources of amino acids, fatty acids, most minerals and most B-vitamins. Based on analysis of feeder insects and reports of nutrient deficiencies in captive insectivores, the nutrients of concern in a captive insectivore feeding program include calcium and the fat soluble vitamins A, D and E. As such, captive insectivores should be fed a mix of invertebrates that have been dusted or gut loaded to provide a wide range of nutrient intakes in an effort to reduce the risk of nutrient deficiencies.

Table 1: Typical amino acid content of some common feeder insects (all values expressed as mg amino acid/g crude protein).

Amino Acid	Mean	Range
Alanine	71.4	40.9 – 101.1
Arginine	56.6	41.4 – 75.5
Aspartic Acid	82.0	65.6 – 95.0
Cystine	9.1	5.6 – 21.3
Glutamic Acid	114.5	96.2 – 138.3
Glycine	54.7	42.1 – 84.4
Histidine	27.8	22.1 – 37.9
Isoleucine	42.6	32.3 – 50.3
Leucine	76.2	48.9 – 106.4
Lysine	56.9	44.3 – 68.0
Methionine	17.0	10.7 – 29.6
Phenylalanine	34.9	26.2 – 43.7
Proline	58.1	33.9 – 77.0
Serine	47.8	35.1 – 77.0
Threonine	36.6	28.4 – 41.8
Tryptophan	9.0	5.2 – 17.3
Tyrosine	56.3	31.2 – 79.9
Valine	56.1	39.8 – 73.7
Taurine	1.5	0.0 – 8.1

Chapter 3

An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption

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Abstract

Background

Greenhouse gas (GHG) production, as a cause of climate change, is considered as one of the biggest problems society is currently facing. The livestock sector is one of the large contributors of anthropogenic GHG emissions. Also, large amounts of ammonia (NH_3), leading to soil nitrification and acidification, are produced by livestock. Therefore other sources of animal protein, like edible insects, are currently being considered.

Methodology/Principal Findings

An experiment was conducted to quantify production of carbon dioxide (CO_2) and average daily gain (ADG) as a measure of feed conversion efficiency, and to quantify the production of the greenhouse gases methane (CH_4) and nitrous oxide (N_2O) as well as NH_3 by five insect species of which the first three are considered edible: *Tenebrio molitor*, *Acheta domesticus*, *Locusta migratoria*, *Pachnoda marginata,* and *Blaptica dubia.* Large differences were found among the species regarding their production of CO_2 and GHGs. The insects in this study had a higher relative growth rate and emitted comparable or lower amounts of GHG than described in literature for pigs and much lower amounts of GHG than cattle. The same was true for CO_2 production per kg of metabolic weight and per kg of mass gain. Furthermore, also the production of NH_3 by insects was lower than for conventional livestock.

Conclusions/Significance

This study therefore indicates that insects could serve as a more environmentally friendly alternative for the production of animal protein with respect to GHG and NH_3 emissions. The results of this study can be used as basic information to compare the production of insects with conventional livestock by means of a life cycle analysis.

Key words: Greenhouse gas emission, edible insect, ammonia, average daily gain, feed conversion efficiency

Introduction

Production of greenhouse gasses (GHG) is considered as an important cause of climate change (Steinfeld et al. 2006; IPCC 2007). The most important GHGs are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). Since the end of the 18th century the atmospheric carbon-dioxide concentration has increased by 30% and CH_4 concentrations by 50% (Kroon, Holtslag et al. 2009). CH_4 and N_2O have considerably greater global warming potentials (GWPs) than CO_2. By assigning CO_2 a value of 1 GWP, the warming potentials of these other gases can be expressed on a CO_2-equivalent basis: CH_4 has a GWP of 25, and N_2O has a GWP of 298 (IPCC 2007). The relative contribution of CO_2 equivalents (CO_2 eq.) of the livestock sector is large, amounting up to 18% of total anthropogenic GHG emissions (Steinfeld et al. 2006). Based on a Life Cycle Assessment (LCA) that takes the entire production process of animal products into account, the global contribution to GHG emissions by the animal sector are: 9% for CO_2 (fertilizer production for feed crops, on-farm energy expenditures, feed transport, animal product processing, animal transport, and land use changes), 35-40% for CH_4 (enteric fermentation in ruminants and from farm animal manure) and 65% for N_2O (farm manure and urine) (Steinfeld et al. 2006). Direct CO_2 production through respiration is not relevant when determining the impact of GHGs as respiration by livestock is not considered a net source of CO_2 (Steinfeld et al. 2006). The respired carbon, which comes from the feed, was first taken up from CO_2 in the air and stored in an organic compound during the production of the feed. However, the ratio between body growth realised and CO_2 production is an indicator of feed conversion efficiency and thereby a relevant indicator for the environmental impact (de Vries and de Boer 2010).

Livestock is also associated with environmental pollution due to ammonia (NH_3) emissions from manure and urine, leading to nitrification and acidification of soil (Aarnink, Keen et al. 1995). Although not considered a GHG, NH_3 can indirectly contribute to N_2O emission (Steinfeld et al. 2006), as conversion takes place by specialized soil bacteria (Wrage, Velthof et al. 2001). Livestock is estimated to be responsible for 64% of all anthropogenic NH_3 emissions (Steinfeld et al. 2006). The main source of gaseous NH_3 is bacterial fermentation of uric acid in poultry manure (Lacey 2002; Fabbri, Valli et al. 2007) and bacterial fermentation of urea in mammals (Cole, Clark et al. 2005). Besides these environmental problems the livestock sector faces challenges regarding resistance to antibiotics, zoonosis and animal welfare (Thorne 2007).

All these problems together illustrate the need to find alternatives for conventional sources of animal protein. Mini-livestock, for instance edible insects, have been suggested as an alternative source of animal protein (Beets 1997).

Production of animal protein in the form of edible insects supposedly has a lower environmental impact than conventional livestock (Nakagaki et al. 1991; Gullan et al. 2005; Ramos-Elorduy 2008). When evaluating the total environmental impact of animal protein production, a LCA, in which all production factors are taken into account, is needed. Differences in environmental impact in a LCA can be explained mainly by three factors: enteric CH_4 emissions, feed conversion efficiencies and reproduction rates (de Vries et al. 2010).

Before performing a LCA, it is necessary to know the GHG production by edible insects. This information is lacking in literature. Therefore, in this study we experimentally quantified the direct production of the GHGs CH_4 and N_2O for five insect species. CO_2 production and average daily gain (ADG) were quantified to provide an estimation of feed conversion efficiency. Additionally, NH_3 emissions were quantified. The results of this study represent a quantification of the insect physiological contribution to GHG production by insects and can in turn be used to create a LCA for insect-derived products.

Materials and methods

Animals and housing

Five insect species were studied: fifth larval stage Yellow mealworms *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), fifth and sixth nymphal stage House crickets *Acheta domesticus* L. (Orthoptera: Gryllidae), third and fourth stage nymphs of Migratory locusts *Locusta migratoria* L. (Orthoptera: Acrididae), third larval stage sun beetles *Pachnoda marginata* Drury (Coleoptera; Scarabaeidae) and a mix of all stages of the Argentinean cockroach *Blaptica dubia* Serville (Dictyoptera: Blaberidae). Currently, *T. molitor*, *A. domesticus* and *L. migratoria* are considered edible, while *P. marginata* and *B. dubia* are not. The latter two species were included since they are a potential source of animal protein, for instance by means of protein extraction. These two species can be bred in large numbers with little time investment and are able to utilise a wide range of substrates as feed (Bruins 2001; Friederich et al. 2004).

Per species three to six repetitions were conducted each for a period of three days. Animals were housed per species in two cages or containers per respiration chamber. These containers were placed in one of two, identical, open circuit climate respiration chambers measuring 80*50*45 cm, with a total volume of 265 L (Verstegen, Van Der Hel et al. 1987). Within these climate respiration chambers, *T. molitor* and *P. marginata* were housed in two stacked plastic containers (50*30*8.7 cm). The three other species were housed in metal wire cages (45*37.5*41 cm; mesh width 1 mm) with a glass cover plate. To increase surface area for *A. domesticus* and *B. dubia*, hollow plastic tubes (20 cm long and 3 cm in diameter), were stacked to a height of 30 cm in the wired cages, while for *L. migratoria*, two V-shaped-folded metal screens (70*15 cm) were entered per cage. Humidity, temperature, and day length were based on rearing conditions used by commercial insect rearing companies (Table 1). All animal masses reported are averages of fresh mass per cage. The starting and final animal mass per cage are provided in Table 1.

Diet

Food was provided for each species at the beginning of each repetition, except when mentioned otherwise.

Tenebrio molitor larvae were reared in 300 g mixed grain substrate (wheat, wheat bran, oats, soy, rye and corn, supplemented with beer yeast) with on top pieces of carrot (± 15*2 cm) weighing a total average of 637 g per repetition.

Acheta domesticus was provided with chicken mash (501 g) with carrot pieces (784 g) on top for each repetition.

Locusta migratoria was provided with wheat bran (70 g; Arie Blok Animal Nutrition, Woerden, The Netherlands) in a metal bowl at the beginning of each repetition. Fresh Perennial ryegrass (*Lolium perenne* L.) was provided daily (463 g in three days). The grass was grown by Unifarm, Wageningen University and Research centre, Wageningen, The Netherlands.

Pachnoda marginata larvae were kept in a peat moss substrate (2.0 kg per respiration chamber) in which chicken mash (285 g) was mixed at the beginning of each three-day repetition. Pieces of carrot (± 15*2 cm) with an average total mass of 161 g per repetition were put on top of the substrate.

Blaptica dubia was provided with a chicken mash diet (199 g) and carrots (559 g), fresh carrot being added during the repetitions.

Peat moss, chicken mash, and carrots, offered to *A. domesticus*, *P. marginata* and *B. dubia* were provided by Kreca V.O.F, Ermelo, The Netherlands. The carrots and mixed grains substrate offered to *T. molitor* were provided by Insectra, Deurne, The Netherlands.

Gas measurements

During the experiment concentrations of CO_2 and CH_4 were measured every 9 min in the ingoing and outgoing air stream of the respiration chambers. The difference in CO_2 and CH_4 concentrations between ingoing and outgoing air thus represents the total production of CO_2 and CH_4 of insects, feed, and substrate. The exact air volumes were measured with a calibrated Schlumberger G1.6 dry gas meter and corrected for measured air temperature and pressure. CO_2 and CH_4 concentrations were measured in dried gas. Gas was dried in a +2 ºC dew-point cooler. Non-dispersive infrared analyzers were used to measure CO_2 (type Uras 3G, Hartmann and Braun, Frankfurt, Germany) and CH_4 (type Uras 10E, Hartmann and Braun, Frankfurt, Germany). The refreshed air volume was set so that CO_2 levels did not exceed 1%. From each climate respiration chamber, as well as from the incoming air, an air sample was taken for N_2O analysis after 24, 48, and 72 h with a 60 ml syringe. The syringes were sealed by a shutoff valve and stored at 20 ºC until analysis (within 48 h). The N_2O concentration was analysed by a gas chromatograph (CE instruments GC8000 Top, Interscience, Breda, The Netherlands) using a Haysep Q 80-100 mesh 2 m x 1/8" SS column, at a constant temperature of 60 ºC. N_2O was detected with an electron capture detector (ECD). Injection volume was 5.0 ml in a fixed loop.

NH_3 concentrations in the climate respiration chambers were determined twice daily (at 12.00 and 24.00 h) by means of a gas detection tube system (Kitagawa, type AP-20; Komyo rikagaku kogyo, Tokyo, Japan; type 105 NH_3 gas detector tubes with a range of 1-20 ppm).

Calculations

Production of N_2O was calculated by subtracting the N_2O concentration from the incoming air from that in the outgoing air. These differences were then used in a formula adapted from Wheeler, K.D. Casey et al. (2003):
ER = Emission rate of N_2O = $[N_2O]$ change (ppm x 10^{-6}) x VV (m^3/day) x 44 (g/mol) / 0.0224 (m^3/mol), where VV = ventilation volume of air in a specified time period. The average concentration difference of the three samples taken during the three-day period was used to determine the average N_2O production in a repetition. The formula used by Wheeler et al. (2003) was also used for the calculation of NH_3 production. A molecular mass of 17 was used and instead of a difference in concentration, the measured concentration was used, leading to a slight overestimation of the actual NH_3 production (between 0 and 0.1 mg/kg BM/day).

CO_2 equivalents were calculated by adding the multiplications of the produced amounts of CH_4 and N_2O with their global warming potential; 25 for CH_4, and 298 for N_2O (IPCC 2007).

Mean body mass was calculated by averaging the body mass at the start of the experiment and the body mass at the end of the experiment. Average daily gain (ADG) was calculated as follows: (((End mass - Start mass)/Start mass)/3)*100%, in which 3 is the number of days the experiment was running.

The ratio between CO_2 production per unit biomass per day and ADG gives an indication of the feed conversion efficiency, in which higher values indicate lower efficiencies.

To determine CO_2 production from feed and substrate, all feeds were independently tested in the same respiration chambers, without the animals. A linear time course of consumption was assumed and CO_2 production was recalculated to kg of live insect.

Statistics

The N_2O and NH_3 assay data were subjected to a two-way analysis of variance (ANOVA) with species and time of sampling (24, 48, or 72 h) as fixed factors to determine whether the time of sampling had an effect. No significant effect of the time of sampling was found for N_2O (Pillai's trace: F= 1.467, P = 0.199). Therefore, the average of the three samples taken during the 3-day trial period was used to determine the change per repetition and to calculate total production. However, NH_3 production was significantly affected by the time of sampling (day or night; Pillai's trace: F= 4.065, P = 0.019) and the day of the repetition (first, second or third; Pillai's trace: F= 17.170, P < 0.001). CO_2 and CH_4 production for all five species were analysed by means of a one way analysis of variance (ANOVA) followed by a Tukey post hoc test. Statistical analysis of all data was done by means of SPSS 15.0.

Results

Production of CO_2 is expressed per kilogram of mean live body mass (BM) per day (24 hours) and per kilogram of mass gain (Table 2) and the average daily gain (ADG) is reported (Table 2). Production of CH_4, N_2O, CO_2 equivalents, and NH_3, are expressed per kilogram of mean live body mass (BM) per day (Table 3) and per kilogram of mass gain (Table 4).

ADG and CO_2 production

ADG varied between 4.0% (*P. marginata*) and 19.6% (*L. migratoria*) with the three other species having an ADG of 6-7%. CO_2 production among the five insect species differed significantly and ranged from 19 (*B. dubia*) to 110 (*L. migratoria*) g per kg BM/day. Also, the CO_2 production per kg of metabolic weight (i.e. the weight of metabolically active body tissue) differed greatly between species (Table 5). CO_2 production expressed per kg of mass gain was intermediary for *L. migratoria* due to the high ADG. Still, the CO_2 production of *L. migratoria* per kg of mass gain was more than double the production of CO_2 by *B. dubia. Pachnoda marginata* had the highest production of CO_2 per kg of mass gain (1,539 g/kg), which was more than double the amount of *L. migratoria*.

CH_4

Production of methane was detected for *P. marginata* and *B. dubia*, but not for the three other species. *Pachnoda marginata* produced more than three times as much CH_4 per kg of mass gain than *B. dubia* (4.9 *vs* 1.4 g). This difference was caused by a higher production of CH_4 per kg BM (0.16 g *vs* 0.08 g) and a lower ADG (4.0% *vs* 6.1%).

N_2O

N_2O was produced only in significant amounts by *T. molitor* and *L. migratoria* (1.5 and 8.0 mg/kg BM/day, respectively). Production of N_2O by *L. migratoria* per kg BM was more than 5-fold the production by *T. molitor,* this difference decreased to almost 2.5-fold when expressed per kg of mass gain, due to a much higher ADG of *L. migratoria.*

NH_3

NH_3 was produced by *A. domesticus, L. migratoria,* and *B. dubia* (3.0-5.4 mg/ kg BM/day), and ranged from 36-142 mg/kg of mass gain (Table 3 and 4). Significant differences (Pillai's trace: F= 4.065, P = 0.019) between daytime (12.00) and night-time (24.00) NH_3 emission levels were found for *A. domesticus* (6.4 and 4.4 mg/kg BM/day), *L. migratoria* (5.6 and 3.9 mg/kg BM/day), and *B. dubia* (3.4 and 2.6 mg/kg BM/day).

Discussion

Insects, being poikilotherms, do not use their metabolism to maintain a body temperature within narrow ranges, contrary to homeothermic animals. This is expected to result in higher feed conversion efficiencies. CO_2 production related to growth has an inverse relationship with feed conversion efficiency in a given situation. CO_2 production by insects depends on the species, stage of development (Bailey and Singh 1977; Terblanche and Chown 2007), temperature (Emekci, Navarro et al. 2004), feeding status (Gouveia, Simpson et al. 2000), and on activity level (Aidley 1976; Armstrong and Mordue 1985). A production of 37 g CO_2 / kg BM/day was reported for *Anabrus simplex* (Orthoptera: Tettigoniidae), 40 g CO_2 /kg BM/day for the locust *Schistocerca americana* (Orthoptera: Acrididae) (Greenlee and Harrison 2004) and 94 g/kg BM/day for adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Emekci, Navarro et al. 2002). All five species in the current study had a fairly high production of CO_2. This might to a large extent be explained by *ad libitum* feeding during the experiment that has been reported to increase oxygen consumption fivefold (Gouveia et al. 2000). Reported CO_2 production for inactive, unfed, Tenebrionid adults ranged between 5.4-13.3 g/kg BM/day (Duncan, Krasnov et al. 2002), which is 5 – 10 times lower than observed for *T. molitor* in this experiment. This can partially be explained by the locomotory activities of *T. molitor* larvae in this experiment (Slansky 1985). Furthermore, growing larvae are expected to have a higher CO_2 production than adults. The range of CO_2 production for *T. molitor* is comparable to the factorial metabolic scope reported for tiger beetles (*Cicindela* spp: Coleoptera; Cicindelidae) of 6.1-16.5 (May, Pearson et al. 1986).

Size differences in animals account for a difference in metabolic rate, and thereby CO_2 production. The relation between metabolic rate (B) and body mass (M) was described by Kleiber (1961) as $B = aM^b$, in which a is a constant and b = 0.75. The value of b has been much debated since (Heusner 1982; Agutter and Wheatley 2004; da Silva, Garcia et al. 2006). For poikilotherms values between 0.67 and 1.0 have been reported and a comparison of several arthropod species suggested b approximates 0.82 (Lighton and Fielden 1995; Chown, Marais et al. 2007). The value chosen for b has a large impact on the metabolic weight and thereby the calculated CO_2 production (Table 5). Applying b=0.75 for pigs and beef cattle and b=0.82 for insects, resulted in a lower CO_2 production based on metabolic weight for the studied insect species (Table 5). For *L. migratoria* CO_2 production was only slightly lower than for beef cattle, however, for the other four species production was between 18% and 54% of that for beef cattle and between 11% and 34% of the CO_2 production of pigs.

The CO_2 production per kg BM of insect species investigated in this study was higher than for pigs or cattle (Table 3). This concurs with Prothero (1979), who reported a higher oxygen consumption per kg of BM for insects than for mammals, assuming the respiratory quotient (CO_2 production / O_2 consumption) has similar values (0.7-1.0) for both animal groups. However, the CO_2 production per kg of mass gain for the five insect species in the current study (337-1,539 g/kg) was either 39% (minimum values) or 129% (maximum values) when compared with pigs (865-1,194 g/kg) and much lower (12%-54% respectively) than cattle (2,835 g/kg). Therefore, CO_2 production per kg of mass gain suggests higher feed conversion efficiencies for insects than for mammalian livestock. These results concur with those of other authors (Slansky 1985; Nakagaki et al. 1991; Collavo et al. 2005; Ramos-Elorduy 2008).

A similar trend was visible for ADG; the ADG for the five insect species studied was 4.0-19.6%, the minimum value of this range being close to the 3.2% reported for pigs, whereas the maximum value was 6 times higher. Compared to cattle (0.3%), insect ADG values were much higher. In general, the rate of ADG depends, amongst others, on life phase. Therefore, where available, literature data on growing animals were used. The fundamental biological differences in growth and development processes between pigs and cattle and the studied insects impeded further synchronization.

CH_4 production for the species studied was in agreement with Hackstein et al. (1994); for insects, only representatives of cockroaches, termites, and scarab beetles produce CH_4. This originates from bacterial fermentation by methanobacteriaceae in the hindgut (Egert, Wagner et al. 2003).

We found large variability for the N_2O emission rates. Earlier studies in laying hens using a similar method for determining N_2O production, concluded that production was either negligible or undetectable (Guiziou and Béline 2005; Fabbri et al. 2007). However, other authors (Wathes, Holden et al. 1997; Chadwick, Sneath et al. 1999) determined a production of 28 mg N_2O/kg BM/day and 52 mg N_2O/ kg BM/day, respectively, indicating the difficulty of accurately determining N_2O production (Lemke, Goddard et al. 2002).

In earlier studies respiration of feed was considered to have a negligible effect on utilisation of dry mass as determined gravimetrically (Waldbauer 1968) and therefore on CO_2 production. Later studies suggested that respiration by plant leaves can be an important source of error in the calculation of insect feed intake using gravimetric methods (Axelsson and Ágren 1979) and can cause major errors in energy budget studies of plant-feeding insects (van Loon, Casas et al. 2005). Our reported CO_2 production includes the respiration of the feed (Table 6). The extremely high contribution to total CO_2 production by the substrate of *P. marginata* (92.5%) was most likely due to large amounts of fungal biomass observed in the mixed feed and substrate when insects were absent in the experiments aimed to obtain correction values for CO_2-production by the substrate. No fungal growth was apparent during the experiments on feeding *P. marginata* larvae, suggesting that the contribution of the substrate to total respiration during the experiment was much lower. We conclude that the interaction between actively feeding *P. marginata* larvae and the substrate suppressed fungal growth through either consumption by the beetle larvae (Li and Brune 2007) of fungal biomass or through unknown chemical or combined chemical/mechanical mechanisms. Such interactions hinder the application of realistic corrections for the contribution of feed and substrate to the total CO_2 production and thus to quantify the CO_2 production arising from insect metabolism separately.

For all other species the relative contribution of the feed to total CO_2 production was minor, varying between 1.3% and 3.6%. Although feed respiration did have an impact on production of CO_2, still the production of CO_2 is much higher for *L. migratoria* than for the other insect species. A likely explanation for this higher production of CO_2 is the 7 °C higher temperature *L. migratoria* was kept at, as a difference of 10 °C is expected to double CO_2 production. Furthermore, the comparatively high ADG of *L. migratoria* is expected to result in higher production of CO_2.

In one of the repetitions for *A. domesticus*, a lower ADG and increased mortality were observed. Excluding this repetition, the emission of CO_2 per kg BM decreased slightly (68 vs 71 g/kg), but the emission of CO_2 per kg mass gain changed considerably (918 vs 1468 g/kg). This difference can for a large part be explained by a decrease in ADG (from 9.0 to 7.2 %). *Acheta domesticus* did not produce CH_4, but N_2O production doubled (from 0.1 to 0.2 mg/kg BM; 1.9 vs 5.3 mg/kg mass gain). The production of CO_2 eq. also increased (0.04 vs 0.05 g CO_2 eq./kg BM and 0.57 vs 1.57 g/kg mass gain). It is well possible that the higher N_2O production measured was caused by saprophytic bacteria utilising the dead *A. domesticus* and producing N_2O (Wrage et al. 2001). Although we included this repetition in the results, it is not clear whether this represents the practical situation best.

Large differences in NH_3 emission have been reported for conventional livestock. Pigs for example emit 4.8-75 mg/kg BM/day (Groot Koerkamp, Metz et al. 1998; Nicks, Laitat et al. 2003; Cabaraux, Philippe et al. 2009), poultry 72-436 mg/kg BM/day (Wathes et al. 1997; Groot Koerkamp et al. 1998; Demmers, Burgess et al. 1999) and cattle 14-170 mg/kg BM/day (Groot Koerkamp et al. 1998; Demmers, Phillips et al. 2001; Harper, Flesch et al. 2009). Several factors influence NH_3 emission, such as temperature, relative humidity, food type, moisture content, pH, wind speed, housing type, and substrate (Casey and Holden 2006; Faulkner and Shaw 2008).

In the current experiment, a clear NH_3 emission pattern was found; higher amounts of NH_3 were emitted during daytime for *A. domesticus*, *L. migratoria* and *B. dubia*, than during night-time. Day-night rhythms for NH_3 excretion have been documented for pigs (Aarnink et al. 1995) and are strongly correlated with activity levels (Blanes-Vidal, Hansen et al. 2008). Quantitatively the differences between day and night emission levels are small; 7-10% with a maximum difference of 25% (Aarnink et al. 1995). In our study this relative difference was approximately 33%. In all cases NH_3 emission levels were higher during the daytime than during the night-time. For *L. migratoria* this is the active period, for the nocturnal *B. dubia* and *A. domesticus* it is not, indicating that a different, unknown variable might influence NH_3 emission patterns in these insects.

NH_3 concentrations in the outgoing air, and consequently calculated NH_3 emission, increased from day one to day three in *B. dubia* (1.57 to 4.29 mg/kg BM/day) and *A. domesticus* (2.46 to 8.01 mg/kg BM/day). This could indicate that NH_3 emissions might be underestimated due to the relatively short time frame of our experiments. For *L. migratoria* NH_3 emission did not increase between day 1 and day 3 (5.57 and 5.05 mg/kg BM/day), suggesting that NH_3 production was stable. This might be caused by the faeces of this species that, contrary to those of *B. dubia* or *A. domesticus*, dry quickly after defecation.

We conclude that *P. marginata* and *T. molitor* probably did not emit NH_3. Poultry deep litter systems (Volkova, Bailey et al. 2009) have higher NH_3 emission rates than battery systems (Faulkner et al. 2008), which is explained by the presence of substrate.

The presence of substrates for *P. marginata* and *T. molitor* in this study corresponded with lower NH_3 emissions. A possible explanation is that gas exchange in the container is inhibited by the substrate and therefore less emission of NH_3 was measured. However, it could also be that these species produce less NH_3.

All insect species in this study produced much lower amounts of NH_3 (3.0 to 5.4 mg/kg BM/day for *A. domesticus, L. migratoria* and *B. dubia*) than conventional livestock (4.8-75 mg/kg BM/day for pigs and 14-170 mg/kg BM/day for cattle; Table 3). Further research is needed to determine for which insect species and to what extent NH_3 emissions increase further when a longer time frame is used.

Conclusions

To the authors' knowledge, the study presented here is the first to report on both GHG and NH_3 emissions of edible insect species. An evaluation of the GHG emissions of edible insect species is most relevant when based on CO_2 eq. per kg of mass gain. In that way a comparison of the selected species with each other and with conventional livestock is based on a cost-benefit principle, in which the GHG production (environmental cost) is directly linked to food production (benefit). GHG emission of four of the five insect species studied was much lower than documented for pigs when expressed per kg of mass gain and only around 1% of the GHG emission for ruminants.

The measured NH_3 emission levels of all insect species in this experiment were lower than reported NH_3 emission levels for conventional livestock.

The ADG of all insect species in this study was higher than for conventional livestock, while CO_2 production expressed as g/kg mass gain was comparable or lower, which indicates higher feed conversion efficiencies for insects.

This study therefore indicates that insects could serve as a more environmentally friendly alternative for the production of animal protein from the perspective of GHG and NH_3 emissions. A complete lifecycle analysis for species of edible insects is lacking at this point in time (de Boer 2008) and should be the focus point of further studies to allow a conclusive evaluation of the sustainability of insects as a protein-rich food source. The data presented in this study are indispensable for conducting a lifecycle analysis for edible insects.

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Table 1: Mean values and standard deviations of temperature, humidity, ventilation, hours of light per day and average start and final weight for five insect species.

	Pachnoda. marginata	*Tenebrio molitor*	*Blaptica dubia*	*Acheta domesticus*	*Locusta migratoria*
Temperature (ºC)	28.0 ± 0	25.0 ± 0	28.0 ± 0	28.0 ± 0	32.0 ± 0
Humidity (%)	84.3 ± 3.3	79.8 ± 0.2	70.0 ± 0.0	69.9 ± 0.1	69.7 ± 0.2
Ventilation (L/min)	6.46 ± 2.06	6.82 ± 1.31	5.16 ± 0.05	11.18 ± 1.80	4.98 ± 0.39
Hours of light per day	0	0	12	12	12
Start weight (kg)	0.99	0.91	1.10	0.96	0.08
Final weight (kg)	1.10	1.10	1.28	1.17	0.13

Table 2: CO_2 production (average ± standard deviation) per kilogram of body mass per day, per kg of mass gain and average daily gain for five insect species, pigs and beef cattle.

Species	CO_2 (g/kg BM/day)	CO_2 (g/kg mass gain)	ADG (%)
Pachnoda marginata (n=4)	50 ± 22 ^{a}	1,539 ± 518 ^{a}	4.0 ± 2.1% ^{a}
Tenebrio molitor (n=4)	61 ± 9 ^{b}	1,031 ± 349 ^{b}	7.3 ± 2.5% ^{b}
Blaptica dubia (n=3)	19 ± 3 ^{c}	337 ± 51 ^{c}	6.1 ± 0.7% ^{c}
Acheta domesticus (n=4)	68 ± 10 ^{d}	1,468 ± 971 ^{a}	7.2 ± 3.4% ^{b}
Locusta migratoria (n=6)	110 ± 21 ^{e}	734 ± 119 ^{d}	19.6 ± 2.1% ^{d}
Pigs	21.6 - 29.6	865 – 1,194	3.2 ± 0.53%
Beef cattle	5.3 - 7.0	2,835	0.3 ± 0.07%

BM = Body Mass

ADG = Average daily gain

Reported values for pigs and beef cattle were obtained from: Aarnink et al., 1995; Groot Koerkamp et al., 1998; Demmers et al., 2001; Nicks et al., 2003; Beauchemin & McGinn, 2005; Cabaraux et al., 2009 and Harper et al., 2009. Mean values bearing different superscripts in a column differ significantly ($P<0.05$).

Table 3: CH_4, N_2O, CO_2 eq. and NH_3 production (average ± standard deviation) per kilogram of body mass per day for five insect species, pigs and beef cattle.

Species	CH_4 (g/kg BM/day)	N_2O (mg/kg BM/day)	CO_2 eq. (g/kg BM/day)	NH_3 (mg/kg BM/day)
Pachnoda marginata (n=4)	0.16 ± 0.085 ^{a}	0.0 ± 0.03 ^{a}	4.00 ± 2.13 ^{a}	0.1 ± 0.16 ^{a}
Tenebrio molitor (n=4)	0.00 ± 0.002 ^{b}	1.5 ± 0.13 ^{b}	0.45 ± 0.04 ^{b}	0.0 ± 0.09 ^{a}
Blaptica dubia (n=3)	0.08 ± 0.021 ^{c}	0.3 ± 0.24 ^{a}	2.12 ± 0.57 ^{c}	3.0 ± 1.63 ^{b}
Acheta domesticus (n=4)	0.00 ± 0.002 ^{c}	0.1 ± 0.13 ^{a}	0.05 ± 0.04 ^{b}	5.4 ± 3.40 ^{c}
Locusta migratoria (n=6)	0.00 ± 0.017 ^{c}	8.0 ± 13.50 ^{b}	2.37 ± 4.02 ^{c}	5.4 ± 1.65 ^{c}
Pigs	0.049 - 0.098	2.7 - 85.6	2.03-27.96	4.8 – 75
Beef cattle	0.239 - 0.283	N/A	5.98-7.08	14-170

BM = Body Mass
N/A = Not Available

Reported values for pigs and beef cattle were obtained from: Aarnink et al., 1995; Groot Koerkamp et al., 1998; Demmers et al., 2001; Nicks et al., 2003; Beauchemin & McGinn, 2005; Cabaraux et al., 2009 and Harper et al., 2009. Mean values bearing different superscripts in a column differ significantly ($P<0.05$).

Table 4: CH_4, N_2O, CO_2 eq. and NH_3 production (average ± standard deviation) per kilogram of mass gain for five insect species, pigs and beef cattle.

Species	CH_4 (g/kg mass gain)	N_2O (mg/kg mass gain)	CO_2 eq. (g/kg mass gain)	NH_3 (mg/day/kg mass gain)
Pachnoda marginata (n=4)	4.9 ± 1.96^{a}	1.03 ± 1.06^{a}	121.86 ± 49.09^{a}	3 ± 4.8^{a}
Tenebrio molitor (n=4)	0.1 ± 0.03^{b}	25.5 ± 7.70^{b}	7.58 ± 2.29^{b}	1 ± 2.0^{a}
Blaptica dubia (n=3)	1.4 ± 0.30^{c}	5.7 ± 4.05^{a}	37.54 ± 8.01^{c}	54 ± 31.1^{a}
Acheta domesticus (n=4)	0.0 ± 0.09^{b}	5.3 ± 6.05^{a}	1.57 ± 1.80^{d}	142 ± 184.5^{b}
Locusta migratoria (n=6)	0.0 ± 0.11^{b}	59.5 ± 104.8^{c}	17.72 ± 31.22^{e}	36 ± 10.8^{a}
Pigs	1.92 - 3.98	106 - 3457	79.59 – 1,130	1140-1920
Beef cattle	114	N/A	2,850	N/A

BM = Body Mass
N/A = Not Available

Reported values for pigs and beef cattle were obtained from: Aarnink et al., 1995; Groot Koerkamp et al., 1998; Demmers et al., 2001; Nicks et al., 2003; Beauchemin & McGinn, 2005; Cabaraux et al., 2009 and Harper et al., 2009. Mean values bearing different superscripts in a column differ significantly ($P<0.05$).

Table 5: CO_2 production (g) per kilogram of metabolic weight per day for five insect species, pigs and beef cattle based on Kleiber's law ($B = aM^b$)

Species	b=0.67	b=0.75	b=0.82
Pachnoda marginata (n=4)	7	11	17
Tenebrio molitor (n=4)	3	7	12
Blaptica dubia (n=3)	2	4	6
Acheta domesticus (n=4)	4	8	14
Locusta migratoria (n=6)	9	17	29
Pigs	63	50	41
Beef cattle	50	31	21

Table 6: Calculated CO_2 production of provided feed for five insect species recalculated per kg of animal body mass.

Species	g CO_2/kg BM of insect	Relative contribution
Pachnoda marginata	46.2	92.46%
Tenebrio molitor	2.2	3.58%
Blaptica dubia	0.4	2.31%
Acheta domesticus	0.9	1.34%
Locusta migratoria	3.3	3.04%

Chapter 4

Environmental impact of the production of mealworms as a human protein source – A life cycle assessment

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Abstract

The demand for animal protein is expected to rise by 70-80% between 2012 and 2050, while the current animal production sector already causes major environmental degradation. Edible insects are suggested as a more sustainable source of animal protein. However, few experimental data regarding environmental impact of insect production are available. Therefore, a lifecycle assessment for mealworm production was conducted, in which greenhouse gas production, energy use and land use were quantified and compared to conventional sources of animal protein. Production of one kg of edible protein from either milk, chicken, pork or beef result in higher greenhouse gas emissions, require similar amounts of energy and require much more land. This study demonstrates that mealworms should be considered a more sustainable source of edible protein.

