From innovation to implementation

Viability of the increased use of insects as food and feed

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Abstract

As global pressures on food systems mount the push for novel solutions, particularly how to source new forms of protein, has intensified. One possible solution, which has garnered attention in recent years, is increasing the use of edible insects as food and feed. Preliminary research has suggested that increasing the use of insects could be beneficial as they require less water, land and feed to rear and thus have a smaller environmental footprint than does any other protein source, whilst also maintaining high nutritional quality. Furthermore, insects have high micronutrient levels and could therefore play a vital role in alleviating many forms of malnutrition. Considering insects as a source of food and feed is not far-fetched as there are more than 2000 documented edible species and 2 billion people globally already regularly consume insects. However, reliable data to substantiate the claims made about the potential of sustainable implementation of insects within food systems are lacking.

This thesis starts with a review (Paper I) of the current opportunities and major hurdles facing the incorporation of edible insects into existing dietary systems. From this It is apparent that whilst there are environmental and nutritional opportunities these are presently hampered by a lack of information on how to adequately rear and process insects. There is also limited data available on the safety of incorporating insects into the diets of animals, including humans, which have not traditionally consumed them.

Papers II and III of this thesis examine key aspects of how to rear insects and what diets would ensure that they would be socially, economically and environmentally sustainable. It has been frequently stated that insects can be farmed easily on bio-waste sources so two trials were done to test products of various composition as feed for *Gryllus bimaculatus* and *Hermetia illucens*, two species commonly used as food or feed. The trial with *H. illucens* showed that the larvae easily survived and thrived on a brewing waste from Uganda, suggesting that this is a viable option. However, a second trial with *G. bimaculatus* showed that they could not achieve adequate survival rates when fed on food waste, beer brewing waste or cow manure and the only way to improve this was to introduce a high quality, non-

waste, feed for at least 1 week prior to being switched to a waste food source. Thus, limiting the sustainability of the system.

As well as considering the production of insects for food, it was also important to look at the nutritional quality and Paper IV examines the effects of heat-processing on the nutritional content of *G. bimaculatus.* This showed a significant effect of drying temperature on the lipid profile of *G.* bimaculatus; crickets that had been freeze-dried containing significantly more polyunsaturated and total fatty acids than those dried at 120℃.

One way of gaining a wide acceptance of insects in the food chain is to use them to rear fish for human consumption. Thus, Paper V reports trials where two meals were created using either *H. illucens* larvae or *G. bimaculatus*. These were analysed for their nutritional content, showing that both met or exceeded the basic nutritional requirements for omnivorous fish. A fish-feeding trial was then conducted with Nila tilapia, whereby the fish were fed on either a fish-meal, plant-based, *H. illucens*-based, or *G. bimaculatus*-based diet for 12 weeks, over which feed consumption, growth and survival were monitored. This demonstrated that the insect-based diets were just as good as the others supporting the view that insects as fish feed are a promising sustainable protein source.

It is evident from the findings presented in this thesis that the use of insects as food and feed is a complex issue that needs to be considered carefully. There are benefits to insects in the form of their favourable nutritional profile and in the success of using them as an omnivorous fish feed component in place of fish-meal. However, it is equally evident that a key area requires significant further exploration. If the rearing of insects is to be sustainable they need to be able to use a sustainable food source such as bio-waste and this will need further optimisation to ensure that it produces the same results as the chicken feed alternative that is being used currently. It will also have to be compatible with the downstream use of the insects interms of geographic location and cultural considerations.

Keywords: edible insects, entomophagy, nutrition, bio-waste, rearing, tilapia

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List of Publications

This doctoral thesis is based on the work contained in the following papers:

I. Dobermann, D., Swift, J. A., & Field, L. M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin*, *42*(4), 293-308.

II. Dobermann, D., Michaelson, L. V., & Field, L. M. (2018). The effect of an initial high-quality feeding regime on the survival of *Gryllus bimaculatus* (black cricket) on bio-waste. *Journal of Insects as Food and Feed. In press.*

III. Dobermann, D., Field, L.M., & Michaelson, L. V. (2019). Using *Hermetia illucens* to process Ugandan waragi waste. *Journal of Cleaner Production,* 211: 303-308.

IV. Dobermann, D., Field, L.M., & Michaelson, L. V. (2019). Impact of heat-processing on the nutritional content of *Gryllus bimaculatus* (black cricket). *Nutrition Bulletin*. *In press.*

V. Dobermann, D., Badr, A., Dickson, M., & Al-Kenawy, D. (2018). Growth of Nile tilapia, *Oreochromis niloticus* (Abbass strain) fed on insect meal, fish meal or plant-based diets in Egypt. *Aquaculture. In review.*

Contribution Report

Paper I: Darja Dobermann (DD) participated in outlining the review, performed the literature search, and wrote the draft manuscript

Paper II: DD designed the study, performed the experimental work, and wrote the draft manuscript

Paper III: DD designed the study, performed the experimental work, and wrote the draft manuscript

Paper IV: DD participated in the design of the study, performed the experimental work, and wrote the draft manuscript

Paper V: DD participated in the design of the study and wrote the draft manuscript

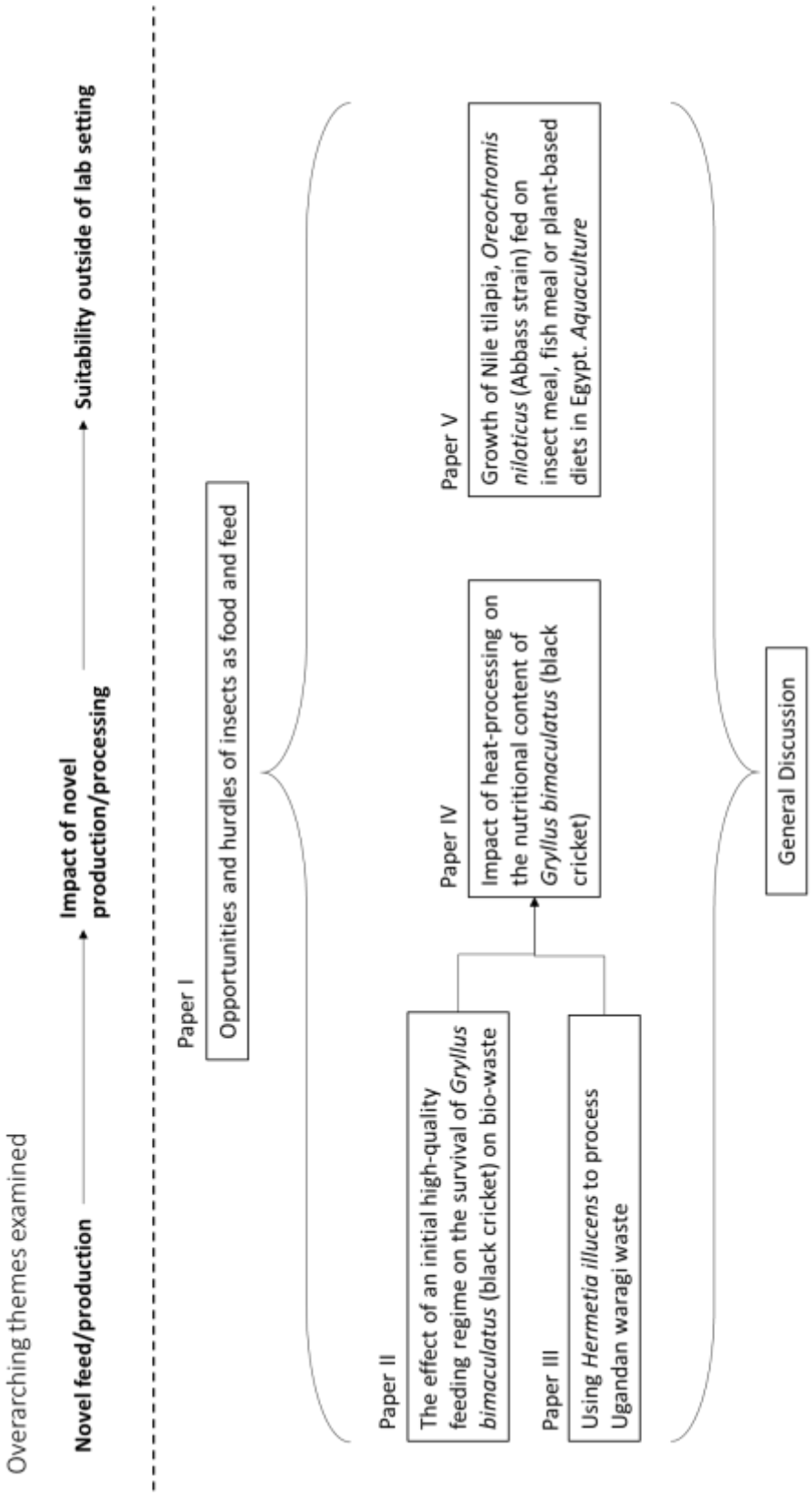
Objectives

Insects are increasingly brought to the table as an innovative solution to a myriad of food system challenges, but research is lacking in key areas to implement these solutions. Thus, an important goal in research is to assess if and how insects can be implemented to alleviate pressure on food systems. The overall aim of the work in this thesis was to investigate two of the primary claims made with regard to edible insects: 1) that they can easily be reared in a sustainable way to a high nutritional quality 2) that they are suitable from a nutritional and safety perspective in food and feed stuffs. The intention being to fill in some of the knowledge gaps to better inform if and how to increase the utilisation of insects within existing food systems.

Specific objectives of the work presented in this thesis were to:

- Review the current position on insects as food and feed (Paper I)
- Test to see if commonly consumed insects can be reared successfully on bio-waste sources (Paper II and III)
- Investigate methods to improve insect survival on bio-waste sources (Paper II)
- Explore how changing processing methods of edible insects potentially impacts their nutritional quality and content (Paper IV)
- Assess the suitability of insects as a feed stuff in aquaculture (Paper V)

Visual Overview



Opportunities and hurdles of edible insects for food and feed

Abstract

Entomophagy, the consumption of insects, is being promoted as an alternative sustainable source of protein for humans and animals. Seminal literature highlights predominantly the benefits, but with limited empirical support and evaluation. We highlight the historical significance of entomophagy by humans and key opportunities and hurdles identified by research to date, paying particular attention to research gaps. It is known that insects present a nutritional opportunity, being generally high in protein and key micronutrients; it is unclear how their nutritional quality is influenced by what they are fed. Research indicates that in ideal conditions insects have a smaller environmental impact than more traditional Western forms of animal protein; less known is how to scale up insect production while maintaining these environmental benefits. Studies overall show that insects could make valuable economic and nutritional contributions to the food or feed systems, but there are no clear regulations in place to bring insects into such supply systems. Future research needs to examine how the nutritional value of insects can be managed systematically, establish clear processing and storage methodology, define rearing practices, and implement regulations with regard to food and feed safety. Each of these aspects should be considered within the specifics of concrete supply and value chains, depending on whether insects are intended for food or for feed, to ensure insects are a sound economic, nutritional and sustainable protein alternative – not just a more expensive version of poultry for food, or soya for feed.