Key words: Life cycle assessment, climate change, land use, fossil fuel depletion, edible insect, mealworm, *Tenebrio molitor*, *Zophobas morio*

Introduction

The demand for food of animal origin is rising globally and is expected to increase by 70-80% between 2012 and 2050 (Steinfeld et al. 2006; Pelletier et al. 2010; Steinfeld 2012). Currently, the livestock sector uses about 70% of all agricultural land (Steinfeld et al. 2006; Foley et al. 2011) and is responsible for about 15% of the total emission of anthropogenic greenhouse gas (GHG) (Steinfeld et al. 2006; Steinfeld 2012). Expansion of agricultural acreage by land clearing is a major source of GHG emissions (Steinfeld et al. 2006; Pan, Birdsey et al. 2011) and one of the largest contributors to global warming (Godfray, Pretty et al. 2011). People's choices for certain diets influence GHG emissions and other environmental parameters (Carlsson-Kanyama 1998; Godfray et al. 2011). A suggested mitigation measure is a shift towards protein from lower impact animal species (McMichael, Powles et al. 2007; Pelletier et al. 2010; Foley et al. 2011). Various authors have suggested insects as an environmentally more friendly alternative to conventional livestock (Mercer 1997; Huis 2003; Ramos-Elorduy 2005). However, little data are available on the environmental impact associated with insect production. Husbandry contributions to GHG emissions is much lower for insects (2-122 g/kg mass gain) than for beef cattle (2850 g/kg mass gain), and in the lower range when compared to pigs (80-1130 g/kg mass gain) (Oonincx, van Itterbeeck et al. 2010b). However, this is only a part of the total GHG emissions in animal production chains. To choose among different sources of animal protein, GHG emissions, and other environmental parameters, such as land or fossil energy use, need to be assessed along the entire life cycle of these products. For mealworms, for instance, this includes GHG emissions related to feed production, or heating of the climate-controlled-rearing facility. Life cycle assessment (LCA) is a widely accepted method to quantify these parameters (Guinee 2001) and has been used for various animal products (de Vries et al. 2010). Although claims that insects are a more sustainable protein source than conventional livestock are widespread, to our knowledge, a LCA of any insect species used as a protein source has ever been published. The objective of this paper, therefore, was to quantify the environmental impact attributed to the production of two tenebrionid species, viz. the Yellow mealworm (*Tenebrio molitor*) and the Superworm (*Zophobas morio*). This impact was then compared to conventional sources of animal protein, such as milk, chicken, pork and beef.

Materials and methods

Through collaboration with a commercial mealworm producer in The Netherlands (van de Ven Insectenkwekerij, Deurne, The Netherlands), insight in the production process of mealworms was acquired. This farm produces two mealworm species in the same way and at equal volume. Therefore, we conducted a combined LCA for both species. First, the system boundary was defined (Figure 1). A cradle-to-farm-gate approach was chosen, which means that the environmental impact was assessed up to the moment that the fresh product leaves the farm gate.

An LCA relates the environmental impact of a product to a functional unit (FU). Comparing LCA results among animal products demands identical FU's. Mealworms, like other animal products, can nutritionally be seen as a source of protein. Therefore, we defined two FUs in our study: 1) kg of fresh product, and 2) kg of edible protein. To compute the amount of edible protein, we first multiplied the kg of fresh product with the average reported dry matter (DM) content (*T. molitor* 38%; *Z. morio* 43%) and the average percentage of reported crude protein in the dry matter (*T. molitor* 53%; *Z. morio* 45%) (Bernard et al. 1997; Barker et al. 1998; Finke 2002; Oonincx et al. 2010a). Subsequently, we multiplied with the edible portion, which we consider to be 100% for mealworms since they are consumed by humans as the whole animal. Protein content (Smil 2002; FAO 2007; de Vries et al. 2010) and edible portion (Smil 2002; de Vries et al. 2010) of conventional production animals vary depending on breed, country of production and other factors. In this study we used the data reported by de Vries et al. (2010).

We quantified three environmental indicators: 1) global warming potential (GWP), 2) fossil energy use (EU), and, 3) land use (LU). Global warming potential was expressed in CO_2-equivalents (CO_2-eq); the sum of CO_2, CH_4, and N_2O emissions. The conversion factor to CO_2-eq is 1 for CO_2, 25 for CH_4 and 298 for N_2O (IPCC 2007). Land use was expressed in m^2 per year, and fossil energy use in mega joules (MJ).

When several products stem from one production process, such as grain and straw, it is called a multifunctional production process. Its environmental impact is then allocated to the various outputs. Multifunctional processes included in our system were: (1) production of feed ingredients and their co-products, and (2) production of mealworms and its co-product manure. We allocated the impact of feed production to its outputs based on their relative economic value, whereas the impact of mealworm production was fully allocated to mealworms.

All inputs of the mealworm production system were quantified in this assessment. The production system, including the diet, is identical for both mealworm species (Figure 1). The diet consisted of fresh carrots and a mixed grain feed (i.e. wheat bran, oats, soy, rye and corn supplemented with beer yeast). For industrial competitive protection the exact composition of the diet is not disclosed. The feed conversion ratio (FCR) for concentrates was calculated by dividing the amount of concentrates used by the amount of live mealworms produced.

Egg cartons are used to increase the surface area for the adult mealworms. For the environmental impact of the egg cartons, data for recycled cardboard was used. A batch of twenty egg cartons was dried at 70ºC until a stable weight was reached and the average dry weight was assumed representative.

In order to create an optimal rearing environment, the climate-controlled rearing facility is heated and ventilated by the usage of natural gas and electricity from the Dutch power grid.

The quantities of all inputs and the output of the production system were disclosed by the mealworm producing company. Quantitative data for each input regarding GWP, EU, and LU (Table 1) were based on Ecoinvent (Ecoinvent 2004) and new data from the Dutch animal feed industry (Blonk and Research 2011). This data includes: the production, processing, and transportation of carrots, feed ingredients, and egg trays as well as the production, transportation and use of natural gas, electricity and water.

The LU of the farm and the direct GHG emissions from the mealworms were added to this data. Direct GHG emission for *Z. morio* and *T. molitor* were assumed equal and data from Oonincx et al. (2010b), in which the same diet was used, were assumed representative.

Finally, the total GWP, EU, and LU were divided by kg of fresh mealworm, or the kg of edible protein produced, resulting in an expression for both FUs.

Results

The Feed Conversion Ratio (FCR) for concentrates was 2.2 kg/kg of live weight in this study. The absolute and relative GWP, EU, and LU for the production of one kilogram of fresh mealworms based on economic allocation are provided in Table 2.

The GWP of one kg of fresh mealworms was 2.7 kg of CO_2-eq, of which 42% results from the production and transport of feed grains, 14% from the production and transport of carrots, 26% from gas used for heating, and 17% from the use of electricity.

The EU of one kg of fresh mealworms was 34 MJ, of which 31% results from the emission of production and transport of feed grains, 13% from the production and transport of carrots, 35% from gas used for heating, and 21% from the use of electricity.

The LU of one kg of fresh mealworms was 3.6 m^2 per year, of which 85% was required to cultivate feed grains, and 14% to produce carrots.

When expressed per kg of edible protein (Figure 2) the GWP was 14 kg of CO_2-eq, the EU was 173 MJ and the LU was 18 m^2. The relative contributions remain the same.

Discussion

Both mass and economic allocation can be used when describing the environmental impact of a product. Since the latter is more commonly used, values in this discussion are based on economic allocation allowing comparison with other food sources of animal origin.

Differences in environmental impact of products from pork, chicken, and beef are caused by three main factors; 1) enteric CH_4 production, 2) reproduction rate and 3) feed conversion efficiency (de Vries et al. 2010).

Mealworms do not produce CH_4 (Oonincx et al. 2010b) and have a high reproduction rate; 160 eggs in 3 months for one female of *T. molitor* and 1500 eggs in one year for a *Z. morio* female. Furthermore, the maturation period is short; *T. molitor* reaches adulthood in 10 weeks and *Z. morio* in 3.5 months (Friederich et al. 2004).

Feed conversion efficiency depends, amongst other things, on the diet provided. The feed conversion ratio (FCR) for concentrates (kg/kg of fresh weight) for the mealworms in this study (2.2) was similar to values reported for chicken (2.3) but lower than for pigs (4.0) and beef cattle (2.7-8.8) (Wilkinson 2011).

Over the last two decades productivity of chickens and pigs has increased annually by 2.3%, due to the application of science and new technologies (Steinfeld and Gerber 2010). Further improvement of the mealworm production system by, for instance, automation, feed optimization or genetic strain selection is expected to increase productivity and decrease the environmental impact. Since these aspects are currently underdeveloped, the potential rate of productivity improvement is expected to be higher for mealworms compared to the more common production animals.

Processing and storage has a large effect on the environmental impact of a food product: over 60% of the GWP for carrots is attributed to storage (Carlsson-Kanyama 1998); emissions during slaughtering, transportation and storage of pork and chicken contribute 17-25% to the total GWP (Carlsson-Kanyama 1998; de Vries et al. 2010). For mealworms, used for human consumption, there is currently no standard method for processing and storing.

We assessed three indicators to provide insight in the sustainability of mealworm production and compared these to literature values (Figure 2). The GWP of mealworms per kg of edible protein is low compared to milk (1.77-2.80x as high), chicken (1.32-2.67x as high), pork (1.51-3.87x as high) or beef (5.52-12.51x as high) (de Vries et al. 2010). The EU of mealworm production per kg of edible protein is higher than for milk (21-83% of the value for mealworm) or chicken (46-88% of the value for mealworm), similar to pork (between 55 and 137% of the value for mealworm) and lower than for beef (1.02-1.58x as high). Mealworms, being poikilothermic, depend on suitable ambient temperatures for growth and development. When ambient temperatures are low, heating is required, increasing energy use. Mitigation measures are being investigated: larger larvae in this system produce a surplus of metabolic heat, which could be used to heat the heat-demanding small larvae.

The LU of the described production system was very low compared to milk (1.81-3.23x as high), chicken (2.30-2.85x as high), pork (2.57-3.49x as high higher) and beef (7.89-14.12x as high).

We consider the low LU of mealworms to be particularly important; effects of GHG emissions can be countered by carbon fixation (Piao, Fang et al. 2009), and forest regrowth and afforestation (Wise, Calvin et al. 2009; Pan et al. 2011), depletion of fossil fuels can be countered by usage of alternative sources of energy (Tilman, Socolow et al. 2009; Graham-Rowe 2011), but land availability is fixed and limited. Expansion of agricultural land is a major source of GHG production especially in tropical regions (Foley et al. 2011; Godfray et al. 2011; Pan et al. 2011). Slowing down the expansion of agricultural land is a critical step towards sustainable agriculture (Foley et al. 2011). The increasing world population will therefore need to be fed using the same area of land that is available now (Godfray et al. 2011). Mealworms require only 43% of the amount of land used for the production of one kg of edible animal protein as milk, and only 10% of the land used for production of beef.

Conclusions

The EU of mealworm production is higher than for milk or chicken and similar to pork and beef. However, mealworm, when considered as a human protein source, produces much less GHG's and requires much less land than chicken, pigs and cattle. With land availability being the most stringent limitation in sustainably feeding the world's population, this study clearly shows that mealworm should be considered as a more sustainable alternative to milk, chicken, pork and beef.

Acknowledgements

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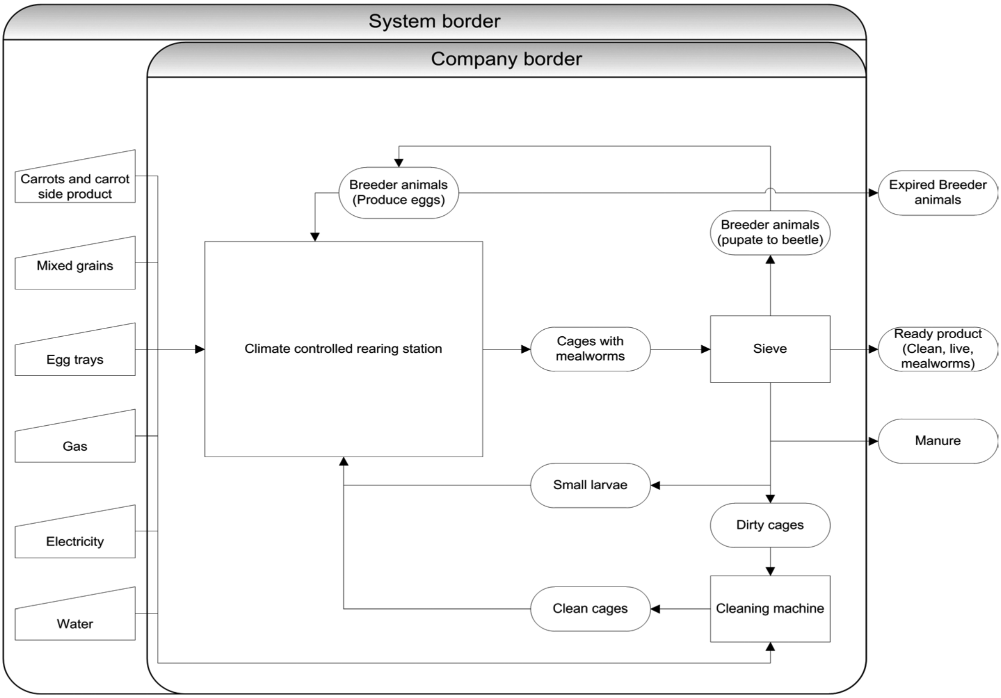

Figure 1: The mealworm production system. Flows entering the company are on the left, centrally the production steps are shown and flows exiting the system are on the right. For flow quantities see Table 1.

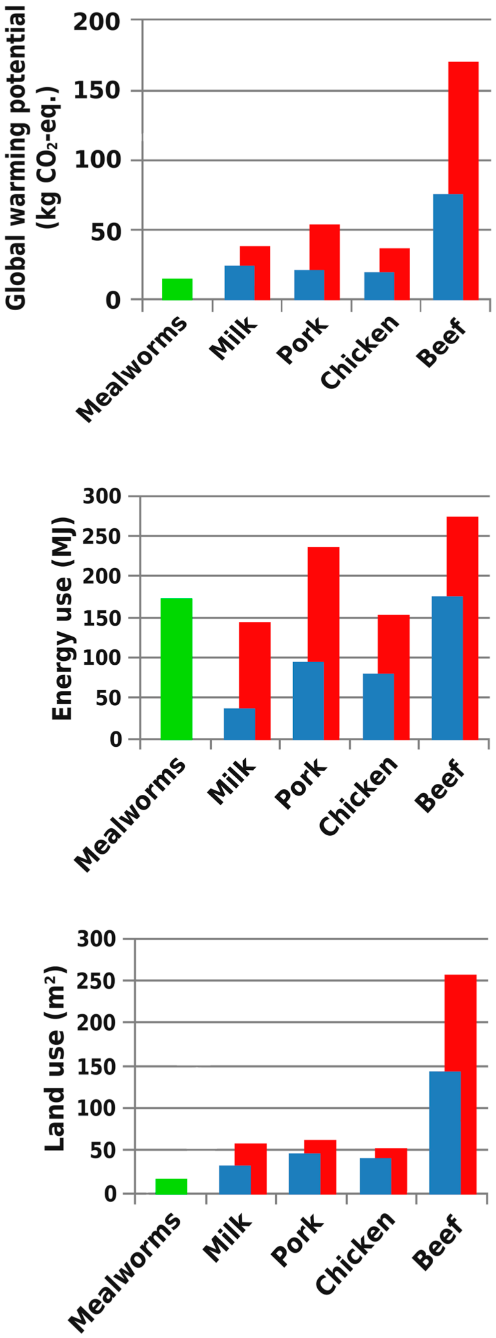

Figure 2: Environmental impact of mealworms compared to other animal products. Global warming potential, energy use, and land use due to the production of one kg of edible protein. Results from this study depicted in green. Minimum (blue) and maximum (red) literature data is adapted from de Vries & de Boer (2010).

Table 1: Resource use per year and environmental impact. Global warming potential (GWP), energy use (EU) and land use (LU) are expressed per unit of input based on economic allocation.

Resource	Turnover / Year	GWP kg CO_2-eq	EU MJ	LU m^2
Carrots (kg)	260000	0.12	1.38	0.16
Mixed grains (kg)	182000	0.51	4.79	1.39
Egg trays (kg)	262	0.98	13.70	0.10
Gas (MJ)	811200	0.07	1.20	0.00
Electricity (MJ)	187200	0.20	3.17	0.00
Water (M3)	211	0.32	5.55	0.04
Animal (kg)	83200	0.01	0.00	0.00
Farm	1	0	0.00	588

Table 2: Environmental impact of inputs in a mealworm production system. Absolute and relative contribution global warming potential (GWP), energy use (EU) and land use (LU) for the production of one kg of fresh mealworms based on economic allocation

	GWP (kg CO_2-eq)		EU (MJ)		LU (m^2)	
Carrots (kg)	0.38	14.27%	4.31	12.80%	0.51	14.39%
Mixed grains (kg)	1.11	41.98%	10.47	31.09%	3.03	85.14%
Gas (MJ)	0.70	26.26%	11.71	34.77%	0.00	0.02%
Egg trays (kg)	0.00	0.12%	0.04	0.13%	0.00	0.01%
Electricity (MJ)	0.45	17.06%	7.13	21.17%	0.01	0.24%
Water (M^3)	0.00	0.03%	0.01	0.04%	0.00	0.00%
Animal (kg)	0.01	0.29%	0.00	0.00%	0.00	0.00%
Farm	0.00	0.00%	0.00	0.00%	0.01	0.20%
Total	2.65	100.00%	33.68	100.00%	3.56	100.00%

Chapter 5

Feed conversion efficiency, survival, crude protein content and lipid composition of four insect species on diets composed of organic by-products

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Submitted

Abstract

Insects are suggested to efficiently convert feed to body mass. Environmental impact of animal production systems is for a large part determined by the type and amount of feed used and the efficiency with which this is converted into body mass. Therefore, insects might be a food and/or feed source with low environmental impact. Newly hatched Argentinean cockroaches, Black soldier flies (BSFs), Yellow mealworms, and House crickets were subjected to four dietary treatments. The first two species are potentially interesting as a feed ingredient, while the latter two are considered edible for humans. Diets were composed from industrial organic by-products and formulated such as to vary in protein and fat content. Feed conversion efficiency, survival, development time, as well as chemical composition (N, P, and fatty acids), were determined. The Argentinean cockroaches and the BSFs converted feed more efficiently than Yellow mealworms and House crickets. The first two were also more efficient than conventional production animals. On three of the four diets feed conversion efficiency for Yellow mealworms and House crickets was similar to that reported for pigs. On the most suitable diet, feed conversion efficiency was similar to that of poultry. All four insect species had a higher N-efficiency than conventional production animals. Offering carrots to Yellow mealworms increased dry matter- and N-efficiency and decreased development time. Survival was affected by diet in all species but BSFs. Development time was strongly influenced by the diet offered. The chemical composition of Argentinean cockroaches proved to be highly dependent on diet. BSFs on the other hand, thrived on all diets, attaining similar body composition. Hence, the investigated species are efficient production animals and interesting as a source of feed or food. The diet provided resulted in compositional differences in the insect species, indicating possibilities to tailor it to meet consumers' needs.

Key words: Feed conversion efficiency, insect, by-product, crude protein, phosphorus, fatty acid, entomophagy

Introduction

Several insect species can be produced for food and/or feed (Bondari and Sheppard 1981; Sheppard et al. 1994; Collavo et al. 2005; Hanboonsong et al. 2013). Conventional animal production systems contribute greatly to anthropogenic greenhouse gas production and use vast amounts of fossil energy and arable land (Steinfeld et al. 2006; Steinfeld 2012). For a large part, these indicators of environmental impact are determined by the amount of feed used during animal production (de Vries et al. 2010; Oonincx and de Boer 2012a). Therefore, the environmental impact associated with production of the feed, and the efficiency with which this feed is converted into body mass, strongly influences the environmental impact of animal production systems. One of the suggested advantages of insects over conventional production animals such as chicken, pigs and cattle, is a higher feed conversion efficiency because they are poikilothermic (Nakagaki et al. 1991; Collavo et al. 2005; Ramos-Elorduy 2008). However, this efficiency depends on a variety of factors, such as the species and the diet consumed. The same diet may result in different feed conversion efficiencies for different species due to differences in digestive systems and nutrient requirements (Cohen 2003). Furthermore, diet composition also affects development rate and body nutrient composition (McFarlane, Neilson et al. 1959; Oonincx et al. 2011; Finke and Oonincx 2013; Gobbi, Martínez-Sánchez et al. 2013). To quantify these variables, four insect species, two edible for humans and two suitable as animal feed, were selected.

When several products originate from one production process, the environmental impact of this process is allocated to the various products, which can be done based on their respective economical values (de Vries et al. 2010). This means that industrial by-products, with lower economic value than the main product, are considered to have lower environmental impact. These industrial by-products are becoming increasingly important feed ingredients (Wilkinson 2011). Their usefulness depends, amongst others, on how efficiently they are converted to body mass by the production animal. Combinations of by-products could make suitable insect diets. Hence, four diets, differing in protein and fat content, were composed from several by-products. The objectives of the experiment were to: 1) compare the feed conversion efficiency of several insect species to be used as production animals, and 2) determine the effects of diet composition on survival, development time, and chemical composition of these species.

Materials and methods

Insects

Four insect species were selected: the Argentinean cockroach (*Blaptica dubia* (Serville); Dictyoptera: Blaberidae), Black soldier fly (*Hermetia illucens* (L.); Diptera: Stratiomyidae), Yellow mealworm (*Tenebrio molitor* (L.); Coleoptera: Tenebrionidae), and the House cricket (*Acheta domesticus* (L.); Orthoptera: Gryllidae). Adult Argentinean cockroaches were provided by a private Dutch insect breeder and were checked daily for new-born offspring. Newly hatched nymphs of the House cricket and larvae of the Black soldier fly (BSF) were taken from colonies maintained at the Laboratory of Entomology, Wageningen University. These two species had been reared on chicken feed for over four years (Opfokmeel farmfood, Agruniek Rijnvallei Voer BV, Wageningen, The Netherlands). Mealworm eggs were provided by Kreca V.O.F. (Ermelo, The Netherlands). For all species, larvae or nymphs were randomly allocated to control and experimental groups within 24 hours of egg hatch (*T. molitor, A. domesticus* and *H. illucens*) or birth (*B. dubia*).

Diet preparations

The following industrial by-products deemed suitable as feed ingredients were selected: beet molasses (Royal Cosun, Breda, The Netherlands), potato steam peelings (Hedimix BV, Boxmeer, The Netherlands), spent grains and beer yeast (Anheuser-Busch, Dommelen, The Netherlands), bread remains (Bakkersland BV, Hedel, The Netherlands), and cookie remains (Banketbakkerij van Strien, Oud-Beijerland, The Netherlands). Bread and cookie remains were cut by means of a Hobart 8145 cutter (Hobart Nederland BV, Woerden, The Netherlands). All ingredients were subsequently freeze-dried in an Edwards Lyofast S 08 Freezedryer (A. de Jong TH BV, Dordrecht, The Netherlands). From these ingredients four experimental diets; with either a high, or a low protein content (HP or LP), and a high, or a low fat content (HF or LF) were formulated (Table 1). Diets were mixed in a Magimix CS 5200 food processor (Magimix LTD, Surrey, UK). Each species had its own control diet, named after the initials of their scientific names. For Yellow mealworms two control diets (TM1 and TM2) were available. Both these diets were used in large scale production, but differences in colour and structure suggested differences in composition. Hence, both were tested. Kreca VOF provided a control diet for the Argentinean cockroaches (BD), Yellow mealworms (TM1) and House crickets (AD). Van de Ven Insectenkwekerij (Deurne, The Netherlands) provided the second control diet for Yellow mealworms

(TM2). For BSFs the chicken feed diet used for the BSF colony at the Laboratory of Entomology of Wageningen University (Wageningen, The Netherlands) served as a control diet (HI). Diets were stored at -20°C.

Experimental setup

Ten Argentinean cockroach nymphs were placed in a plastic container (17.5 x 9.3 x 6.3 cm) with aeration slits on the sides. A piece of egg carton was placed in the container to provide a hiding place for the nymphs. Nymphs were provided with four grams of either an experimental diet, or control diet (BD). Moisture was provided three times per week by applying a few drops of tap water in a corner of the container.

One hundred BSF larvae were placed in a plastic container (17.8 x 11.4 x 6.5 cm) of which the sides were manually perforated to allow air flow. Larvae were provided with four grams of either an experimental diet, or control diet (HI). For each gram of diet, approximately two ml of water was added by means of a syringe.

Fifty Yellow mealworm larvae were placed in a plastic container (17.5 x 9.3 x 6.3 cm) with aeration slits on the sides. Larvae were provided with one gram of either an experimental diet or a control diet (TM1or TM2). In some rearing facilities, this species is provided with carrot as a source of moisture (Oonincx et al. 2012a). Therefore, the effect of providing carrot was tested for each experimental and control diet. For the larvae that were allocated to a treatment with carrot, 0.30 g of carrot was provided, three times per week.

Fifty House cricket nymphs were housed in a plastic cage (35.6 x 23.4 x 22.8 cm; Faunarium type pt2665, Hagen, Holm, Germany), with aeration slits in the lid. To increase surface area, two layers of hollow plastic tubes (20 cm long and 3 cm in diameter) were placed in each cage. Nymphs were provided with one gram of either an experimental diet or control diet (AD). Furthermore, a water dispenser (Gebroeders de Boon, Gorinchem, The Netherlands), with a piece of tissue paper placed in the opening to prevent drowning, was placed in each cage.

For each species, six replicate containers per dietary treatment were set up, after which the containers were placed in a climate chamber at 28ºC with a relative humidity of 70% and a photoperiod of 12 hours.

Three times per week, all containers were checked visually. If the feed provided was almost depleted, as indicated by changes in colour and particle size, more was added to ensure *ad libitum* feeding. Water for the House crickets was replenished when required.

All insects were harvested per container when the first (pre-)pupa (BSF or Yellow mealworm), or adult (Argentinean cockroaches and House crickets) was observed. This moment was chosen because most specimens per container would be expected to be in their last larval or nymphal stage, which is when BSFs and Yellow mealworms are normally sold, and when House crickets have the highest edible portion (Nakagaki et al. 1991). The same moment was chosen for Argentinean cockroaches.

Chemical analysis

Nitrogen (N) and phosphorus (P) content of the diets, insects and faeces were determined according to Novozamsky, Houba et al. (1984), unless insufficient sample was available. In that case N content was determined according to Patton and Kryskalla (2003), and P content according to Rowland and Haygarth (1997). Fatty acids were extracted according to Folch, Lees et al. (1957) and the fatty acid profiles were determined according to Raes, De Smet et al. (2001).

Calculations and statistics

There are different ways in which feed conversion efficiency can be expressed. The most common measure in animal production systems is the Feed Conversion Ratio (FCR), which is the amount of feed needed (in kg), for one kg of weight increase of the production animal. Entomologists, however, commonly use Efficiency of Conversion of Ingested food (ECI) as a measure for feed conversion efficiency on a dry matter (DM) basis. ECI is calculated as (weight gained / weight of ingested food) * 100% (Waldbauer 1968). For all species it was assumed that all provided feed had been ingested. Both the FCR and the ECI can be calculated on a fresh and a DM basis, and can also be used for specific nutrient conversion efficiencies. In this paper, FCR is expressed on a fresh weight basis, whereas ECI is expressed on a DM basis. FCRs for concentrates (feeds with a high nutrient density) exclude the weight of provided carrots.

Crude protein (CP) content was calculated by multiplying the nitrogen content by 6.25. Total fatty acid content (TFA) was calculated by summing the contents of individual fatty acids. Nitrogen conversion efficiency (N-ECI) was calculated as the amount of N in the insects at the moment of harvest, divided by the total amount of N ingested, calculated by multiplying the N-content of the diet by the total weight of diet provided.

Significant differences between treatments ($P < 0.05$) were determined by means of a Kruskall-Wallis test and subsequently a Scheffé test was used for posthoc testing. If only one sample was analysed for a dietary treatment, this diet was excluded from the dataset for post-hoc testing. Correlations between parameters were determined by Pearson correlation tests. Statistical analysis for all data was performed using SPSS 19.0.

Results

Diet composition

The DM content of all diets was 88-95% of the fresh mass (Table 2). The carrots, used as a source of moisture for mealworms in the carrot (C) treatments, had a DM content of 9%. Diets differed considerably in CP and TFA contents and FA profiles (Table 3). Crude protein content was 22-23% DM in the HP diets, 17-19% DM in the control diets and 13-14% DM in the LP diets. The TFA content was 10-15% DM in the HF diets, 3-5% DM in the control diets , and 1-2% DM in the LF diets. The most prevalent FAs in the diets were C13:0, C16:0, C18:0, C18:1n9c, and C18:2 n6c, with the latter being especially abundant in control diets (30-48% of TFA; Table 3). The main FA in the HPLF diet was C13:0 (62% of TFA). In the control diets it accounted for 12-22% of TFA, and only 4-6% of TFA in the HF diets. In the HF diets, C14:0 was present in larger concentration (5-9% of TFA) than in the other diets (< 1%).

Feed conversion ratio and ECI

The FCR of Argentinean cockroaches was higher on BD than on HF and LPLF diets (Table 4). On the LPHF diet the Argentinean cockroaches had the highest ECI of all species. Both this species and the BSFs generally had low FCRs and concomitantly high ECIs compared to the other two species. The BSFs tended to use the HP diets and the HI diet more efficiently than the LP diets ($P = 0.051$). Yellow mealworms had higher FCRs on all diets than Argentinean cockroaches and BSFs. Provision of carrot increased FCRs on the LP diets, and ECIs on all diets. For House crickets, FCRs did not vary due to dietary treatment. Their FCRs were similar to mealworms, but the ECI of the House crickets was the lowest of the species studied. Nitrogen conversion efficiency (N-ECI) differed greatly between species and between dietary treatments. Argentinean cockroaches provided with the LPHF diet had the highest N-ECI (87%). On the other diets N-ECI for this species was between 51 and 66%, which is slightly higher than for BSFs (43-55%). Yellow mealworms had a N-ECI between 22 and 31% without carrot supplementation, but when carrot was provided it increased to 35-38%. For House crickets N-ECI was lower on the HPHF diet than on the AD diet (23 *vs.* 41%).

Survival rates and development time

The survival rate of Argentinean cockroaches, and Yellow mealworms were strongly affected by diet (Table 4). For Argentinean cockroaches on the HPLF diet, the survival rate was much lower than on the HPHF diet (47 *vs.* 80%). In contrast, survival of BSFs was high on all five diets (72-86%). Survival rate of Yellow mealworms was highest on the HP and TM1 diets. Addition of carrot resulted in uniform survival rates ≥ 80%, except for the LPHF diet, which resulted in very low survival (> 20%). For House crickets, survival rate was low on all diets, except for the AD diet, in which 55% survived. Development times were strongly affected by diet (Table 4). For Argentinean cockroaches development took ca. seven months on the HPHF and BD diets, while this was prolonged to almost ten months on the HPLF diet. BSFs developed in three to five weeks, with HPHF and HI diets resulting in shorter development times than the LP diets. Yellow mealworms developed in 12 to 32 weeks. Addition of carrot shortened development time on all diets. Regardless of supplementation with carrot, the HP diets and the TM1 diet resulted in shorter development times than LP diets. The development time of House crickets in our study varied substantially and was between 7 and 24 weeks (AD and LPHF diet, respectively). Higher survival rates correlated with shorter development times for the Argentinean cockroaches ($R = -0.708$; $P < 0.001$), Yellow mealworms ($R = -0.524$; $P < 0.001$), and House crickets ($R = -0.718$; $P = 0.001$), but not for BSFs.

Dry matter and nutrient content

The DM content for Argentinean cockroaches varied between 28 and 39% of the fresh matter (Table 5). For Yellow mealworms a similar range was found, with lower DM percentages on diets with carrots. For House crickets the DM content was lower than for the other species (~25%).

Crude protein content differed between species ($P < 0.001$). It was the lowest in BSFs, followed by Yellow mealworms, whereas Argentinean cockroaches and House crickets had higher CP contents. On the HPLF and BD diets, CP content of Argentinean cockroaches was almost twice as high as on the LPHF diet, while the HPHF and LPLF diets resulted in intermediate CP contents. For BSFs, CP content was less variable, although on the HPHF diet CP content was higher than on the LP diets. For Yellow mealworms, CP content was similar on most diets.

However, the LPHF diet supplemented with carrot, resulted in a lower CP content than the HP and TM1 diets (irrespective of the provision of carrot). For House crickets, data on CP were available only for the HPHF and AD diet which resulted in similar levels (58-59% DM). When analysed for the four species together, CP and P content are not correlated ($P = 0.572$). They are, however, in the Argentinean cockroach ($R = 0.776$; $P = 0.001$), BSF ($R = 0.827$; $P < 0.001$), and Yellow mealworm ($R = 0.546$; $P = 0.001$), but this was not shown in House crickets ($P = 0.11$).

Argentinean cockroaches had a lower P content than the other species. For this species the lowest P content was found on the LPHF diet. The P content of BSF on the HI diet was similar to HP diets but higher than those reared on LP diets. For Yellow mealworms P content differed only between the TM1 and TM2 diets, when supplemented with carrots.

No species differences were found regarding TFA content. Within species, however, dietary treatment did affect TFA content. For instance, Argentinean cockroaches on the LPHF diet contained double the amount of fatty acids compared to the other diets. For BSFs the TFA content did not vary over dietary treatments, and values were in the same range as for Yellow mealworms. For the latter species the TM2 diet, irrespective of carrot provision, resulted in a higher TFA content than the HPLF diet and the HP diets supplemented with carrot. House crickets had a low TFA content, values being similar to Argentinean cockroaches on most diets, except for the LPHF diet. The ratios between the TFA and CP content were strongly influenced by diet, but also species differences were apparent (Figure 1).

Fatty acids contributing > 0.5% to TFA are reported in Table 6. Due to a limited amount of sample, FA data on House crickets is available for only three diets. None of the insects species had detectable levels of C4:0, C6:0, C8:0, C11:0, or C22:1n9. C10:0 was only detected in BSFs (0.8-1.3% of TFA). Although C12:0 was present in all insect samples, in BSFs they contributed between a third to half to TFA, while this was ≤ 0.5% in the other species. While C16:0 concentrations were affected by diet, similar ranges were found for all species. The contribution of C18:0 to TFA was highest in House crickets, followed by Argentinean cockroaches and Yellow mealworms, while for BSFs this was lowest ($P < 0.001$). The main FA in Argentinean cockroaches and Yellow mealworms was C18:1n9c, while the relative concentration was much less in BSFs, and House crickets ($P < 0.001$). Large variation due to dietary treatment was apparent for C18:2n6c in all species, but especially for the Argentinean cockroaches and BSFs. In House crickets the main FA was C18:2 n6, although C16:0 and C18:1n9 were also present in high concentrations. Together these made up ≥ 75% of TFA.

In House crickets C20:3n3 contributed 0.4% to TFA on the three diets analysed, and C22:6n3 contributed 0.1% to TFA on the HP diets, whereas these

FAs were not detected in other species (Data not shown). Furthermore, both House crickets on HPHF, and BSFs on LPHF contained 0.1% C20:5n3, while in the other species this FA was not detected.

In all species investigated, n3 FAs were present in concentrations of 1.5%, or less, while n6 FAs were present in higher concentrations (Table 6). The diets provided in our experiment differed in their n6/n3 ratio, and resulted in different n6/n3 ratios in the insects tested (Table 7). Besides dietary effects, species specific effects were apparent; e.g. on most diets BSFs had a relatively low n6/n3 ratio. While provision of carrot decreased the n6/n3 ratio in the Yellow mealworm, they had the highest n6/n3 ratio on all diets tested.