Published as: Dobermann, D., Swift, J. A., & Field, L. M. (2017). Opportunities and hurdles of edible insects for food and feed. *Nutrition Bulletin*, *42*(4), 293-308.

1. Introduction

Entomophagy, the eating of insects, is not a new phenomenon for humans, with archaeological evidence demonstrating that humans have evolved as an entomophagous species (Sutton, 1995; Raubenheimer & Rothman 2011). More recently, for several hundreds of years, humans have been making use of a range of insects as a food source, which is naturally high in protein and micronutrients. In parts of Central Africa, at times, up to 50% of dietary protein comes from insects and their market value is higher than many alternative sources of animal protein (Paoletti & Dreon 2005; Raubenheimer & Rothman 2011). It has been estimated that entomophagy is practiced in at least 113 countries with over 2000 documented edible insect species (Jongema 2017), and the United Nations has recommended the practise as a potential solution to the shortage of world food supplies (van Huis *et al.*, 2013).

Whether the wider adoption of entomophagy could help alleviate growing pressure on the environment from food production, and reduce malnutrition in both developed and developing countries, is a topic of extensive debate. This narrative review aims to highlight some of the opportunities and hurdles associated with entomophagy with respect to malnutrition and food security. The paper will explore the history, culture and customs surrounding the collection and eating of insects; the main nutritional and environmental benefits of entomophagy; and the barriers to the widespread implementation of entomophagy and the steps necessary to counter them. It is intended as a narrative review and as such is not wholly exhaustive of the literature. Rather it provides a general overview of the state of research on edible insects and current challenges due to the lack of research.

2. History of insect consumption by humans

Insect species that have become the most commonly consumed are popular due to their size and availability (Bukkens 1997). Insects need to be large enough to make the effort of catching them worthwhile and easy to locate, preferably in predictably large quantities. Thus, popular insects species for consumption fall into the following categories: beetles (Coleoptera, 31%); caterpillars (Lepidoptera, 18%); bees, wasps and ants (Hymenoptera, 14%); grasshoppers, locusts and crickets (Orthoptera, 13%); cicadas, leafhoppers, plant-hoppers, scale insects, and true bugs (Hemiptera, 10%); termites (Isoptera, 3%); dragonflies (Odonata, 3%); and flies (Diptera, 2%)(Jongema 2017). Insects are consumed at a variety of

their life-stages and with various methods of preparation including raw, fried, boiled, roasted or ground.

In Thailand, for example, 150 different insect species, mostly wild-harvested, are consumed and are a vital staple in the diet (Yhoung-Aree 2010). Entomophagy has also been shown to be a successful method of crop pest control. In 1978, a locust (*Patanga succincta*) outbreak in Thailand resulted in a government campaign promoting the locust's edibility. They became such a popular snack that they are now no longer a crop pest and their market value means that some farmers now grow crops specifically to feed them (Hanboonsong 2010). An increasing demand for insects as food in Thailand has resulted in a shift from wild collection to the development of mass rearing facilities, with crickets being the most commonly reared species by individual farmers for whom they provide a valuable source of additional income (Hanboonsong 2010). Similarly, in Kenya and Burkina Faso, there is a rich history of consuming insects, the most popular in Kenya being palm weevil (*Rhynchophrus Phoenicis*) larvae, which are both wild harvested and semi-cultivated by the chopping down of raffia trees (Kelemu *et al.* 2015). In Burkina Faso, the most commonly consumed insect is the Shea tree caterpillar (*Cirina butyrospermi*), which is considered a pest to the tree plantations that are grown for the production of shea butter (Anvo *et al.* 2016)**.** The larvae are usually boiled in water and then fried in butter for immediate use or boiled and sun-dried to be sold in the market.

Native human populations in the majority of Africa and Asia, and in large parts of South America, consume various species of insects in a multitude of dishes. However, until recently, the consumption of insects was generally decreasing, partly due to the spread of Western views of insects as a source of fear and disgust, particularly in the context of food (Looy *et al.* 2014). Insects were seen as a 'starvation food,' to be eaten only in times of extreme food shortage (Kinyuru *et al.* 2011; Looy, *et al.* 2014). Individuals engaging in entomophagy have been considered as 'rural', 'barbarian' or engaging in 'primitive peoples' practice' (Megido & Sablon 2014; Verbeke 2015). Thus, the negative portrayal and failure to embrace entomophagy by Western expats and the media has resulted in a decline in insect consumption, with negative health consequences for communities that relied on the nutrition that insects provided (Hanboonsong *et al.* 2013; Verbeke 2015). However, new data suggests that in certain countries, such as Thailand and Laos, the demand for insects is

on the rise (Durst & Hanboonsong 2015). These changing attitudes may be due, at least in part, to the increased acceptance that consumption of insects does not only occur in developing countries in times of starvation (Kinyuru *et al.* 2011).

There are many examples of insects being eaten as part of everyday diets. For example, in Japan insects form part of the traditional diet (Bukkens 1997), with 55 edible species documented in 1919. Although these numbers have dropped, due to environmental and social changes, there are still a handful of species being eaten today, often as delicacy/luxury food items (Nonaka 2010). Wasps are the chosen delicacy in Japan and deeply entrenched in the food culture, with an annual festival celebrating their consumption during which individuals compete to see who has the biggest wasp nest, either wild-harvested or cultivated. Communities come together to celebrate the wasp harvest, exchange knowledge on collection and cultivation methods and eat various wasp delicacies (Nonaka 2010). Farmers also continue to make efforts to improve on wasp husbandry methods, which are often unsuccessful and costly, indicating that enjoyment one of the primary motivators for the keeping of wasps (Payne & Evans 2017).

3. Opportunities

3.1 Nutrition

Insects are a source of energy, protein, fat, minerals and vitamins (Rumpold & Schlüter 2013a), with the energy content being on a par with other fresh meat sources (per fresh weight), with the exception of pork due to its high fat content (Rumpold & Schlüter 2013a). Mean estimates show energy levels to be around 400-500 kcal per 100 g of dry matter, making it comparable with other protein sources (Payne *et al.* 2016).

3.1.1 Macronutrients

Levels of protein, fat, and energy vary across insect species and also within a species depending on what the insects have fed on, stage of development, sex and environmental factors (Bukkens 1997; Ramos-Elorduy *et al.* 2002; Finke & Oonincx 2013). However, general ranges can be estimated as shown in Table 1.

	Protein (% dry matter)	Fat (% dry matter)	Energy (kcal/100 g)
Coleoptera (adult beetles, larvae)	**40.69**	**33.4**	**490.3**
Rhynchophorus phoenicis (palm weevil larvae)	32.86	36.86	478.87
Tenebrio molitor (mealworm larvae)	48.35	38.51	557.12
Diptera (flies)	**49.48**	**22.75**	**409.78**
Hemiptera (true bugs)	**48.33**	**30.26**	**478.99**
Hymenoptera (ants, bees)	**46.47**	**25.09**	**484.45**
Oecophylla smaragdina (weaver ant)	53.46	13.46	
Isoptera (termites)	**35.34**	**32.74**	
Lepidoptera (butterflies, moths)	**45.38**	**27.66**	**508.89**
Bombyx mori (silkworm larvae)	61.8	8.81	389.6
Cirina forda (shea caterpillar)	47.48	11.5	359
Galleria mellonella (waxworm larvae)	38.01	56.65	650.13
Samia ricinii (ailanthus silkworm pupae)	54.7	25.6	463.63
Odonata (dragonflies, damselflies)	**55.23**	**19.83**	**431.33**
Orthoptera (crickets, grasshoppers, locusts)	**61.23**	**13.41**	**426.25**
Acheta domesticus (house cricket adult)	65.04	22.96	455.19
Schistocerca sp.	61.05	17	427
Sphenarium purpuracens (Chapulin adult)	61.33	11.7	404.22
Ruspolia differens (longhorn grasshopper)	44.3	46.2	

Table 1. Protein, fat and energy content of some insects. *Data from* (Rumpold & Schlüter 2013a). Specific species were selected as examples if they deviated significantly from the average or are one of the most popularly consumed species. If there was more than one entry for a specific species, the average was calculated.

Protein is a significant component of edible insects, comprising between 30% - 65% of the total dry matter. The quality of protein is determined by both the amino acid composition and the digestibility of the protein, expressed as a percentage of that of an 'ideal' protein (Belluco *et al.* 2013). Between 46% and 96% of all amino acids are present in insect protein, although there are limited amounts of tryptophan and lysine (Bukkens 1997; Ramos-Elorduy *et al*. 1997), and digestibility is estimated to be between 77% and 98% for most species (Ramos-Elorduy *et al.* 1997). The suitability of insect protein for human nutrition is yet to be assessed but studies with juvenile rats have demonstrated that crickets (*Acheta domesticus*) offer a superior source of protein when compared to a plant source (soy protein) (Belluco *et al.* 2013). Human trials of insect consumption remain a significant research gap and definitive recommendations regarding insects as nutritionally suitable for humans cannot currently be made.

After protein, fat is the next main component of insects. The unsaturated fatty acid profile is similar to that of poultry and fish but contains more poly-unsaturated fatty acids (PUFAs) than either poultry or red meat (Rumpold & Schlüter 2013a). Omega-3 fatty acids, EPA (C20:5) and DHA (C22:6) being the primary types, are essential for normal cellular functioning and must be supplied by the diet. Insects contain little to no traces of EPA and DHA but do contain linoleic acid (C18:2) and occasionally linolenic acid (C18:3), which humans can synthesise to make arachidonic acid (C20:4) and EPA (Rumpold & Schlüter 2013a; Calder 2017). It must be noted that the fat profiles of insects are highly dependent on their feedstuff. For example, a study has shown that levels of EPA and DHA can be increased in black soldier flies by feeding them fish offal (St-Hilaire, Cranfill & McGuire 2007). More research is needed to draw clear conclusions on the availability of specific fats in insects.

Overall fat content is also highly variable among insects, ranging from 7-77 g/100 g of dry weight, with larvae generally having a higher overall content than adults (Ramos-Elorduy *et al.* 1997). Insect larvae and some soft-bodied adult insects, such as termites, have the highest levels of fat and insects with a hard exoskeleton, such as crickets and grasshoppers, are at the lower end (Bukkens 1997).

3.1.2 Micronutrients

Although micronutrient levels vary greatly across insect species, some species do have consistently higher levels of certain micronutrients. Table 2 summarises the average levels of minerals and vitamins in a range of commonly consumed insect species. A large variation is often seen within a single species due to environmental factors and contaminants, particularly metal, acquired during processing.

Species	Calcium	Potassium	Magnesium	Phosphorus	Sodium	Iron	Zinc	Manganese	Copper	Vitamin A
Rhynchophorus phoenicis (African Palm Weevil Larvae)	131.05	1617	82.7	518.5	48.4	22.75	21.15	2.15	1.6	11.25
Tenebrio molitor (mealworm larvae)	45.77	828.28	215.89	722.74	133.16	5.46	12.53	1.14	1.62	
Oecophylla smaragdina Fabricius (weaver ant)	63.85	749	96.05	726.5	225	65.4	13.5	7.68	1.52	
Agro (termites)	132					161	14.3			
Bombyx mori (silkworm larvae)	102.31	1826.59	287.86	1369.94	274.57	9.54	17.75	2.49	2.08	273.99
Cirina forda Westwood (shea caterpillar)	17.48	1100.91	34.72	480.92	130.39	23.93	6.44	7		2.99
Galleria mellonella (waxworm larvae)	59.28	532.53	83.07	834.94	39.76	6.57	7	0.32	0.62	4.5
Samia ricinii (silkworm pupae)	72.2		182.5	577		23.7	7.13	2.58	1.78	
Acheta domesticus (house cricket adult)	171.07	1126.62	94.71	832.9	435.06	8.75	20.22	3.35	1.43	24.33
Sphenarium purpuracens (Chapulin adult)	112	377	424		609	18	42			
Ruspolia differens (longhorn grasshopper brown)	24.5	259.7	33.1	121	229.7	13	12.4	2.5	0.5	280

Table 2. Mineral and vitamin A content in popularly consumed insect species. All minerals in mg/100 g dry matter except vitamin A (µg/100 g dry matter). *Data from* Rumpold & Schlüter (2013a)

Iron has been shown to range from 18 -1562 mg/100 g dry matter across insect species, with low levels in ants, mid-levels in termites and the highest levels in crickets (Christensen *et al.* 2006). Metal contaminants may account for some of the reported variation in levels of iron. Although iron levels in insects are high, especially when compared to plant-based food sources, which are generally consumed in place of meat, no research has identified the type of iron found in insects. 'Haem' iron, present in animals (with a blood circulatory systems and haemoglobin), is more bioavailable and absorbed more uniformly in the human body than the 'non-haem' iron found in plants, (Kongkachuichai & Napatthalung 2002; Hurrell & Egli 2010). Insects do have a circulatory system but this does not involve haemoglobin and so the availability of their iron is unknown, although it has been suggested that it may have a bioavailability more like that of the iron found in meat rather than in plants. For example, one study examined the bio-availability of iron in maize-field grasshopper (*Sphenarium purpurascens)*, black crickets (*Gryllus bimaculatus)*, mealworms (*Tenebrio molitor)*, and buffalo worms (*Alphitobius diaperinus)*. It found that buffalo worms and sirloin meat displayed the highest levels of iron bioavailability, with buffalo worms slightly higher than sirloin meat, while the other insects displayed a mid-range of iron bioavailability between the highest and lowest samples (whole wheat) tested (Latunde-Dada & Yang 2016). It should be noted these studies were done *in vitro* in a Caco-2 cell model and no human or animal trials have been conducted to date.

Zinc, calcium and vitamin A have all been found in insects but data on the quantities present is limited. Crickets have been reported to have zinc in the range of 8-25 mg/100 g dry matter (Christensen *et al.* 2006), and ants, termites and crickets have calcium in the range of 33 - 341 mg/100 g dry matter, with crickets having the highest levels. Vitamin A has been found to range from 3-273 µg/100 g dry matter across insect species (Christensen *et al.* 2006).

Overall, the nutritional status of insects is highly variable, depending on different species, diets and life-stages. Additionally, there is little known about the digestibility and availability of nutrients from insects for humans or for animals.

3.2 Environmental considerations

The environmental impact of food production is increasingly being brought to the forefront of sustainability debates, particularly surrounding the reduction of carbon dioxide (CO_2) emissions. However, there are two other important environmental factors which are often ignored: water and land use. It is predicted that by 2025, at least 1.8 million people will be living in regions without adequate fresh water supplies and a further two thirds of the global population will be in areas under pressure from dwindling water resources (FAO 2012). Freshwater is a finite resource, of which an estimated 70% is used by the livestock and agriculture industries (Doreau *et al.*2012). Agriculture uses water directly to grow crops and indirectly to grow fodder for the production of livestock.

3.2.1 Land and water use

Land availability is an issue which frequently arises in the discussion of sustainable agriculture. As the demand for meat grows, there is increasing pressure on producers to farm more livestock, which requires more land. The increase in livestock requires more feed, which in turns leads to farmers increasing the amount of land being cropped, often involving deforestation or an increase in fertiliser use. Currently the livestock sector uses about 70% of available agricultural land worldwide (Oonincx & de Boer 2012). Figure 1 shows the maximum documented levels of land, feed and water use for the three main groups of livestock and two insect species, *Locusta migratoria* and *Tenebrio molitor*. For insects, data are estimates based on available feed conversion values and calculations of land and water needed to produce the feed.

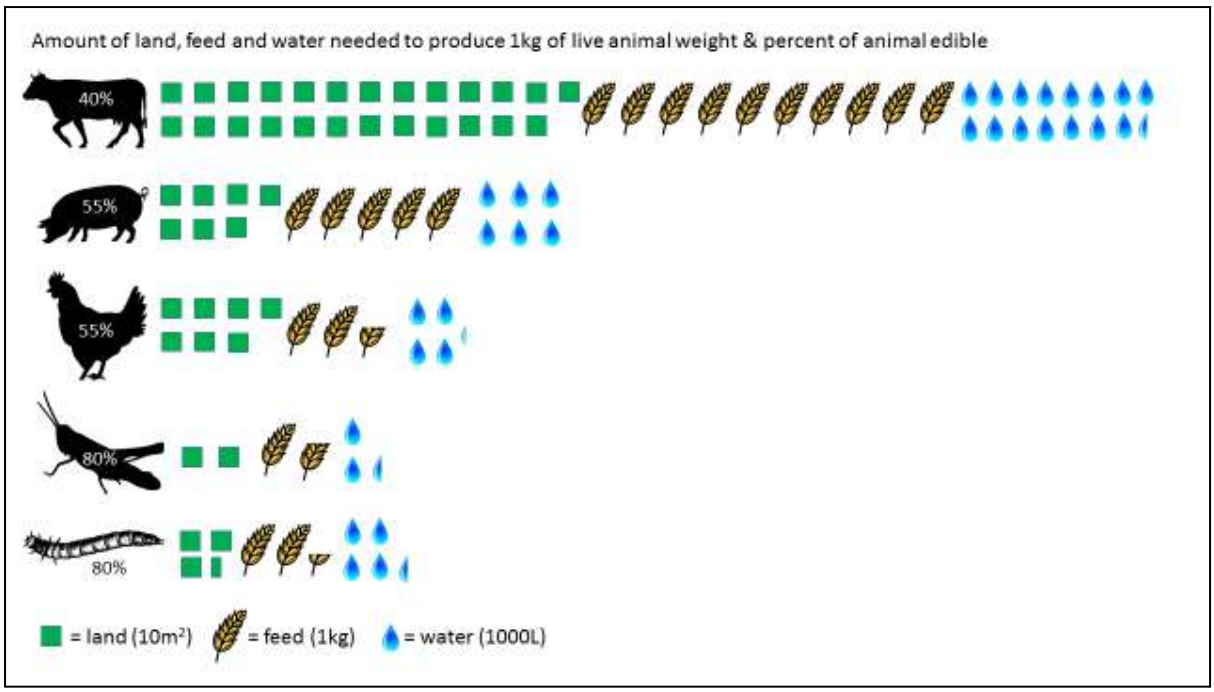


Figure 1. *Data adapted from* Hoekstra (2012), Hoekstra & Mekonnen (2008), Mekonnen & Hoekstra (2010, 2012), Oonincx & de Boer (2012) and Arnold van Huis et al. (2013)

The key variable in how much water is needed to produce livestock is the 'feed conversion efficiency', which measures the amount of food needed to produce a given amount of the final product (meat, eggs *etc*.). It is evident that insects are significantly more efficient than other livestock in terms of feed conversion because they are cold-blooded and rely on their environment to control metabolic processes, such as body temperature (van Huis *et al.* 2013). To date, one study has examined the water footprint, taking into account the entire production system, of commercially produced insects. Miglietta and colleagues (2015) found that for mealworms, within a commercial system, the water footprint per ton was larger than that for production of pigs and chickens. This data must be looked at within the context of the percentage of the animal which is edible, as insects are considered to be 80-100% edible, compared with other livestock at 40-50% (Lundy & Parrella 2015). When the data are re-examined, taking into account the percentage of the animal which is edible, mealworms have a lower water footprint than the other livestock (Miglietta *et al.* 2015). Only limited data is available about the feed conversion efficiency, land requirements and water use of insects and more data is required for commonly farmed species before recommendations can be made.

3.2.2 Emissions

There is consensus that the biggest contributor to global climate change is greenhouse gas emissions (GHGs), predominantly CO_2, nitrous oxide and methane, from fossil fuels and agricultural and industrial processes (Sachs 2015). The agricultural sector contributes the most to GHG emissions, with livestock accounting for an overall 18% of CO_2 equivalents (van Huis *et al.* 2013; Sachs 2015). Studies, considering only husbandry conditions, have found that insects perform favourably when compared to beef cattle and pigs (Figures 2 and 3).