Discussion

Most feed conversion studies describe the provision of different diets within a species. This is the first study that compares feed conversion efficiency on four diets for four insect species, and reports on life history traits, as well as on effects on chemical composition.

Feed conversion efficiency

The FCRs of the species used as animal feed (Argentinean cockroaches and BSFs) was lower than for the species suitable for human consumption (Yellow Mealworms and House crickets). For conventional production animals, FCRs for concentrates are reported to be 2.3 for poultry, 4.0 for pigs, and 8.8 for cereal beef (Wilkinson 2011). The Argentinean cockroaches and BSFs had more favourable FCRs than these conventional production animals and offer potential economic and environmental benefits as a protein source in animal feed. Their added value would, however, strongly depend on whether their diet could also be provided directly to the conventional production animals, and how efficiently it would be used by these animals. The FCR for concentrates of Yellow mealworms was between 1.8 (HPHF with carrots), and 3.1 (HPLF with carrots), and similar to commercially produced mealworms (2.2) provided with carrots (Oonincx et al. 2012a). These FCRs, and the FCR of House crickets on the AD diet are similar to poultry, which are efficient production animals (Wilkinson 2011). Diet composition is the main variable determining feed conversion efficiency for a given insect species (Scriber and Slansky 1981). For over 65 species, ECIs between 6 and 43% were reported (Slansky 1985), which is a similarly large range as found in our experiment. In our experiment N-ECI > ECI for all species and dietary treatments, indicating that N was more efficiently converted to body mass than other diet components. Compared to conventional production animals (12% for cereal beef and 33% for poultry), the N-ECI of all four species studied was high (Wilkinson 2011). With a 50% increase in demand of animal based protein expected by 2050, this high N-ECI might well be the most relevant benefit of insects over conventional production animals (Herrero and Thornton 2013b). Whereas in conventional production animals energy content of the feed determines growth rates and efficiencies, in insects protein composition and density seem to be more important (Friend 1958; House 1961; Herrero, Havlík et al. 2013a). Indeed, HP diets resulted in lower FCRs and higher ECIs for most species. However, in the Argentinean cockroaches, the HF diets resulted in the most efficient weight gain. The Argentinean cockroaches had a high N-ECI , especially on the LPHF diet. Both Argentinean and American cockroaches (*Periplaneta americana*) produce methane by means of endosymbionts (Hackstein et al. 1994; Oonincx et al. 2010b). The cockroach endosymbiont *Blattabacterium* converts nitrogenous waste products, such as uric acid, to amino acids, and vitamins, which explains the high N-ECI in American cockroaches (51-83%) and possibly in our Argentinean cockroaches (Mullins and Cochran 1975). In the Yellow mealworms addition of carrot to the diet greatly increased their N-ECI and ECI, while FCR was similar on most diets. The high water content of carrots might be expected to increase the FCR for all carrot-supplemented diets, but this was only evident for the LP diets. In these diets protein content probably limited development rate, resulting in longer periods of carrot provision, and hence the provision of larger amounts of carrot. Furthermore, development time of the Yellow mealworms was shortened in all our treatments, compared to treatments where no carrot was provided. More efficient and faster development was previously reported in Yellow mealworms when a source of water was provided (Fraenkel 1950). Without carrots, Yellow mealworm FCRs were similar to those of House crickets. Published FCR values for House crickets (1.7-2.8) (Collavo et al. 2005; Ramos-Elorduy 2008) were low compared to our results. Furthermore, their low DM content result in the lowest ECIs of the species studied, which were also low compared to literature values for last stage nymphs and adults (20-38%) (Woodring et al. 1977; Woodring et al. 1979; Clifford and Woodring 1990). This could indicate that water provision was suboptimal, and affected the ECI similarly as in the Yellow mealworms. However, it could also be due to a densovirus (AdDNV), present in most European and North-American House crickets production facilities, which interferes with nutrient absorption, increases mortality and decreases growth rates (Liu, Li et al. 2011; Szelei, Woodring et al. 2011; Pham, Yu et al. 2013).

Development time and survival rate

Development time and survival rate are indicators of the nutritional quality of diets (Cohen 2003), hence it is expected that these parameters are strongly correlated. Whereas all diets were accepted by the four species, development time and survival differed within species depending on dietary treatment. In BSFs the survival rate was high on all diets and dietary quality only affected development time. On a high quality diet such as chicken feed, or a diet designed for houseflies, BSF larvae develop in two to three weeks (Furman, Young et al. 1959; Diener, Zurbrugg et al. 2009). This is similar to our HPHF and HI diet, but on the other diets this was extended to approximately five weeks, indicating a lower dietary quality (Cohen 2003; Gobbi et al. 2013). The Yellow mealworms developed quicker and survival was higher on HP diets than on LP diets, while the TM diets were intermediary. This suggests that protein content is a determining factor for development and survival. A further factor could be the inclusion of beer yeast in the HP diets, which works as a feeding stimulant (Murray 1960), and contains important growth factors for Yellow mealworms (Fraenkel 1950).
The development times and survival of Yellow mealworm larvae on the TM and HPHF diets were similar to those reported by Urs and Hopkins (1973b) and Urs and Hopkins (1973a) (64-154 days and 69-81%, respectively). In this species development time was shortened and survival increased when carrot was provided as a water source. The increases in survival and shortening of development time could be due to the provision of moisture as was shown by Urs et al. (1973a), although nutrients such as β-carotene could also have influenced these parameters (Cohen 2003).

Development times of the House crickets on the AD and HPHF diet were similar to literature values (31-80 days) (McFarlane 1962; Patton 1967; Patton 1978; Clifford et al. 1990; Collavo et al. 2005), but was strongly prolonged on the other diets. Furthermore, survival rates were low in this species on all diets, with the possible exception of the AD diet. Whereas older studies report that House crickets do well on most animal feeds or poultry mashes (Clifford et al. 1990), that a 20% CP content is sufficient to support growth (Woodring et al. 1979), and that survival can be up to 80% (McFarlane 1962), a more recent study reports survival rates similarly low (24-47.5%) as our study (Collavo et al. 2005). These differences might be due to the previously mentioned densovirus.

Crude protein, total fatty acids and phosphorus content

Crude protein and TFA content varied between species and depended on the diet. BSFs and Yellow mealworms had a low CP content and had considerable variation in their TFA content (Figure 1). House crickets and, on most diets, the Argentinean cockroaches, had a high CP content and a concomitant low TFA content. On the LPHF diet, however, the Argentinean cockroaches had a much lower CP content and a high TFA content. In American cockroaches uric acid (which contains N) is stored when provided with high protein diets (Mullins et al. 1975). If this is also the case in Argentinean cockroaches, CP determinations (which are based on N content), would lead to an overestimate of true protein, especially on HP diets. No comparative compositional data are available for Argentinean cockroaches, but the plasticity in TFA content shown in this study is much larger than in the other three species. For those three species CP and TFA contents were within published values, however, the P content of BSFs were lower, and for Yellow mealworms they were higher (Table 8).

Although CP and P content were correlated within a species, they were not over species, which suggests species-specific CP and P ratios. Because in most diets higher CP contents coincided with higher P contents, this could also have been a dietary effect. Further studies are needed to divulge the relationship between CP and P.

Fatty acid composition

Fatty acid profiles depended on species and diet (Figure 2). The most striking difference is the high concentration of C12:0 in the BSFs, whereas the other species contained only low levels of this FA. Fatty acids with a 16-carbon chain are suggested to predominate in Dipterans (Fast 1970), however, in the BSFs in this study C12:0 was dominant. Also C14:0 was present in higher levels than in the other species investigated. Overall, FA profiles of BSFs in this study were similar to published values (Table 8), although C12:0 concentrations were higher and C18:1n9c concentrations were lower. The FA profiles of the BSFs did not follow the dietary FA pattern. BSFs on the HF diets, rich in C18:1n9c, did retain more of this FA than on the other diets, but the LPLF diet, resulted in relatively low concentrations of C18:1n9c. It appears in BSFs a large proportion of FAs is metabolized to C12:0 when lower amounts of fat are provided, whereas FAs are stored in their dietary form when higher amounts are provided, indicating limited possibilities to tailor the FA profile of BSFs.

In Argentinean cockroaches C18:1n9c was the main FA, which concurs with FA profiles published by Fast (1970) for several cockroach species (30-55% of TFA), although the concentration of C16:0 was higher in that study (23-40% of TFA). For a large part FA profiles reflected the FA profile of the diet in this species, although C18:1n9c seemed to have been selectively accumulated on the HPLF diet.

Also Yellow mealworms and House crickets were rich in C18:1n9c, although the House crickets contained more C18:2n6c. Our findings were similar to published FA profiles for these two species (Table 8). Whereas the provision of carrot strongly influenced N-ECI in Yellow mealworms, it did not influence their general FA profile, which agrees with the results of Urs et al. (1973b) on water provision to Yellow mealworms. Yellow mealworm fat composition seems to be fairly constant.

Although we determined the FA profiles for House crickets only on the AD, HPHF and HPLF diets, we found large differences in their C18:2n6 and C18:3n3 concentrations on these three diets. Together with the study of Collavo et al. (2005) this suggests large plasticity in the content of these FAs. House crickets can convert C18:1n9 into C18:2n6 (Cripps, Blomquist et al. 1986; de Renobales et al. 1987), but probably require one of these in a dietary form. The low dietary intake of these FAs in the HPLF treatment might have caused the prolonged development and low survival on that diet. On the three dietary treatments analysed, a small proportion of the House cricket FAs consisted of C20:3n3 (0.4% of TFA), and C22:6n3 (~0.1% of TFA). These FAs were not detected in the other species analysed, or in any of the diets. Whereas conversion from C18:3n3 to C20:5n3 was already known (Blomquist et al. 1991), the apparent *de novo* synthesis of C20:3n3 and C22:6n3, is new.

The content of n3 FAs, as well as the n6/n3 ratio is relevant for human and animal health. A n6/n3 ratio < 5 is considered optimal for human health (Kouba et al. 2011). In most animal products this ratio is between 10 and 15, but in monogastric animals it can be altered through diet (Kouba et al. 2011). Similarly, the FA profile of insects is considered to reflect the FA composition of the diet (Cohen 2003), although this is not true for all species (Howard and Stanley-Samuelson 1996). Even on the HPLF diet, which had a n6/n3 ratio of 4.9, none of the species investigated attained a n6/n3 ratio < 5 (Table 7). The lowest n6/n3ratios were present in the Argentinean cockroaches and BSFs, followed by the House crickets. Although it seems provision of carrot decreases the n6/n3ratio in Yellow mealworms, their n6/n3ratio was the highest on all diets.
In order to optimize n6/n3 ratios in insect-derived food or feed further experiments on the plasticity of fat content and FA profile are needed.

Conclusions

This study shows that 1) insects can be produced on diets entirely composed of organic by-products, 2) Argentinean cockroaches and BSFs use feed more efficiently than conventional production animals , 3) Yellow mealworms and House crickets were equally efficient in converting feed as poultry, 4) N-ECI varied over species and diets, but was higher than for conventional production animals, and 5) the composition of insect species can be altered through their diet.

Further studies should focus on finding optimised combinations of insect species and diet composition, in order to efficiently produce insects that meet the nutritional requirements of humans and animals.

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Table 1: Inclusion percentage of feed ingredients to experimental diets

Diet	Spent grains	Beer yeast	Cookie remains	Potato steam peelings	Beet molasses	Bread
HPHF	60%	20%	20%			
HPLF		50%		30%	20%	
LPHF			50%			50%
LPLF				30%	20%	50%

Table 2: Dry matter (DM)%, crude protein, total fatty acid and Phosphorus content (% of DM) of diets with high (HP) and low (LP) protein, and high (HF) and low (LF) fat contents.

Diet	DM%	Protein (%DM)	Total fatty acids (%DM)	Phosphorus (%DM)
HPHF	95.0%	21.9	9.5	0.56
HPLF	95.1%	22.9	1.0	0.53
LPHF	89.1%	12.9	14.6	0.22
LPLF	89.1%	14.4	2.1	0.21
TM 1	89.3%	17.5	4.9	0.25
TM 2	89.3%	17.1	4.2	0.54
AD	89.9%	17.2	4.0	0.66
HI	90.0%	19.1	3.5	0.67
BD	88.0%	18.4	2.7	0.60
Carrot	9.1%	5.9	1.6	0.25

Control diets for: *Tenebrio molitor* (TM), *Blaptica dubia* (BD), *Acheta domesticus* (AD), and *Hermetia illucens* (HI)

Table 3: Fatty acid composition (as % of total fatty acids*) of diets with high (HP) or low (LP) protein, and high (HF) or low (LF) fat contents, and control diets provided to *Blaptica dubia* (BD), *Hermetia illucens* (HI), *Tenebrio molitor* (TM), and *Acheta domesticus* (AD).

Diet	C8:0	C10:0	C12:0	C13:0	C14:0	C16:0	C16:1	C18:0	C18:1t11	C18:1n9c	C18:2n6c	C18:3n3	C20:4n6	C23:0
HPHF	0.48	1.38	1.94	6.29	4.94	23.60	0.99	4.56	1.10	20.86	27.19	2.50	0.14	0.03
HPLF	0.00	1.19	0.00	62.10	0.00	14.60	4.80	3.91	0.00	5.33	5.30	1.36	1.41	0.00
LPHF	1.05	2.35	3.80	4.12	8.76	21.96	1.36	7.29	2.00	30.05	10.36	0.73	0.07	0.02
LPLF	0.00	0.00	0.00	28.55	0.86	11.09	0.37	3.01	0.00	23.11	26.81	4.32	0.00	0.00
TM 1	0.00	0.00	0.64	14.19	0.40	14.56	0.15	0.97	0.00	15.19	47.98	4.33	0.00	0.00
TM 2	0.00	0.00	0.00	12.30	0.00	14.00	0.19	1.82	0.00	22.93	43.88	3.25	0.00	0.00
AD	0.49	0.00	1.00	14.87	0.66	16.10	0.18	2.40	0.20	21.34	38.52	2.35	0.00	0.00
HI	0.00	0.00	0.00	16.89	0.19	16.77	0.00	2.17	0.00	21.57	39.37	1.96	0.00	0.00
BD	0.00	0.00	0.00	21.67	0.23	12.79	0.00	1.62	0.00	17.15	41.98	2.90	0.00	0.00
Carrot	0.00	0.00	0.00	50.40	0.00	11.73	0.00	1.20	0.00	2.12	29.79	2.34	0.00	1.00

* Fatty acids ≤ 1% of total fatty acids are excluded

Table 4: Survival rate, development time, Feed Conversion Ratio (FCR), Conversion of ingested food (ECI), and nitrogen efficiency (N-ECI), of *Blaptica dubia* (BD), *Hermetia illucens* (HI), *Tenebrio molitor* without (TM) and with carrot (TM-C), and *Acheta domesticus* (AD) on different diets (Mean ± SD). No superscripts in common for a species in the same column, means differ significantly (Kruskal Wallis followed by Scheffé's posthoc test; P<0.05)

Species	N	Diet	Survival rate (%)	Development time (days)	FCR (FM)	ECI (DM;%)	N-ECI (%)
BD	6	HPHF	80 ± 17.9 ^{a}	200 ± 28.8 ^{a}	1.7 ± 0.24 ^{a}	21 ± 3.0 ^{b}	58 ± 8.3 ^{a}
	6	HPLF	47 ± 16.3 ^{b}	294 ± 33.5 ^{c}	2.3 ± 0.35 ^{bc}	16 ± 2.7 ^{ab}	51 ± 8.7 ^{a}
	6	LPHF	53 ± 13.2 ^{ab}	266 ± 29.3 ^{bc}	1.5 ± 0.19 ^{a}	30 ± 3.9 ^{c}	87 ± 11.4 ^{b}
	6	LPLF	51 ± 12.2 ^{ab}	237 ± 14.9 ^{ab}	1.7 ± 0.15 ^{ab}	18 ± 1.9 ^{ab}	66 ± 6.7 ^{a}
	6	BD	75 ± 21.7 ^{ab}	211 ± 18.7 ^{a}	2.7 ± 0.47 ^{c}	14 ± 2.1 ^{a}	52 ± 8.1 ^{a}
HI	6	HPHF	86 ± 18.0 ^{a}	21 ± 1.4 ^{ab}	1.4 ± 0.12 ^{a}	24 ± 1.5 ^{a}	51 ± 3.2 ^{a}
	6	HPLF	77 ± 19.8 ^{a}	33 ± 5.4 ^{bc}	1.9 ± 0.20 ^{a}	20 ± 1.3 ^{a}	51 ± 32.5 ^{a}
	5	LPHF	72 ± 12.9 ^{a}	37 ± 10.6 ^{c}	2.3 ± 0.56 ^{a}	18 ± 4.8 ^{a}	55 ± 14.6 ^{a}
	6	LPLF	74 ± 23.5 ^{a}	37 ± 5.8 ^{c}	2.6 ± 0.85 ^{a}	17 ± 5.0 ^{a}	43 ± 12.8 ^{a}
	6	HI	75 ± 31.0 ^{a}	21 ± 1.1 ^{a}	1.8 ± 0.71 ^{a}	23 ± 5.3 ^{a}	52 ± 12.2 ^{a}
TM	6	HPHF	79 ± 7.0 ^{ab}	116 ± 5.2 ^{abc}	3.8 ± 0.63 ^{a}	12 ± 2.7 ^{abcd}	29 ± 6.7 ^{abc}
	6	HPLF	67 ± 12.3 ^{ac}	144 ± 13.0 ^{cd}	4.1 ± 0.25 ^{a}	10 ± 1.0 ^{abc}	22 ± 2.3 ^{a}
	6	LPHF	19 ± 7.3 ^{d}	191 ± 21.9 ^{ef}	5.3 ± 0.81 ^{a}	8 ± 0.8 ^{ab}	28 ± 2.8 ^{ab}
	6	LPLF	52 ± 9.2 ^{ce}	227 ± 26.9 ^{f}	6.1 ± 0.62 ^{a}	7 ± 1.0 ^{a}	23 ± 3.1 ^{ab}
	6	TM1	84 ± 9.9 ^{ab}	145 ± 9.3 ^{cd}	4.8 ± 0.14 ^{a}	9 ± 0.2 ^{abc}	28 ± 0.6 ^{abc}
	6	TM2	34 ± 15.0 ^{de}	151 ± 7.8 ^{cde}	4.1 ± 0.49 ^{a}	11 ± 1.5 ^{abcd}	31 ± 4.2 ^{abc}

Species	N	Diet	Survival rate (%)	Development time (days)	FCR (FM)	EC (DM;%)	N-ECI (%)
TM-C	6	HPHF	88 ± 5.4 ^{ab}	88 ± 5.1 ^{a}	4.5 ± 0.17 ^{a}	19 ± 1.6 ^{ef}	45 ± 4.5 ^{d}
	6	HPLF	82 ± 6.4 ^{ab}	83 ± 6.5 ^{a}	5.8 ± 0.48 ^{a}	15 ± 0.9 ^{de}	35 ± 2.2 ^{bcd}
	6	LPHF	15 ± 7.4 ^{d}	135 ± 17.3 ^{bcd}	19.1 ± 5.93 ^{c}	13 ± 2.7 ^{bcd}	45 ± 9.2 ^{de}
	6	LPLF	80 ± 5.6 ^{ab}	164 ± 32.9 ^{de}	10.9 ± 0.61 ^{b}	13 ± 1.4 ^{bcd}	41 ± 4.6 ^{cd}
	6	TM1	93 ± 9.3 ^{ab}	91 ± 8.5 ^{a}	5.5 ± 0.49 ^{a}	14 ± 3.3 ^{cde}	45 ± 2.4 ^{d}
	6	TM2	88 ± 3.1 ^{ab}	95 ± 8.0 ^{ab}	5.0 ± 0.48 ^{a}	21 ± 2.6 ^{f}	58 ± 7.3 ^{e}
AD	6	HPHF	27 ± 19.0 ^{ab}	55 ± 7.3 ^{a}	4.5 ± 2.84 ^{a}	8 ± 4.9 ^{a}	23 ± 13.4 ^{a}
	1	HPLF	6	117	10.0	3	
	3	LPHF	7 ± 3.1 ^{a}	167 ± 4.4 ^{c}	6.1 ± 1.75 ^{a}	5 ± 1.3 ^{a}	
	2	LPLF	11 ± 1.4 ^{a}	121 ± 2.8 ^{b}	3.2 ± 0.69 ^{a}	9 ± 2.2 ^{a}	
	6	AD	55 ± 11.2 ^{c}	48 ± 2.3 ^{a}	2.3 ± 0.57 ^{a}	12 ± 3.2 ^{a}	41 ± 10.8 ^{b}

Table 5: Dry matter (DM), crude protein (CP), and phosphorus (P) content, and total fatty acids (TFA), of *Blaptica dubia* (BD), *Hermetia illucens* (HI), *Tenebrio molitor* without (TM) and with carrot (TM-C), and *Acheta domesticus* (AD) on different diets (Mean ± SD). No superscripts in common in a column for a species, means differ significantly (Kruskal Wallis followed by Scheffé's posthoc test; P<0.05)

Species	Diet	DM (%FM)	CP (% DM)	P (g/kg DM)	TFA (% DM)
BD	HPHF	32.7 ± 2.72 ^{ab}	60.7 ± 1.59 ^{a}	6.0 ± 0.16 ^{a}	19.6 ± 0.59 ^{ab}
	HPLF	33.7 ± 1.53 ^{bc}	72.5 ± 1.25 ^{b}	5.8 ± 0.31 ^{a}	16.1 ± 1.81 ^{ab}
	LPHF	38.5 ± 5.09 ^{c}	37.5 ± 0.99 ^{c}	4.7 ± 0.28 ^{b}	40.2 ± 2.69 ^{c}
	LPLF	27.6 ± 1.71 ^{a}	53.9 ± 0.88 ^{d}	5.9 ± 0.08 ^{a}	20.5 ± 0.30 ^{b}
	BD	31.6 ± 1.36 ^{ab}	69.8 ± 1.91 ^{b}	6.2 ± 0.45 ^{a}	15.2 ± 1.38 ^{a}
HI	HPHF	32.9 ± 1.86 ^{a}	46.3 ± 0.93 ^{a}	8.5 ± 0.28 ^{ab}	24.7 ± 0.38 ^{a}
	HPLF	35.6 ± 2.45 ^{a}	43.5 ± 3.00 ^{ab}	8.6 ± 0.90 ^{ab}	25.5 ± 3.80 ^{a}
	LPHF	35.1 ± 1.97 ^{a}	38.8 ± 2.56 ^{b}	6.7 ± 1.34 ^{a}	28.0 ± 7.42 ^{a}
	LPLF	35.3 ± 2.36 ^{a}	38.3 ± 1.41 ^{b}	6.4 ± 0.32 ^{a}	33.5 ± 3.17 ^{a}
	HI	33.9 ± 2.28 ^{a}	43.8 ± 0.24 ^{ab}	9.7 ± 1.13 ^{b}	25.4 ± 3.99 ^{a}
TM	HPHF	41.5 ± 0.37 ^{a}	53.6 ± 0.45 ^{b}	8.9 ± 0.31 ^{ab}	26.5 ± 1.10 ^{ab}
	HPLF	36.7 ± 3.65 ^{abc}	53.5 ± 1.25 ^{b}	8.8 ± 0.15 ^{ab}	23.0 ± 1.31 ^{a}
	LPHF	37.2 ± 2.76 ^{abc}	44.4 ***	8.8***	26.8 ± 1.89 ^{ab}
	LPLF	38.2 ± 2.85 ^{ab}	47.5 ± 1.26 ^{ab}	8.2 ± 0.06 ^{ab}	28.5 ± 0.71 ^{abc}
	TM1	39.8 ± 0.97 ^{ab}	52.4 ± 0.36 ^{b}	9.7 ± 0.26 ^{b}	27.0 ± 1.02 ^{ab}
	TM2	39.2 ± 1.27 ^{ab}	49.2 ± 1.01 ^{ab}	7.7 ± 0.40 ^{a}	30.9 ± 0.37 ^{bc}
TM-C	HPHF	32.3 ± 2.90 ^{cd}	51.3 ± 1.09 ^{b}	8.3 ± 0.20 ^{ab}	22.6 ± 1.36 ^{a}
	HPLF	35.1 ± 0.80 ^{bcd}	53.3 ± 1.13 ^{b}	8.4 ± 0.25 ^{ab}	23.6 ± 1.59 ^{a}
	LPHF	34.8 ± 2.39 ^{bcd}	44.1 ± 4.86** ^{a}	7.8 ± 1.70 ^{ab}	27.2 ± 0.99 ^{ab}
	LPLF	30.2 ± 1.29 ^{d}	48.3 ± 0.00** ^{ab}	7.9 ± 0.06 ^{ab}	24.8 ± 2.08 ^{ab}
	TM1	35.0 ± 2.05 ^{bcd}	50.4 ± 1.94 ^{b}	9.2 ± 0.27 ^{ab}	24.8 ± 1.41 ^{ab}
	TM2	36.0 ± 0.96 ^{abc}	47.8 ± 0.22 ^{ab}	7.9 ± 0.24 ^{ab}	34.5 ± 3.27 ^{c}
AD	HPHF	25.7 ± 2.67 ^{a}	59.2 ± 5.57** ^{a}	8.5 ± 0.86 ^{a}	20.8 ± 3.44 ^{a}
	HPLF	24.0***	*	*	20.8 ± 1.50 ^{a}
	LPHF	25.1 ± 5.24 ^{a}	*	*	*
	LPLF	24.8 ± 0.98 ^{a}	*	*	*
	AD	24.1 ± 1.52 ^{a}	57.8 ± 2.78 ^{a}	8.9 ± 0.26 ^{a}	17.4 ± 1.61 ^{a}

For DM% n=6, for CP, P & TFA n=3 unless indicated otherwise; * Insufficient sample, ** n=2, *** n=1.

Table 6: continued

Species	Diet	C14:1	C16:0	AI-C17:0	C16:1
BD	HPHF	0.1 ± 0.02 ^{a}	17.7 ± 1.04 ^{a}	1.0 ± 1.34 ^{a}	1.6 ± 1.39 ^{a}
	HPLF	0.0 ± 0.04 ^{b}	21.6 ± 0.37 ^{b}	0.2 ± 0.02 ^{a}	8.6 ± 0.22 ^{b}
	LPHF	0.2 ± 0.02 ^{c}	22.2 ± 0.62 ^{b}	0.2 ± 0.00 ^{a}	8.0 ± 0.89 ^{b}
	LPLF	0.1 ± 0.02 ^{b}	20.9 ± 0.37 ^{b}	0.2 ± 0.01 ^{a}	7.7 ± 1.46 ^{b}
	BD	0.0 ± 0.01 ^{b}	15.7 ± 0.81 ^{a}	0.2 ± 0.04 ^{a}	2.0 ± 0.08 ^{a}
HI	HPHF	0.4 ± 0.01 ^{a}	17.0 ± 0.16 ^{a}	0.5 ± 0.06 ^{ab}	2.9 ± 0.21 ^{a}
	HPLF	0.9 ± 0.10 ^{b}	11.8 ± 0.84 ^{b}	0.1 ± 0.03 ^{c}	6.6 ± 0.90 ^{b}
	LPHF	0.6 ± 0.04 ^{c}	14.4 ± 1.74 ^{ab}	0.6 ± 0.21 ^{b}	3.4 ± 0.11 ^{ac}
	LPLF	0.7 ± 0.03 ^{c}	11.6 ± 1.24 ^{b}	0.2 ± 0.01 ^{ac}	4.7 ± 0.51 ^{c}
	HI	0.3 ± 0.01 ^{a}	12.7 ± 0.91 ^{b}	0.2 ± 0.02 ^{ac}	3.4 ± 0.06 ^{ac}
TM	HPHF	0.0 ± 0.00 ^{ab}	15.5 ± 0.33 ^{a}	1.1 ± 0.05 ^{ab}	2.0 ± 0.01 ^{ab}
	HPLF	0.0 ± 0.01 ^{cd}	17.2 ± 0.24 ^{bcd}	1.1 ± 0.06 ^{ab}	2.9 ± 0.17 ^{c}
	LPHF	0.0 ± 0.00 ^{b}	16.4 ± 0.57 ^{abc}	1.1 ± 0.09 ^{b}	1.4 ± 0.05 ^{d}
	LPLF	0.0 ± 0.00 ^{cde}	16.6 ± 0.29 ^{abcd}	0.9 ± 0.03 ^{abcd}	1.7 ± 0.11 ^{abd}
	TM1	0.0 ± 0.00 ^{cde}	16.0 ± 0.36 ^{ab}	0.7 ± 0.05 ^{cdef}	1.8 ± 0.04 ^{abd}
	TM2	0.0 ± 0.00 ^{cde}	15.3 ± 0.23 ^{a}	0.8 ± 0.11 ^{acdef}	2.1 ± 0.10 ^{b}
TM-C	HPHF	0.1 ± 0.00 ^{f}	20.2 ± 0.29 ^{e}	0.6 ± 0.04 ^{ef}	1.7 ± 0.04 ^{abd}
	HPLF	0.0 ± 0.01 ^{cde}	17.8 ± 0.26 ^{d}	0.9 ± 0.02 ^{abcdf}	2.8 ± 0.05 ^{c}
	LPHF	0.0 ± 0.00 ^{ad}	17.0 ± 0.58 ^{bcd}	1.0 ± 0.14 ^{abd}	1.6 ± 0.19 ^{ad}
	LPLF	0.0 ± 0.01 ^{ce}	17.7 ± 0.20 ^{cd}	0.8 ± 0.04 ^{cdef}	2.0 ± 0.16 ^{b}
	TM1	0.0 ± 0.00 ^{e}	17.4 ± 0.36 ^{bcd}	0.6 ± 0.09 ^{ef}	1.7 ± 0.07 ^{abd}
	TM2	0.0 ± 0.01 ^{ce}	16.4 ± 0.14 ^{abc}	0.7 ± 0.03 ^{cef}	1.9 ± 0.07 ^{ab}
AD	HPHF	0.1 ± 0.01 ^{a}	26.5 ± 0.84 ^{a}	0.3 ± 0.04 ^{a}	1.5 ± 0.16 ^{a}
	HPLF	0.0 ± 0.04 ^{b}	24.6 ± 2.87 ^{a}	0.4 ± 0.21 ^{a}	1.9 ± 0.84 ^{a}
	LPHF	*	*	*	*
	LPLF	*	*	*	*
	AD	0.0 ± 0.00 ^{b}	25.1 ± 0.42 ^{a}	0.3 ± 0.03 ^{a}	0.8 ± 0.09 ^{a}

Table 6: Fatty acid composition (% of total fatty acids) of *Blaptica dubia* (BD), *Hermetia illucens* (HI), *Tenebrio molitor* without (TM) and with carrot (TM-C), and *Acheta domesticus* (AD), on different diets (Mean ± SD). If for a species superscripts in the same column have no letters in common, means differ significantly (Kruskal Wallis test followed by Scheffé's posthoc test; P<0.05)

Species	Diet	C10:0	C12:0	C13:0	C14:0	Iso-C15:0
BD	HPHF	0.0 ± 0.04 ^{a}	0.3 ± 0.09 ^{a}	3.1 ± 0.09 ^{ab}	2.6 ± 0.20 ^{a}	0.1 ± 0.01 ^{ab}
	HPLF	0.0 ± 0.00 ^{a}	0.2 ± 0.04 ^{b}	3.8 ± 0.45 ^{ad}	1.7 ± 0.13 ^{b}	0.0 ± 0.03 ^{c}
	LPHF	0.0 ± 0.00 ^{a}	0.5 ± 0.03 ^{c}	1.5 ± 0.10 ^{c}	3.9 ± 0.07 ^{c}	0.1 ± 0.01 ^{a}
	LPLF	0.0 ± 0.00 ^{a}	0.2 ± 0.01 ^{b}	2.9 ± 0.06 ^{b}	1.4 ± 0.09 ^{bd}	0.1 ± 0.01 ^{abc}
	BD	0.0 ± 0.00 ^{a}	0.2 ± 0.04 ^{b}	4.0 ± 0.37 ^{d}	1.1 ± 0.07 ^{d}	0.1 ± 0.01 ^{bc}
HI	HPHF	0.7 ± 0.09 ^{a}	28.9 ± 1.01 ^{a}	2.4 ± 0.04 ^{a}	7.4 ± 0.16 ^{a}	0.0 ± 0.02 ^{a}
	HPLF	1.3 ± 0.07 ^{b}	48.4 ± 1.54 ^{bc}	2.4 ± 0.37 ^{a}	9.5 ± 0.36 ^{b}	0.0 ± 0.01 ^{a}
	LPHF	0.8 ± 0.09 ^{a}	38.4 ± 6.46 ^{ab}	2.3 ± 0.68 ^{a}	7.8 ± 0.36 ^{a}	0.2 ± 0.02 ^{b}
	LPLF	1.2 ± 0.04 ^{b}	50.7 ± 4.18 ^{c}	1.8 ± 0.16 ^{a}	9.0 ± 0.14 ^{b}	0.0 ± 0.01 ^{a}
	HI	0.9 ± 0.15 ^{a}	46.6 ± 1.52 ^{bc}	2.4 ± 0.39 ^{a}	9.2 ± 0.35 ^{b}	0.1 ± 0.02 ^{c}
TM	HPHF	0.0 ± 0.00 ^{a}	0.3 ± 0.01 ^{a}	2.3 ± 0.09 ^{a}	4.5 ± 0.08 ^{abcde}	0.1 ± 0.02 ^{ab}
	HPLF	0.0 ± 0.00 ^{a}	0.5 ± 0.25 ^{a}	2.7 ± 0.16 ^{a}	4.9 ± 0.12 ^{abc}	0.2 ± 0.04 ^{abc}
	LPHF	0.0 ± 0.00 ^{a}	0.3 ± 0.06 ^{a}	3.3 ± 1.30 ^{a}	5.5 ± 0.54 ^{c}	0.8 ± 0.09 ^{d}
	LPLF	0.0 ± 0.00 ^{a}	0.3 ± 0.03 ^{a}	2.1 ± 0.05 ^{a}	4.8 ± 0.32 ^{abcd}	0.4 ± 0.03 ^{ce}
	TM1	0.0 ± 0.00 ^{a}	0.4 ± 0.02 ^{a}	2.2 ± 0.09 ^{a}	4.7 ± 0.12 ^{abcde}	0.1 ± 0.01 ^{ab}
	TM2	0.0 ± 0.00 ^{a}	0.3 ± 0.03 ^{a}	2.0 ± 0.04 ^{a}	4.4 ± 0.29 ^{abde}	0.5 ± 0.11 ^{e}
TM-C	HPHF	0.0 ± 0.00 ^{a}	0.3 ± 0.03 ^{a}	2.7 ± 0.15 ^{a}	4.7 ± 0.23 ^{abcde}	0.1 ± 0.01 ^{a}
	HPLF	0.0 ± 0.00 ^{a}	0.3 ± 0.02 ^{a}	2.6 ± 0.16 ^{a}	3.7 ± 0.03 ^{de}	0.2 ± 0.00 ^{ab}
	LPHF	0.0 ± 0.00 ^{a}	0.4 ± 0.06 ^{a}	2.2 ± 0.10 ^{a}	5.1 ± 0.43 ^{bc}	0.5 ± 0.01 ^{e}
	LPLF	0.0 ± 0.00 ^{a}	0.3 ± 0.01 ^{a}	2.4 ± 0.23 ^{a}	3.9 ± 0.17 ^{ade}	0.3 ± 0.03 ^{bc}
	TM1	0.0 ± 0.00 ^{a}	0.3 ± 0.02 ^{a}	2.4 ± 0.12 ^{a}	3.6 ± 0.40 ^{e}	0.1 ± 0.04 ^{ab}
	TM2	0.0 ± 0.00 ^{a}	0.3 ± 0.05 ^{a}	1.8 ± 0.16 ^{a}	4.2 ± 0.08 ^{abde}	0.2 ± 0.01 ^{abc}
AD	HPHF	0.0 ± 0.00 ^{a}	0.2 ± 0.04 ^{a}	2.9 ± 0.44 ^{a}	2.5 ± 0.22 ^{a}	0.1 ± 0.01 ^{a}
	HPLF	0.0 ± 0.00 ^{a}	0.1 ± 0.13 ^{a}	10.8 ± 10.87 ^{a}	1.4 ± 1.46 ^{a}	0.0 ± 0.08 ^{a}
	LPHF	*	*	*	*	*
	LPLF	*	*	*	*	*
	AD	0.0 ± 0.00 ^{a}	0.1 ± 0.03 ^{a}	3.5 ± 0.35 ^{a}	0.7 ± 0.03 ^{a}	0.0 ± 0.00 ^{a}