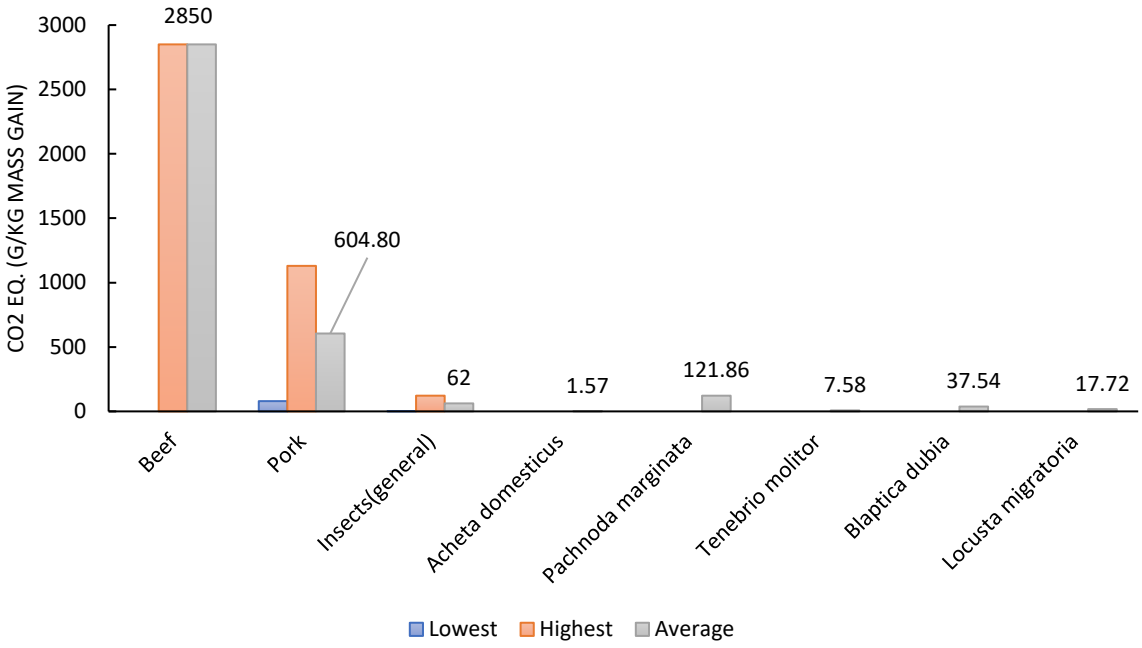


Figure 2. Production of CO_2 equivalents during rearing of livestock and insects. *Data adapted from* Oonincx et al. (2010)

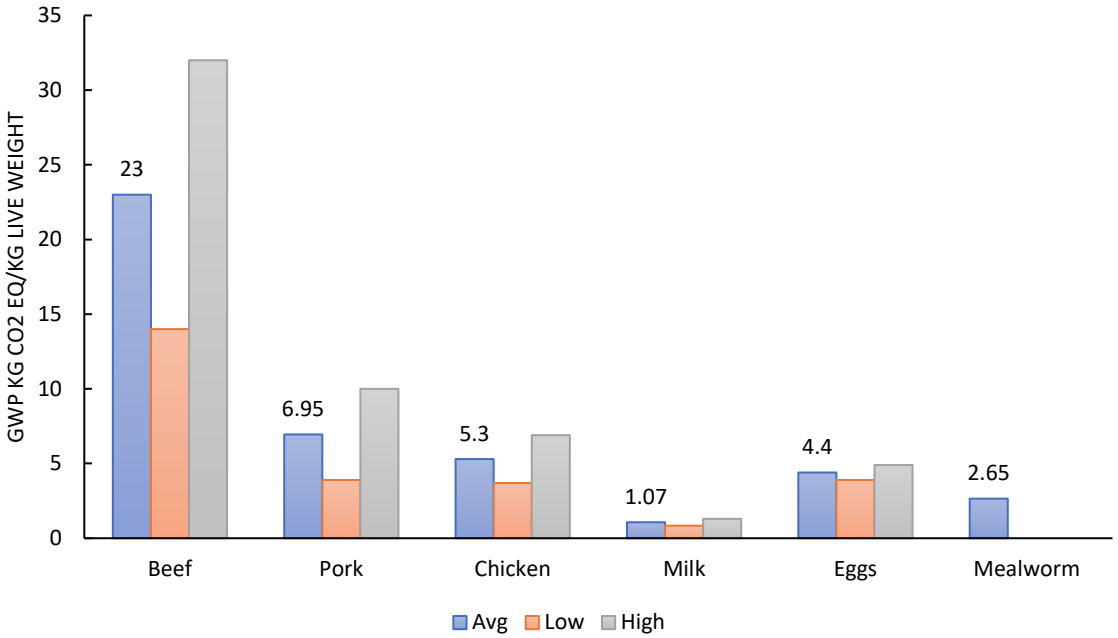


Figure 3. Global warming potential of production of staple foods and an insect (mealworm, T. molitor). *Data adapted from* de Vries & de Boer (2010) and Oonincx & de Boer (2012)

Overall, preliminary results suggest that insects produce far fewer GHGs than standard large livestock and are approximately on par with chickens on a per kg basis. However, studies of larger scale production have reported less optimistic figures and shown that values are largely dependent on the type of feed. A life-cycle assessment of a commercial cricket farm in Thailand found that cricket production had a smaller environmental footprint than did broiler chicken farms, and that the largest footprint hotspots were in relation to grain feed production for both systems (Halloran *et al.* 2017). No other data is currently available regarding GHGs from insect production and concrete statements regarding their environmental benefit over other livestock cannot be made. This is in part due to industrial-scale insect production relying on the same grain feed used for livestock and it has been suggested by Lundy and Parrella (2015) that feeding bio-waste could make insect production more environmentally viable. Lundy and Parrella (2015) used three organic waste sources as feed: a low-quality unprocessed food waste, a predominantly straw-based feed and a filtrate from food waste processed via enzymatic digestion. It was found that crickets did not grow on the two unprocessed feeds, but the processed filtrate gave harvestable size insects with food efficiency equal to, or better than, chickens. Insects can only be competitive as an alternative protein source if they can outperform chickens. It has been demonstrated that black soldier flies can be reared on faecal waste, making them ideal

for aiding manure disposal (van Huis *et al.* 2013). Overall, organic waste is probably the best option for insect rearing but the specific sources need to be determined for each species.

Another factor to consider in the livestock industry is the global warming potential associated with transport, slaughter and storage of meat, which contributes 17-25% of GHG (Oonincx & de Boer 2012). As there is currently no uniform method for processing insects, such equivalent values cannot be assessed. It is also unclear whether regulations would restrict insect slaughter at the production facility (as they do with livestock), which would introduce transport costs.

3.3 Use in animal feed

The cost of producing meat, fish and soybean meal as feed for animals accounts for 70% of the production costs of livestock (van Huis *et al.* 2013). Insects, on the other hand, are comparably high in nutrients, have a low environmental impact, require less space and are already part of the natural diets of pigs, poultry and fish, making them an ideal feed alternative (Rumpold & Schlüter 2013b). Incorporating insects into broiler poultry feeds has been reported to result in no reduction in growth rates and in some cases increased chick growth rates (Rumpold & Schlüter 2013b). Replacement of soybean oil with black soldier fly larvae has been shown to have no influence on growth or performance of broiler chickens, suggesting it is a viable alternative (Schiavone *et al.* 2017).

In laying hens, better feed conversion was seen in hens with insect meal in their diet; however, there was more variation in egg sizes (Marono *et al.* 2017). Similar results of increased feed conversion and growth rates on insect meal diets compared to a standard soybean control was seen in Barbary partridges (Loponte *et al.* 2017).

Aquaculture (the farming of fish, crustaceans and other aquatic animals) is one of the fastest growing industries. However, a major hurdle to sustainable growth of the industry is the cost of feeds, particularly fishmeal and fish oil (van Huis *et al.* 2013). Approximately 10% of fish production is recycled into fishmeal, and ocean fish stocks are being depleted by overfishing to provide the feed. Increasing restrictions on unregulated fishing and catch quotas have forced the aquaculture industry to search for alternative high-value protein sources for feed, which is where insects can play a valuable role. The use of insects in fish feed is not new and is widely practised by small-holder farms in Africa and Asia, who feed

insects (when seasonally available) or hang lights over fish ponds to attract insects (van Huis *et al.* 2013). Predominantly, black soldier flies, housefly larvae, silkworms and mealworms have been used in aquaculture feeds, but feeding trials have given mixed results, in relation to both protein content and the ratio of EPA and DHA lipids to other nutrients.

Fresh water fish with omnivorous diets appear to do better on insect diets, with trials that have replaced 25% of fishmeal with black soldier flies or locusts, showing no adverse growth effects for tilapia (van Huis *et al.* 2013; Makkar *et al.* 2014). Similarly, more recent trials have replaced up to 75% of fishmeal in Nile tilapia diets with housefly maggot meal without any adverse effects (Wang *et al.* 2017). Results with mealworms fed to catfish have shown successful replacement of 40%-80% of the normal diet without adverse effects (Makkar *et al.* 2014). Replacing fishmeal in carnivorous fish diets has proven more difficult. Trials with seabass show success when up to 19.5% of the fishmeal is replaced with black soldier fly larvae (Magalhães *et al.,* 2017) and for gilthead sea bream a replacement of up to 25% or 50% of fishmeal with mealworm meal gave positive results (Iaconisi *et al.* 2017; Piccolo *et al.* 2017).

3.4 Economics

In addition to the environmental and nutritional benefits of feeding insects to humans or livestock, there are also economic benefits. To date, the clearest economic picture of the edible insect trade comes from Southeast Asia, where there are well-established farms and trade routes (Figure 4) and where researchers have documented the trade most thoroughly, particularly in Thailand.