* Insufficient sample

Table 6: continued

Species	Diet	C18:2n6c		C18:3n3		CLA9c11tr		C20:4n6		C24:1	
BD	HPHF	19.5 ± 2.08	a	1.1 ± 0.10	a	0.7 ± 0.04	a	0.1 ± 0.08	ab	0.1 ± 0.07	a
	HPLF	1.7 ± 0.13	b	0.3 ± 0.02	b	0.0 ± 0.01	b	0.0 ± 0.02	a	0.0 ± 0.00	a
	LPHF	7.3 ± 0.17	c	0.4 ± 0.01	b	1.0 ± 0.01	c	0.1 ± 0.01	ab	0.0 ± 0.00	a
	LPLF	7.0 ± 0.17	c	0.7 ± 0.03	c	0.0 ± 0.00	b	0.1 ± 0.02	ab	0.0 ± 0.00	a
	BD	17.2 ± 1.48	a	0.8 ± 0.09	c	0.1 ± 0.01	d	0.2 ± 0.01	b	0.0 ± 0.04	a
HI	HPHF	17.1 ± 0.35	a	1.5 ± 0.02	a	0.3 ± 0.01	a	0.2 ± 0.01	a	0.0 ± 0.00	a
	HPLF	3.6 ± 1.59	b	0.6 ± 0.17	b	0.0 ± 0.05	b	0.6 ± 0.16	b	0.0 ± 0.00	a
	LPHF	8.3 ± 0.86	cd	0.8 ± 0.05	bc	0.3 ± 0.03	a	0.1 ± 0.01	a	0.0 ± 0.00	a
	LPLF	6.0 ± 0.62	bc	1.0 ± 0.11	c	0.0 ± 0.00	b	0.1 ± 0.03	a	0.0 ± 0.00	a
	HI	9.4 ± 0.56	d	0.6 ± 0.08	b	0.0 ± 0.01	b	0.0 ± 0.00	a	0.5 ± 0.92	a
TM	HPHF	25.4 ± 0.82	ab	0.8 ± 0.05	a	0.2 ± 0.00	a	0.3 ± 0.04	a	0.0 ± 0.00	a
	HPLF	12.8 ± 0.92	c	0.1 ± 0.02	b	0.0 ± 0.00	b	0.8 ± 0.01	b	0.1 ± 0.05	b
	LPHF	16.3 ± 0.62	de	0.2 ± 0.03	bc	0.2 ± 0.03	ac	0.0 ± 0.00	c	0.0 ± 0.00	a
	LPLF	17.5 ± 0.64	ef	0.4 ± 0.06	cd	0.0 ± 0.01	b	0.0 ± 0.02	c	0.0 ± 0.00	a
	TM1	25.5 ± 0.26	ab	0.9 ± 0.02	a	0.0 ± 0.00	b	0.0 ± 0.00	c	0.1 ± 0.02	abc
	TM2	24.5 ± 0.46	a	0.5 ± 0.07	de	0.0 ± 0.00	b	0.0 ± 0.01	c	0.0 ± 0.00	
TM-C	HPHF	21.3 ± 0.06	fg	0.9 ± 0.02	a	0.2 ± 0.02	ac	0.3 ± 0.02	a	0.1 ± 0.01	bc
	HPLF	14.0 ± 0.47	cd	0.2 ± 0.01	bc	0.0 ± 0.00	b	0.8 ± 0.08	b	0.1 ± 0.02	bc
	LPHF	18.2 ± 0.46	e	0.3 ± 0.02	bcd	0.2 ± 0.02	c	0.0 ± 0.01	c	0.0 ± 0.00	a
	LPLF	19.2 ± 0.24	ef	0.5 ± 0.04	de	0.0 ± 0.00	b	0.0 ± 0.00	c	0.0 ± 0.01	a
	TM1	27.9 ± 1.78	b	1.3 ± 0.17	f	0.0 ± 0.00	b	0.0 ± 0.03	c	0.1 ± 0.02	ac
	TM2	23.9 ± 0.31	ag	0.7 ± 0.01	ae	0.0 ± 0.00	b	0.0 ± 0.00	c	0.0 ± 0.04	a
AD	HPHF	30.1 ± 1.19	ab	1.5 ± 0.10	a	0.4 ± 0.03	a	0.0 ± 0.03	a	0.1 ± 0.03	a
	HPLF	23.6 ± 6.30	a	0.4 ± 0.14	b	0.1 ± 0.25	a	0.0 ± 0.02	a	0.0 ± 0.00	b
	LPHF	*		*		*		*		*	
	LPLF	*		*		*		*		*	
	AD	34.9 ± 1.12	b	1.2 ± 0.09	c	0.1 ± 0.04	a	0.0 ± 0.00	a	0.1 ± 0.01	a

Table 6: continued

Species	Diet	C18:0		C18:1t11		C18:1n9c		C18:1c11	
BD	HPHF	6.5 ± 0.26	^{a}	0.7 ± 0.07	^{a}	42.5 ± 0.91	^{a}	0.5 ± 0.03	^{a}
	HPLF	4.2 ± 0.07	^{b}	0.0 ± 0.03	^{b}	56.6 ± 0.08	^{b}	0.2 ± 0.01	^{b}
	LPHF	3.7 ± 0.32	^{b}	0.3 ± 0.31	^{ab}	48.5 ± 0.96	^{c}	0.4 ± 0.00	^{c}
	LPLF	4.4 ± 0.61	^{b}	0.0 ± 0.02	^{b}	53.1 ± 1.17	^{d}	0.3 ± 0.02	^{d}
	BD	7.7 ± 0.44	^{c}	0.1 ± 0.03	^{b}	48.8 ± 0.17	^{c}	0.4 ± 0.01	^{ac}
HI	HPHF	2.8 ± 0.09	^{a}	0.2 ± 0.04	^{a}	15.9 ± 0.32	^{a}	0.3 ± 0.02	^{ab}
	HPLF	2.0 ± 0.05	^{a}	0.0 ± 0.02	^{b}	10.8 ± 1.12	^{b}	0.5 ± 0.07	^{b}
	LPHF	2.4 ± 1.08	^{a}	0.2 ± 0.04	^{a}	18.1 ± 1.78	^{a}	0.2 ± 0.04	^{c}
	LPLF	1.8 ± 0.36	^{a}	0.0 ± 0.00	^{b}	10.3 ± 0.95	^{b}	0.2 ± 0.07	^{ac}
	HI	2.1 ± 0.23	^{a}	0.0 ± 0.00	^{b}	10.2 ± 0.30	^{b}	0.7 ± 0.04	^{d}
TM	HPHF	3.1 ± 0.06	^{a}	0.1 ± 0.02	^{a}	43.1 ± 0.61	^{ab}	0.2 ± 0.01	^{ab}
	HPLF	4.1 ± 0.36	^{ab}	0.0 ± 0.00	^{b}	51.7 ± 1.25	^{c}	0.1 ± 0.01	^{c}
	LPHF	4.0 ± 0.50	^{ab}	0.1 ± 0.03	^{ac}	49.5 ± 0.41	^{cde}	0.1 ± 0.01	^{c}
	LPLF	4.0 ± 0.02	^{ab}	0.0 ± 0.00	^{b}	50.6 ± 0.78	^{cde}	0.1 ± 0.01	^{c}
	TM1	3.5 ± 0.21	^{ab}	0.0 ± 0.00	^{b}	43.0 ± 0.60	^{abf}	0.2 ± 0.03	^{a}
	TM2	3.2 ± 0.28	^{a}	0.0 ± 0.01	^{bc}	45.4 ± 0.23	^{bg}	0.2 ± 0.02	^{a}
TM-C	HPHF	4.4 ± 0.13	^{b}	0.2 ± 0.03	^{d}	40.3 ± 0.33	^{af}	0.3 ± 0.01	^{b}
	HPLF	4.0 ± 0.07	^{ab}	0.0 ± 0.00	^{b}	51.5 ± 0.62	^{ce}	0.1 ± 0.02	^{c}
	LPHF	3.8 ± 0.33	^{ab}	0.0 ± 0.03	^{bc}	48.7 ± 0.62	^{deh}	0.1 ± 0.01	^{c}
	LPLF	3.7 ± 0.23	^{ab}	0.0 ± 0.00	^{b}	48.3 ± 0.51	^{gh}	0.1 ± 0.01	^{c}
	TM1	3.3 ± 0.34	^{a}	0.0 ± 0.00	^{b}	40.1 ± 1.56	^{f}	0.3 ± 0.04	^{ab}
	TM2	3.2 ± 0.06	^{a}	0.0 ± 0.00	^{b}	45.8 ± 0.19	^{bgh}	0.2 ± 0.02	^{ab}
AD	HPHF	6.3 ± 0.38	^{a}	0.5 ± 0.03	^{a}	24.6 ± 1.51	^{a}	0.5 ± 0.03	^{a}
	HPLF	7.4 ± 0.86	^{ab}	0.3 ± 0.34	^{ab}	26.9 ± 6.30	^{a}	0.4 ± 0.10	^{a}
	LPHF	*		*		*		*	
	LPLF	*		*		*		*	
	AD	7.9 ± 0.27	^{b}	0.2 ± 0.05	^{b}	23.6 ± 0.90	^{a}	0.4 ± 0.05	^{a}

Table 7: Ratios between n6 and n3 fatty acids in diets with high (HP) and low (LP) protein, and high (HF) and low (LF) fat, and control diets provided to *Blaptica dubia*, *Hermetia illucens*, *Tenebrio molitor* (without and with carrot), and *Acheta domesticus*.

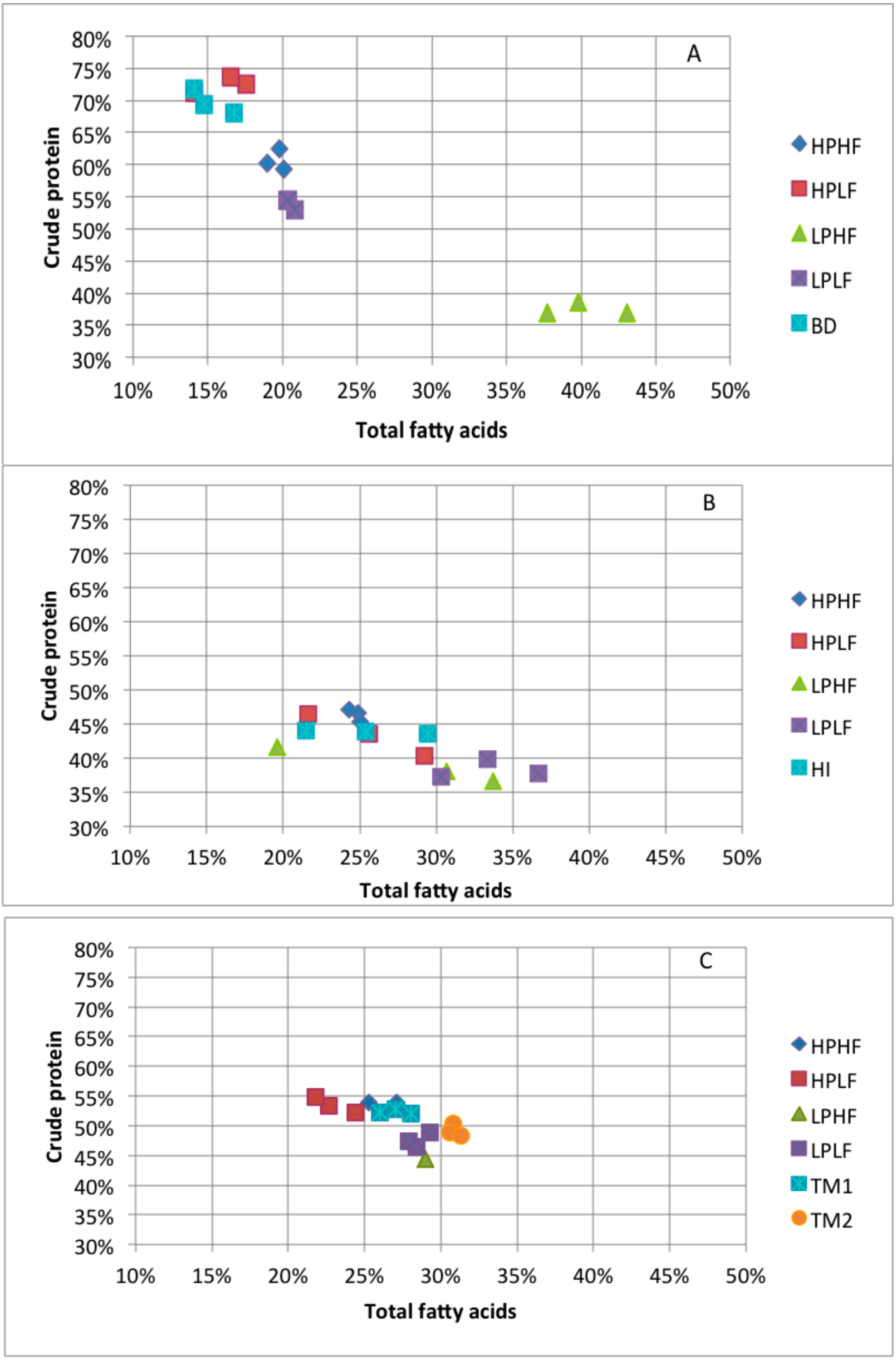
Treatment	Diet	*Blaptica dubia*	*Hermetia illucens*	*Tenebrio molitor*	*Tenebrio molitor* with carrot	*Acheta domesticus*
HPHF	10.7	16.2	11.1	32.1	23.7	15.3
HPLF	4.9	5.8	7.2	102.1	66.0	29.0
LPHF	13.5	18.2	9.1	79.1	57.7	
LPLF	6.2	10.4	6.1	40.6	35.4	
TM 1	11.1			26.6	20.9	
TM 2	13.5			45.2	32.4	
AD	16.4					22.2
HI	20.1		15.1			
BD	14.5	22.1				
Carrot	12.8					

Control diets for: *Tenebrio molitor* (TM), *Blaptica dubia* (BD), *Acheta domesticus* (AD), and *Hermetia illucens* (HI)

Table 8: Comparative data on crude protein (CP), fat and phosphorus (P) content (%DM), and the main fatty acids (as a % of total fatty acids) for *H. illucens, T. molitor* and *A. domesticus.*

	CP	Fat	P	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1n9c	C18:2n6c	C18:3n3
H. illucens	38-46	21-35	0.9-1-5	21-37	2.9-8.6	12-20	3.8-6.3	1.8-6.5	23-32	2.1-6.8	0.0-0.5
T. molitor	45-69	27-36	0.7	0.2-1.3	1.1-8.2	11-23	2.6-4.7	1.0-4.5	40-61	15-26	0.3-1.0
A. domesticus	52-70	6.5-35	1.0	0.2-0.4	0.6-2.9	23-32	0.7-5.4	8.4-8.8	20-29	20-41	0.9-5.1

Data adapted from: (Hale 1973; Newton et al. 1977; Woodring et al. 1977; Bondari et al. 1981; Nakagaki 1987; Clifford et al. 1990; Bernard et al. 1997; Barker et al. 1998; Finke 2002; Collavo et al. 2005; Newton et al. 2005a; Newton et al. 2005b; St-Hilaire et al. 2007; Diener et al. 2009; Ghaly et al. 2009; Oonincx et al. 2010a; Li et al. 2011; Sealey et al. 2011, Finke 2013)


A
Crude protein
80%
75%
70%
65%
60%
55%
50%
45%
40%
35%
30%
Total fatty acids
10%
15%
20%
25%
30%
35%
40%
45%
50%
◆ HPHF
■ HPLF
▲ LPHF
■ LPLF
■ BD
B
Crude protein
Total fatty acids
◆ HPHF
■ HPLF
▲ LPHF
■ LPLF
■ HI
C
Crude protein
Total fatty acids
◆ HPHF
■ HPLF
▲ LPHF
■ LPLF
■ TM1
● TM2

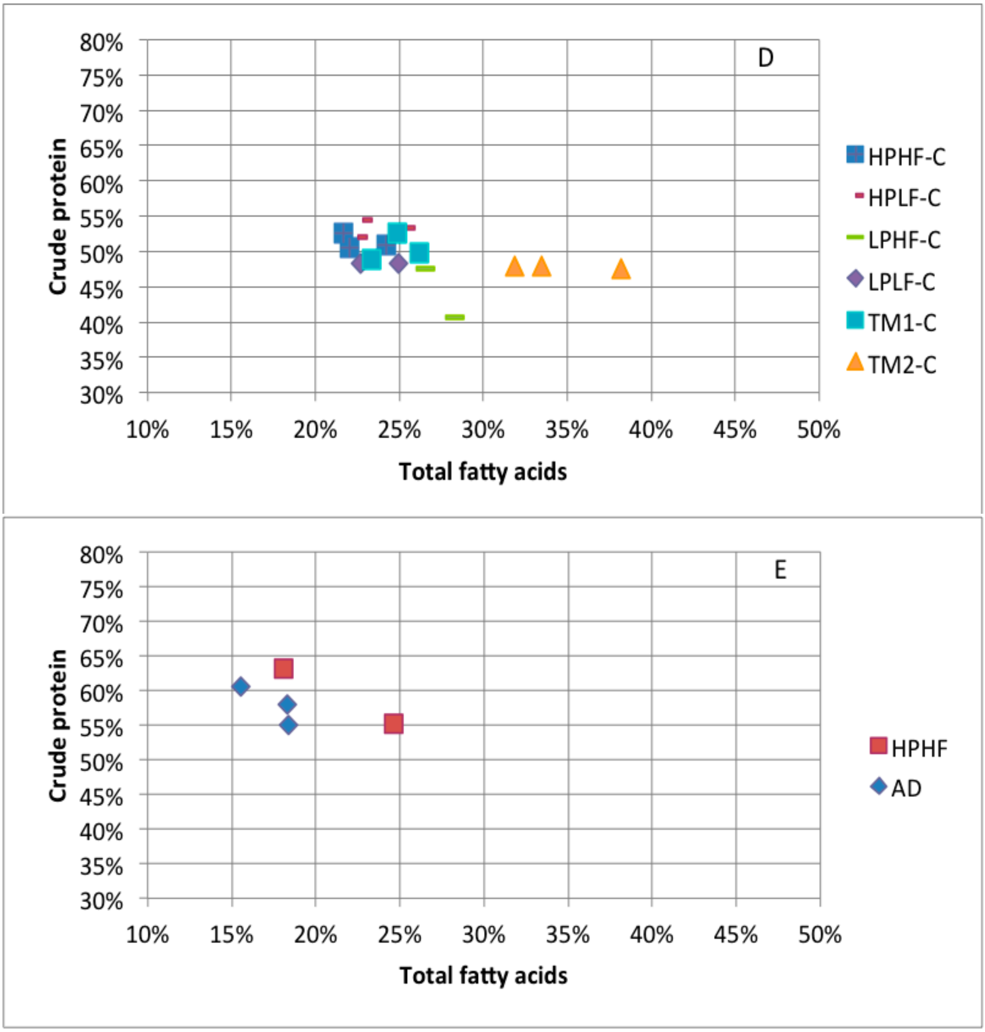


Figure 2: Average fatty acids composition (as a % of TFA) of *Blaptica dubia* (BD), *Hermetia illucens* (HI), *Tenebrio molitor* (TM), and *Acheta domesticus* (AD) reared on different diets

Chapter 6

Nutrient utilisation by Black soldier flies fed with chicken, pig, or cow manure

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Submitted

Abstract

An experiment was conducted to compare the suitability of chicken, pig, and cow manure as feed for larvae of the Black soldier fly (*Hermetia illucens* (L.); Diptera: Stratiomyidae). Newly hatched larvae were inoculated on moistened manure (33% DM). Water and dried manure were added three times per week, until the first prepupae appeared. Survival was between 82 and 97%, indicating that the tested substrates were suitable. However, development time was much longer than on the control diet (144-215 days *vs.* 20 days). Efficiency of conversion of ingested nitrogen (N-ECI) was higher on pig manure than on chicken and cow manure, while the ECI for phosphorus was highest on cow manure. Substrate nitrogen content decreased in chicken manure, but were stable in pig and cow manure. Phosphorus concentration and N:P-ratio decreased in all treatments. Since a large proportion of the nitrogen from the manure (23-78%) was lost, the production system would require an air washer for instance, to make it ecologically sound. Shorter development times are required in order to improve economic viability. Possibly this could be achieved by providing a more nutritious starter diet to larvae, allowing more rapid development.

Key words: *Hermetia illucens*, manure, nitrogen reduction, phosphorus reduction, nitrogen efficiency

Introduction

Animal production systems have a considerable environmental impact (Steinfeld 2012). In certain countries, for instance The Netherlands, there is insufficient farmland to produce the required amount of feed for the livestock sector. This results in large scale imports of animal feed, and consequently in surpluses of nitrogen (N) and phosphorus (P) in the form of manure and urine. Concomitant ammonia (NH_3) emissions lead to nitrification and acidification of soil, and eutrophication of water bodies (Steinfeld et al. 2006). More than half of the nutrients contained in feed are excreted as manure (Steinfeld 2012). In natural systems, manure and other decaying organic materials are broken down by a variety of organisms, among which the larvae of the Black soldier fly (BSF) (*Hermetia illucens* (L.); Diptera: Stratiomyidae) (May 1961; Larde 1990; Myers, Tomberlin et al. 2008). This species can digest pig manure (Sheppard et al. 1994), chicken manure (Bondari et al. 1981), and dairy manure (Myers et al. 2008). Furthermore, BSF larvae have a high protein (32-64% of dry matter), and fat content (20-40% of dry matter) (Bondari et al. 1981; Sheppard et al. 1994; Dierenfeld et al. 2008; Sealey et al. 2011), and can be used as animal feed (Newton, Booram et al. 1977; Bondari et al. 1981; Bondari and Sheppard 1987; Elwert, Knips et al. 2010; Sealey et al. 2011). As a diet ingredient, BSF larvae and prepupae have been found to support growth in rainbow trout (St-Hilaire, Cranfill et al. 2007a), channel catfish (Bondari et al. 1987), blue tilapia (Sheppard, Newton et al. 2008), pigs (Newton et al. 1977), and poultry (Hale 1973; Elwert et al. 2010). Animal studies indicate that their protein quality is similar to fish meal, which is an important ingredient in animal feed (Bondari et al. 1981). The price of fish meal has doubled from January 2008 to January 2013 (Barrientos and Soria 2013). Due to marine overexploitation, availability of fish meal is steadily decreasing (Steinfeld et al. 2006; FAO 2012), which is expected to lead to further price increases. If BSF larvae are reared on manure from livestock production, and subsequently reused as animal feed, this could decrease the environmental impact of the livestock sector. Furthermore, this would decrease the need for feed imports, and thereby the pressure on unsustainable sources of dietary protein currently used, such as fishmeal or soybean meal. Several studies have reported on the provision of manure to BSF larvae. However, differences in experimental design prevent direct comparison of the suitability of manures as feed for BSFs. Furthermore, these studies focused on properties of either the BSF or the manure. For these reasons, a study was conducted to quantify the suitability of chicken, pig, and cow manure as feed for BSF larvae.

Materials and methods

Diet preparation

Fresh chicken, pig, and cow manure (faeces) was collected at the animal experiment facilities of Wageningen University, Wageningen, The Netherlands. In order to homogenize the manure, it was oven-dried at 60°C until constant weight and subsequently ground in a cross beater mill (Peppink AN200, Machinefabriek G.R. Veerman BV, Olst, The Netherlands).

Animals, housing and treatment

Dried manure (2 g) was placed in a plastic container (17.8 x 11.4 x 6.5 cm), and mixed with 4 ml of tap water in order to create a diet with a water content of ~66%. The sides of the containers were manually perforated to allow ventilation, and the top was sealed with a lid. Egg masses were collected from the BSF colony maintained at the Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands. This colony was reared for over 4 years on a chicken feed diet (Opfokmeel farmfood, Agruniek Rijnvallei Voer BV, Wageningen, The Netherlands). Egg masses were incubated in a climate chamber at 28ºC at a relative humidity of 70%, and a photoperiod of 12 h. One hundred emerged larvae, less than 24 h old, were placed in a container. There were six replicates for each treatment *i.e.* the three manure types offered as diet. Furthermore, chicken feed (n=2) served as a control for larval quality and suitability of abiotic factors. The containers were placed in a climate chamber under the before mentioned conditions, which are considered appropriate for this species (May 1961; Holmes, Vanlaerhoven et al. 2012). In the containers, relative humidity increased to approximately 85% during the experiment. A photoperiod of 12 hours was provided by an array of fluorescent tubes (TLD 840, Phillips, Eindhoven, The Netherlands). The containers were placed in stacked plastic trays (50*30*8.7 cm), with one empty tray on top. This resulted in a light intensity in the plastic container, depending on the exact position in the tray, of 500 to 1000 lux (Luxmeter LX1010BS, Uzman Import-export GMBH, Bocholt, Germany). Three times per week at regular intervals each box was visually inspected and after each inspection the containers were rotated in the tray. During inspection, changes in colour and structure of the manure were evaluated and used as indicators of feed consumption. If most manure had been consumed, more was added to allow continuous feeding. The amount of dried manure to be added per occasion (0.25-1.5 g) was based on the amount consumed since the last feeding and the estimated larval mass present, *i.e.* more manure was added for larger larvae than for smaller ones. For each gram of manure provided to the larvae, approximately 2 ml of tap water was added. If the manure in the plastic containers appeared too dry, water was provided in order to attain a consistency visually similar to fresh manure. Prepupae were distinguished from larvae based on colour; larvae have a white cuticle, whereas prepupae have a melanised cuticle (May 1961). As soon as the first prepupa was found in a particular container, this container was removed from the experiment and the date noted. All animals from that container were harvested with forceps, counted, and weighed collectively. Subsequently, residues of feed and faeces adhering to their integument were removed by washing in a standard kitchen sieve under running water (Lardé 1989). Cleaned BSF larvae and prepupae were put on paper tissues for a few seconds to absorb water adhering to their integument, weighed, and this mass was noted as BSF yield (g fresh matter). The difference between the two weights was considered to be residual material (faeces and leftover feed). The BSFs were then frozen at -20ºC.

To determine the survival rate, the number of live BSFs at the end of the experiment was divided by the initial number of larvae per replicate (100). The development time was considered to be the number of days between the start of the experiment, and the day the first prepupa was observed.

From the container, a representative sample of residual material (at least 80%) was taken. This sample and the container with residual material were weighed and stored at -20ºC until all replicates were finished. Then, the container was dried, weighed, cleaned with water, dried, and weighed again, in order to determine the fresh and the dry matter (DM) weight of the residual material in the container. The fresh weight of the residual material in the container, the sample of the residual material, together with the weight of the residual material that adhered to the larvae, was considered the total residual material. DM content of the manure provided, the sampled residual material, and the BSF yield was determined by freeze drying (Model GRI 20-85 MP 1996, GRInstruments, Wijk bij Duurstede, The Netherlands), until constant weight. All samples were subsequently ground with a batch mill (Ika Labortechnik, Staufen, Germany) and stored at -20°C until further analysis.

Chemical analysis

Total nitrogen (N) and total phosphorus (P) content of the manure, residual material, and harvested BSFs was analysed at the Chemical Biological Soil Laboratory (Wageningen UR, Wageningen, The Netherlands) as described by Novozamsky et al. (1984), unless insufficient sample was available. In that case N content was determined according to Patton et al. (2003) and P content according to Rowland et al. (1997).

Calculations and Statistics

Crude protein content was assumed to equal N content * 6.25, and P content was calculated by dividing P_2O_5 content by 2.3.

Reduction percentages were calculated by [1- (the amount present in the residual material / the amount offered as feed)] * 100%.

Similar to Diener et al. (2009), it was assumed that all feed offered had been consumed. The efficiency of conversion of ingested food to body substance (ECI) was calculated by (weight gained / weight of ingested food) * 100% (Waldbauer 1968).

A Kruskall-Wallis test, in which manure type was used as factor, followed by a post-hoc Scheffé test was performed to determine significance of differences ($P < 0.05$). Because the manure was obtained from a single farm, replicates were not independent, hence we used a conservative test to infer conclusions from the data. Correlation coefficients were determined by a Pearson Product Moment Correlation. All statistical analyses were performed using SPSS 19.0.

Results

BSF larvae were provided with 34-51 g of dry manure (Table 1). The survival rate of the BSF larvae was higher on pig manure than on chicken manure ($P = 0.019$), but similar to that on cow manure (Table 1). The development time was 144 days for BSF larvae on chicken and pig manure, but when fed on cow manure this was longer, *viz.* 215 days ($P = 0.003$). No significant differences in BSF yield were found among manure types (Table 1). Also, there was no difference regarding average fresh (0.068-0.083 g) or dry weight at harvest (0.014-0.017 g), or their DM content (Table 2). In the control treatment survival was also high (86.5%), however, less feed (16.2 g DM) was provided and development time was shorter (20 days). The yield on this chicken feed diet was also higher (11.6 g fresh weight, 4.21 g DM), as was the DM% (36.25%).

BSF larvae fed on chicken manure had a lower N-content (6.5% DM) than on the other treatments (6.9% DM; $P < 0.01$ for both), whereas P-content was highest on pig manure (1.99% DM), followed by chicken (1.65% DM; $P < 0.01$) and then cow manure (1.27% DM; $P < 0.01$ for both). Phosphorus content of BSF larvae was correlated with manure P-content (Pearson's $R = 0.892$; $P < 0.001$). Their N:P ratio was higher on cow manure (5.44) than on pig (3.49), and chicken manure

(3.97; Table 2).

Chicken manure had a higher N-content than pig and cow manure (P < 0.001, Table 2). Pig manure had the highest P content (2.58% of DM), followed by chicken manure (1.24% of DM), and cow manure (0.64% of DM; P < 0.001 for all groups). N:P ratio was highest for chicken manure, followed by cow manure and lowest for pig manure (Table 2). The composition of the residual material differed between the three manure types (P < 0.001) regarding dry matter-, N- and P-content (Table 2). The N:P ratio varied between 0.99 and 4.27 in the manure provided. It was similar in the residual material from the chicken and pig manure treatments (0.66-0.76), but much higher in the residual material from the cow manure treatment (3.06).

The DM weight of the manure was reduced by ~37% in all three treatments (Table 3). However, N reduction was different between manure types (P = 0.001); 82% for chicken manure, 37% for pig manure, and 30% for cow manure.

DM-ECI was higher on pig than on cow manure (P = 0.012), while it was intermediate for chicken manure. Nitrogen-ECI was highest on pig manure (P = 0.001), while P-ECI was highest on cow manure (P = 0.003).

Discussion

Life history characteristics of BSFs, such as survival rate and development time, are determined by a variety of factors such as temperature (May 1961), relative humidity (Holmes et al. 2012), feed availability (Furman et al. 1959; Diener et al. 2009), and feed composition (Gobbi et al. 2013). The survival rates in our study were high compared to literature values (Table 4), indicating that the tested manure types were suitable as feed.

However, the BSF larvae on our substrates reached the prepupal stage much later than in most other studies (Table 4). In part, this could be an effect of low dietary quality (Gobbi et al. 2013). A second factor could be the age of the larvae; we used neonate larvae, whereas in other studies larvae were first reared on a housefly medium for four days (Myers et al. 2008), or on an unreported diet for ten days (Li, Zheng et al. 2011), before they received a manure diet. In the latter study most prepupated and crawled out of the manure within 21 days. BSF larvae reared on dairy manure from hatching, took approximately 120 days to develop in prepupae (Sealey et al. 2011). These data indicate that larval age at transfer to manure, and dietary quality during early development could be a key factor for the development time of BSF larvae. The use of specialized starter diets (diets for optimal early development) is common in conventional production animals (Hansen, Nelssen et al. 1993; Gorka, Kowalski et al. 2009; Delcroix, Gatesoupe et al. 2014; Serbester, Çakmakçi et al. 2014). Also in BSF production systems starter diets have been used, however, the duration differs between producers and depends on the composition of the subsequent diet (Drew 2014; Katz 2014). Further studies should determine which diet should be provided for which period of time to reduce development time in large scale manure processing.

A third explanation for prolonged development in our study could be the feeding regime. The amount of feed provided was not quantified in Sealey et al. (2011). However, Li et al. (2011) provided 48 grams of cow manure (DM basis) per 100 larvae in a single feeding, and Myers et al. (2008) provided 32.8-72.4 g in 26-30 days (assuming the DM content of their manure was the same as ours). Whereas the amounts are similar to our study we provided this total amount distributed over a longer period of time, which could mean that feed was restricted during our study. Feed restriction leads to higher ECIs, while a surplus of feed leads to lower ECIs (Diener et al. 2009). Similar to Diener et al. (2009) we assumed for our ECI calculation that all provided feed was ingested. Although this assumption has not been checked, the validity was supported by observed changes in colour and texture of the residual material, compared to the manure provided. The DM-ECIs in our study were higher than for BSF larvae on coffee pulp (1.8%) (Lardé 1989), and lower than for BSF larvae on chicken feed (6.0-16.1%) (Diener et al. 2009). These ECIs seem to reflect substrate nutritional quality, a trend generally observed in insect nutrition (Slansky and Rodriguez 1987). For the DM-ECI of BSF larvae on manure limited data is available; an estimation of 4.2-7.8% was reported for chicken manure (Sheppard et al. 1994), and a DM-ECI of 12-16% on pig manure was suggested by Newton, Sheppard et al. (2005). However, how the latter values were obtained was not reported. Li et al. (2011) did not report the initial weight of their 10 day old larvae reared on cow manure, but if we would assume a negligible initial weight, their DM-ECI would be 12.2%.

These values indicate that our DM-ECIs might be considered low, which would indicate that a surplus of feed was provided. Such a surplus would lead to lower larval N contents, while feed restriction leads to higher N contents (Diener et al. 2009). Our N contents were similar to most published values, except for Dierenfeld et al. (2008) (Table 5). The approximately 50% higher N content in that study probably was due to feed restriction prior to analysis, during shipping of the commercially obtained BSF larvae. Larvae show feed searching behaviour (wandering) when they are deprived of feed. This was observed a few times (≥4 per replicate) during our study, indicating that feed was depleted on a limited number of occasions. Based on these observations, the ECIs calculated, and the larval N contents, we assume that in general our feeding regime approximated *ad* *libitum* feeding, although sometimes limited feed restriction occurred.