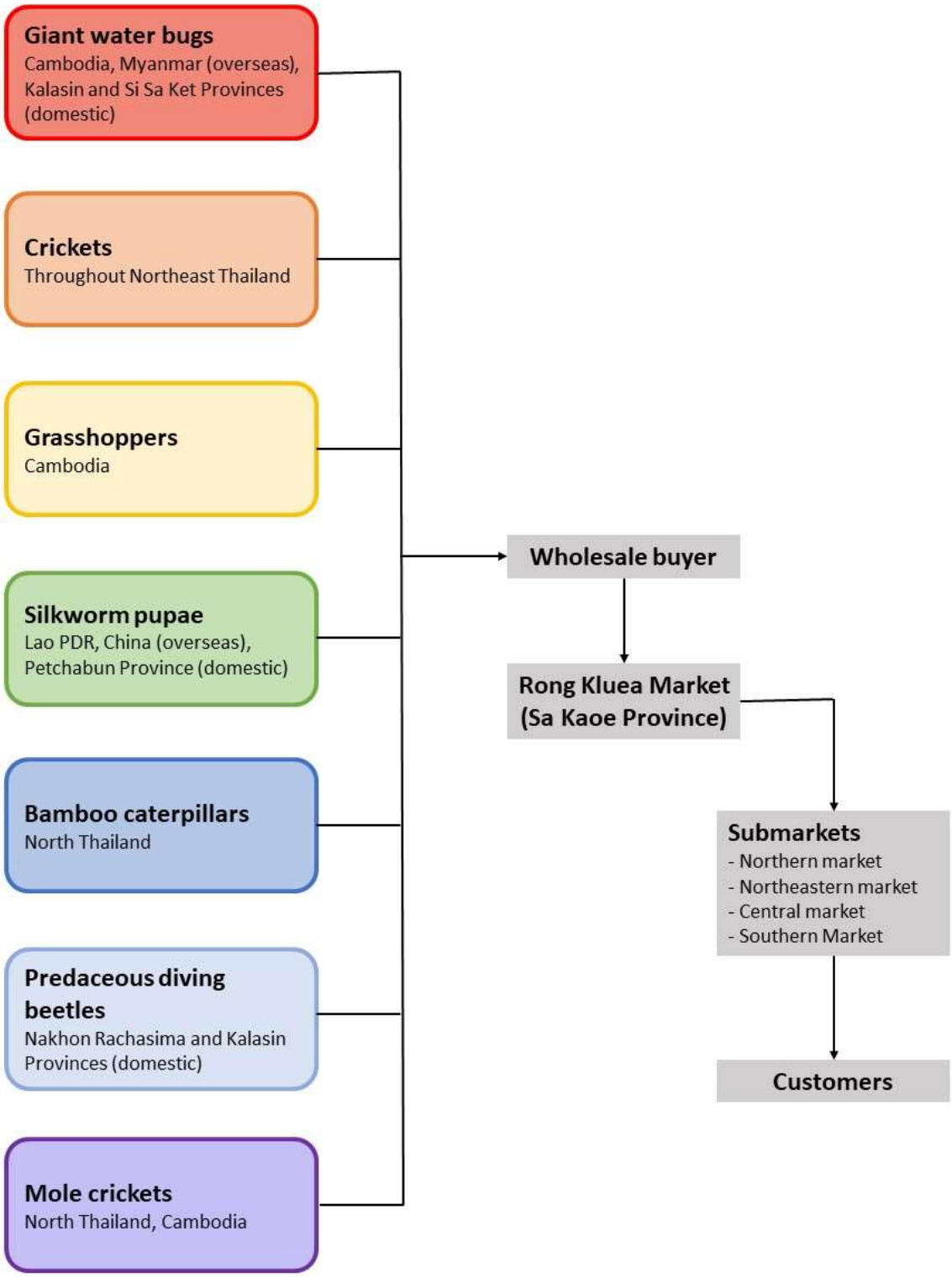


Figure 4. Collection and distribution chains of insects in Southeast Asia, reproduced with permission (Hanboonsong, Jamjanya and Durst, 2013).

Export and import of insects for food plays a strong economic role throughout Southeast Asia - the import market in Thailand alone is valued at 1.14 million USD/year (Hanboonsong

et al. 2013). Figure 5 shows the market values for various commonly consumed insect species in Thailand compared to the market values for various other staple food sources.

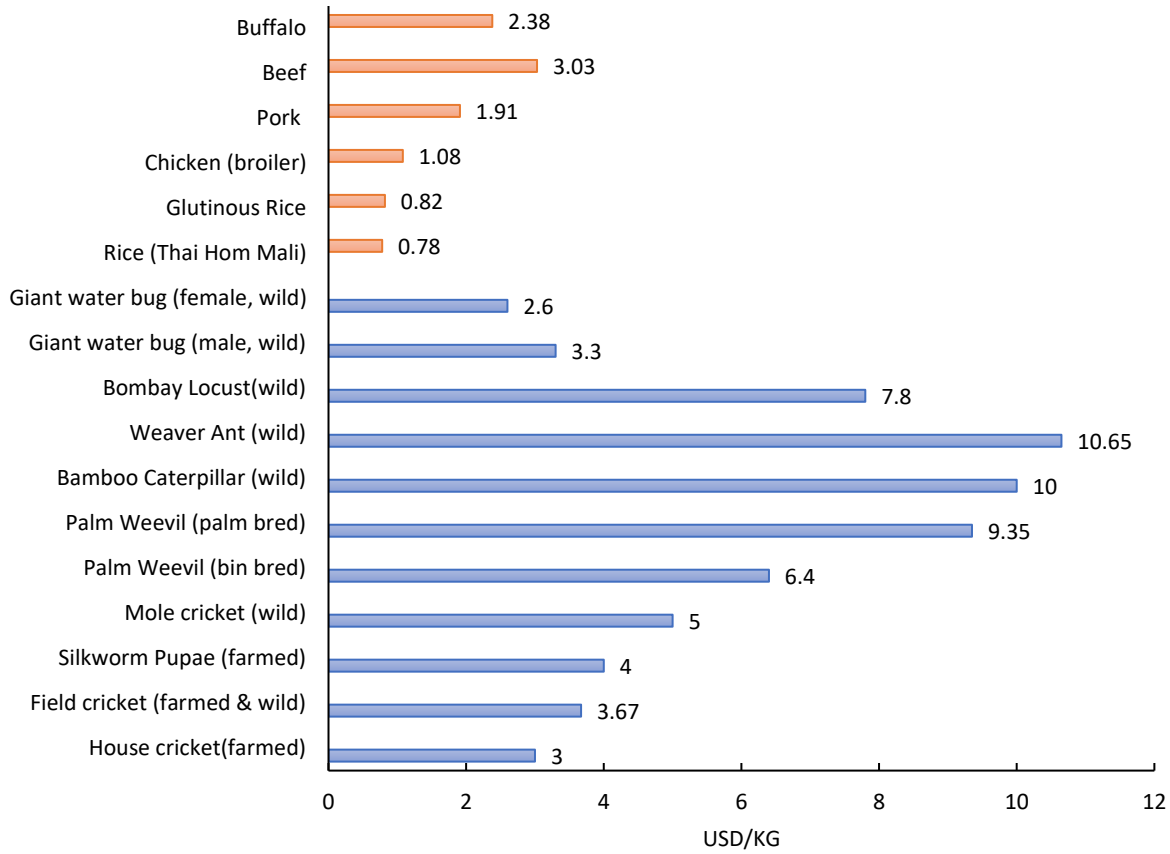


Figure 5. Market value of insects and staple foods in Thailand. Insect data from Hanboonsong et al. (2013), rice prices from Thai Rice Exporters Association on March 2, 2016, and meat prices from Thai Office of Agricultural Economics, values for the week of Feb. 22, 2016.

Given that the market value for insects often exceeds that of other standard protein sources, insect farming can provide a stable income for established farmers. Medium sized farms, which produce roughly 500-750 kg of crickets, four to five times a year, can get net incomes of 4270 to 9970 USD in a country where the average yearly gross national income (GNI) per capita is approximately 5640 USD (World Bank 2016). Although data on the insect market in Africa is limited, the financial benefits of insect trading can be seen in Namibia where collections and sales of mopane caterpillars (*Gonimbrasia belina*) provide valuable income (a 50 kg bag sells on average for 71.43 USD) or act as a barter item (Thomas 2013). Estimates place the value of insects as food and feed for the combined market in the US, Belgium, France, UK, Netherlands, China, Thailand, Vietnam, Brazil, and Mexico at 25.1

million GBP for 2015, with a predicted growth to 398 million GBP by 2023. This growth is predicted to be largely driven by increased consumer awareness and acceptance of insects as food, as well as use in animal feed (Global Market Insights Inc. 2015). In South Korea the market, which includes insects for food, feed and medicine, is valued at 109 million GBP for 2017 with predications that by 2020 it will have quadrupled to 348 million GBP, predicted to be driven by increased acceptance from the global community that insects play a vital role in global food security (Han *et al.* 2017). These growth predictions present a great opportunity for new businesses, particularly in developing countries.

4. Hurdles to the use of insects as food and feed

Although a myriad of opportunities exists for entomophagy, there are significant hurdles to overcome as a result of the lack of research and the need for innovation within the sector. Major issues include the possibility that insects may contain 'anti-nutrient' properties, concerns around food safety related to storage and allergic reactions, consumer acceptability and ambiguous or non-existent regulation.

4.1 Anti-nutrient properties

Chitin is a structural nitrogen-based carbohydrate found in the exoskeleton of insects, which may have 'anti-nutrient' properties due to potential negative effects on protein digestibility (Belluco *et al.* 2013). One study of seven insect species found 2.7 to 49.8 mg chitin per kg fresh weight and 11.6 to 137.2 mg/kg in dry matter (Finke 2007). A study comparing dried honey bees and honey bee protein concluded that the removal of chitin improved the quality of the insect protein as measured through protein digestibility, amino acid content, protein efficiency ratio and net protein utilisation (Ozimek *et al.* 1985). On the other hand, chitin is notably high in fibre and chitin extracts from the exoskeletons of shellfish have been approved by relevant authorities and are readily used in Japan as a source of fibre in cereals (DeFoliart 1992). Although chitin is usually considered to be indigestible by humans (Bukkens 1997), recently chitinolytic enzymes, produced by bacteria from human gastrointestinal tracts, have been found, suggesting that chitin and chitosan can be digested (Paoletti *et al.* 2007; Dušková *et al.* 2011; Rumpold & Schlüter 2013a).

In a 2-week trial with healthy adult males, chitosan, a derivative of chitin, ingested at a dose of 3-6g/day induced a significant decrease in total serum cholesterol and an increase in

serum HDL-cholesterol (Koide 1998)**.** It has also been demonstrated that for poultry, chitin has a positive effect on the immune system and thus may reduce the need for antibiotic use (van Huis *et al.* 2013). However, the effect of long-term ingestion of chitin is unknown (Koide 1998) and more research is required in this area to understand the impact of chitin on human and animal health.