Variable values of prepupal fresh weight of BSFs have been reported: on chicken feed it was between 0.089 and 0.157 g (Diener et al. 2009); on dairy manure it was between 0.137 and 0.179 g when 40% reached the prepupal stage (Myers et al. 2008). Depending on the amount of feed available, prepupae weighed 0.11-0.22 g on chicken manure (Sheppard et al. 1994). In our study, the average weight was similar between treatments, but at the low end of the above mentioned values. This could be due to our feeding regime or because we did not first provide a starter diet to the neonate larvae. Furthermore, we harvested the prepupae when the first ones were found, meaning that most of the BSFs in our study had not yet reached the prepupal stage.

The average DM content of the BSFs in our study (~20%) was on the low end of reported values (17-40%; Table 5) except when reared on chicken feed (36%). This indicates that a large variation in DM content can occur on different diets, hence yields should be reported both as fresh and dry weight.

P-content of BSFs varied between treatments, but was similar to most published values (Table 5). While P content reported by Newton et al. (2005) for BSF larvae reared on chicken manure was similar to our study, it differed for pig manure (Table 5). Since the P-content of the pig manure used in that study was also lower than in our study (0.68 *vs.* 2.58%), and because P-content of BSF larvae was strongly correlated with the P-content of the manure provided, differences in P-content could in part be due to material present in the gastro-intestinal tract at the moment of sampling. Further studies should be conducted to provide accurate estimates of the relative contribution of the gut content to the total fresh and dry weight of BSF larvae.

The N:P ratio was higher for BSF larvae on cow manure than on the other two manure types ($P = 0.002$). In all treatments these were at the low end of the published range, as presented in Table 5.

Before drying, the DM content of dairy manure was 12% which is lower than reported values (19-47%) (Myers et al. 2008; Li et al. 2011; den Boer, Reijneveld et al. 2012). DM content of the chicken manure used (29.7%) was lower than reference values for Dutch farms (57.3%), while DM content of pig manure was higher (34.4 *vs*. 26%) (den Boer et al. 2012). In our study, water was added to the dried manure to prevent larval desiccation so that a DM% ~33% was obtained at the beginning of the experiment for all three manure types. This is lower than other studies with chicken feed and housefly medium (40-48% DM) (Furman et al. 1959; Diener et al. 2009). The addition of water at the beginning and during the experiment makes it impossible to evaluate the change in DM% between the manure and the residual material, due to BSF processing. While most studies with BSF larvae on chicken and dairy manure report increased DM percentages

(Sheppard 1983; Li et al. 2011), it has also been suggested that BSF larvae decrease the DM% of chicken manure (Axtell and Arends 1990). Factors such as evaporation and larval transpiration, together with the intake of DM and larval metabolism determine the final DM%.

During our experiment, the total amount of DM was reduced by ~37%. A larger DM reduction was reported for chicken manure (42-56%) (Sheppard 1983). However, DM reduction seems very variable (Table 4). Possibly, differences in DM reduction are partially due to differences in the suitability of the substrate for BSF larvae, and for micro-organisms. In our study micro-organism incidence (or abundance) might have been lower than for Sheppard (1983) because they used fresh manure, whereas ours had been dried.

In our, as well as in other studies, DM is much more reduced than DM-ECI. This is due to the metabolism of the larvae and possibly due to microbial activity. In our study, ~33% of the dry matter was metabolised, which seems fairly high compared to other studies but is in line with longer development time (Table 4).

Chicken manure generally contains more N than manure from other livestock (Nahm 2003). Total N content of the chicken manure in our study was similar to reference values for the Dutch livestock sector (4.8 *vs.* 4.47% DM); as was the case for pig manure (2.55 *vs*. 3.04% DM), and cow manure (2.74 *vs*. 2.73% DM) (den Boer et al. 2012). The latter value is higher than certain studies with cow manure (1.5-1.7%) (Eghball, Power et al. 1997; Li et al. 2011). Our three types of manure were collected from one farm, which was not necessarily representative. However, it seems that N content in our manures was representative for Dutch manures.

Approximately 70% of N in chicken manure is in the form of uric acid and 30% is in the form of undigested protein (Groot-Koerkamp 1994). If oxygen and water are present, uric acid is converted to volatile ammonia (Nahm 2003), which may explain the high N reduction (82.1%) found for chicken manure in our experiment. Because part of the N in pig manure is in the form of ammonia (Canh, Verstegen et al. 1997), it is plausible that in our study the difference between N-ECI and N-reduction (24%) was due to ammonia volatilisation. In a study where blowfly larvae were reared on pig manure, 22.5% of the N was volatilised (Nuov, Little et al. 1995). The reduction of N in cow manure in our study was on the low end of the range reported by Myers et al. (2008) (30 *vs.* 30-50%) .

Nitrogen-ECI was higher for BSF larvae reared on pig manure, than when reared on chicken or cow manure, which indicates that pig manure could be more suitable than cow or chicken manure. Larvae of the blowfly *Lucilia serricata* on fresh pig manure had a higher N-ECI (21%) (Nuov et al. 1995).

In our study, chicken manure contained less P than manure from pigs or cows (Table 2), contrary to the findings of Nahm (2003), and den Boer et al.

(2012). Accurate quantification of P reduction proved difficult in our study, as indicated by the large standard deviations. Phosphorus-ECI and the P reduction percentage were similar in our study. Because P, contrary to N, does not volatilize, P-ECI (amount of P retained in BSF larvae, divided by the amount of P in the manure provided) seems a good estimate for P reduction.

An ECI of 62-71% for available P has been reported for BSF larvae on dairy manure (Myers et al. 2008). However, total P, as we quantified in our study, was not reported in that study, and due to possibly large variations in the proportion of available P (22-56% of total P), a direct comparison cannot be made (Eghball et al. 1997; Malley, McClure et al. 2005).

The P concentration in the manure was increased by approximately 50% in the residual material for the three types of manure. This can be explained by the higher utilisation of other manure constituents by the larvae, and the volatilisation of N in the form of ammonia.

The residual material (manure after processing by BSF larvae) can be used as fertilizer in agriculture. In Nitrate Sensitive Areas, *i.e.* areas where the nitrate concentration in groundwater and surface water exceeds European limits, the application rate of animal manure is limited to 170 kg total N per ha per year. In many EU member states less than 170 kg total N per ha can be applied if the N content of a manure is associated with an amount of P that exceeds the agronomic demands (Schröder, Smit et al. 2011). In that situation mineral N fertilizer can be purchased, while manure with an excess of P is sold (Schröder and Verloop 2010). To prevent this, farms producing both animals and crops prefer manures with varying N:P ratio and a high N availability. On the other hand, arable farms prefer manures with a narrow N:P ratio and a somewhat slower release of N. Such manures allow for application of the manure as a source of P and organic matter on cereal stubbles in late summer, with little loss of N to the environment during the winter. Processing by BSF larvae changes the characteristics of manure, including the N:P ratio and possibly N availability, and could hence provide means to alter the composition to specific needs of farmers. While this processing decreases the total amount of N, it increases the N concentration in the residual material of cow and chicken manure. Increased P concentration after manure processing by BSF larvae could enable farmers to use the processed manure as a substitute for mineral P fertilizer (Schröder et al. 2010). Further studies should be conducted in order to determine to what extent the N fraction in the residual material is suitable as a fertilizer.

Conclusions

BSF larvae can develop on the three manure types studied. However, their development time was considerably extended compared to optimal diets. If a more nutritious starter diet decreases development time, this would increase the manure processing capacity of the system. Furthermore, increased processing speed would decrease N volatilization. Other measures to decrease N-loss, for instance by an air washer, should be taken to prevent air pollution. These measures would help to make manure processing with BSFs environmentally sound, and economically viable.

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Table 1: Survival rate, development time, and yield of Black soldier flies reared on either chicken, pig or cow manure (n=6)

Manure	Survival rate (%)	Development time (Days)	Yield (g fresh weight)
Chicken	82.2 ± 13.50^{a}	144.0 ± 33.12^{a}	5.68 ± 1.603^{a}
Pig	97.0 ± 4.73^{b}	144.0 ± 52.80^{a}	6.90 ± 1.400^{a}
Cow	87.8 ± 5.00^{ab}	214.5 ± 21.56^{b}	7.43 ± 1.414^{a}

Table 2: Original dry matter (DM, % of fresh weight), nitrogen (N) and phosphorus (P) content as a percentage of DM and N:P in manure provided (n=3), residual material (n=6), and in Black soldier fly larvae, and prepupae harvested (BSF) (n=6).

	Manure			Residual material			
	N	P	N:P	DM	N	P	N:P
Chicken	4.78 ± 0.156^{a}	1.24 ± 0.026^{a}	3.85 ± 0.100^{a}	40.0 ± 2.15^{a}	1.35 ± 0.077^{a}	1.86 ± 0.074^{a}	0.73 ± 0.014^{a}
Pig	2.55 ± 0.012^{b}	2.58 ± 0.062^{b}	0.99 ± 0.027^{b}	24.3 ± 1.29^{b}	2.59 ± 0.085^{b}	3.94 ± 0.148^{b}	0.66 ± 0.037^{a}
Cow	2.74 ± 0.087^{b}	0.64 ± 0.022^{c}	4.27 ± 0.037^{c}	20.8 ± 0.64^{c}	3.03 ± 0.178^{c}	0.99 ± 0.023^{c}	3.06 ± 0.144^{b}
				BSF			
				DM	N	P	N:P
Chicken				20.6 ± 0.98^{a}	6.53 ± 0.177^{a}	1.65 ± 0.124^{a}	3.97 ± 0.336^{a}
Pig				20.2 ± 0.46^{a}	6.90 ± 0.215^{b}	1.99 ± 0.190^{b}	3.49 ± 0.400^{a}
Cow				20.3 ± 0.29^{a}	6.87 ± 0.107^{b}	1.27 ± 0.066^{c}	5.44 ± 0.324^{b}

Table 3: Manure provided, ECI, and reduction of dry matter (DM), nitrogen (N), and phosphorus (P) as a percentage of DM, for Black soldier flies on three types of manure (n=6).

	Manure provided	ECI			Reduction		
	(g DM)	DM	N	P	DM	N	P
Chicken	35.4 ± 11.59^{a}	3.4 ± 0.49^{ab}	4.6 ± 0.59^{a}	4.5 ± 0.69^{a}	36.7 ± 3.45^{a}	82.1 ± 0.89^{a}	5.1 ± 3.77^{a}
Pig	34.3 ± 14.17^{a}	4.5 ± 1.37^{b}	12.4 ± 4.04^{b}	3.5 ± 0.87^{a}	37.7 ± 2.59^{a}	36.7 ± 4.22^{b}	5.0 ± 3.38^{a}
Cow	51.0 ± 10.38^{a}	2.9 ± 0.19^{a}	7.4 ± 0.48^{a}	5.8 ± 0.47^{b}	36.8 ± 1.43^{a}	30.0 ± 4.29^{c}	2.3 ± 2.69^{a}

Table 4: Comparative data on survival rate, development time, amount of feed provided, reduction of dry matter (DM) and metabolized DM for Black soldier fly larvae on various substrates and temperatures.

	Substrate	Temperature	Survival rate	Development time (days)	Grams of dry feed / surviving larva	DM reduction	DM metabolized
Diener et al. (2009)	Chicken feed	27	-	42	0.21	42%	26%
Diener et al. (2009)	Chicken feed	27	-	33	0.33	38%	25%
Diener et al. (2009)	Chicken feed	27	-	20	0.4	44%	33%
Diener et al. (2009)	Chicken feed	27	-	17	0.68	39%	30%
Diener et al. (2009)	Chicken feed	27	-	16	1.28	23%	17%
Gobbi et al. (2013)	Meat meal	25	40%	33	0.70	~31%	-
Gobbi et al. (2013)	Hen feed	25	93%	15	0.45	~70%	-
Lardé (1990)	Coffee pulp	25	-	-	-	30%	28%
Li et al. (2011)	Dairy manure	27	-	<31	0.48*	53%	27%
May (1961)	House fly medium	28	-	18	-	-	-
Myers et al. (2008)	Dairy manure	27	71-85%	26-30	3.85-7.09**	33-58%	-
Sealey et al. (2009)	Dairy manure	-	-	~120	-	-	-

- Not reported, * Not corrected for survival, ** Fresh matter basis

Table 5: Nitrogen and phosphorus content and their ratio of Black soldier flies on various substrates.

	Substrate	DM (% of live weight)	N (%DM)	P (%DM)	N:P
Bondari & Shephard (1981)	Chicken manure	17	6.1-6.4	-	-
Diener et al. (2009)	Chicken feed	33-40	5.1-7.4	-	-
Dierenfeld & King (2008)	Commercial diet	26.8	9.1-10.3	1.28-2.00	5.17-7.13
Newton et al. (1977)	Beef manure	-	6.74	1.50	4.49
Newton et al. (2005)	Poultry manure	-	6.74	1.51	4.46
Newton et al. (2005)	Swine manure	-	6.91	0.88	7.85

- Not reported

Chapter 7

General discussion

D.G.A.B. Oonincx

Introduction

Fundamental entomology has focused on insect molecular biology, genomics, physiology, interactions between insects and plants and multitrophic relationships, while applied entomology focused on topics such as beneficial insects for biological control, plant resistance, integrated pest management and development of methods to control disease vectors such as the malaria mosquito. Whereas the notion of insects as a source of nutrition has been around for millennia, until recently this topic received little interest. Probably the first scientist to publish on entomophagy was Aristotle in 350 BC (Thompson 1910). Up till the latter part of the 20th century the scientific community considered entomophagy mainly as a non-Western, ethno-biological phenomenon (Chapter 1). However, in recent years the attention for this topic from both the scientific community and the general public has increased. The number of scientific papers increases each year (Larsen and von Ins 2010), but the increase of papers on entomophagy has steeply increased. According to Scopus, there was less than one publication per year on 'entomophagy' between 1977 and 1996. This increased to 2.3 publications per year between 1997 and 2006, and increased further to 7 publications per year between 2007 and 2013.

The reasons for this increased interest is the urgency to sustainably respond to the increased demand for protein-rich food. This increased demand is caused by the expected growth of the human population from 7 to 9 billion by 2050, as well as increased prosperity in developing countries, expected to lead to an increased per capita consumption of 25% (FAO 2009; Herrero et al. 2013b). These factors in turn lead to increased prices of traditional protein sources, thereby fueling the search for alternatives, such as insects.

As mentioned in Chapter 1, the main arguments why insects could form a good alternative source of feed or food are: 1) reproductive capacity, 2) nutritional quality, 3) sustainability of insect production, 4) high feed conversion efficiency, and 5) possibility to use waste as feed.

It has to be taken into account that due to the number of insect species (~2000 edible species) (Jongema 2013), and the large differences between these species the aforementioned arguments cannot be valid for all species. However, I will give some generalized statements valid for a great number of species. Insect species that can be used as mini-livestock were selected as examples, and where possible compared to three conventional production animals; chickens, pigs and cows. These species were selected because they provide the bulk of animal protein in the Western diet.

Reproductive capacity

The reproductive capacity of an animal is determined by the number of progeny per female, the period required to produce this number of progeny (generation time), the survival rate of the progeny, and the ratio between males and females (Birch 1948). Reproductive capacity in natural systems is limited by the available amount of feed and space, and by predation. If feed and space are not limiting the reproductive rate would approach the reproductive capacity of a species.

Most of the parameters determining reproductive capacity were not experimentally quantified in this thesis. However, for many insect species the number of offspring per female outpaces the number of offspring per female of conventional production animals, such as cows, pigs and chicken (Table 1). This is even more true for the generation time of the insect species in Table 1, compared to the three conventional production animals.

Survival for animal species depends on many factors, for instance the production system or the feed composition. While for conventional production animals these elements have been optimized, this is not yet the case for many of the insect production systems. When provided with a feed that can be considered suitable, survival was above 80% for most species studied in chapter 5, except for the House crickets (55%). The latter might well have been caused by the expected presence of a densovirus in that species, which increases mortality (Szelei et al. 2011). For the conventional production animals mortality during rearing are low; between four and seven percent for chicken (Ross 2011), approximately three percent in pigs, and around 13% for cows (Vermeij 2013). However, this does not mean that all surviving animals are considered suitable for reproduction; for instance 32% of the sows is excluded (Vermeij 2013). Currently, suitability for reproduction is not considered in insect production. However, selection of the most fit individuals would increase the productivity of an insect production system, and improve the genetic makeup of the reared colony.

The sex ratio also influences the reproductive capacity of a species. For the conventional production animals the male to female ratios are approximately 1:1, and this seems the case for insects as well. However, sex ratios can be skewed due to genetic (Werren and Beukeboom 1998), or environmental conditions (Korpelainen 1990), as well as due to the presence of certain symbionts (Narita, Kageyama et al. 2007; Cordaux, Bouchon et al. 2011). In Yellow mealworms the sex ratio under normal circumstance is 1:1, however it becomes female biased when they are reared under low oxygen concentrations (Loudon 1988). Also, for Black Soldier Flies (BSFs), a female-biased population structure has been described, however, the cause of this is not reported (Gobbi et al. 2013).

The values listed in Table 1 show that many insect species produce more offspring in less time than the conventional production animals. Assuming a 1 to 1 sex ratio, and similar mortality, insects have a high reproductive capacity compared to conventional production animals.

Table 1: Number of offspring and generation time for a selection of insect species and chicken, pigs and cows

Species	Number of offspring	Generation time (days)
House cricket	200-300	84
Yellow mealworm	150-167	112
Migratory locust	150-300	90
Black soldier fly	400-1475	35
House fly	800-1500	15-23
Chicken	136-182	420-448
Pig	64-67	1097-1138
Cow	5	2738

(Jacobs et al. 1988; Nakagaki et al. 1991; Koketsu et al. 1999; Friederich et al. 2004; Ross 2011; Agrovision 2013; Cobb-Vantress 2013; Gobbi et al. 2013; Vermeij 2013; DAFF 2014; Chapter 5; Vermeij 2014)

Nutritional quality

Nutritional quality of insects is determined by their chemical composition, together with their digestibility in the consuming species, and its nutrient requirements. Several factors influence the composition of insects (Chapter 2). The most obvious factor, and likely the most influential is diet. In Chapter 5 diets differing in protein and fat content were provided to four insect species. Their composition was affected by the diet provided, for instance a diet high in protein increased their protein content, although the magnitude of the effect is species dependent. This enables producers to alter the chemical composition of insects through adapting their diet so that they best match the requirements of the animals or humans that consume them.

The digestibility of insects is the extent to which nutrients from the insect are absorbed. This depends on the species, its development stage, its chemical composition and the digestive system of the consumer. For some combinations of species this parameter has been quantified. Digestibility of nine insect species was determined in an *in vitro* setup, simulating a canine digestive system (Bosch, Zhang et al. 2014). Organic matter and protein digestibility of most insects (78-92%) was similar to poultry meat meal, fishmeal and soybean meal (81-95%). That study also showed that the organic matter and protein digestibility of BSF pupae (68 and 78%) was much lower than for larvae (84 and 90%). The quality of BSF larvae for growing chicks has been reported as equal to soybean or meat scraps (Hale 1973), and the protein quality of mealworms tested in growing rats was similar to casein and soybean (75-79%) (Goulet et al. 1978). An *in vivo* trial comparing BSFs with House crickets in a frog species reported a protein efficiency of 77 and 95%, respectively (Dierenfeld et al. 2008). These examples illustrate that differences occur between species and also between developmental stages of an insect species.

The nutrient requirement of the consumer also determines the nutritional quality of insects. Nutritional quality can be determined by multiplying the chemical composition with the digestibility coefficient per nutrient (if available), and comparing the resulting profile with the requirements of the consumer. This approach is suitable if insects, or even a single insect species, make up the complete diet of the target species. However, if we take humans as an example, a direct comparison as mentioned above would result in a diet that is too high in protein and fat, and too low in carbohydrates. Since in most cases the insect will only be a part of the diet, a more appropriate approach would be to compare the nutrient profile with a currently used dietary ingredient with a similar nutritional purpose. An example of this can be found in Table 2, in which the chemical composition of Yellow mealworms, House crickets and Migratory locusts is compared to beef, based on the nutrient requirements of humans. These data are not corrected for differences in digestibility due to a lack of reliable data. The match between the chemical composition of the insects and the nutrient requirement of people is similar to beef. However, there are large differences between insect species when evaluated on specific nutrients. Assuming similar digestibility of insects and beef in humans, the nutritional quality of insects can be considered as high as for beef.

Table 2: Percentage of dietary requirements for adult males (70 kg) fullfilled by a portion of 50 g DM

Dietary component	Yellow Mealworm	House Cricket	Migratory Locust	Beef
Protein	49.1	66.6	65.0	55.0
Fat	54.1	34.0	28.6	63.1
Metabolizable energy	9.8	8.3	9.2	11.0
Minerals				
Calcium	2.6	7.8	4.5	1.1
Phosphorus	59.8	76.6	52.2	34.6
Magnesium	30.9	16.1	12.8	7.1
Sodium	6.7	20.7	9.5	38.5
Potassium	16.3	20.5	18.7	12.1
Chloride	15.1	22.7	NA	NA
Iron	28.9	33.5	80.8	29.8
Zinc	55.7	88.9	70.2	51.0
Copper	35.6	44.7	75.1	5.2
Manganese	29.7	81.2	NA	NA
Iodine	15.4	23.5	NA	NA
Selenium	57.1	53.6	NA	35.8
Vitamins				
Vitamin E	ND	30.5	NA	2.7
Vitamin C	3.2	9.7	NA	ND
Thiamin	30.0	6.2	NA	8.9
Riboflavin	78.7	410.1	NA	16.9
Pantothenic acid	68.8	74.7	NA	NA
Niacin	29.7	34.6	NA	21.5
Pyridoxine	62.5	22.0	NA	20.8
Folic acid	103.0	121.8	NA	3.7
Biotin	71.6	50.2	NA	NA
Vitamin B12	35.3	498.1	NA	146.8
Choline	44.0	44.8	NA	19.0
Vitamin K	NA	NA	NA	2.5

NA = Not available. ND = Not detected. Data adapted from Garrow and James 2000; Finke 2002; FAO and WHO 2007; Oonincx and van der Poel 2011, and Haytowitz et al. 2012.

Sustainability

Differences in environmental impact of products from conventional production animals can be explained by three main factors: 1) differences in reproduction rates, 2) differences in enteric methane production, and 3) differences in feed conversion efficiency (de Vries et al. 2010). Because the first and last factor are often mentioned as separate arguments for entomophagy, they are discussed elsewhere in this chapter.

The direct production of methane was determined experimentally in Chapter 3 (Oonincx et al. 2010b). From that, and previous studies (Hackstein et al. 1994; Egert et al. 2003), it is clear that certain groups of insects, such as cockroaches, scarabs, and termites, produce methane due to bacterial symbionts. Methane is a potent greenhouse gas (GHG), which can greatly contribute to the Global Warming Potential (GWP; the impact factor for the effects of GHGs) associated with animal production systems (de Vries et al. 2010). The Argentinean cockroach (*Blaptica dubia)* and larvae of the Sun beetle (*Pachnoda marginata*) produce methane, resulting in a GWP respectively five and 16 times greater than for Yellow mealworms, when expressed per kg of mass gain (Oonincx et al. 2010b). Similarly large differences occurred for N_2O showing that large differences in GHG emissions occur between insect species. However, when expressed in CO_2 equivalents, for all five insect species the GHG production was low compared to pigs and beef cattle (Oonincx et al. 2010b). The ammonia production of the insect species was also far lower than for pigs (10-1000 times) when expressed per kg of mass gain.

The relevance of the direct GHG production is limited when considered in the light of the entire production process. This is shown in Chapter 4, in which the direct GHG production of the insect was quantified to be 0.29% of the total GWP of mealworm production (Oonincx et al. 2012a). The main sources of GHG emissions of mealworm production are associated with energy use (43%) and feed production (56%). The high energy usage is due to the need of providing a high ambient temperature. Feed utilization of insects is discussed in the following paragraph. Since the environmental impact of insect production has been quantified for only one insect farm, it is currently unwarranted to evaluate the sustainability of insect production in general.

Feed conversion efficiency

Feed conversion efficiency is a major determinant for the environmental impact of conventional production animals (de Vries et al. 2010), as well as for mealworms (Oonincx et al. 2012a). It has a strong impact on GHG emissions and land use (Herrero et al. 2013a). How efficiently feed is converted to body mass is determined by the composition of a feed, and its digestibility within the target animal (Herrero et al. 2013a). For conventional production animals energy density is an important factor (Herrero et al. 2013a). It seems that in insects protein composition and density are of greater importance for their growth, development and fecundity (Friend 1958; House 1961). A possible explanation for this is their relatively low metabolic rate (Oonincx et al. 2010b), leading to lower energy requirements for their resting metabolism than for conventional production animals. There are indications that a minimal content of body protein is required to reach adulthood in insects, whereas the lipid content is variable (Oonincx et al. 2011).

Feed conversion ratios (FCRs) can be expressed in different ways. For comparison reasons, in this chapter it is defined as the amount of feed (grams) required to increase the body mass (grams). Both values are expressed on a fresh matter basis unless mentioned otherwise. Variation in the dry matter (DM) content of the feed can greatly influence the FCR. The DM content of a meal used as feed often approximates 90% (Chapter 5), while the DM content of a live insect ranges from 15-40% (Finke et al. 2013). Hence, when water is provided separately and therefore not accounted for in the FCR, an FCR lower than unity is possible. Ranges for FCRs of a selection of insect species and conventional production animals are provided in Table 3. For cows the FCR is presented on a DM basis because representative fresh weight values were not available. The reported FCR for Migratory locusts is higher than for the other insect species because its feed had a DM content of ~10% (Simpson 1982). Correcting the DM content of the feed to 90%, as is the case for the other insect species, results in an FCR of 0.73-0.92.

No studies have been published in which the same diet was provided to an insect and a conventional production animal. A direct comparison in feed conversion efficiency can therefore not be made. The FCR provides some insight in the efficiency of feed utilization, but without taking compositional differences into account conclusions are unwarranted. However, the general notion that insects utilize their feed efficiently is supported by the values listed in Table 3. It seems that the FCR of these insects are most similar to chicken, on the low side of pigs, and lower than for beef cattle. Also the rate of conversion is of importance in animal production. This can be expressed as average daily gain (ADG (%)), which was determined for five insect species in Chapter 3. These five species had higher

ADGs (4.0-19.6%) than pigs (3.2%), and beef cattle (0.3%) (Oonincx et al. 2010b). For broilers the ADG is approximately 5.5% (Ross 2011), which is similar to four of the insect species studied (4.0-7.3%), whereas the Migratory locusts had a much higher ADG (19.6%) (Oonincx et al. 2010b).

When insects are used as food another relevant factor is the edible portion. This is higher for insects than for conventional production animals (Table 3). This means that the feed is converted to food more efficiently in insects than in conventional production animals than can be deducted from the FCR alone.

Table 3: Feed conversion Ratio (FCR) and edible portion of a selection of insect species, chicken, pigs and cows

Species	FCR	Edible portion (%)
House cricket	0.92-2.3	80
Yellow mealworm	1.8-3.1	100
Migratory locust	6.7-8.3	80 **
Black soldier fly	1.4-2.6	100
Chicken	1.8	56-72
Pig	2.86	53
Cow	4.21*	40-46

Chapter 5,(Simpson 1982; Nakagaki et al. 1991; de Vries et al. 2010; Ross 2011; Cobb-Vantress 2013; Vermeij 2014)

* DM basis, mother excluded, ** Extrapolated from Nakagaki and deFoliart (1991)

“Waste” as feed

Cereal grains are regularly used as feed, however, these could also be directly used as food (Wilkinson 2011). Therefore it is more sensible to use other ingredients for insect diets. Certain species, for instance the BSF, the House fly, and certain cockroach species can be grown on a large variety of substrates. However, many other species, for instance Migratory locusts, accept a much smaller range of dietary constituents (Bernays, Chapman et al. 1976). In Chapter 5 four insect species were provided with diets composed of industrial by-products. Based on the reported survival and developmental times, some of these diets were suitable for the species selected. Since these by-products are widely used as animal feed ingredients they should not be considered waste. If waste is seen as resources out of place (Kollikkathara et al. 2009), animal manure does fall under that definition in the current Dutch context. The suitability of manure as feed for BSFs was evaluated in Chapter 6. Although the BSF larvae had a low mortality, the developmental period was five to ten times longer than for BSFs provided with diets composed of industrial by-products (Chapter 5), or BSFs provided with manure for a part of their developmental period (Myers et al. 2008). The latter indicates that manure could be used as a part (either during a certain period, or as an ingredient) of the larval diet. However, within the European Union, products such as manure, or parts of animals that are not, or not anymore, intended for human consumption, are banned as feed (Regulation 1069/2009).

In summary, the argument that insects can use waste as feed, is valid for a limited number of insects species and depends strongly on the definition of waste adopted. Furthermore, legislation in certain cases prohibits the use of waste as insect feed. These prohibitions should be justified by scientific studies, or lifted to aid the sustainable development of the insect producing sector.

The future

The five arguments described in the introductory chapter and discussed above, indicate that insects have the potential to form a good alternative source of feed or food. The abundance of insect species should be used to determine which species might form an addition to the currently produced species. When selecting species, this can be based on productivity characteristics and body composition, but also on the capability to up-cycle organic side streams. Besides selection of species, genetic selection within a produced species can lead to strains with different nutrient requirements, development times and other properties (House 1961; Leclercq 1963; Urs et al. 1973a; Urs et al. 1973b; Zhou, Tomberlin et al.

2013; Francuski, Djurakic et al. 2014). Selective breeding can thus make species more suitable as production animals within a specific production system.

Further implementation of insects as a source of feed or food will require more research. In many cases the objectives of such studies will have strong parallels with studies on conventional production animals. Technical improvements such as improving animal diets will likely lead to increased productivity and efficiency, and thereby increased sustainability (Herrero et al. 2013b). Therefore, insect nutrition will require further studies, for instance regarding optimization of diets during the starting, growing and finishing phase. This is common in poultry, pig and beef production, and can likely be applied in insects. This could mean that insects that are required for the colony are reared in a different way than those that are to be sold. For the first, reproductive output would be important, while for the latter the composition would be important. The finishing diet could serve to adapt body composition in order to produce insects that are tailored towards providing nutrients to their consumer (human or animals). Subsequently, *in vitro* and *in vivo* digestibility trials should determine which proportion is digested by the consuming species. Currently insects are considered mainly as a source of high quality proteins. Other components, for instance fat, should also be considered as relevant. These could serve as nutrients for humans and animals, but could also be used for other purposes, such as bio fuels (Yang, Li et al. 2012; Zheng, Hou et al. 2012; Zheng, Li et al. 2012; Zheng, Hou et al. 2013; Yang and Liu 2014), or antibiotics (Choi, Yun et al. 2012; Mohtar, Yusof et al. 2014; Park, Chang et al. 2014). Lastly, before insects are used as a source of food or feed on a large scale within the Western world, methods to guarantee feed and food safety need to be developed (Veldkamp, Duinkerken et al. 2012; Belluco, Losasso et al. 2013; Caparros Megido, Sablon et al. 2013; Lv, Liu et al. 2013; Spiegel, Noordam et al. 2013; van Huis, Van Itterbeeck et al. 2013). This also includes studies on the prevalence and intensity of allergic reactions due to insect consumption (Barre, Caze-Subra et al. 2014; Van der Brempt and Moneret-Vautrin 2014; Verhoeckx, van Broekhoven et al. 2014).

Progress in research on the abovementioned topics will aid in the further development of the insect producing sector. This development should be fueled by three types of stakeholders; producers, knowledge institutes, and governments, sometimes collectively called “the golden triangle”. An example of how this golden triangle has functioned in the Netherlands is the so-called Greendeal “Insects for feed, food and pharma”. Knowledge institutions develop methods which are subsequently applied by insect producers, while the government removes unnecessary legal hurdles. Joined efforts of these partners will materialise the potential of insects as production animals.

References

Aarnink, A. J. A., Keen, A., Metz, J. H. M., Speelman, L. and Verstegen, M. W. A. (1995). Ammonia emission patterns during the growing periods of pigs housed on partially slatted floors. Journal of Agricultural Engineering Research 62: 105-116.

Abasiekong, S. F. (1997). Effects of termite culture on crude protein, fat and crude fibre contents of fibrous harvest residues. Bioresource Technology 62: 55-57.

Adamolekun, B. (1993). *Anaphe venata* entomophagy and seasonal ataxic syndrome in southwest Nigeria. The Lancet 341: 629.

Adamolekun, B., McCandless, D. W. and Butterworth, R. F. (1997). Epidemic of seasonal ataxia in Nigeria following ingestion of the African silkworm *Anaphe venata*: role of thiamine deficiency? Metabolic Brain Disease 12: 251-258.

Africanus, L. (1896). The History and Description of Africa, and of the notable Things therein contained. London, The Hakluyt society, pp 1-9.

Agea, J. G., Biryomumaisho, D., Buyinza, M. and Nabanoga, G. N. (2008). Commercialization of *Ruspolia nitidula* (Nsenene grasshoppers) in Central Uganda. African Journal of Food Agriculture and Development 8: 319-332.

Aguilar-Miranda, E. D., Lopez, M. G., Escamilla-Santana, C. and Barba de la Rosa, A. P. (2002). Characteristics of maize flour tortilla supplemented with ground *Tenebrio molitor* larvae. Journal of Agricultural and Food Chemistry 50: 192-195.

Agutter, P. S. and Wheatley, D. N. (2004). Metabolic scaling: Consensus or controversy? Theoretical Biology and Medical Modelling 1: 13-23

Aidley, D. J. (1976). Increase in respiratory rate during feeding in larvae of armyworm, *Spodoptera exempta*. Physiological Entomology 1: 73-75.

Akinnawo, O. O., Abatan, M. O. and Ketiku, A. O. (2002). Toxicological study on the edible larva of *Cirina Forda* (Westwood). African Journal of Biomedical Research 5: 43-46.

Akman Gündüz, N. E. and Gülel, A. (2002). Effect of temperature on development, sexual maturation time, food consumption and body weight of *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae). Türk Zooloji Dergisi 26: 223-227.

Aldama-Aguilera, C., Lianderal-Cazares, C., Soto-Hernandez, M. and Castillo-Marquez, L. E. (2005). Cochineal (*Dactylopius coccus* Costa) production in prickly pear plants in the open and in microtunnel greenhouses. Agrociencia 39: 161-171.

Aldrovandi, U., Ferroni, C. and Salmincio, S. (1638). De Animalibus insectis libri septem: cum singulorum iconibus ad viuum expressis, Apud Clementem Ferronium, pp 767.

Ali, M. and Ewiess, M. A. (1977). Photoperiodic and temperature effects on rate of development and diapause in the green stink bug, *Nezara viridula* L. (Heteroptera: Pentatomidae). Zeitschrift für Angewandte Entomologie 84: 256-264.

Ali, M. F., Mashaly, A. M. A., Mohammed, A. A. and El -Magd Mahmoud Mohammed, A. (2011). Effect of temperature and humidity on the biology of *Attagenus fasciatus* (Thunberg) (Coleoptera: Dermestidae). Journal of Stored Products Research 47: 25-31.

Allen, M. E. and Oftedal, O. T. (1989). Dietary manipulation of the calcium content of feed crickets. Journal of Zoo and Wildlife Medicine 20: 26-33.

Allotey, J. and Mpuchane, S. (2003). Utilization of useful insects as food source. African Journal of Food, Agriculture, Nutrition and Development 3: 1-6.

Anderson, S. J. (2000). Increasing calcium levels in cultured insects. Zoo Biology 19: 1-9.

Angilletta Jr, M. J., Steury, T. D. and Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44: 498-509.

Anonymous. (2002, 10-07-2002). “Why not bugs?” Stalking the wild The Magazine of Outdoor Discovery. Retrieved 10-07-2002, from http://www.stalkingthewild.com/why_not_bugs.htm.

Armstrong, G. and Mordue, W. (1985). Oxygen consumption of flying locusts. Physiological Entomology 10: 353-358.

Asp, E. H. (1999). Factors affecting food decisions made by individual consumers. Food Policy 24: 287-294.

Axelsson, B. and Ágren, G. I. (1979). A correction for food respiration in balancing energy budgets. Entomologia Experimentalis et Applicata 25: 260-266.

Axtell, R. and Arends, J. (1990). Ecology and management of arthropod pests of poultry. Annual Review of Entomology 35: 101-126.

Backwell, L. R. and d’Errico, F. (2001). Evidence of termite foraging by Swartkrans early hominids. Proceedings of the National Acadamy of Sciences of the United States of America 98: 1358-1363.

Bailey, C. G. and Singh, N. B. (1977). Energy budget for *Mamestra configurata* (Lepidoptera-Noctuidae). Canadian Entomologist 109: 687-693.

Banjo, A. D., Lawal, O. A. and Songonuga, E. A. (2006). The nutritional value of fourteen species of edible insects in southwestern Nigeria. African Journal of Biotechnology 5: 298-301.

Barbehenn, R. V. (2003). Antioxidants in grasshoppers: higher levels defend the midgut tissues of a polyphagous species than a graminivorous species. Journal of Chemical ecology 29: 683-702.

Barker, D., Fitzpatrick, M. P. and Dierenfeld, E. S. (1998). Nutrient composition of selected whole invertebrates. Zoo Biology 17: 123-134.

Barlow, J. S. (1965). Composition of the fats in pupae of *Agria affinis* (Fallén) (Diptera: Sarcophagidae). Canadian Journal of Zoology 43: 291-295.

Barre, A., Caze-Subra, S., Gironde, C., Bienvenu, F., Bienvenu, J., et al. (2014). Entomophagy and the risk of allergy. Revue Française d'Allergologie 54: 315-321.

Barrientos, M. and Soria, C. (2013). "Fishmeal Monthly Price." Retrieved 18-02-2013, 2013, from www.indexmundi.com.

Barwa, E. (2009). Increasing household protein consumption through minilivestock production in Nigeria. The Information Manager 9: 10-13.

Baskes, J. (2005). Colonial institutions and cross-cultural trade: Repartimiento credit and indigenous production of cochineal in eighteenth-century Oaxaca, Mexico. Journal of Economic History 65: 186-210.

Basseri, H. R., Emmami, N., Haji-Hosseini, R., Abolhasani, M. and Moradi, A. (2008). Biological transmission of bacteria inhibit by hemolymph lectins of American cockroach. Iranian Journal of Public Health 37: 75-82.

Beenakkers, A. M. T., Meisen, M. A. H. Q. and Scheres, J. M. J. C. (1971). Influence of temperature and food on growth and digestion in fifth instar larvae and adults of *Locusta*. Journal of Insect Physiology 17: 871-880.

Beenakkers, A. M. T. and Scheres, J. M. J. C. (1971). Dietary lipids and lipid composition of the fat-body of *Locusta migratoria*. Insect Biochemistry 1: 125-129.

Beenakkers, M. T. A., Van der Horst, D. J. and Van Marrewijk, W. J. A. (1985). Insect lipids and lipoproteins, and their role in physiological processes. Progress in Lipid Research 24: 19-67.

Beets, W. C. (1997). The need for an increased use of small and mini-livestock in integrated smallholder farming systems. Ecology of Food and Nutrition 36: 237-245.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., et al. (2013). Edible insects in a food safety and nutritional perspective: A critical review. Comprehensive Reviews in Food Science and Food Safety 12: 296-313.

Bender, D. A. (2002). Introduction to nutrition and metabolism. London, CRC Press, pp 450.

Bennett, G., Selby, J., Marsh, K., Vantrappen, G., Geboes, K., et al. (1993). Pest control: Cockroaches as carriers of bacteria. The Lancet 341: 732-732.

Bequaert, J. (1921). Insects as food. How they have augmented the food supply of mankind in early and recent years. The American Museum Journal 21: 191-200.

Berenbaum, M. R. (1995). Bugs in the system. Helix books, Addison-Wesley Publishing Company, Reading.

Berenbaum, M. R. and Miliczky, E. (1984). Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. American Midland Naturalist: 64-68.

Berkvens, N., Bonte, J., Berkvens, D., Tirry, L. and De Clercq, P. (2008). Influence of diet and photoperiod on development and reproduction of European populations of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). BioControl 53: 211-221.

Bernard, J. B. and Allen, M. E. (1997). Feeding captive insectivorous animals: Nutrional aspects of insects as food. Nutrition Advisory Group handbook 1997: 1-7.

Bernays, E. A., Chapman, R. F., MacDonald, J. T. and Salter, J. E. R. (1976). The degree of oligophagy in *Locusta migratoria* (L.). Ecological Entomology 1: 223-230.

Birch, L. C. (1948). The intrinsic rate of natural increase of an insect population. Journal of Animal Ecology 17: 15-26.

Blanes-Vidal, V., Hansen, M. N., Pedersen, S. and Rom, H. B. (2008). Emissions of ammonia, methane and nitrous oxide from pig houses and slurry: Effects of rooting material, animal activity and ventilation flow. Agriculture, Ecosystems & Environment 124: 237-244.

Bloch, G., Francoy, T. M., Wachtel, I., Panitz-Cohen, N., Fuchs, S., et al. (2010). Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees. Proceedings of the National Academy of Sciences of the United States of America 107: 11240-11244.

Blomquist, G. J., Borgeson, C. E. and Vundla, M. (1991). Polyunsaturated fatty acids and eicosanoids in insects. Insect Biochemistry 21: 99-106.

Blonk, M. A. and Research, W.-L. (2011). Drafts of data reports carbon footprint of animal nutrition. Gouda/Lelystad, the Netherlands

Bodenheimer, F. S. (1951). Insects as human food; a chapter of the ecology of man. The Hague, Dr. W. Junk, Publishers, pp 352

Bodnaryk, R. P. (1981). The biosynthesis, function and fate of taurine during the metamorphosis of the noctuid moth *Mamestra configurata* Wlk. Insect Biochemistry 11: 199-205.

Bondari, K. and Sheppard, D. C. (1981). Soldier fly larvae as feed in commercial fish production. Aquaculture 24: 103-109.

Bondari, K. and Sheppard, D. C. (1987). Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquaculture and Fisheries Management 18: 209-220.

Borgeson, C. E. and Blomquist, G. J. (1993). Subcellular location of the [Delta]12 desaturase rules out bacteriocyte contribution to linoleate biosynthesis in the house cricket and the American cockroach. Insect Biochemistry and Molecular Biology 23: 297-302.

Borgeson, C. E., Kurtti, T. J., Munderloh, U. G. and Blomquist, G. J. (1991). Insect tissues, not microorganisms, produce linoleic-acid in the house cricket and the American cockroach. Experientia 47: 238-241.

Bosch, G., Zhang, S., Oonincx, D. G. A. B. and Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. Journal of Nutritional Science 3: 1-4.

Bostock, J. and Riley, H. (1855). Pliny the Elder. The Natural History.

Brower, L. P. (1969). Ecological Chemistry. Scientific American: 22-29.

Bruins, E. (2001). Geïllustreerde Terrariumencyclopedie. Lisse, The Netherlands., Rebo Productions BV, pp 320.

Bukkens, S. G. F. (1997). The nutritional value of edible insects. Ecology of Food and Nutrition 36: 287-319.

Bureš, S. and Weidinger, K. (2003). Sources and timing of calcium intake during reproduction in flycatchers. Oecologia 137: 634-647.

Cabaraux, J.-F., Philippe, F.-X., Laitat, M., Canart, B., Vandenheede, M., et al. (2009). Gaseous emissions from weaned pigs raised on different floor systems. Agriculture, Ecosystems & Environment 130: 86-92.

Calvez, B. (1975). Effect of nutritional level of diet on chemical composition of organs and their interrelations in silkworm *Bombyx mori* L. Annales de la Nutrition et de l’ Alimentation 29: 259-269.

Canh, T., Verstegen, M., Aarnink, A. and Schrama, J. (1997). Influence of dietary factors on nitrogen partitioning and composition of urine and feces of fattening pigs. Journal of Animal Science 75: 700-706.

Caparros Megido, R., Sablon, L., Geuens, M., Brostaux, Y., Alabi, T., et al. (2013). Edible insects acceptance by Belgian consumers: Promising attitude for entomophagy development. Journal of Sensory Studies 29: 14-20.

Carlsson-Kanyama, A. (1998). Climate change and dietary choices - how can emissions of greenhouse gases from food consumption be reduced? Food Policy 23: 277-293.

Casey, J. W. and Holden, N. M. (2006). Greenhouse gas emissions from conventional, agri-environmental scheme, and organic Irish suckler-beef units. Journal of Environmental Quality 35: 231-239.

Cauchie, H. M. (2002). Chitin production by arthropods in the hydrosphere. Hydrobiologia 470: 63-96.

Cazemier, A. E., Hackstein, J. H. P., Op den Camp, H. J. M., Rosenberg, J. and van der Drift, C. (1997). Bacteria in the intestinal tract of different species of arthropods. Microbial Ecology 33: 189-197.

Cerda, H., Martinez, R., Briceno, N., Pizzoferrato, L., Manzi, P., et al. (2001). Palm worm: (*Rhynchophorous palmarum*) traditional food in Amazonas, Venezuela - nutritional composition, small scale production and tourist palatibility. Ecology of Food and Nutrition 40: 13-32.

Cervellon, M.-C. and Dubé, L. (2005). Cultural influences in the origins of food likings and dislikes. Food Quality and Preference 16: 455-460.

Chadwick, D. R., Sneath, R. W., Phillips, V. R. and Pain, B. F. (1999). A UK inventory of nitrous oxide emissions from farmed livestock. Atmospheric Environment 33: 3345-3354.

Choi, W. H., Yun, J. H., Chu, J. P. and Chu, K. B. (2012). Antibacterial effect of extracts of *Hermetia illucens* (Diptera: Stratiomyidae) larvae against Gram-negative bacteria. Entomological Research 42: 219-226.

Chown, S. L., Marais, E., Terblanche, J. S., Klok, C. J., Lighton, J. R. B., et al. (2007). Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21: 282-290.

Clifford, C. W. and Woodring, J. (1990). Methods for rearing the house cricket, *Acheta domesticus* (L.), along with baseline values for feeding rates, growth rates, development times, and blood composition. Journal of Applied Entomology 109: 1-14.

Cobb-Vantress (2013). Cobb 500 SF Breeder Management Supplement. Siloam Springs, AR, USA.

CodexAlimentarius, C. (2008). Guideline for the Validation of Food Safety Control Measures. Rome, pp 16.

Cohen, A. C. (2003). Insect Diets: Science and Technology, CRC Press, London, UK, pp 324.

Cole, N. A., Clark, R. N., Todd, R. W., Richardson, C. R., Gueye, A., et al. (2005). Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure. Journal of Animal Science 83: 722-731.

Collavo, A., Glew, R. H., Huang, Y.-S., Chuang, L.-T., Bosse, R., et al. (2005). House cricket small-scale farming. Ecological Implications of Minilivestock: potential of insects, rodents, frogs and snails. M. G. Paoletti. Enfield, New Hampshire, U.S.A., Science Publishers Inc: pp. 519-544.

Cookman, J. E., Angelo, M. J., Slansky Jr, F. and Nation, J. L. (1984). Lipid content and fatty acid composition of larvae and adults of the velvetbean caterpillar, *Anticarsia gemmatalis*, as affected by larval diet. Journal of Insect Physiology 30: 523-527.

Cordaux, R., Bouchon, D. and Grève, P. (2011). The impact of endosymbionts on the evolution of host sex-determination mechanisms. Trends in Genetics 27: 332-341.

Cowan, F. (1865). Curious Facts in the History of Insects, including Spiders and Scorpions, Lippincott & Co., pp 396.

Crippen, T. L., Sheffield, C. L., Esquivel, S. V., Droleskey, R. E. and Esquivel, J. F. (2009). The acquisition and internalization of Salmonella by the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Vector-Borne and Zoonotic Diseases 9: 65-71.

Crippen, T. L., Zheng, L., Sheffield, C. L., Tomberlin, J. K., Beier, R. C., et al. (2012). Transient gut retention and persistence of *Salmonella* through metamorphosis in the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Applied Microbiology 112: 920-926.

Cripps, C., Blomquist, G. J. and de Renobales, M. (1986). De novo biosynthesis of linoleic acid in insects. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 876: 572-580.

Cunningham, E. (2001). Entomophagy: What is it and why are people doing it? Journal of the American Dietetic Association 101: 785-785.

da Silva, J. K. L., Garcia, G. J. M. and Barbosa, L. A. (2006). Allometric scaling laws of metabolism. Physics of Life Reviews 3: 229-261.

Das, M., Ganguly, A. and Haldar, P. (2009). Space requirement for mass rearing of two common Indian Acridid adults (Orthoptera: Acrididae) in laboratory condition American-Eurasian J. Agric. & Environ. Sci. 6: 313-316.

Dashefsky, H. S., Anderson, D. L., Tobin, E. N. and Peters, T. M. (1976). Face fly pupae: A potential feed supplement for poultry. Environmental Entomology 5: 680-682.

Davies, R. H. and Wray, C. (1995). Contribution of the lesser mealworm beetle (*Alphitobius diaperinus*) to carriage of *Salmonella enteritidis* in poultry. The Veterinary record 137: 407-408.

de Boer, I. J. M. (2008). Personal Communication. D. Oonincx. Wageningen.

De Jesús Méndez-Gallegos, S., Panzavolta, T. and Tiberi, R. (2003). Carmine Cochineal *Dactylopius coccus* Costa (Rhyncota: Dactylopiidae): Significance, production and use. Advances in horticultural science 17: 165-171.

de Réaumur, R. A. F. (1736). Mémoires pour servir à l'histoire des insectes, Imprimerie Royale.

de Renobales, M., Cripps, C., Stanley-Samuelson, D. W., Jurenka, R. A. and Blomquist, G. J. (1987). Biosynthesis of linoleic acid in insects. Trends in Biochemical Sciences 12: 364-366.

de Vries, M. and de Boer, I. J. M. (2010). Comparing environmental impacts for livestock products: A review of life cycle assessments. Livestock Science 128: 1-11.

DeFoliart, G. (1990). Insects as food in indigenous populations. In: Proceedings of the First International Congress of Ethnobiology (Belem, 1988), Vol. 1, Belem, Museu Paraense Emilio Goeldi, 1990, p. 145-150.

DeFoliart, G. (1992). Insect as human food; Gene DeFoliart discusses some nutritional and economic aspects. Crop Protection 11: 395-399.

DeFoliart, G. R. (1989). The human use of insects as food and as animal feed. Bulletin of the Entomological Society of America 35: 22-35.

DeFoliart, G. R. (1995). Edible insects as minilivestock. Biodiversity and Conservation 4: 306-321.

DeFoliart, G. R. (1999). Insects as food: Why the western attitude is important. Annual Review of Entomology. 44: 21-50.

DeFoliart, G. R. (2002). The human use of insects as food resource: a bibliographic account in progress. http://www.food-insects.com/book7_31/The%20 human%20use%20of%20insects.

DeFoliart, G. R. (2005). An overview of role of edible insects in preserving biodiversity. Ecological Implications of Minilivestock: Potential of Insects, Rodents, Frogs, and Snails. . M. G. e. Paoletti. New Hampshire, U.S.A., Science Publishers, Inc. Enfield: 141-161.

Delcroix, J., Gatesoupe, F. J., Desbruyères, E., Huelvan, C., Le Delliou, H., et al. (2014). The effects of dietary marine protein hydrolysates on the development of sea bass larvae, *Dicentrarchus labrax*, and associated microbiota. Aquaculture Nutrition.

Demmers, T. G. M., Burgess, L. R., Short, J. L., Phillips, V. R., Clark, J. A., et al. (1999). Ammonia emissions from two mechanically ventilated UK livestock buildings. Atmospheric Environment 33: 217-227.

Demmers, T. G. M., Phillips, V. R., Short, L. S., Burgess, L. R., Hoxey, R. P., et al. (2001). SE--Structure and environment: Validation of ventilation rate measurement methods and the ammonia emission from naturally ventilated dairy and beef buildings in the United Kingdom. Journal of Agricultural Engineering Research 79: 107-116.

den Boer, D. J., Reijneveld, J. A., Schröder, J. J. and van Middelkoop, J. C. (2012). Mestsamenstelling in Adviesbasis Bemesting Grasland en Voedergewassen. Wageningen, Wageningen UR Livestock Research, pp 24.

Diener, S., Zurbrugg, C. and Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management & Research 27: 603-610.

Dierenfeld, E. S. and King, J. (2008). Digestibility and mineral availability of Phoenix Worms (*Hermetia illucens*) ingested by Mountain Chicken Frogs (*Leptodactylus fallax*). Journal of Herpetological Medicine and Surgery 18: 100-105.

Dikeman, C. L., Plesuk, S. D., Klimek, D. L. and Simmons, L. G. (2007). Effect of gut-loading time on nutrient content of adult feeder crickets. Journal of Animal Science 85: 195.

Dominy, N. J., Davoust, E. and Minekus, M. (2004). Adaptive function of soil consumption: an in vitro study modeling the human stomach and small intestine. Journal of Experimental Biology 207: 319-324.

Donoghue, S. (2006). Reptile medicine and surgery. D. R. Mader, Saunders: 251-298.

Donovan, E. and Westwood, J. O. (1842). Natural history of the insects of China: Containing upwards of two hundred and twenty figures and descriptions, Henry G. Bohn, pp 216.

Downer, R. G. H. and Matthews, J. R. (1976). Patterns of lipid distribution and utilisation in insects. Integrative and Comparative Biology 16: 733-745.

Drew, D. (2014). Personal communication.

Dreyer, J. J. and Wehmeyer, A. S. (1982). On the nutritive value of mopanie worms. South African Journal of Science 78: 33-35.

Dufour, D. L. (1987). Insects as food: A case study from the Northwest Amazon. American Anthropologist 89: 383-397.

Duncan, F. D., Krasnov, B. and McMaster, M. (2002). Metabolic rate and respiratory gas-exchange patterns in tenebrionid beetles from the Negev Highlands, Israel. Journal of Experimental Biology 205: 791-798.

Ecoinvent (2004). Final Reports Ecoinvent 2000. S. C. f. L. C. Inventories. Dubendorf, Switzerland, Swiss Centre for Life Cycle Inventories.

Eeva, T., Helle, S., Salminen, J.-P. and Hakkarainen, H. (2010). Carotenoid composition of invertebrates consumed by two insectivorous bird species. Journal of chemical ecology 36: 608-613.

Egert, M., Wagner, B., Lemke, T., Brune, A. and Friedrich, M. W. (2003). Microbial community structure in midgut and hindgut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Applied and Environmental Microbiology 69: 6659-6668.

Eghball, B., Power, J. F., Gilley, J. E. and Doran, J. W. (1997). Nutrient, carbon, and mass loss during composting of beef cattle feedlot manure. Journal of Environmental Quality 26: 189-193.

Ekoue, S. E. and Hadzi, Y. A. (2000). Production d'asticots comme source de proteines pour jeunes volailles au togo - observations preliminaires. Tropicultura 18: 212-214.

Ekpo, K. E., Onigbinde, A. O. and Asia, I. O. (2009). Pharmaceutical potentials of the oils of some popular insects consumed in southern Nigeria. African Journal of Pharmacy and Pharmacology 3: 051-057.

El Boushy, A. R. (1991). House-fly pupae as poultry manure converters for animal feed: A review. Bioresource Technology 38: 45-49.

El Boushy, A. R. and van der Poel, A. F. B. (2000). Handbook of Poultry Feed from Waste, Processing and Use. Dordrecht, The Netherlands, Kluwer Academic Publishers, pp 409.

Elias, S. (2010). The use of insect fossils in archaeology. Advances in Quaternary Entomology: 12: 89-121.

Elwert, C., Knips, I. and Katz, P. (2010). A novel protein source: Maggot meal of the black soldier fly (*Hermetia illucens*) in broiler feed. 11. Tagung Schweine- und Geflügelernährung, Lutherstadt Wittenberg, Institut für Agrar- und Ernährungswissenschaften, Universität Halle-Wittenberg, pp 140-142, pp 140-142.

Emekci, M., Navarro, S., Donahaye, E., Rindner, M. and Azrieli, A. (2002). Respiration of *Tribolium castaneum* (Herbst) at reduced oxygen concentrations. Journal of Stored Products Research 38: 413-425.

Emekci, M., Navarro, S., Donahaye, E., Rindner, M. and Azrieli, A. (2004). Respiration of *Rhyzopertha dominica* (F.) at reduced oxygen concentrations. Journal of Stored Products Research 40: 27-38.

Ernst, W. H. O. (1992). Nutritional aspects in the development of *Bruchidius sahlbergi* (Coleoptera: Bruchidae) in seeds of *Acacia erioloba*. Journal of Insect Physiology 38: 831-838.

Fabbri, C., Valli, L., Guarino, M., Costa, A. and Mazzotta, V. (2007). Ammonia, methane, nitrous oxide and particulate matter emissions from two different buildings for laying hens. Biosystems Engineering 97: 441-455.

FAO (2007). Meat processing technology for small- to medium-scale producers. Bangkok, FAO, pp 456.

FAO (2009). How to Feed the World in 2050. Rome, pp 35.

FAO (2012). The state of world fisheries and aquaculture. Rome, FAO, pp 174.

Farina, L., Demey, F. and Hardouin, J. (1991). Production de termites pour l'aviculture villageoise au Togo. Tropicultura 9: 181-187.

Fast, P. G. (1970). Insect lipids. Progress in the chemistry of fats and other lipids 11: 181-242.

Faulkner, W. B. and Shaw, B. W. (2008). Review of ammonia emission factors for United States animal agriculture. Atmospheric Environment 42: 6567-6574.

Ferguson, G. W., Jones, J. R., Gehrmann, W. H., Hammack, S. H., Talent, L. G., et al. (1996). Indoor husbandry of the panther chameleon *Chamaeleo [Furcifer] pardalis*: Effects of dietary vitamins A and D and ultraviolet irradiation on pathology and life-history traits. Zoo Biology 15: 279-299.

Fielding, D. J. and Defoliart, L. S. (2008). Discriminating tastes: self-selection of macronutrients in two populations of grasshoppers. Physiological Entomology 33: 264-273.

Finke, M. D. (1984). The use of nonlinear models to evaluate the nutritional quality of insect protein. PhD thesis, University of Wisconsin.

Finke, M. D. (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21: 269-285.

Finke, M. D. (2003). Gut loading to enhance the nutrient content of insects as food for reptiles: A mathematical approach. Zoo Biology 22: 147-162.

Finke, M. D. (2005). Nutrient composition of bee brood and its potential as human food. Ecology of Food and Nutrition 44: 257-270.

Finke, M. D. (2007). Estimate of chitin in raw whole insects. Zoo Biology 26: 105-115.

Finke, M. D. (2013). Complete nutrient content of four species of feeder insects. Zoo biology 32: 27-36.

Finke, M. D., DeFoliart, G. R. and Benevenga, N. J. (1987). Use of a four-parameter logistic model to evaluate the protein quality of mixtures of Mormon cricket meal and corn gluten meal in rats. Journal of Nutrition 117: 1740-1750.

Finke, M. D., DeFoliart, G. R. and Benevenga, N. J. (1989). Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats. Journal of Nutrition 119: 864-871.

Finke, M. D., Dunham, S. D. and Kwabi, C. A. (2005). Evaluation of four dry commercial gut loading products for improving the calcium content of crickets, *Acheta domesticus* Journal of Herpetological Medicine and Surgery 15: 7-12.

Finke, M. D. and Oonincx, D. G. A. B. (2013). Insects as Food for Insectivores. Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens. J. A. Morales-Ramos, M. G. Rojas and D. I. Shapiro-Ilan. London, UK, Academic Press: 583-616.

Finke, M. D., Sunde, M. L. and DeFoliart, G. R. (1985). An evaluation of the protein quality of mormon crickets (*Anabrus simplex* Haldeman) when used as a high protein feedstuff for poultry. Poultry Science 64: 708-712.

Finlay, B. J., Thomas, J. A., McGavin, G. C., Fenchel, T. and Clarke, R. T. (2006). Self-similar patterns of nature: Insect diversity at local to global scales. Proceedings of the Royal Society B: Biological Sciences 273: 1935-1941.

Folch, J., Lees, M. and Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226: 497-509.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., et al. (2011). Solutions for a cultivated planet. Nature 478: 337-342.

Fontaneto, D., Tommaseo-Ponzetta, M., Galli, C., Risé, P., Glew, R. H., et al. (2011). Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. Ecology of Food and Nutrition 50: 351-367.

Fraenkel, G. (1950). The nutrition of the mealworm, *Tenebrio molitor* L. (Tenebrionidae, Coleoptera). Physiological Zoology 23: 92-108.

Francuski, L., Djurakic, M., Ludoški, J., Hurtado, P., Pérez-Bañón, C., et al. (2014). Shift in phenotypic variation coupled with rapid loss of genetic diversity in captive populations of *Eristalis tenax* (Diptera: Syrphidae): Consequences for rearing and potential commercial use. Journal of Economic Entomology 107: 821-832.

Friederich, U. and Volland, W. (2004). Breeding Food Animals: Live Food for Vivarium Animals. Malabar, Florida, Krieger publishing company, pp 178.

Friend, W. (1958). Nutritional requirements of phytophagous insects. Annual Review of Entomology 3: 57-74.

Frye, F. L. and Calvert, C. C. (1989). Preliminary information on the nutritional content of mulberry silk moth (*Bombyx mori)* larvae. Journal of Zoo and Wildlife Medicine 20: 73-75.

Fujimoto, W., Suzuki, M., Kimura, K. and Iwanaga, T. (2002). Cellular expression of the gut chitinase in the stomach of frogs *Xenopus laevis* and *Rana catesbeiana*. Biomedical Research 23: 91-99.

Furman, D. P., Young, R. D. and Catts, P. E. (1959). *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* (Linnaeus). Journal of Economic Entomology 52: 917-921.

Geissler, P. W., Shulman, C. E., Prince, R. J., Mutemi, W., Mnazi, C., et al. (1998). Geophagy, iron status and anaemia among pregnant women on the coast of Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 92: 549-553.

German, D. P., Nagle, B. C., Villeda, J. M., Ruiz, A. M., Thomson, A. W., et al. (2010). Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): Effects on gut size and digestive physiology. Physiological and Biochemical Zoology 83: 1-18.

Gist, C. S. and Crossley, D. A., Jr. (1975). The litter arthropod community in a Southern Appalachian hardwood forest: numbers, biomass and mineral element content. American Midland Naturalist 93: 107-122.

Gobbi, P., Martínez-Sánchez, A. and Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). European Journal of Entomology 110: 461-468.

Godfray, H. C. J., Pretty, J., Thomas, S. M., Warham, E. J. and Beddington, J. R. (2011). Linking Policy on Climate and Food. Science 331: 1013-1014.

Goldsmith, T. H. and Warner, L. T. (1964). Vitamin A in the vision of Insects. The Journal of General Physiology 47: 433-441.

Gomez, P. A., Halut, R. and Collin, A. (1961). Production de proteines animales au Congo. Bulletin Agricole du Congo 52: 689-700 + 788+786.

Gorka, P., Kowalski, Z., Pietrzak, P., Kotunia, A., Kiljanczyk, R., et al. (2009). Effect of sodium butyrate supplementation in milk replacer and starter diet on rumen development in calves. Journal of Physiology and Pharmacology 60, Suppl 3 47-53.

Goulet, G., Mullier, P., Sinave, P. and Brisson, G. J. (1978). Nutritional evaluation of dried *Tenebrio molitor* larvae in the rat. Nutrition Reports International 18: 11-15.

Gouveia, S. M., Simpson, S. J., Raubenheimer, D. and Zanotto, F. P. (2000). Patterns of respiration in *Locusta migratoria* nymphs when feeding. Physiological Entomology 25: 88-93.

Graham-Rowe, D. (2011). Agriculture: Beyond food versus fuel. Nature 474: S6-S8.

Graveland, J. and van Gijzen, T. (1994). Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. Ardea 82: 299-314.

Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana* I. Across-instar effects. Journal of Experimental Biology 207: 497-508.

Groot-Koerkamp, P. W. G. (1994). Review on emissions of ammonia from housing systems for laying hens in relation to sources, processes, building design and manure handling. Journal of Agricultural Engineering Research 59: 73-87.

Groot Koerkamp, P. W. G., Metz, J. H. M., Uenk, G. H., Phillips, V. R., Holden, M. R., et al. (1998). Concentrations and emissions of ammonia in livestock buildings in Northern Europe. Journal of Agricultural Engineering Research 70: 79-95.

Guinee, J. (2001). Handbook on life cycle assessment - Operational guide to the ISO standards. International Journal of Life Cycle Assessment 6: 255-255.

Guiziou, F. and Béline, F. (2005). In situ measurement of ammonia and greenhouse gas emissions from broiler houses in France. Bioresource Technology 96: 203-207.

Gullan, P. J. and Cranston, P. S. (2005). The insects: an outline of entomology, Blackwell Publishing, Oxford, pp 528.

Hackstein, J. H. and Stumm, C. K. (1994). Methane production in terrestrial arthropods. Proceedings of the National Academy of Sciences of the United States of America 91: 5441-5445.

Haglund, O., Wallin, R., Wretling, S., Hultberg, B. and Saldeen, T. (1998). Effects of fish oil alone and combined with long chain (n-6) fatty acids on some coronary risk factors in male subjects. The Journal of Nutritional Biochemistry 9: 629-635.

Hale, O. M. (1973). Dried *Hermetia illucens* larvae (Diptera; Stratiomyidae) as a feed additive for poultry Journal of the Georgian Entomological Society 8: 16-20.

Hamilton, A. G. (1936). The relation of humidity and temperature to the development of three species of African locusts -*Locusta migratoria migratoioides* (R. & F.), *Schistocerca gregaria* (Forsk.), *Nomadacris septemfasciata* (Serv.). Transactions of the Royal Entomological Society of London 85: 1-60.

Hamilton, H. C. and Falconer, W. (1892). The geography of Strabo: Literary translated, with notes, G. Bell & sons, pp 490.

Han, R. D., Parajulee, M., Zhong, H. and Feng, G. (2008). Effects of environmental humidity on the survival and development of pine caterpillars, *Dendrolimus tabulaeformis* (lepidoptera: lasiocampidae). Insect Science 15: 147-152.

Hanboonsong, Y., Jamjanya, T. and Durst, P. B. (2013). Six-legged livestock: Edible insect farming, collection and marketing in Thailand. Bangkok, FAO.

Hansen, J., Nelssen, J., Goodband, R. and Weeden, T. (1993). Evaluation of animal protein supplements in diets of early-weaned pigs. Journal of Animal Science 71: 1853-1862.

Hanson, B. J., Cummins, K. W., Cargill, A. S. and Lowry, R. R. (1985). Lipid content, fatty acid composition, and the effect of diet on fats of aquatic insects. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 80: 257-276.

Hanson, B. J., Cummins, K. W., Ii, A. S. C. and Lowry, R. R. (1983). Dietary effects on lipid and fatty acid composition of *Clistoronia magnifica* (Trichoptera:Limnephilidae). Freshwater Invertebrate Biology 2: 2-15.

Hardouin, J. (1995). Minilivestock: from gathering to controlled production. Biodiversity and Conservation 4: 220.

Hardouin, J., Thys, É., Joiris, V. and Fielding, D. (2003). Mini-livestock breeding with indigenous species in the tropics. Livestock Research for Rural Development 15: 3-4.

Harein, P. (1972). Microbial relationship between the lesser mealworm and its associated environment in a turkey brooder house. Environmental Entomology 1: pp 189-194.

Harper, L. A., Flesch, T. K., Powell, J. M., Coblentz, W. K., Jokela, W. E., et al. (2009). Ammonia emissions from dairy production in Wisconsin. Journal of Dairy science 92: 2326-2337.

Hatt, J. M., Hung, E. and Wanner, M. (2003). The influence of diet on the body composition of the house cricket (*Acheta domesticus*) and consequences for their use in zoo animal nutrition. Zoologische Garten 73: 238-244.

Hayes, W. A., Smith, P. G. and Morris, A. E. J. (1998). The production and quality of tomato concentrates. Critical Reviews in Food Science and Nutrition 38: 537-564.

Heinrich, M. and Prieto, J. M. (2008). Diet and healthy ageing 2100: Will we globalise local knowledge systems? Ageing Research Reviews 7: 249-274.

Herrero, M., Havlík, P., Valin, H., Notenbaert, A., Rufino, M. C., et al. (2013a). Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. Proceedings of the National Academy of Sciences of the United States of America 110: 20888-20893.

Herrero, M. and Thornton, P. K. (2013b). Livestock and global change: Emerging issues for sustainable food systems. Proceedings of the National Academy of Sciences 110: 20878-20881.

Heusner, A. A. (1982). Energy metabolism and body size I. Is the 0.75 mass exponent of Kleiber’s equation a statistical artifact? Respiration Physiology 48: 1-12.

Hill, D. S. (1997). The Economic Importance of Insects. London, Chapman & Hall, pp 45-50.

Hoby, S., Wenker, C., Robert, N., Jermann, T., Hartnack, S., et al. (2010). Nutritional metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptratus*) and its prevention. Journal of Nutrition 140: 1923-1931.

Hocking, B. and Matsumura, F. (1960). Bee brood as food. Bee World 41: 113-120.

Hoffmann, K. H. (1973). Effects of temperature on chemical composition of crickets (*Gryllus*, orthopt.) (In German). Oecologia 13: 147-175.

Hoffmann, K. H. (1974). Effects of constant and varying temperatures on life-span, food utilization and fertility of adult crickets, *Gryllus bimaculatus.* (Wirkung von konstanten und tagesperiodisch alternierenden Temperaturen auf Lebensdauer, Nahrungsverwertung und Fertilität adulter *Gryllus bimaculatus*). Oecologia 17: 39-54.

Holmes, L. A., Vanlaerhoven, S. L. and Tomberlin, J. K. (2012). Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). Environmental Entomology 41: 971-978.

Holt, V. M. (1995). Why not eat insects? Oxford, Thornton's, 67 pp. Text reset from the original 1885 edition by Daniel H. Meeuws, Oxford July/August 1993.

House, H. L. (1961). Insect nutrition. Annual Review of Entomology 6: 13-26.

House, H. L., Riordan, D. F. and Barlow, J. S. (1958). Effects of thermal conditioning and of degree of saturation of dietary lipids on resistance of an insect to a high temperature. Canadian Journal of Zoology 36: 629-632.

Howard, L. O. (1915). The edibility of insects. Scientific notes. Journal of Economic Entomology, 8: 549.

Howard, L. O. (1916). *Lachnosterna* larvae as a possible food supply. Journal of Economic Entomology, 9: 389-392.

Howard, R. W. and Stanley-Samuelson, D. W. (1996). Fatty acid composition of fat body and Malpighian tubules of the tenebrionid beetle, *Zophobas atratus*: Significance in eicosanoid-mediated physiology. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 115: 429-437.