The potential toxicity of some compounds in insects is also of concern. There are two categories of toxic insects: cryptotoxics and phanerotoxics. Cryptotoxics contain toxic substances from either direct synthesis or by accumulation from their diet. Phanerotoxics are have specific organs that synthesise toxins (Belluco *et al.* 2013). Commonly consumed insect species are not in either category and studies of hydrocyanide, oxalate, phytate, phenol and tannins in edibles insect species have shown low levels of these compounds, with values falling well below levels of toxicity for human consumption (Ekop *et al.* 2010; Shantibala & Lokeshwari 2014). Analysis of the larvae of *Cirina forda* has confirmed that oxalate and phytic acid levels are well within safe ranges and that they contain no tannins (Omotoso 2006). A further clinical trial which fed Sprague-Dawley rats varying levels of freeze-dried mealworm powder over a 90-day period found no toxic effects (Han *et al.* 2016). Overall, data on anti-nutrient properties of edible insects is limited and more research is required.

4.1.1 Microbial risks

Spore forming bacteria and enterobacteriaceae have been reported in mealworms and crickets, with higher levels found in insects which had been crushed - likely due to the release of bacteria from the gut (Klunder *et al.* 2012). For the species examined (*Gryllotalpa africana, Rhynchophorus phoenicis, Bematistes alcinoe*), the main bacteria identified were from the genera *Bacillus* and *Staphylococcus* and the majority of the microbes were saprophytes (Amadi & Kiin-Kabari 2016). Analysis of edible insects for the Belgian market identified that all untreated fresh insect samples had an aerobic mesophilic microorganism, yeast and mould count higher than the Good Manufacturing Practice limits for raw meat (FDA 2016); however, introducing a simple blanching step in the processing reduced levels to below accepted limits (Megido *et al.* 2017). Further research indicates that treating insects the same as other foodstuffs of animal origin during processing (*i.e*. washing and

thorough heating) sufficiently reduces the risk of bacteria borne disease (Grabowski &Klein 2016).

While harmful bacteria such as *Salmonella* have been detected in insects that were in close contact with livestock (Belluco *et al.* 2013), research suggests that the majority of the contamination comes from the gut microbiota of the insect (Rumpold *et al.* 2014). Starving insects for 24-48 hours prior to slaughter has been suggested as a way to reduce harmful bacteria in the gut, although the one published study in this area reported that this approach had no significant impact on microbiota levels (Wynants *et al.* 2017) and unpublished studies also support this finding (Larouche *et al.* 2017). Some risk of mycotoxins has been identified, but this has been studied only in the two emperor moth species (*Imbrasia belina* and *Bucnaea alcinoe*) with strains identified predominantly in the intestinal track or from outside contamination (Simpanya & Allotey 2000; Braide & Oranusi 2011). It was concluded these risks could likely be mitigated with evisceration and appropriate processing steps, as is done with other meat sources.

Studies on the level of organic and metal contaminants (*e.g.* polychlorinated biphenyl, DDT, dioxin compounds, heavy metals) in both whole edible insects and insect-based food items in Belgium found that all contaminant levels were generally lower than what was found in other common animal products (Poma *et al.* 2017). This study indicates that consuming insects presents no more of a microbial or contaminant risk than consuming other meat sources, when the same good practice standards of preparation are applied.

Little is known about how to safely store insects to reduce microbial risk. Research has shown that freshly boiled insects spoil rapidly at room temperature (28℃) but remain stable at 3-5℃ over a 2-week period; microbial levels in dried insects have also been reported to be stable at room temperature (Klunder *et al.* 2012).

The European Food Safety Authority (EFSA) published a risk profile examining hazards relating to insects as food and feed, taking the entire production chain into account. EFSA came to the overall conclusions that if the currently permitted feed materials are used as the growth substrate for the insects, the possible occurrence of significant microbial hazards is comparable to other sources of protein of animal origin (EFSA 2015). Further systematic

work is needed to establish the safe shelf life of edible insects, both for human and animal consumption.

4.1.2 Allergens

Many arthropods, which includes insects, arachnids, myriapods and crustaceans, are known to induce allergic reactions in susceptible individuals, caused by the presence of tropomyosin, arginine kinase, glyceraldehyde 3-phosphate dehydrogenase and hemocyanin (Belluco *et al.* 2013; Srinroch *et al.* 2015). Cross-reactive allergies have been identified in crustaceans, cockroaches and dust mites. One study identified a positive cross-reaction between mealworm proteins and individuals with known dust mite and crustacean allergies (Verhoeckx *et al.* 2013; Broekhoven *et al.* 2016). A study on crickets (*Gryllus bimaculatus*) showed a cross reaction to crickets in individuals with known prawn allergies (Srinroch *et al.* 2015). In this study, an additional novel allergen was identified in the cricket, hexamerin1B (Srinroch *et al.* 2015)**.** A recent systematic review of studies examining cross-reactivity/sensitivity with insects in individuals with known arthropod allergies has demonstrated that the all patients demonstrated allergic reactions to insects (Ribeiro & Cunha 2017). In addition to direct consumption allergies, there is evidence to support contact allergy sensitivity in individuals who are frequently exposed to insects, for example breeding farm workers (Jensen-Jarolim & Pali-Schöll 2015)**.**

The data on allergen risk to insects is limited as the majority of trials to date have been conducted with a small number of participants (n <20); however, these studies point in the direction that individuals with crustacean allergies will react negatively to insects, and that there may be several additional novel insect allergens to consider.

4.2 Mass production

For insects to be considered a viable micro-livestock, it must be possible to produce them on a large scale in a sustainable, safe and efficient manner. It is frequently forgotten that large scale domestic rearing of insects has been occurring for over 7000 years for sericulture (silk), apiculture (honey), biological control of pests and the production of medicinal products and shellac (Rumpold & Schlüter 2013b). Significant advances have been made with artificial rearing diets and controlled conditions for mass rearing. However, there are still several hurdles preventing insect farming for human and animal consumption from being scaled up. First and foremost, an ideal candidate insect species for mass rearing must be identified.

Domestication has occurred for several thousand years with silkworms, and it has been documented that domestic silkworms can no longer effectively cling to branches and would die in the wild (Defoliart 1995). Crickets and palm weevils are mass reared in Thailand but they are not the ideal species, as they are reared on high quality chicken feed. The ideal insect species would have high egg production, high egg hatch, a short larval stage, optimum synchronisation of pupation, high weights of larvae or pupae, a high productivity (*i.e.* high conversion rate and high potential of biomass increase per day), low feed costs, low vulnerability to diseases, ability to live in high densities and a high quality protein content (Rumpold and Schlüter 2013b). The search for such an insect continues, although the black soldier fly (*Hermetia illucens*) does meet the above criteria, the issue of optimisation of farming techniques remains.

The majority of livestock and agricultural production systems have some level of automation, reducing the expense of manual labour. This is not the case for the majority of insect farms where manual labour is still required to complete tasks such as feeding, collection, cleaning and rehousing (Rumpold & Schlüter 2013b). This dependence on manual labour means that farm-reared insects are expensive, even when feed costs are low. There are a handful of exceptions to this where companies have developed automation but these are still in trial phases. Thus, in order for insects to be an attractive alternative to protein sources such as beef and poultry, automation must be further developed to bring down the price of the end product.

In addition to the labour costs, the conditions in the rearing facility such as temperature, light, humidity, ventilation, rearing containers, population density, oviposition sites, feed and water availability, feed composition and quality and microbial contaminants must be controlled at the optimum levels for successful mass production (Rumpold & Schlüter 2013b).

4.2.1 Processing

As with any livestock system, farmed insects must be processed for consumption or use in feed products. There are a myriad of standards governing the processing of conventional livestock but there are no best practices in place for insects, largely due to the lack of data on the impact of different processing techniques on food safety and nutritional content. As discussed above, trials have examined aspects of microbial safety; however, there is still no

standard processing procedure in place for insect farmers. Ultimately, an ideal insect production system would be as shown in Figure 6 (*i.e*. an efficient system with a clear set of standards and production protocols).

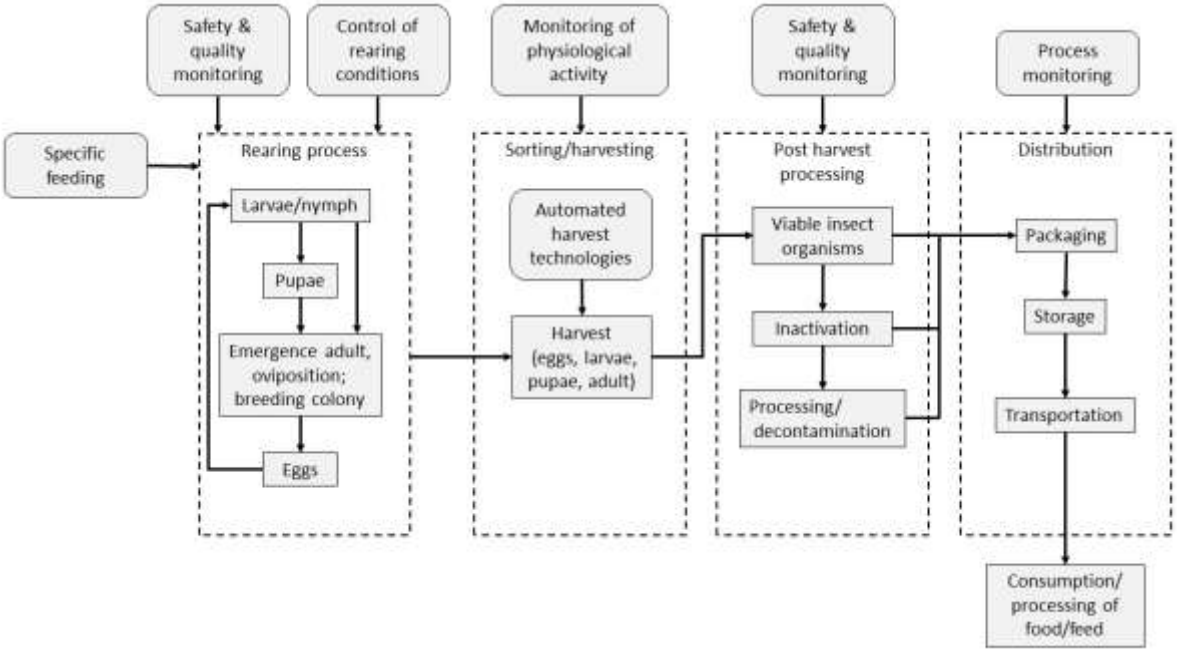


Figure 6. Model insect production system, reproduced with permission from (Rumpold and Schlüter, 2013b)

The only component not considered in Figure 6 is the identification of ideal feeds and this can be a major hurdle to the sustainable mass production of insects. At present, established insect farms operate using predominantly poultry feed, which results in a system not significantly superior to poultry production. Utilising bio-waste sources, particularly food waste, has been lauded as the ideal for insect farming. However, aside from one trial (Lundy & Parrella 2015), research to date on bio-waste feed sources for commonly consumed edible insects has used small colony sizes, not reflective of mass rearing practices (*e.g.* Oonincx *et al*. 2015).