Huis, A. v. (2003). Insects as food in Sub-Saharan Africa. Insect Science and its Application, 23(3): 163-185.

Hunt-Coslik, A., Ward, A. M. and McClements, R. D. (2009). Gut-loading as a method to effectively supplements crickets with calcium and vitamin A. 8th Conference on Zoo and Wildlife Nutrition, Tulsa OK, AZA Nutrition Advisory Group, pp p 163-171.

Illgner, P. and Nel, E. (2000). The geography of edible insects in sub-Saharan Africa: A study of the mopane caterpillar. Geographical Journal 166: 336-351.

IPCC (2007). Summary for Policymakers. . Cambridge, United Kingdom and New York, NY, USA., IPCC, pp 18.

Isaksson, C. and Andersson, S. (2007). Carotenoid diet and nestling provisioning in urban and rural great tits Parus major. Journal of Avian Biology 38: 564-572.

Jones, L. D., Cooper, R. W. and Harding, R. S. (1972). Composition of Mealworm *Tenebrio molitor* Larvae. The Journal of Zoo Animal Medicine 3: 34-41.

Jongema, Y. (2013). "World list of edible species." Retrieved 10-07-2014, 2014, from http://www.wageningenur.nl/upload_mm/7/e/6/c79e66db-00d5-44c9-99cb-f38943723db6_LIST%20Edible%20insects%201st%20of%20April.pdf.

Kammann, C., Hepp, S., Lenhart, K. and Müller, C. (2009). Stimulation of methane consumption by endogenous CH4 production in aerobic grassland soil. Soil Biology and Biochemistry 41: 622-629.

Katayama, N., Ishikawa, Y., Takaoki, M., Yamashita, M., Nakayama, S., et al. (2008). Entomophagy: A key to space agriculture. Advances in Space Research 41: 701-705.

Katayama, N., Yamashita, M., Kishida, Y., Liu, C.-C., Watanabe, I., et al. (2008). Azolla as a component of the space diet during habitation on Mars. Acta Astronautica 63: 1093-1099.

Katayama, N., Yamashita, M., Wada, H. and Mitsuhashi, J. (2005). Entomophagy as part of a space diet for habitation on Mars. Journal of Space Technology Science 21-22: 27-38.

Katz, H. (2014). Personal communication.

Kinfu, A. and Erko, B. (2008). Cockroaches as carriers of human intestinal parasites in two localities in Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene 102: 1143-1147.

Kitsa, K. (1989). Contribution des insectes comestibles a l'amelioration de la ration alimentaire au Kasai-Occidental. Zaire-Afrique 239: 511-519.

Kiuchi, M. and Tamaki, Y. (1990). Future of edible insects. Farming Japan 24-26.

Klarsfeld, J. and Mitchell, M. (2005). An evaluation of the gray cricket, *Acheta domestica*, as a source of oxyurids for reptiles. Journal of Herpetological Medicine and Surgery 15: 18-20.

Klasing, K. C., Thacker, P., Lopez, M. A. and Calvert, C. C. (2000). Increasing the calcium content of mealworms (*Tenebrio molitor*) to improve their nutritional value for bone mineralization of growing chicks. Journal of Zoo and Wildlife Medicine 31: 512-517.

Kleiber, M. (1961). The fire of life: an introduction to animal energetics New York, London, John Wiley & Sons, Inc., pp 454.

Klunder, H., Wolkers-Rooijackers, J., Korpela, J. and Nout, M. (2012). Microbiological aspects of processing and storage of edible insects. Food Control 26: 628-631.

Koc, Y. and Gulel, A. (2008). Age and sex related variations in protein and carbohydrate levels of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) in constant lightness and darkness. Pakistan Journal of Biological Sciences 11: 733-739.

Kodondi, K. K., Leclercq, M. and Gaudin-Harding, F. (1987). Vitamin estimations of three edible species of Attacidae caterpillars from Zaire. International Journal of Vitamins and Nutrional Research 57: 333-334.

Kok, R. (1983). The production of insects for human food. Journal de l'Institut Canadien de Science et Technologie Alimentaire 16: 5-18.

Kollikkathara, N., Feng, H. and Stern, E. (2009). A purview of waste management evolution: Special emphasis on USA. Waste Management 29: 974-985.

Korpelainen, H. (1990). Sex ratios and conditions required for environmental sex determination in animals. Biological Reviews 65: 147-184.

Kouba, M. and Mourot, J. (2011). A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie 93: 13-17.

Kramer, K. J., Hopkins, T. L. and Schaefer, J. (1995). Applications of solids NMR to the analysis of insect sclerotized structures. Insect Biochemistry and Molecular Biology 25: 1067-1080.

Krengel, S., Stangl, G. I., Brandsch, C., Freier, B., Klose, T., et al. (2012). A comparative study on effects of normal versus elevated temperatures during preimaginal and young adult period on body weight and fat body content of mature *Coccinella septempunctata* and *Harmonia axyridis* (coleoptera: Coccinellidae). Environmental Entomology 41: 676-687.

Kroon, L. J. M., Holtslag, A. A. M. and Krol, M. C. (2009). Inleiding atmosfeer (in Dutch). Wageningen, Wageningen University.

Kruse, M. and Kwon, C. (2004). Edible insects, important source of protein in central africa. FAO newsroom.

Lacey, R. E., J.S. Redwine & C.B. Parnell Emission Factors for Broiler Production Operations: A Stochastic Modeling Approach. In: (2002). Emission Factors for Broiler Production Operations: A Stochastic Modeling Approach. 2002 ASAE Annual International Meeting / CIGR XVth World Congress. . Chicago, Illinois. Paper No 024212.

Lamiaa, B., Mariam, L. and Ahmed, A. (2007). Bacteriological analysis of *Periplaneta americana* L. (Dictyoptera; Blattidae) and *Musca domestica* L. (Diptera; Muscidae) in ten districts of Tangier, Morocco. African Journal of Biotechnology 6: 2038-2042.

Larde, G. (1990). Recycling of coffee pulp by *Hermetia Illucens* (Diptera, Stratiomyidae) larvae. Biological Wastes 33: 307-310.

Lardé, G. (1989). Investigation on some factors affecting larval growth in a coffee-pulp bed. Biological Wastes 30: 11-19.

Larsen, P. O. and von Ins, M. (2010). The rate of growth in scientific publication and the decline in coverage provided by Science Citation Index. Scientometrics 84: 575-603.

Lease, H. M. and Wolf, B. O. (2010). Exoskeletal chitin scales isometrically with body size in terrestrial insects. Journal of Morphology 271: 759-768.

Lease, H. M. and Wolf, B. O. (2011). Lipid content of terrestrial arthropods in relation to body size, phylogeny, ontogeny and sex. Physiological Entomology 36: 29-38.

Leclercq, J. (1963). Artificial selection for weight and its consequences in *Tenebrio molitor* L. Nature 198: 106-107.

Lemke, R., Goddard, T., Hahn, D., Burton, D., Ellert, B., et al. (2002). An inter-laboratory comparison of nitrous oxide analysis in western Canada. Communications in Soil Science and Plant Analysis 33: 2705-2713.

Levi, L., Ziv, T., Admon, A., Levavi-Sivan, B. and Lubzens, E. (2012). Insight into molecular pathways of retinal metabolism, associated with vitellogenesis in zebrafish. American Journal of Physiology - Endocrinology and Metabolism 302: 626-644.

Levy, R. and Cromroy, H. L. (1973). Concentration of some major and trace elements in forty-one species of adult and immature insects determined by atomic absorption spectroscopy. Annals of the Entomological Society of America 66: 523-526.

Lewandowski, I. and Faaij, A. P. C. (2006). Steps towards the development of a certification system for sustainable bio-energy trade. Biomass & Bioenergy 30: 83-104.

Li, H., Vaughan, M. J. and Browne, R. K. (2009). A complex enrichment diet improves growth and health in the endangered Wyoming Toad (*Bufo baxteri*). Zoo Biology 28: 197-213.

Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J. K., et al. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Management 31: 1316-1320.

Li, X. Z. and Brune, A. (2007). Transformation and mineralization of soil organic nitrogen by the humivorous larva of *Pachnoda ephippiata* (Coleoptera : Scarabaeidae). Plant and Soil 301: 233-244.

Lighton, J. R. B. and Fielden, L. J. (1995). Mass scaling of standard metabolism in ticks - a valid case of low metabolic rates in sit-and-wait strategists. Physiological Zoology 68: 43-62.

Liu, K., Li, Y., Jousset, F.-X., Zadori, Z., Szelei, J., et al. (2011). The *Acheta domesticus* densovirus, isolated from the European house cricket, has evolved an expression strategy unique among parvoviruses. Journal of Virology 85: 10069-10078.

Lizot, J. (1977). Population, resources and warfare among the Yanomami. Man 12: 497-517.

Looy, H., Dunkel, F. V. and Wood, J. R. (2013). How then shall we eat? Insect-eating attitudes and sustainable foodways. Agriculture and Human Values: 1-11.

Lorenz, M. W. and Anand, A. N. (2004). Changes in the biochemical composition of fat body stores during adult development of female crickets, *Gryllus bimaculatus*. Archives of Insect Biochemistry and Physiology 56: 110-119.

Loudon, C. (1988). Development of *Tenebrio molitor* in low oxygen levels. Journal of Insect Physiology 34: 97-103.

Luoba, A. I., Geissler, P. W., Estambale, B., Ouma, J. H., Magnussen, P., et al. (2004). Geophagy among pregnant and lactating women in Bondo District, western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene 98: 734-741.

Lv, X., Liu, C., Li, Y., Gao, Y., Guo, B., et al. (2013). Bioaccumulation and excretion of enantiomers of myclobutanil in *Tenebrio molitor* larvae through dietary exposure. Chirality 25: 890-896.

Machin, J. (1975). Water balance in *Tenebrio molitor*, L. Larvae; the effect of atmospheric water absorption. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 101: 121-132.

Madsen, D. B. and Kirkman, J. E. (1988). Hunting hoppers. American Antiquity 53: 593-604.

Majumder, U. K. and Sengupta, A. (1979). Triglyceride composition of chrysalis oil, an insect lipid. Journal of the American Oil Chemists' Society 56: 620-623.

Malley, D., McClure, C., Martin, P., Buckley, K. and McCaughey, W. (2005). Compositional analysis of cattle manure during composting using a field portable near infrared spectrometer. Communications in Soil Science and Plant Analysis 36: 455-475.

Martin, R. D., Rivers, J. P. W. and Cowgill, U. M. (1976). Culturing mealworms as food for animals in captivity. International Zoo Yearbook 16: 63-70.

Martines-Filho, J., Burnquist, H. L. and Vian, C. E. F. (2006). Bioenergy and the rise of sugarcane-based ethanol in Brazil. Choices 21: 91-96.

Massie, H. R., Williams, T. R. and DeWolfe, L. K. (1989). Changes in taurine in aging fruit flies and mice. Experimental gerontology 24: 57-65.

May, B. (1961). The occurrence in New Zealand and the life history of the soldier fly *Hermetia illucens* (L.)(Diptera: Stratiomyidae). New Zealand Journal of Science 4: 55-65.

May, M. L., Pearson, D. L. and Casey, T. M. (1986). Oxygen-consumption of active and inactive adult tiger beetles. Physiological Entomology 11: 171-179.

McAllister, J. C., Steelman, C. D., Skeeles, J. K., Newberry, L. A. and Gbur, E. E. (1996). Reservoir competence of *Alphitobius diaperinus* (Coleoptera:Tenebrionidae) for *Escherichia coli* (Eubacteriales:Enterobacteriaceae). Journal of Medical Entomology 33: 983-987.

McClements, R. D., Lintzenich, B. A. and Boardman, J. (2003). A Zoo-wide evaluation into the current feeder insect supplementation program at the Brookfield Zoo. Proceedings of the NAG 4th Conference on Zoo and Wild Nutrition, pp 91-94.

McComb, A. (2010). Evaluation of vitamin A supplementations for captive amphibian species. MSc. Thesis, North Carolina State University, pp 129.

McFarlane, J., Neilson, B. and Ghouri, A. (1959). Artificial diets for the house cricket, *Acheta domesticus* (L.). Canadian Journal of Zoology 37: 913-916.

McFarlane, J. E. (1962). A comparison of the growth of the House chricket (Orthoptera:Gryllidae) reared singly and in groups. Canadian Journal of Animal Science 40: 559-560.

McMichael, A. J., Powles, J. W., Butler, C. D. and Uauy, R. (2007). Food, livestock production, energy, climate change, and health. Lancet 370: 1253-1263.

McMillian, W. W., Widstrom, N. W. and Wilson, D. M. (1981). Rearing the Maize Weevil on maize genotypes when aflatoxin-producing *Aspergillus flavus* and *A. parasiticus* isolates were present. Environmental Entomology 10: 760-762.

Menzel, P. and D'Aluisio, F. (1998). Man eating bugs: the art and science of eating insects. Berkely, Ten Speed Press, pp 191.

Mercer, C. W. L. (1997). Sustainable production of insects for food and income by New Guinea villagers. Ecology of Food and Nutrition 36: 151-157.

Meyer-Rochow, V. B. (1973). Edible insects in 3 different ethnic groups of Papua and New-Guinea. American Journal of Clinical Nutrition 26: 673-677.

Meyer-Rochow, V. B. (2005). Traditional food insects and spiders in several ethnic groups of Northeast India, Papua New Guinea, Australia and New Zealand. Ecological Implications of Minilivestock: rodents frogs. snails and insects fo sustainable development M. G. Paoletti. New Hampshire, U.S.A., Science Publishers, Inc. Enfield: 385-409.

Mignon, J. (2002). L'entomophagie: une question de culture? Tropicultura, 20(3): 151-155.

Miller, E. A., Green, S. L., Otto, G. M. and Bouley, D. M. (2001). Suspected hypovitaminosis A in a colony of captive green anoles (*Anolis carolinensis*). Journal of the American Association for Laboratory Animal Science 40: 18-20.

Mohtar, J. A., Yusof, F. and Ali, N. M. H. (2014). Screening of novel acidified solvents for maximal antimicrobial peptide extraction from *Zophobas morio* Fabricius. Advances in Environmental Biology 8: 803-809.

Mollenhorst, H., Berentsen, P. B. M. and De Boer, I. J. M. (2006). On-farm quantification of sustainability indicators: an application to egg production systems. British Poultry Science 47: 405 - 417.

Mollenhorst, H. and de Boer, I. J. M. (2004). Identifying sustainability issues using participatory SWOT analysis - A case study of egg production in the Netherlands. Outlook on Agriculture 33: 267-276.

Morgan, T. D., Baker, P., Kramer, K. J., Basibuyuk, H. H. and Quicke, D. L. (2003). Metals in mandibles of stored product insects: do zinc and manganese enhance the ability of larvae to infest seeds? Journal of stored products research 39: 65-75.

Mouffet, T. (1634). Theater of insects London William Iaggard.

Mpuchane, S., Gashe, B. A., Allotey, J., Siame, B., Teferra, G., et al. (2000). Quality deterioration of phane, the edible caterpillar of an emperor moth *Imbrasia belina*. Food Control 11: 453.

Müller, K., Raila, J., Altenkamp, R., Schmidt, D., Dietrich, R., et al. (2012). Concentrations of retinol, 3,4-didehydroretinol, and retinyl esters in plasma of free-ranging birds of prey. Journal of Animal Physiology and Animal Nutrition 96: 1044-1053.

Mullins, D. E. and Cochran, D. G. (1975). Nitrogen metabolism in the American cockroach—I. An examination of positive nitrogen balance with respect to uric acid stores. Comparative Biochemistry and Physiology Part A: Physiology 50: 489-500.

Munyuli Bin Mushambanyi, T. (2000). Étude préliminaire orientée vers la production des chenilles comsommables par l’élevage des papillons (*Anaphe infracta*: Thaumetopoeidae) à Lwiro, Sud-Kivu. République Démocratique du Congo. Tropicultura 18: 208-211.

Munyuli Bin Mushambanyi, T. and Balezi, N. (2002). Utilisation des blattes et des termites comme substituts potentiels de la farine de viande dans l’alimentation des poulets de chair au Sud Kivu , République. Démocratique, du Congo . Tropicultura 20: 10-16

Murray, D. R. P. (1960). The stimulus to feeding in larvae of *Tenebrio molitor* L. Journal of Insect Physiology 4: 80-91.

Myers, H. M., Tomberlin, J. K., Lambert, B. D. and Kattes, D. (2008). Development of Black Soldier Fly (Diptera: Stratiomyidae) larvae fed dairy manure. Environmental Entomology 37: 11-15.

Nahm, K. (2003). Evaluation of the nitrogen content in poultry manure. World's Poultry Science Journal 59: 77-88.

Nakagaki, B. J. and deFoliart, G. R. (1991). Comparison of diets for mass-rearing *Acheta domesticus* (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. Journal of Economic Entomology, 84(3): 891-896.

Nakagaki, B. J., Sunde, M. L. and Defoliart, G. R. (1987). Protein quality of the house cricket, *Acheta domesticus*, when fed to broiler chicks. Poultry Science 66: 1367-1371.

Nakasone, S. and Ito, T. (1967). Fatty acid composition of the silkworm, *Bombyx mori* L. Journal of Insect Physiology 13: 1237-1246.

Narita, S., Kageyama, D., Nomura, M. and Fukatsu, T. (2007). Unexpected mechanism of symbiont-induced reversal of insect sex: feminizing *Wolbachia* continuously acts on the butterfly *Eurema hecabe* during larval development. Applied and environmental microbiology 73: 4332-4341.

Nation, J. L. (2008). Insect Physiology and Biochemistry. Boca Raton, Florida, CRC press, pp 496.

Nchito, M., Wenzel Geissler, P., Mubila, L., Friis, H. and Olsen, A. (2004). Effects of iron and multimicronutrient supplementation on geophagy: a two-by-two factorial study among Zambian schoolchildren in Lusaka. Transactions of the Royal Society of Tropical Medicine and Hygiene 98: 218-227.

Nedvěd, O. and Kalushkov, P. (2012). Effect of air humidity on sex ratio and development of ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). Psyche Article ID 173482: 1-5.

Nestel, D., Papadopoulos, N. T., Liedo, P., Gonzales-Ceron, L. and Carey, J. R. (2005). Trends in lipid and protein contents during medfly aging: An harmonic path to death. Archives of Insect Biochemistry and Physiology 60: 130-139.

Newton, G. L., Booram, C. V., Barker, R. W. and Hale, O. M. (1977). Dried *Hermetia illucens* larvae meal as supplement for swine. Journal of Animal Science 44: 395-400.

Newton, L., Sheppard, C., Watson, D. W. and Burtle, G. (2005). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. North Carolina, Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, Nc. , pp 17.

Ngoka, B. M., Kioko, E. N., Raina, S. K., Mueke, J. M. and Kimbu, D. M. (2008). Semi-captive rearing of the African wild silkmoth *Gonometa postica* (Lepidoptera: Lasiocampidae) on an indigenous and a non-indigenous host plant in Kenya. International Journal of Tropical Insect Science 27: 183-190.

Nicks, B., Laitat, M., Vandenheede, M., Desiron, A., Verhaeghe, C., et al. (2003). Emissions of ammonia, nitrous oxide, methane, carbon dioxide and water vapor in the raising of weaned pigs on straw-based and sawdust-based deep litters. Animal Research 52: 299-308.

Nielsen, N. K. (2000). Food, hunting, and taboo: Cultural heritage in practice. Ethnologia Scandinavica, 30: 62-75.

Nishimune, T., Watanabe, Y., Okazaki, H. and Akai, H. (2000). Thiamin is decomposed due to *Anaphe* spp. entomophagy in seasonal ataxia patients in Nigeria. Journal of Nutrition 130: 1625-1628.

Nonaka, K. (2009). Feasting on insects. (Special Issue: Trends on the edible insects in Korea and Abroad). Entomological Research 39: 304-312.

Novozamsky, I., Houba, V., Temminghoff, E. and Van der Lee, J. (1984). Determination of total N and total P in a single soil digest. Netherlands Journal of Agricultural Science 32: 322-324.

Nuov, S., Little, D. and Yakupitiyage, A. (1995). Nutrient flows in an integrated pig, maggot and fish production system. Aquaculture Research 26: 601-606.

Ødegaard, F. (2000). How many species of arthropods? Erwin's estimate revised. Biological Journal of the Linnean Society 71: 583-597.

Ogilvy, V., Fidgett, A. L. and Preziosi, R. F. (2012). Differences in carotenoid accumulation among three feeder-cricket species: implications for carotenoid delivery to captive insectivores. Zoo Biology 31: 470-478.

Oladipo, I. O. (1996). Palm grub culture and domestication. Semestrial Bulletin BEDIM 5: 10-11.

Olson, J. A. (1989). Provitamin A function of carotenoids: the conversion of beta-carotene into vitamin A. Journal of Nutrition 119: 105-108.

Onifade, A. A., Oduguwa, O. O., Fanimo, A. O., Abu, A. O., Olutunde, T. O., et al. (2001). Effects of supplemental methionine and lysine on the nutritional value of housefly larvae meal (*Musca domestica*) fed to rats. Bioresource Technology 78: 191-194.

Oonincx, D. G. A. B. and de Boer, I. J. M. (2012a). Environmental impact of the production of mealworms as a protein source for humans – A Life Cycle Assessment. PLoS One 7: e51145.

Oonincx, D. G. A. B. and Dierenfeld, E. S. (2012b). An investigation into the chemical composition of alternative invertebrate prey. Zoo Biology 31: 40-54.

Oonincx, D. G. A. B., Stevens, Y., van den Borne, J. J. G. C., van Leeuwen, J. P. T. M. and Hendriks, W. H. (2010a). Effects of vitamin D3 supplementation and UVb exposure on the growth and plasma concentration of vitamin D3 metabolites in juvenile bearded dragons (*Pogona vitticeps*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 156: 122-128.

Oonincx, D. G. A. B. and van der Poel, A. F. B. (2011). Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). Zoo Biology 30: 9-16.

Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J., van den Brand, H., van Loon, J. J., et al. (2010b). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One 5: e14445.

Osbaldeston, T. A. and Wood, R. (2000). Dioscorides: De materia medica, Ibidis Press, Johannesburg.

Oyarzun, S. E., Crawshaw, G. J. and Valdes, E. V. (1996). Nutrition of the tamandua: I. Nutrient composition of termites (*Nasutitermes spp.*) and stomach contents from wild tamanduas (*Tamandua tetradactyla*). Zoo Biology 15: 509-524.

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., et al. (2011). A large and persistent carbon sink in the world's forests. Science 333: 988-993.

Paoletti, M. G. and Dufour, D. L. (2005). Edible Invertebrates among Amazonian Indians: A Critical Review of Disappearing Knowledge. Ecological implications of minilivestock: potential of insects, rodents, frogs and snails. M. G. Paoletti. Enfield, New Hampshire, Science Publishers Inc.: 293-342.

Park, S. I., Chang, B. S. and Yoe, S. M. (2014). Detection of antimicrobial substances from larvae of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Entomological Research 44: 58-64.

Patton, C. J. and Kryskalla, J. R. (2003). Methods of analysis by the US Geological Survey National Water Quality Laboratory: Evaluation of alkaline persulfate digestion as an alternative to kjeldahl digestion for determination of total and dissolved nitrogen and phosphorus in water, US Department of the Interior, US Geological Survey.

Patton, R. (1978). Growth and development parameters for *Acheta domesticus*. Annals of the Entomological Society of America 71: 40-42.

Patton, R. L. (1967). Oligidic diets for *Acheta domesticus* (Orthoptera: Gryllidae). Annals of the Entomological Society of America 60: 1238-1242.

Pelletier, N. and Tyedmers, P. (2010). Forecasting potential global environmental costs of livestock production 2000-2050. Proceedings of the National Academy of Sciences of the United States of America 107: 18371-18374.

Pemberton, R. W. (1988). The use of the giant waterbug, *Lethocerus indicus* (Hemiptera: Belostomatidae), as human food in California. Pan-Pacific Entomology 64: 81-82.

Pemberton, R. W. (1994). The revival of rice-field grasshoppers as human food in South Korea. Pan-Pacific Entomologist 70(4: 323-327.

Pemberton, R. W. (1995). Catching and eating dragonflies in Bali and elsewhere in Asia. American Entomologist 41: 97-99.

Pennino, M., Dierenfeld, E. S. and Behler, J. L. (1991). Retinol, alpha-tocopherol, and proximate nutrient composition of invertebrates used as food. International Zoo Yearbook 30: 143-149.

Pereira, N. R., Ferrarese Filho, O., Matsushita, M. and Souza, N. E. d. (2003). Proximate composition and fatty acid profile of *Bombyx mori* L. chrysalis toast. Journal of Food Composition and Analysis. 16: 451-457.

Pessier, A. P., Linn, M., Garner, M. M., Raymond, J. T., Dierenfeld, E. S., et al. (2005). Suspected hypovitaminosis A in captive toads (*Bufo spp.*). AAZV, AAWV, AZA/NAG Joint conference, Omaha, NE, pp 57.

Pham, H. T., Yu, Q., Bergoin, M. and Tijssen, P. (2013). A novel ambisense densovirus, *Acheta domesticus* mini ambidensovirus, from crickets. Genome announcements 1: e00914-00913.

Piao, S., Fang, J., Ciais, P., Peylin, P., Huang, Y., et al. (2009). The carbon balance of terrestrial ecosystems in China. 458: 1009-1013.

Pliner, P. and Pelchat, M. L. (1991). Neophobia in humans and the special status of foods of animal origin. Appetite 16: 205-218.

Premalatha, M., Abbasi, T., Abbasi, T. and Abbasi, S. A. (2011). Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects Renewable and Sustainable Energy Reviews 15: 4357- 4360.

Pretorius, Q. (2011). The evaluation of larvae of *Musca domestica* (common housefly) as protein source for broiler production MSc, Stellenbosch University, pp 107.

Prothero, J. W. (1979). Maximal oxygen-consumption in various animals and plants. Comparative Biochemistry and Physiology A: Physiology 64: 463-466.

Punzo, F. (2003). Nutrient composition of some insects and arachnids. Florida Scientist 66: 84-98.

Quin, P. J. (1959). Food and feeding habits of the Pedi with special reference to identification, classification, preparation and nutritive value of the respective foods. Thesis, Witwatersrand University Press, Johannesburg. 278 pp.

Raes, K., De Smet, S. and Demeyer, D. (2001). Effect of double-muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on conjugated linoleic acid and polyunsaturated fatty acids. Animal Science 73: 253-260.

Ramos-Elorduy de Conconi, J. and M., J. M. P. (1979). Insectos comestibles del Valle del Mezquital y su valor nutritivo. Zoologia 1: 563-574.

Ramos-Elorduy, J. (1989). Valor nutritivo de insectos comestibles en las antiguas culturas de Mexico. Los Insectos Comestibles en el Mexico Antiguo. J. Ramos-Elorduy and J. M. Pino Moreno: 47-56.

Ramos-Elorduy, J. (1990). Edible insects: barbarism or solution to the hunger problem? Ethnobiology: Implications and Applications, Museu Paraense Emílio Goeldi, Bélem, Proceedings of the First International Congress of Ethnobiology, 1988, pp 151-158.

Ramos-Elorduy, J. (1996). Insect consumption as a means of national identity. IV International Congres of Ethnobiology, Lucknow, Deep publications, New Delhi, pp 9-12.

Ramos-Elorduy, J. (1997a). The importance of edible insects in the nutrition and economy of people of the rural areas of Mexico. Ecology of Food and Nutrition, 36: 347-366.

Ramos-Elorduy, J. (1997b). Insects: a sustainable source of food? Ecology of Food and Nutrition 36: 246-276.

Ramos-Elorduy, J. (2005). Insects: a hopeful food source. Ecological Implications of minilivestock. Potential of insects, rodents, and frogs. M. G. Paoletti. Enfield, New Hampshire, Science Publishers, Inc. Chapter 14: 263-291.

Ramos-Elorduy, J. (2006). Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. Journal of Ethnobiology and Ethnomedicine 2: 1-10.

Ramos-Elorduy, J. (2008). Energy supplied by edible insects from Mexico and their nutritional and ecological importance. Ecology of Food and Nutrition 47: 280-297.

Ramos-Elorduy, J., Gonzalez, E. A., Hernandez, A. R. and Pino, J. M. (2002). Use of *Tenebrio molitor* (Coleoptera : Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. Journal of Economic Entomology 95: 214-220.

Ramsay, S. L. and Houston, D. C. (2003). Amino acid composition of some woodland arthropods and its implications for breeding tits and other passerines. Ibis 145: 227-232.

Raubenheimer, D. and Simpson, S. J. (2003). Nutrient balancing in grasshoppers: Behavioural and physiological correlates of dietary breadth. The Journal of Experimental Biology 206: 1669-1681.

Ravindran, V. and Blair, R. (1993). Feed resources for poultry production in Asia and the Pacific. Animal protein sources. World's Poulty Science Journal 49: 219-235.

Reichle, D. E., Shanks, M. H. and Crossley, D. A. (1969). Calcium, potassium, and sodium content of forest floor arthropods. Annals of the Entomological Society of America 62: 57-62.

Rich, C. N. and Talent, L. G. (2008). The effects of prey species on food conversion efficiency and growth of an insectivorous lizard. Zoo Biology 27: 181–187.

Rivault, C., Cloarec, A. and Guyader, A. L. (1993). Bacterial load of cockroaches in relation to urban environment. Epidemiology and Infection 110: 317-325.

Roe, R. M., Clifford, C. W. and Woodring, J. P. (1980). The effect of temperature on feeding, growth, and metabolism during the last larval stadium of the female house cricket, *Acheta domesticus*. Journal of Insect Physiology 26: 639-644.

Roe, R. M., Clifford, C. W. and Woodring, J. P. (1985). The effect of temperature on energy distribution during the last-larval stadium of the female house cricket, *Acheta domesticus*. Journal of Insect Physiology 31: 371-378.

Ross (2011). Ross308 Parent stock: Performance Objectives. Newbridge, Scotland, Ross Breeders Limited 12.

Rowland, A. P. and Haygarth, P. M. (1997). Determination of total dissolved phosphorus in soil solutions. Journal of Environmental Quality 26: 410-415.

Ruddle, K. (1973). The human use of insects: examples from the Yukpa. Biotropica 5: 94-101.

Russo, A., Cocuzza, C. E., Vasta, M. C., Simola, M. and Virone, G. (2006). Life fertility tables of *Piophila casei* L. (Diptera : Piophilidae) reared at five different temperatures. Environmental Entomology 35: 194-200.

Santos Oliveira, J. F., Passos De Carvalho, J., Bruno De SousaR, F. X. and Simao, M. M. (1976). The nutritional value of four species of insects consumed in Angola. Ecology of Food and Nutrition 5: 91-97.

Schaff, P. and Wace, H. (1890). A select library of Nicene and post-Nicene Fathers of the Christian Church: second series, Christian literature Company, pp 736.

Schmitz, G. and Ecker, J. (2008). The opposing effects of n-3 and n-6 fatty acids. Progress in Lipid Research 47: 147-155.

Schröder, J. and Verloop, J. (2010). Slurry separation could allow a wider use of manure within the EU Nitrates Directive. Proceedings Ramiran 2010 Conference, Lisboa, Portugal, Lissabon< http://www. ramiran. net/ ramiran2010/docs/Ramiran2010_0047_final. pdf.

Schröder, J. J., Smit, A. L., Cordell, D. and Rosemarin, A. (2011). Improved phosphorus use efficiency in agriculture: A key requirement for its sustainable use. Chemosphere 84: 822-831.

Scriber, J. M. and Slansky, F. j. (1981). The nutritional ecology of immature insects. Annual Review of Entomology 26: 183.

Sealey, W. M., Gaylord, T. G., Barrows, F. T., Tomberlin, J. K., McGuire, M. A., et al. (2011). Sensory analysis of Rainbow Trout, *Oncorhynchus mykiss*, fed enriched Black Soldier Fly prepupae, *Hermetia illucens*. Journal of the World Aquaculture Society 42: 34-45.

Sebek, L., Kuikman, P. and Vriesekoop, P. (2008). Klimaat en Veehouderij. Wageningen, Wageningen UR, pp 16.

Seki, T., Isono, K., Ozaki, K., Tsukahara, Y., Shibata-Katsuta, Y., et al. (1998). The metabolic pathway of visual pigment chromophore formation in *Drosophila melanogaster*: All-trans (3S)-3-hydroxyretinal is formed from all- trans retinal via (3R)-3-hydroxyretinal in the dark. European Journal of Biochemistry 257: 522-527.

Senar, J. C., Møller, A. P., Ruiz, I., Negro, J. J., Broggi, J., et al. (2010). Specific appetite for carotenoids in a colorful bird. PLoS One 5: e10716.

Serbester, U., Çakmakçi, C., Göncü, S. and Görgülü, M. (2014). Effect of feeding starter containing butyrate salt on pre- and post-weaning performance of early or normally weaned calves. Revue de Medecine Veterinaire 165: 44-48.

Shearer, P. W. and Jones, V. P. (1996). Diel feeding pattern of adult female Southern Green Stink Bug (Hemiptera: Pentatomidae). Environmental Entomology 25: 599-602.

Sheppard, C. (1983). House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environmental Entomology 12: 1439-1442.

Sheppard, C. D., Larry Newton, G., Thompson, S. A. and Savage, S. (1994). A value added manure management system using the black soldier fly. Bioresource Technology 50: 275-279.

Sheppard, D. C., Newton, G. L. and Burtle, G. (2008). Black Soldier Fly Prepupae A Compelling Alternative to Fish Meal and Fish Oil, pp 116.

Simpson, S. J. (1982). Changes in the efficiency of utilization of food throughout the 5th instar nymphs of *Locusta migratoria*. Entomologia Experimentalis Et Applicata 31: 265-275.

Simpson, S. J. and Abisgold, J. D. (1985). Compensation by locusts for changes in dietary nutrients: behavioral mechanisms. Physiological Entomology 10: 443-452.

Simpson, S. J. and Raubenheimer, D. (2001). The geometric analysis of nutrient - allelochemical interactions: A case study using locusts. Ecology 82: 422-439.

Simpson, S. J., Raubenheimer, D., Behmer, S. T., Whitworth, A. and Wright, G. A. (2002). A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. The Journal of Experimental Biology 205: 121-129.

Slansky, F. and Rodriguez, J. (1987). Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. New York, John Wiley, pp 1016.

Slansky, F. and Scriber, J. M. (1985). Food consumption and utilization. Comprehensive insect physiology biochemistry and pharmacology. G. A. Kerkut and L. I. Gilbert. Oxford, Pergamon Press 4: 87–163.

Slansky, F. j. (1985). Food utilization by insects: Interpretation of observed differences between dry weight and energy efficiencies. Entomologia Experimentalis et Applicata 39: 47.

Smil, V. (2002). Worldwide transformation of diets, burdens of meat production and opportunities for novel food proteins. Enzyme and Microbial Technology 30: 305-311.

Smith, W. C. and Goldsmith, T. H. (1990). Phyletic aspects of the distribution of 3-hydroxyretinal in the class Insecta. Journal of Molecular Evolution 30: 72-84.

Sönmez, E. and Gülel, A. (2008). Effects of different temperatures on the total carbohydrate, lipid and protein amounts of the bean beetle, *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). Pakistan Journal of Biological Sciences 11: 1803-1808.

Spiegel, M., Noordam, M. and Fels-Klerx, H. (2013). Safety of novel protein sources (Insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehensive Reviews in Food Science and Food Safety 12: 662-678.