4.3 Regulations

For countries where eating insects is traditional there are no regulatory hurdles to their production, marketing and consumption. However, in Western cultures regulations present a significant barrier to the use of insects in both feed and food. EFSA has stated that all insect products for human consumption will be considered a 'Novel Food' and must be submitted for Novel Food approval by 2018, with a two-year transition period allowing

already approved products to stay on the market until 2020 (IPIFF 2017). However, some European Union (EU) Member States have their own legislation to circumvent this requirement. The Netherlands is working closely with researchers to draft legislation and in Belgium the Federal Agency for Safety of the Food Chain has endorsed certain insect species to be allowed as food (Belluco *et al.* 2017). With the occurrence of Brexit, it is unclear whether the UK will still follow the EFSA ruling or proceed with their own approval process under the Food Standards Agency (FSA), where decisions would presumably no longer need to be ratified by EFSA, perhaps giving more legislative freedom.

Similar rules surrounding novel food approval are in place in North America. In Canada, regulatory approval must come from the Canadian Food Inspection Agency and Health Canada, and in the US from the Food and Drug Administration, as well as the Ingredients Definition Committee of the Association of American Feed Control Officials for feed ingredients.

The use of insects in animal feed has less challenging regulations to overcome and as of ^{t} July 2017 insect proteins have already been approved for use in fish feed within the EU. These insects must still be raised according to conventional livestock regulations, meaning that they cannot be fed any form of waste product, reducing the environmental benefit of rearing insects as discussed above.

The regulatory issue of insect welfare is also one of uncertainty. There is ample research and regulation regarding the necessary welfare conditions for traditional livestock but these regulations explicitly exclude invertebrates, leading to debate about how to proceed with farming insect ethically (Goede *et al*. 2013). A recent review by Lähteenmäki-Uutela *et al.* (2017) examined the regulatory issues facing the EU, Canada, the US, Mexico, Australia and China, outlining that at this stage rules on safety, marketing, and farming are largely missing. If a clear set of rules regarding insects for food and feed can be established, it will facilitate the sustainable growth of the insect business; however, for now, with no clear rules, the insect business is left in limbo.

4.4 Consumer acceptability

There are two distinct psychological reactions to insects as a food source for humans. In countries where entomophagy is the norm, they are seen as a valued protein source and

knowledge on which species are edible is considered local wisdom passed down between generations. Conversely, in Western cultures, insects can invoke visceral negative reactions: 'deeply embedded in the Western psyche is a view of insects as dirty, disgusting and dangerous' (Looy *et al.* 2014). This view of insects as inedible is perpetuated by the Western media through TV shows such as 'Fear Factor' and 'I'm A Celebrity…Get Me Out Of Here!' where contestants are forced to eat raw insects to advance in the competition and show their daring. One study reported that in Western societies only 12.8% of males and 6.3% of females were likely to adopt insects as a substitute for meat (Verbeke 2015) and another that 19% of individuals were prepared to eat insects as a meat substitute (Hartmann & Siegrist 2017). This presents the additional hurdle of how to increase acceptance of entomophagy in Western cultures.

To date, no socio-demographic factors have been linked to the willingness to eat insects (Hartmann & Siegrist 2017). Rather, the main influential factors seem to be neophobia, familiarity, interest in the environment, convenience and attachment to meat (Verbeke 2015; Gere *et al.* 2017). The more neophobic, uninterested in the environment and attached to a diet that contains meat the person is, the less likely they are to be prepared to eat insects. However, if insects are presented in a convenient, appropriate and familiar form (*e.g*. insect flour in a cookie), the more willing an individual may be to try it (Megido *et al.* 2016; Tan, Verbaan & Stieger 2017). Verneau and colleagues (2016) demonstrated, in Denmark and Italy, that presenting information about the benefits of eating insects increases consumers' willingness to eat them and with the effect persisting for at least 2 weeks after the experiment. However, another study showed that providing false information about insect flour being an ingredient in bread led to lower scores of the bread's flavour, even though the bread did not actually contain the flour (Barsics *et al.* 2017).

Although acceptance of insects as a human food in Western cultures is low, there is significantly more support for insects as an animal feed. Two thirds of 415 farmers surveyed in Belgium found it acceptable to use insects in animal feed (Verbeke *et al.* 2015). The *PROteINSECT* project reported that 66% of consumers consider fly larvae as suitable feed stuff, over 80% want to know more about insects as feed and that 75% are happy to eat

animals fed on insects (PROteINSECT 2016). Perhaps the first step to increasing consumer acceptability of entomophagy is through increased use in animal feed.

5. Conclusion

Research to date indicates that insects could play an important role in addressing the impending protein supply crisis. Overall insects contain sufficient levels of protein, fats and micronutrients to contribute to improvements in global health and food security, both via direct consumption and indirect use in feeds. In addition, research has demonstrated that insects have can have a smaller environmental footprint and a higher economic value than other livestock protein sources; they are unlikely to pose significant microbial risks if consumed; they appear to cause allergic reactions in individuals with known arthropod allergies; and the majority of people from Western societies are comfortable with insects being used as animal feed but hesitant about consuming them directly.

Future research should address questions related to the scaling up of insect production to commercial levels, such as: how can insect nutritional profiles be improved in a systematic and consistent manner?; how can the environmental footprint of insects remains small when scaled up commercially?; how can insects be fed on a commercial scale in a sustainable way?; what do the regulations around insect farming, processing, and storage need to cover?; are there any harmful effects to human or animal populations from the consumption of large quantities of insects?; what do insect supply and value chains look like depending on the specific end uses?; how can the economic value of established insect supply chains be protected so as not to damage livelihoods?. Ultimately, only if insects are able to compete with traditional Western livestock, particularly chickens, or livestock feed within the supply-value chain and environmentally, will they be considered a viable alternative.

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The effect of an initial high-quality feeding regime on the survival of *Gryllus bimaculatus* (black cricket) on bio-waste

Abstract

Previous studies have led to claims that insects can offer a solution to several food security hurdles, one of which is the processing of food waste. However, although it has been demonstrated that some insects survive well on bio-waste (e.g. *Hermetia illucens*), no study, has to date, demonstrated success rearing species more commonly used for human consumption, such as crickets, on biowaste from hatching. This trial aimed to establish if the black cricket, *Gryllus bimaculatus*, can be reared successfully on bio-waste from hatching. Since, in other livestock sectors it has been established that nutritional requirements vary with age and that diet must be altered accordingly to achieve the best growth, e.g. chick feed to layer mash in chickens, the present trial used a similar feeding regime of an initially high-quality feed to see if this allowed the subsequent survival of crickets on low quality bio-waste products. Pilot trials have demonstrated poor to no survival on beer waste and cow manure and mid-level survival on unprocessed vegetable waste with chicken feed as the control. Based on this, feed regimes of either 1 or 2 weeks high quality feed (chicken feed) and then either 2 or 3 weeks of low quality feed (beer waste or vegetable waste) were tested. Results showed that even 1 week of high quality feed makes a significant difference in survival and end size of crickets subsequently reared on low-quality bio-waste.

Keywords: insect production, bio-waste feed, survival, growth, crickets

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1. Introduction

The environmental impact of food production is increasingly being brought to the forefront of sustainability debates, particularly surrounding the reduction of carbon dioxide (CO_2) emissions. However, there are two equally important environmental factors which are sometimes ignored, water and land use. It is predicted that by 2025, at least 1.8 billion people will be living in regions without adequate fresh water supplies and a further two thirds of the global population will be in areas feeling pressure from dwindling water resources (FAO, 2012). Freshwater is a finite resource, of which an estimated 70% is used by the livestock and agriculture industries (Doreau et al., 2012). Agriculture uses water directly to grow crops and indirectly to grow fodder to produce livestock. Land availability is an issue which frequently arises in the discussion of sustainable agriculture. As the demand for meat grows there is increasing pressure on producers to farm more livestock which requires more land. It also requires more feed, which in turns leads to farmers increasing the amount of land being cropped, often via deforestation or an increase in fertilizer use. Currently the livestock sector uses about 80% of available agricultural land worldwide (Herrero et al., 2015).

Insects have been proposed an innovative alternative to farming traditional livestock by the United Nations (van Huis et al., 2013), as they are high in protein but assumed to require less water, land and feed input. Data looking at the water footprint of a commercial mealworm farm also found that when water use is controlled for percentage of edible protein (insects are 80-100% edible, while traditional livestock is 40-50% edible; Lundy and Parrella, 2015) mealworms have a lower water footprint than traditional livestock (Miglietta et al., 2015). One of the key arguments for the use of insects as food is that they have a better feed conversion efficiency because they are cold-blooded and depend on their environment to control metabolic processes (van Huis et al., 2013). Another argument also frequently attached to insects is their ability to make use of bio-waste feeds. Trials with *Tenebrio molitor* and *Teleogryllus testaceus* have demonstrated that diets can be successfully formulated from bio-waste sources (Caparros Megido et al., 2016; Miech et al., 2016; Ramos-Elorduy et al., 2002). However, to date only *Hermetia illucens* has been reared successfully on anything but chicken feed directly from hatching. For other insects such as *Acheta domesticus*, commercial scale trials have shown limited success with bio-waste products as feed (Lundy & Parrella, 2015) and lab scale trials with *Blaptia dubia*, *Tenebrio molito*, and *A. domesticus* found that

survival and development rates were significantly negatively impacted on bio-waste feeds (Oonincx et al., 2015a).

Bio-waste feed trials have demonstrated that black crickets, *Gryllus bimaculatus,* fed on a variety of bio-waste diets do not perform well (unpublished data). These trials found low to no survival in the two lowest quality feed groups (spent beer waste and cow manure), and for unprocessed vegetable waste the crickets only achieved 50% of the colony size of a control group fed on chicken feed. These findings were in line with previous findings from Lundy and Parrella (2015). Thus, alternative approaches are needed to make cricket rearing more sustainable successful, this is of particular importance because crickets are one of the most popular insects for human consumption second only to mealworms.