St-Hilaire, S., Cranfill, K., Mcguire, M. A., Mosley, E. E., Tomberlin, J. K., et al. (2007a). Fish offal recycling by the Black Soldier Fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38: 309-313.

St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., et al. (2007b). Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 38: 59-67.

Starkie, W. J. M. (1909). The Acharnians of Aristophanes, Macmillan and Company, limited.

Steinfeld, H. (2012). Sustainability Issues in Livestock Production. Exploratory Workshop Sustainable Protein Supply. Amsterdam.

Steinfeld, H. and Gerber, P. (2010). Livestock production and the global environment: Consume less or produce better? Proceedings of the National Academy of Sciences of the United States of America 107: 18237-18238.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., et al. (2006). Livestock's long shadow. Environmental issues and options. Rome, FAO, pp 319.

Strzelewicz, M., Ullrey, D., Schafer, S. and Bacon, J. (1985). Feeding insectivores: increasing the calcium content of wax moth (*Galleria mellonella*) larvae. The Journal of Zoo Animal Medicine: 25-27.

Studier, E. H., Keeler, J. O. and Sevick, S. H. (1991). Nutrient composition of caterpillars, pupae, cocoons and adults of the eastern tent moth, *Malacosoma americanum* (Lepidoptera: Lasiocampidae). Comparative Biochemistry and Physiology A: Physiology 100: 1041-1043.

Studier, E. H. and Sevick, S. H. (1992). Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. Comparative Biochemistry and Physiology A: Physiology 103: 579-595.

Sturtevant, W. C. (1978). Handbook of North American Indians, Volume 4: History of Indian-White Relations, Smithsonian Institution, pp 10.

Sullivan, K. E., Livingston, S. and Valdes, E. V. (2009). Vitamin A supplementation via cricket dusting : the effects of dusting fed and fasted crickets of three sizes using two different supplements on nutrient content 8th Conference on Zoo and Wildlife Nutrition, Tulsa OK, AZA Nutrition Advisory Group, pp 160-162.

Sutton, M. Q. (1990a). Aboriginal Tasmanian entomophagy. Proceedings of the First International Congress of Ethnobiology, Museu Paraense Emilio Goeldi, Belém, Brazil, pp 209-217.

Sutton, M. Q. (1990b). Insect resources and Plio-Pleistocene hominid evolution. Proceedings of the First International Congress of Ethnobiology, Museu Paraense Emilio Goeldi, Belém, Brazil, pp 195-207.

Sutton, M. Q. (1995). Archaeological aspects of insect use. Journal of Archaeological Method and Theory 2: 253-298.

Szelei, J., Woodring, J., Goettel, M. S., Duke, G., Jousset, F. X., et al. (2011). Susceptibility of North-American and European crickets to *Acheta domesticus* densovirus (AdDNV) and associated epizootics. Journal of Invertebrate Pathology 106: 394-399.

Tchibozo, S., van Huis, A. and Paoletti, M. G. (2005). Notes on edible insects of south Benin: A source of protein. Ecological Implications of Minilivestock (Role of Rodents, Frogs, Snails, and Insects for Sustainable Development). M. G. Paoletti. Enfield, New Hampshire, Science publishers, INC.,: 245-251.

Terblanche, J. S. and Chown, S. L. (2007). The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. Physiological Entomology 32: 175-180.

Thompson, D. (1910). The works of Aristotle. Vol. 4. Historia Animalium, Oxford: Section 30.

Thompson, S. N. (1973). Review and comparative characterization of fatty-acid compositions of 7 insect orders. Comparative Biochemistry and Physiology 45: 467-482.

Thorne, P. S. (2007). Environmental health impacts of concentrated animal feeding operations: Anticipating hazards - Searching for solutions. Environmental Health Perspectives 115: 296-297.

Thurnham, D. I., Bender, D. A., Scott, J. and Halsted, C. H. (2000). Water-soluble vitamins. Human nutrition and dietetics. J. S. Garrow, W. P. T. James and A. Ralph. London, Churchill Livingstone: 249-287.

Tietz, A. and Stern, N. (1969). Stearate desaturation by microsomes on the locust fat-body. FEBS Letters 2: 286-288.

Tilman, D., Socolow, R., Foley, J. A., Hill, J., Larson, E., et al. (2009). Beneficial biofuels—The food, energy, and environment trilemma. Science 325: 270-271.

Tommaseo-Ponzetta, M. (2005). Insects; food for human evolution. Ecological Implications of minilivestock. Potential of insects, rodents, and frogs. M. G. Paoletti. Enfield, New Hampshire, Science Publishers, Inc. Chapter 8: 141-161.

Tordoff, M. G. (2001). Calcium: Taste, intake, and appetite. Physiological Reviews 81: 1567-1597.

Trofimov, Y. (2000). As a cheese turns, so turns this tale of many a maggot. the Wall Street Journal CCXXXVI: 1.

Trusk, A. and Crissey, S. (1987). Comparison of calcium and phosphorus levels in crickets fed a high-calcium diet versus those dusted with supplement. Proceedings of the Sixth and Seventh Dr. Scholl Conferences on the Nutrition of Captive Wild Animals. Chicago, IL: Lincoln Park Zoological Gardens. p, pp 93-99.

Urs, K. C. D. and Hopkins, T. L. (1973a). Effect of moisture on growth rate and development of two strains of *Tenebrio molitor* L.(Coleoptera, Tenebrionidae). Journal of Stored Products Research 8: 291-297.

Urs, K. C. D. and Hopkins, T. L. (1973b). Effect of moisture on the lipid content and composition of two strains of *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). Journal of Stored Products Research 8: 299-305.

Vahabi, A., Rafinejad, J., Mohammadi, P. and Biglarian, F. (2007). Regional evaluation of bacterial contamination in hospital environment cockroaches. Iranian Journal of Environmental Health Science & Engineering 4: 57-60.

Valkenburgh, S., R. van Oosterom, O. Stenvers, M. Aalten, M. Braks, et al. (2007). Zoonoses and zoonotic agents in humans, food, animals and feed in the Netherlands 2003-2006. Bilthoven, pp 140.

Van der Brempt, X. and Moneret-Vautrin, D. A. (2014). The allergic risk of *Tenebrio molitor* for human consumption. Revue Française d'Allergologie 54: 34-36.

van Huis, A. (1996). The traditional use of arthropods in Sub Saharan Africa. The Netherlands Entomological Society (N.E.V.), Amsterdam, pp 3-20.

van Huis, A. (2005). Insects eaten in Africa (Coleoptera, Hymenoptera, Diptera, Heteroptera, Homoptera). Ecological Implications of Minilivestock. M. G. Paoletti. Enfield, New Hampshire, Science publishers, INC.,: 231-244.

van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., et al. (2013). Edible insects: future prospects for food and feed security. FAO Forestry Paper (FAO).

Van Itterbeeck, J. and van Huis, A. (2012). Environmental manipulation for edible insect procurement: a historical perspective. Journal of Ethnobiology and Ethnomedicine 8: 1-19.

van Loon, J. J. A., Casas, J. and Pincebourde, S. (2005). Nutritional ecology of insect plant-interactions: persistent handicaps and the need for innovative approaches. Oikos 108: 194-201.

Veldkamp, T., Duinkerken, G. v., Huis, A. v., lakemond, C. M. M., Ottevanger, E., et al. (2012). Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. Wageningen, The Netherlands, Wageningen University, pp 1-62.

Verbeke, W. (2015). Profiling consumers who are ready to adopt insects as a meat substitute in a Western society. Food Quality and Preference 37.

Verhoeckx, K. C. M., van Broekhoven, S., den Hartog-Jager, C. F., Gaspari, M., de Jong, G. A. H., et al. (2014). House dust mite (Der p 10) and crustacean allergic patients may react to food containing Yellow mealworm proteins. Food and Chemical Toxicology 65: 364-373.

Vermeij, I. (2013). Kwantitatieve Informatie Veehouderij 2012-2013, Wageningen UR, Livestock research.

Vermeij, I. (2014). Kwantitatieve Informatie Veehouderij 2013-2014, Wageningen UR, Livestock research.

Verstegen, M. W. A., Van Der Hel, W., Brandsma, H. A., Henken, A. M. and Bransen, A. M. (1987). The Wageningen respiration unit for animal production research: A description of the equipment and its possibilities Energy Metabolism in Farm Animals: Effects of Housing, Stress and Disease, pp. 21-48 M. W. A. Verstegen and A. M. Henken. Dordrecht, Martinus Nijhoff Publishers: 21-48.

Volkova, V. V., Bailey, R. H. and Wills, R. W. (2009). *Salmonella* in broiler litter and properties of soil at farm location. PLoS ONE 4: e6403.

von Lintig, J. (2012). Metabolism of carotenoids and retinoids related to vision. Journal of Biological Chemistry 287: 1627-1634.

von Lintig, J. and Vogt, K. (2000). Filling the gap in vitamin A research. Journal of Biological Chemistry 275: 11915-11920.

Waldbauer, G. P. (1968). The consumption and utilization of food by insects. Advances in Insect Physiology 5: 229-288.

Wallace, A. R. (1854). On the insects used for food by the Indians of the Amazon (EXCERPT ONLY). Transactions of the Royal Entomological Society London 2: 241-244.

Wang, D., S, W. Zhai, C.X. Zhang, Q. Zhang and H., C. (2007). Nutrition value of the Chinese grasshopper *Acrida cinerea* (Thunberg) for broilers. Animal Feed Science and Technology 135: 66-74.

Wathes, C. M., Holden, M. R., Sneath, R. W., White, R. P. and Phillips, V. R. (1997). Concentrations and emission rates of aerial ammonia, nitrous oxide, methane, carbon dioxide, dust and endotoxin in UK broiler and layer houses. British Poultry Science 38: 14 - 28.

Werren, J. H. and Beukeboom, L. W. (1998). Sex determination, sex ratios, and genetic conflict. Annual Review of Ecology and Systematics 29: 233-261.

Wheeler, E. F., K.D. Casey, J.S. Zajaczkowski, Topper, P. A., Gates, R. S., et al. (2003). Ammonia emissions from U.S. poultry houses: Part III - Broiler houses Proceedings of the 3rd International Conference on Air Pollution from Agricultural Operations, Research Triangle Park, NC.

Whitaker Jr, J. O., Dannelly, H. K. and Prentice, D. A. (2004). Chitinase in insectivorous bats. Journal of Mammalogy 85: 15-18.

Wilkinson, J. M. (2011). Re-defining efficiency of feed use by livestock. Animal 5: 1014-1022.

Winn, D., Dunham, S. and Mikulski, S. (2003). Food for insects and insects as food: Viable strategies for achieving adequate calcium. Journal of Wildlife Rehabilitation 26: 4-13.

Winterbottom, T. (1803). Account of the native Africans in the neighbourhood of Sierra Leone: To which is added, an account of the present state of medicine among them, Cambridge University Press.

Wise, M., Calvin, K., Thomson, A., Clarke, L., Bond-Lamberty, B., et al. (2009). Implications of limiting CO_2 concentrations for land use and energy. Science 324: 1183-1186.

Woodring, J. P., Clifford, C. W. and Beckman, B. R. (1979). Food utilization and metabolic efficiency in larval and adult house crickets. Journal of Insect Physiology 25: 903-912.

Woodring, J. P., Roe, R. M. and Clifford, C. W. (1977). Relation of feeding, growth, and metabolism to age in the larval, female house cricket. Journal of Insect Physiology 23: 207-212.

Wrage, N., Velthof, G. L., van Beusichem, M. L. and Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry 33: 1723-1732.

Xia, Z., Wu, S., Pan, S. and Kim, J. M. (2012). Nutritional evaluation of protein from *Clanis bilineata* (Lepidoptera), an edible insect. Journal of the Science of Food and Agriculture 92: 1479-1482.

Yang, L.-F., Siriamornpun, S. and Li, D. (2006). Polyunsaturated fatty acid content of edible insects in Thailand. Journal of Food Lipids 13: 277-285.

Yang, S., Li, Q., Zeng, Q., Zhang, J., Yu, Z., et al. (2012). Conversion of solid organic wastes into oil via *Boettcherisca peregrine* (Diptera: Sarcophagidae) larvae and optimization of parameters for biodiesel production. PloS one 7: e45940.

Yang, S. and Liu, Z. (2014). Pilot-scale biodegradation of swine manure via *Chrysomya megacephala* (Fabricius) for biodiesel production. Applied Energy 113: 385-391.

Yhoung-Aree, J., Puwastien, P. and Attig, G. A. (1997). Edible insects in Thailand: an unconventional protein source? Ecology of Food and Nutrition 36: 133-149.

Yhoung-Aree, J. and Viwatpanich, K. (2005). Edible insects in the Laos PDR, Myanmar, Thailand, and Vietnam. Ecological Implications of Minilivestock: Potential of Insects, Rodents, Frogs, and Snails. M. G. Paoletti. Enfield, New Hampshire, Science Publishers, Inc. : 415-440.

Yu, X., Liu, H. and Tong, L. (2008). Feeding scenario of the silkworm *Bombyx mori*, L. in the BLSS. Acta Astronautica 63: 1086-1092.

Zhang, Z., Song, X., Wang, Q. and Lu, X. (2012). Cd and Pb contents in soil, plants, and grasshoppers along a pollution gradient in Huludao City, Northeast China. Biological Trace Element Research 145: 403-410.

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., et al. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy 47: 225-229.

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., et al. (2013). Exploring the potential of grease from yellow mealworm beetle (*Tenebrio molitor*) as a novel biodiesel feedstock. Applied Energy 101: 618-621.

Zheng, L., Li, Q., Zhang, J. and Yu, Z. (2012). Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. Renewable Energy 41: 75-79.

Zhi-Yi, L. (1997). Insects as food in China. Ecology of Food and Nutrition 36: 201-207.

Zhou, F., Tomberlin, J. K., Zheng, L., Yu, Z. and Zhang, J. (2013). Developmental and waste reduction plasticity of three black soldier fly strains (Diptera: Stratiomyidae) raised on different livestock manures. Journal of medical entomology 50: 1224-1230.

Zhou, X., Honek, A., Powell, W. and Carter, N. (1995). Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. Entomologia Experimentalis et Applicata 75: 99-107.

Summary

Interest in the usage of insects as a source of food and feed is increasing. This is due to an expected increase in demand of 70-80% between 2012 and 2050 for animal-based protein, and because of sustainability issues with conventional animal production systems. These systems currently use about 70% of all arable land and are responsible for approximately 15% of the total emissions of anthropogenic greenhouse gases (GHG). Insects are suggested to have properties which make them more suitable production animals.

This thesis starts with a historical perspective on insects as food and feed, and introduces five suggestions why insects would be more suitable production animals (Chapter 1). Insects are suggested to have a high reproductive capacity, a high nutritional quality, a high feed conversion efficiency, they can use waste as feed, and insect production systems are suggested to be more sustainable than conventional animal production systems.

In Chapter 2, the nutritional quality of insects is discussed. This chapter emphasizes on a variety of insects that are commonly used as feed or food. The nutrient content of commercially raised insects is reviewed and compared to data available for wild insects. Furthermore, these data are discussed in the light of the nutrient requirements for domestic animals to identify nutrients of concern. Additionally, various environmental and dietary factors which are known to significantly affect insect chemical composition are reviewed. Techniques that are currently used to enhance the nutrient content of commercially produced insects including gut loading and dusting are described. Lastly, possible negative factors including pathogenic microorganisms, toxins, and anti-nutritional compounds that might be important when feeding captive insectivores are discussed.

Chapter 3 focuses on five insect species; Yellow mealworms, House crickets, Migratory locusts, Sun beetles and Argentinean cockroaches. The first three species are considered edible, while the latter two can be used as feed. Their direct GHG and ammonia production are quantified. Furthermore, carbon dioxide (CO_2) production and average daily gain (ADG) were quantified as a measure of feed conversion efficiency. Large differences were found among the species regarding their production of GHGs (expressed as CO_2 equivalents). Sun beetles and Argentinean cockroaches produced methane (CH_4), a powerful GHG, which resulted in a higher GHG production per kg of mass gain than for the other insect species. However, all insect species in this study had a higher ADG, and emitted comparable or lower amounts of GHG than described in literature for pigs, and much lower amounts of GHG than cattle. The same was true for CO_2 production

per kg of metabolic weight and per kg of mass gain, which indicates a higher feed conversion efficiency. Furthermore, also the production of ammonia (NH_3) by insects was lower than for conventional livestock. This chapter therefore indicates that insects are a potentially more environmentally friendly alternative for the production of animal protein with respect to GHG and ammonia emissions.

The results for Yellow mealworms from Chapter 3 were subsequently used in a Life Cycle Assessment (LCA) in Chapter 4. LCA is a holistic method to evaluate the environmental impact of a product during the entire production chain. For a production system producing both Yellow mealworms and Superworms, total GHG production, energy use, and land use were quantified and compared to conventional sources of animal protein. Production of one kg of edible protein from milk, chicken, pork or beef result in higher GHG emissions, require similar amounts of energy and require much more land. This chapter demonstrates that mealworms should be considered a more sustainable source of edible protein. Furthermore, it shows that a large part of the environmental impact of mealworm production is due to the type and amount of feed used and the efficiency with which this is converted into body mass. In LCAs environmental impact is often allocated based on economic value. Industrial by-products are therefore considered to have a lower environmental impact than main products.

In Chapter 5 four diets formulated such as to vary in protein and fat content were composed from industrial organic by-products. These diets were offered to four insect species (Argentinean cockroaches, Black soldier flies (BSFs), Yellow mealworms, and House crickets) and their feed conversion efficiency was determined. Diets used in large scale production systems served as controls. Addition of carrots, serving as a source of moisture, was taken as an extra variable for the Yellow mealworms. Besides feed conversion efficiency, the survival, development time and chemical composition (nitrogen, phosphorous, and fatty acids) were determined. Diet affected survival in all species but BSFs. Development time was strongly influenced by the diet offered. Availability of carrots to Yellow mealworm greatly decreased development time, and increased dry matter- and nitrogen conversion efficiency. The chemical composition of Argentinean cockroaches proved to be highly dependent on diet. BSFs on the other hand, thrived on all diets, and their composition was similar on the diets tested. The Yellow mealworm and the House cricket converted feed less efficiently than the Argentinean cockroach and the BSF. The latter two were also more efficient than conventional production animals. Feed conversion efficiency for Yellow mealworms and House crickets was similar to pigs and lower than for poultry. However, due to their high edible portion these two species converted feed more efficiently to food than conventional production animals. The investigated species are efficient production animals and can therefore be considered interesting for the production of feed or food. Furthermore, different diets resulted in compositional differences in the insect species, indicating possibilities to tailor the composition of these insects to best meet consumers' needs.

By-products are considered to have a lower environmental impact than main products, and the production of by-products with a negligible, or even negative economic value, are considered to have a negligible environmental impact. In the Dutch context, manure is an example of a by-product with a negative economic value. Hence, if an insect is reared on manure there would be no environmental impact allocated to the feed used by the insect. In Chapter 6 the suitability of chicken, pig, and cow manure was compared as feed for larvae of the BSF. Newly hatched larvae were directly inoculated on moistened manure that subsequently was added three times per week, until the first prepupae appeared. High survival indicated that the tested substrates could be considered suitable. However, development time was greatly prolonged compared to the control diet. Efficiency of conversion of ingested nitrogen (N-ECI) was higher on pig manure than on chicken and cow manure, whereas the ECI for phosphorous was highest on cow manure. Substrate nitrogen content decreased in the chicken manure, but were stable in pig and cow manure. Phosphorous concentrations increased, and N:P-ratios decreased in all treatments. Since a large proportion of the nitrogen from the manure was lost, the production system would require a way to prevent this in order to make it ecologically sound. It was also concluded that shorter development times are required to improve economic viability. Possibly this could be achieved by providing a more nutritious starter diet to larvae, allowing more rapid development after switching to manure.

In Chapter 7 the five suggestions why insects would make suitable production animals are evaluated based on both literature data, and data gathered in this thesis. Indeed insects have a higher reproductive capacity, a similarly high nutritional quality, and a high feed conversion efficiency compared to conventional production animals. While certain insects can use waste as feed, this is true for a limited number of insect species only, and depends strongly on the adopted definition of waste. The sustainability of insect production systems in general cannot be evaluated, because insufficient data is available. This thesis confirms that certain insect species are at least as suitable production animals as conventional production animals. In the last section emphasis is put on which knowledge needs to be made available, and which legislation adaptations are needed for the further development of the insect production sector.

Samenvatting

De interesse in het gebruik van insecten als bron van voedsel voor mens en dier neemt wereldwijd toe. Dit komt door een verwachte groei van 70-80% in de vraag naar dierlijke eiwitten in de periode van 2012 tot 2050 en door duurzaamheidsproblemen met de huidige dierlijke productiesystemen. Deze systemen gebruiken momenteel 70% van de beschikbare landbouwgrond en zijn verantwoordelijk voor ongeveer 15% van emissie van antropogene broeikasgassen. Verscheidene auteurs hebben gesuggereerd dat insecten eigenschappen hebben die ze geschikter maken als productiedier dan gangbare productiedieren.

Dit proefschrift begint met een historisch perspectief op insecten als voedsel en diervoeding, en introduceert vijf suggesties waarom insecten geschikt zouden kunnen zijn als productiedier: de hoge reproductie capaciteit; een hoge voedingswaarde; een hoge voerefficiëntie; gebruik van organisch afval als voedsel; en duurzaamheid van insectenproductiesystemen (Hoofdstuk 1).

In Hoofdstuk 2 wordt de voedingswaarde van insecten besproken. Dit hoofdstuk legt de nadruk op insectensoorten die vaak gebruikt worden als voedsel of diervoeder. Het nutriëntgehalte van commercieel geproduceerde insecten wordt besproken en vergeleken met data over wilde insecten. Vervolgens worden deze gegevens vergeleken met de nutriëntbehoefte van gedomesticeerde dieren om eventuele te verwachte nutriënttekorten bij de consumptie van insecten te kunnen identificeren. Daarbij worden verschillende omgevings- en dieet-factoren, waarvan bekend is dat ze de chemische samenstelling van insecten beïnvloeden, besproken. Technieken die momenteel gebruikt worden om de voedingswaarde van commercieel geproduceerde insecten te verbeteren, inclusief “gutloading” en bepoederen, worden beschreven. Ten slotte worden mogelijke negatieve factoren, waaronder pathogene micro-organismen, toxines, en anti-nutritionele factoren, welke mogelijk relevant zijn bij het voeren van in gevangenschap levende insectivoren besproken.

Hoofdstuk 3 richt zich op de productie van broeikasgassen en ammonia (NH_3) van een vijftal insectensoorten; gewone meelwormen, huiskrekels, treksprinkhanen, rozenkevers en Argentijnse kakkerlakken. De eerste drie soorten zijn eetbaar, terwijl de laatste twee als diervoeder gebruikt kunnen worden. Ook de productie van koolstofdioxide (CO_2) en de groeisnelheid werden gekwantificeerd als maatstaf voor de voerefficiëntie. Er waren grote verschillen voor wat betreft de productie van broeikasgassen (uitgedrukt in CO_2 equivalenten). Rozenkevers en Argentijnse kakkerlakken produceren methaan (CH_4), waardoor hun broeikasgas productie per kg gewichtstoename hoger is dan de andere insectensoorten. Echter, alle insectensoorten in deze studie hadden een hogere groeisnelheid, en produceerden vergelijkbare of lagere hoeveelheden broeikasgas dan in de literatuur beschreven voor varkens en veel minder dan dat geproduceerd wordt door rundvee. Dat gold ook voor de CO_2-productie per kg metabool gewicht en per kg gewichtstoename, wat duidt op een hogere voerefficiëntie. Daarbij was ook de NH_3-productie van insecten lager dan die van de gangbare productiedieren. Dit hoofdstuk laat zien dat insecten mogelijk een milieuvriendelijk alternatief zijn voor de productie van dierlijk eiwit voor wat betreft broeikasgas- en NH_3-emissies.

De resultaten voor de gewone meelwormen uit Hoofdstuk 3 zijn vervolgens gebruikt in een Life Cycle Assessment (LCA) in Hoofdstuk4. LCA is een holistische methode om de milieueffecten van een product te evalueren gedurende de gehele productieketen. Voor een productiesysteem, dat zowel gewone meelwormen als morio-wormen produceert, werden de broeikasgasproductie, het energieverbruik en het landgebruik gekwantificeerd en vergeleken met gangbare bronnen van dierlijke eiwitten. De productie van een kilogram eetbaar eiwit in de vorm van melk, kip, varken of rundvlees gaat gepaard met een hogere uitstoot van broeikasgassen, een vergelijkbaar energieverbruik en een hoger landgebruik dan bij insecten. Dit hoofdstuk toont aan dat meelwormen gezien mogen worden als een meer duurzame bron van eetbaar eiwit. Ook toont deze studie aan dat een groot deel van de milieueffecten veroorzaakt worden door het type en de hoeveelheid voer dat gebruikt wordt, en de efficiëntie waarmee dit wordt omgezet in lichaamsmassa.

In LCA’s worden milieueffecten vaak toegewezen op basis van de economische waarde. Industriële bijproducten zijn derhalve minder milieubelastend dan hoofdproducten. In Hoofdstuk 5 werden uit industriële organische bijproducten vier diëten zo geformuleerd dat ze verschilden in eiwit en vetgehalte. Deze diëten werden aan vier insectensoorten aangeboden (Argentijnse kakkerlakken, wapenvliegen, gewone meelwormen en huiskrekels) en hun voerefficiëntie werd gekwantificeerd. Diëten gebruikt in grootschalige productiesystemen werden als controle gebruikt. Het toevoegen van wortelen, welke diende als een bron van vocht, werd als extra variabele meegenomen voor de diëten van de meelwormen. Naast voerefficiëntie werden de overleving, ontwikkelingsduur en de chemische samenstelling (stikstof- en fosforgehalte, en het vetzuurgehalte en -profiel) bepaald. Het dieet beïnvloedde de overleving van alle soorten, behalve van de wapenvlieg. Ontwikkelingsduur werd sterk beïnvloed door het aangeboden dieet. Beschikbaarheid van wortel voor gewone meelwormen verkortte de ontwikkelingsduur en verhoogde de omzettingsefficiëntie van droge stof en stikstof. De chemische samenstelling van Argentijnse kakkerlakken was sterk afhankelijk van het aangeboden dieet. De wapenvliegen deden het daarentegen goed op alle vier de diëten en vertoonde een vergelijkbare chemische samenstelling. De gewone meelwormen en de huiskrekels hadden een lagere voerefficiëntie dan de Argentijnse kakkerlakken en de wapenvliegen. De laatste twee soorten waren ook efficiënter dan gangbare productiedieren. De voerefficiëntie van de gewone meelwormen en de huiskrekels was vergelijkbaar met die van varkens en lager dan die voor kippen. Echter, door de hogere eetbare fractie, waren beide insectensoorten efficiënter in het omzetten van voer naar eetbaar product dan gangbare productiedieren. De onderzochte soorten zijn efficiënte productiedieren en kunnen derhalve interessant zijn voor de productie van voedsel voor mens en dier. Daarbij resulteerden de diëten in verschillen in de chemische samenstelling van de insectensoorten, wat duidt op de mogelijkheid deze te beïnvloeden en aan te passen aan de behoeften van mens of dier.

Organische bijproducten zijn minder milieubelastend dan primaire producten. De milieubelasting van bijproducten met een verwaarloosbare, of zelfs negatieve waarde, wordt als neutraal gezien. In de Nederlandse context is mest een voorbeeld van een bijproduct met een negatieve economische waarde. Als een insect dus gevoed zou worden met mest, dan zouden er geen milieueffecten aan het voer worden toegekend. In Hoofdstuk 6 wordt de geschiktheid van kippen-, varkens- en rundermest vergeleken als voedsel voor larven van de wapenvlieg. Net uitgekomen larven werden direct geïnoculeerd op bevochtigde mest en driemaal per week bijgevoerd totdat de eerste pre-pop zichtbaar was. Een hoge overleving van de larven liet zien dat de geteste substraten geschikt waren. Echter, de ontwikkelingsduur werd sterk verlengd in vergelijking met het controle dieet. De stikstofefficiëntie was hoger op varkensmest dan op kippen- en rundermest, terwijl de efficiëntie voor fosfor het hoogste was op rundermest. In de kippenmest werd de stikstofinhoud in het resterend substraat verlaagd door de larven, terwijl dit gelijk bleef in varkens- en rundermest. Fosfor-concentraties waren verhoogd terwijl N:P ratios verlaagd waren in de resterende substraten. Een groot deel van de stikstof uit de mest ging verloren. Daarom zijn er aanpassingen aan het systeem nodig om het ecologisch verantwoord te maken. Ook dient de ontwikkelingsduur verkort te worden om het systeem economisch levensvatbaar te maken. Mogelijk zou dit gedaan kunnen worden door de larven een voedzaam startdieet te geven, waardoor ze zich sneller ontwikkelen wanneer ze overgeschakeld worden op mest.

In Hoofdstuk 7 worden de vijf redenen waarom insecten geschikte productiedieren zouden kunnen zijn geëvalueerd op basis van zowel literatuur als data verzameld in dit proefschrift. Insecten hebben in vergelijking met gangbare productiedieren een hogere reproductieve capaciteit, een vergelijkbare voedingswaarde en een hoge voerefficiëntie. Alhoewel sommige insecten organisch afval kunnen gebruiken als voedsel, geldt dit slechts voor een beperkt aantal soorten en dit is zeer afhankelijk van de gehanteerde definitie van afval. De duurzaamheid van insectenproductiesystemen in het algemeen kan niet geëvalueerd worden omdat er onvoldoende data voor handen is. Dit proefschrift bevestigt dat bepaalde insectensoorten ten minste zo geschikt zijn als productiedier als gangbare productiedieren. In dit hoofdstuk wordt ook de nadruk gelegd op welke kennis ontwikkeld moet worden en welke veranderingen in wetgeving nodig zijn voor de verdere ontwikkeling van de insectenproductiesector.

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Publications

Peer-reviewed:

Bosch, G., S. Zhang, **D. G. A. B. Oonincx** and W. H. Hendriks (2014). Protein quality of insects as potential ingredients for dog and cat foods. Journal of Nutritional Science 3 (e29): 1-4.

Finke, M. D. and **D. G. A. B. Oonincx** (2013). Insects as food for insectivores. In: Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens. (eds) J. A. Morales-Ramos, M. G. Rojas and D. I. Shapiro-Ilan. London, UK, Academic Press.

Oonincx, D. G. A. B., M. D. van de Wal, G. Bosch, J. B. G. Stumpel, A. C. Heijboer, *et al.* (2013). Blood vitamin D3 metabolite concentrations of adult female bearded dragons (*Pogona vitticeps*) remain stable after ceasing UVb exposure. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 165(3): 196-200.

Oonincx, D. G. A. B. and I. J. M. de Boer (2012). Environmental impact of the production of mealworms as a protein source for humans – a Life Cycle Assessment. PLoS One 7(12): e51145.

Oonincx, D. G. A. B. and E. S. Dierenfeld (2011). An investigation into the chemical composition of alternative invertebrate prey. Zoo Biology 31(1): 40-54

Oonincx, D. G. A. B. and A. F. van der Poel (2011). Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). Zoo Biology 30(1): 9-16.

Oonincx, D. G. A. B., Y. Stevens, J. J. van den Borne, J. P. van Leeuwen and W. H. Hendriks (2010). Effects of vitamin D3 supplementation and UVb exposure on the growth and plasma concentration of vitamin D3 metabolites in juvenile bearded dragons (*Pogona vitticeps*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 156(2): 122-128.

Oonincx, D. G. A. B., J. van Itterbeeck, M. J. Heetkamp, H. van den Brand, J. J. A. van Loon, *et al.* (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One 5(12): e14445.

Popular:

Oonincx, D. G. A. B. (2010). Op zoek naar drakenbloed (In Dutch). Lacerta 68(5): 172-181.

Oonincx, D. G. A. B. (2009). Preferred Hatching Time; Een interessant fenomeen met mogelijke implicaties voor de incubatie van hagedisseneieren (In Dutch). Lacerta 67(6): 259-262.

Submitted:

Oonincx, D. G. A. B., S. van Broekhoven, A. van Huis and J. J. A. van Loon. Feed conversion efficiency, survival, crude protein content and lipid composition of four insect species on diets composed of organic by-products.

Oonincx, D. G. A. B., A. van Huis and J. J. A. van Loon. Nutrient utilisation by Black soldier flies fed with chicken, pig, or cow manure.

Oonincx, D. G. A. B., J. P. van Leeuwen, W. H. Hendriks and A. F. B. van der Poel. The diet of free-roaming Australian Central Bearded Dragons (*Pogona vitticeps*).

van Zanten, H. H. E., H. Mollenhorst, **D. G. A. B. Oonincx**, P. Bikker, B. G. Meerburg, *et al.* From environmental nuisance to environmental opportunity: housefly larvae convert waste to livestock feed.

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Insects as human food source; past, present and future (2009)

Writing of project proposal (4.5 ECTS)

- An assessment of commercially reared insects as a human food source

Post-graduate courses (5.9 ECTS)

- Basic statistics; PE&RC (2008)
- Advanced food analysis; VLAG (2010)
- Innovation for sustainability: bringing theory into practice; PE&RC (2010)

Laboratory training and working visits (1.5 ECTS)

- Rearing methods for edible insects in the Netherlands; VENIK participants (2008-2001)
- Rearing methods for black soldier flies (*Hermetia illucens*); Universidad de Alicante (2012)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Journal of Animal Physiology and Animal Nutrition: high calcium intake affects the apparent digestibility of crude nutrients and energy growing dogs (2009)
- African Journal of Biotechnology: nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile (2011)
- Zoobiology: the silkworm *Bombyx mori* can sustain the growth of the leopard gecko *Eublepharis macularius* until sexual maturity as only food (2014)

Deficiency, refresh, brush-up courses (0.3 ECTS)

- Fundamentals PBPPE (2008)

Competence strengthening / skills courses (4.5 ECTS)

- PhD Competence assessment; PE&RC (2008)
- Scientific publishing; WGS (2008)

- The art of writing; WUR Language Services (2009)
- Science the press and the general public; SENSE (2009)
- Interpersonal communication for PhD students; EPS (2009)
- Teaching and supervising thesis students; DO (2010)
- Writing grant proposals; WUR Language Services (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC Weekend (2008)
- PE&RC Day 4 times (2008-2011)

Discussion groups / local seminars / other scientific meetings (6 ECTS)

- PhD Lunch meetings; Entomology (2008-2012)
- Nederlandse Entomologendag (2009)
- LCA Meetings (2011-2012)

International symposia, workshops and conferences (9 ECTS)

- 39th Animal Nutrition Research Forum; Melle, Belgium (2009)
- 9th International Food Database Composition; Norwich, UK (2011)
- Assessing the potential of insects as food and feed in assuring food security; FAO Expert meeting; Rome, Italy (2012)
- 43th Animal Nutrition Research Forum; Utrecht, the Netherlands (2014)
- Insects to feed the world; Ede, the Netherlands (2014)
- The art, Culture, Science & Business of Entomophagy; Montreal, Canada (2014)

Lecturing / Supervision of practical's / tutorials (3 ECTS)

- Companion animals (2009-2014)
- Insect plant interactions (miniproject) (2010)
- Fundamentals of plant breeding, plant pathology and entomology (2009-2010)
- Ecophysiology (2010-2011)

Supervision of a MSc students

- Greenhouse gas emissions by insects
- Conversion of spent grains and DDGS by black soldier flies
- Fulfilling water requirements of *Alphitobius diaperinus* larvae