In traditional livestock production it has been demonstrated that nutrition in young animals has a significant impact on later development (Blaxter, 1957; Kilpatrick and Steen, 1995; Pordomingo, 2002). For both livestock and domestic pets, it is standard practice to provide feed of varying nutritional content for different life stages, namely: infant, adolescent, adult, and senior (Kellems and Church, 2009). Crickets similarly have distinct life stages, egg, nymph and adult (Masaki and Walker, 1987) and research on diet restriction and impact on compensatory growth, aging and reproduction (Dmitriew and Rowe, 2011; Jobling, 2009; Lyn et al., 2011) shows that these too have differing nutritional requirements. Research has also shown that different life stages of the cricket, *Acheta domesticus*, metabolise food differently (Woodring et al., 1979).

Thus, it seems likely that using different feeds for different life-stages of crickets would allow a sustainable production system. This has not been tested experimentally and in this study, we report a feeding trial aimed at establishing if feeding crickets on a transition diet from high quality to low quality feed gives improved growth and survival when compared to the use of single low-quality feeding regimes.

2. Materials and Methods

2.1 Feed source and composition

All feeds were analysed for crude protein (Dumas), crude fibre, total oil and ash by Sciantec Analytical Services (North Yorkshire, United Kingdom).

The chicken feed was Fancy Feed Layers Pellets (Fancy Feed Company, Essex, UK) composed predominantly of wheat and soya bean meal comprised of 19.0% protein, 3.9% oil, 4.0% fibre and 5.9% ash.

The millet beer waste was sourced from local breweries in Burkina Faso, dried and frozen prior to transport and then ground for use as feed. Millet beer waste was chosen as the intended target of this research is developing communities, which are predominantly located in tropical climates where insect breeding is easier to facilitate and where millet beer is the brew of choice. It was comprised of 23.7% protein, 9.4% oil, 11.1% fibre and 5.3% ash.

The vegetable waste was 'designed' based on global composition records of food waste (WRAP, 2009). It consisted of 63.7% vegetables (56.0% root vegetables, 16.0% stem-leaf vegetables, 11.0% fruit-seed vegetables, 17.0% other vegetables e.g. leeks, peppers) and 36.3% fruit (28.0% bananas, 23.0% apples, 16.0% citrus fruit, 33.0% other fruit e.g. berries, melon and stone-fruit). All the fruits and vegetables were dried at 35℃ for 36 hours and then ground to a homogenous mixture. It was comprised of 9.1% protein, 1.7% oil, 5.1% fibre and 6.0% ash.

All feeds were stored at -20℃ when not in use to prevent spoilage.

2.2 Feeding trial

Each feeding trial was run for four weeks as this is the time it takes the crickets to reach sexual maturity and a size suitable for harvest, under ideal conditions according to current large-scale producers (private correspondence). The details are given in Table 1. The high-quality feed was chicken feed (CF) and the low quality was either dried and ground mixed vegetable waste (veg) or spent millet beer waste (BW) which in previous trials had given less than 50% colony survival.

Name	N	Treatment Diet
$High_{CF}$ (H_{CF})	6	4 weeks on chicken feed
Low_{BW} (L_{BW})	6	4 weeks on spent millet beer waste
Low_{Veg} (L_{Veg})	5*	4 weeks on dried and ground vegetable waste
$1WH_{CF}$ $3WL_{BW}$	9	1 week on chicken feed followed by 3 weeks on millet beer waste
$2WH_{CF}$ $2WL_{BW}$	9	2 weeks on chicken feed followed by 2 weeks on millet beer waste
$1WH_{CF}3WL_{Veg}$	6	1 week on chicken feed followed by 3 weeks on ground dried vegetable waste
$2WH_{CF}2WL_{Veg}$	6	2 weeks ofnchicken feed followed by 2 weeks on ground dried vegetable waste

*One replicate was lost due to cage failure

Table 1. List of trial treatment conditions and replicate numbers

For all treatments, hatchling (less than 24 hours old) *G. bimaculatus* which had not fed were supplied by Monkfield Nutrition Ltd (Ely, United Kingdom) and immediately subjected to treatment. Approximately 3.5 grams (±10%) of crickets were placed in large plastic cages (42cm H x 59cm W x 39cm D) covered with insect-proof mesh netting.

Each cage contained four egg cartons (30cm x 30cm x 30cm) to increase crawl space. Cages were kept in a climate-controlled chamber maintained at 30℃ with 12-hour day/night and 30% humidity. Water was provided *ad libitum* in all cages via plastic tubes (50ml) with cotton balls placed in the end. Food was also provided *ad libitum* in the bottom of the cage.

After four weeks the crickets were killed by placing cages in a -20℃ freezer. Then the following data were collected or calculated for each replicate of each treatment: total number of individuals, percentage survival, total colony weight, percent biomass accumulation and weight of 10 randomly selected individuals. To calculate percentage survival a starting number of individuals per treatment was needed and this was calculated based on the finding that on chicken feed 55% of the starting population survives (Miech et al., 2016; Oonincx et al., 2015a). One H_{CF} group was run with each treatment and the final number to survive on H_{CF} was used in the following equation to estimate the starting number.

$$EH_{CF} \div R = SH_{CF}$$

Where EH_{CF} is the end survival number of the H_{CF} group, R is the known survival rate on H_{CF} (0.55), and SH_{CF} is the starting number of individuals present. This equation was calculated

for SH_{CF} for 6 replicates of H_{CF} and the average taken for the survival rate calculation. As all replicates had the same starting weight of crickets and they were too young to display any significant size differences the same SH_{CF} result was used for all replicates.

2.3 Feed conversion efficiency

Feed conversion efficiency in animal production systems can be expressed in multiple ways but the most common is the Feed Conversion Ratio (FCR), which is the amount of feed (kg) needed to produce one kg of weight increase of the animal. For insect production the Efficiency of Conversion of Ingested food (ECI) is preferred which gives information on how efficiently feed is converted (Waldbauer, 1968). ECI is calculated as (weight gained/weight of consumed food) * 100%. In ideal scenarios one wants a high ECI (100% is perfect conversion), and a low FCR (1 is perfect conversion).

Both FCR and ECI calculations assume all provided feed is consumed, can be calculated on a fresh or dry matter basis and can be used for specific nutrient conversions. In this paper, FCR and ECI are both expressed on a fresh weight basis. An additional FCR was calculated for protein (P-FCR), using the amount (kg) of protein of a given feed, needed to produce one kg of cricket protein. The amount of protein was calculated as the amount of nitrogen (N) in each sample (as determined by the Dumas method) multiplied by the appropriate nitrogen-to-protein conversion factor for the feed source (Mariotti et al., 2008). For the crickets, 5.6 was used as the conversion factor based on previous research which has established that the conventional factor of 6.25 results in an overestimate of protein (Janssen and Vincken, 2017).

2.4 Statistical Analysis

Data were analysed using IBM SPSS Statistics 22 and Microsoft Excel to look for differences between groups, using one-way between subjects analysis of variance (ANOVA), Welch's F was used when Levene's test of homogeneity of variance was violated. The Scheffe test was used for post-hoc testing. The degrees of freedom were the number of compared treatments minus one. Standard deviation (SD) or error (SE) is reported where appropriate for averages.

3. Results and Discussion

To date the main problem in trying to rear crickets on bio-waste has been the very low survival rates coupled with low biomass accumulation. In the present study, we aimed to test whether early stage feeding with high energy feeds, before moving on to bio-waste could improve the production of crickets. This used a range of feeding regimes (see Table 1) and the results for survival across the groups and biomass accumulation are shown in Figures 1 and Table 2.

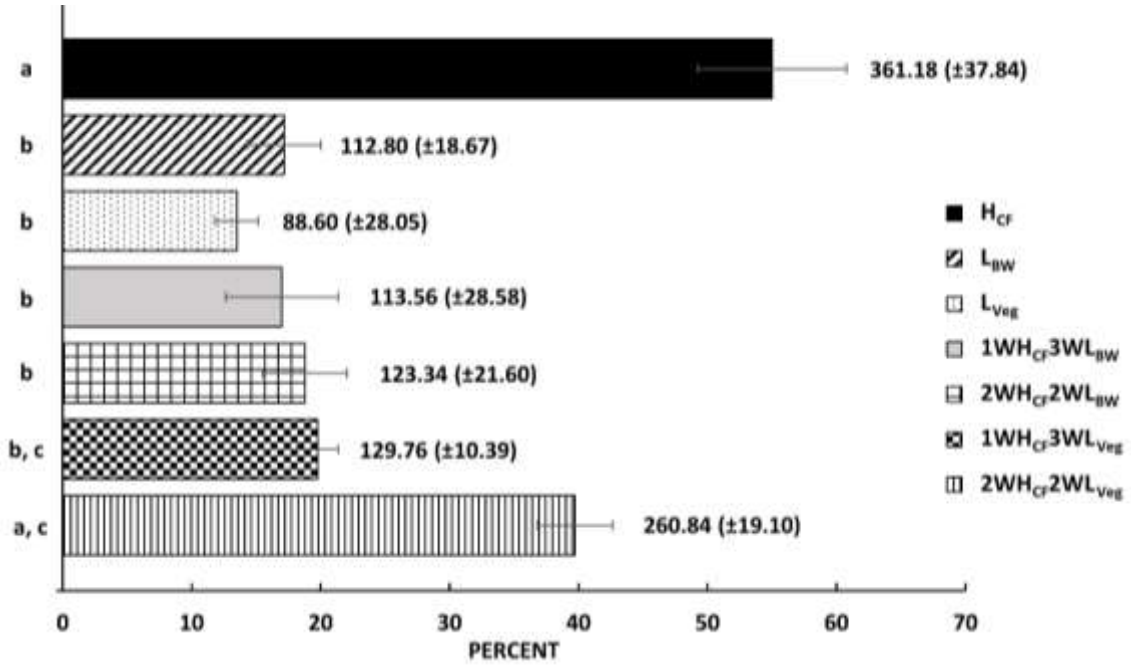

Figure 1. Average survival rate (%) of treatment groups with error bars and average survival number (standard error) listed by treatment, significant differences indicated with letters ($p < .05$

Cricket colonies kept on the high-quality diet for all four weeks had a significantly higher survival rate (55% with ca. 360 individuals surviving) than all of the other treatment groups. However, the $2WH_{CF}2WL_{Veg}$ group had the second highest survival rate, with almost 40% and ca. 260 individuals surviving. Although this is approximately 15% behind the survival of the H_{CF} group, it is a step towards increased survival of crickets on bio-waste sources. There also appears to be a trend towards difference between $1WH_{CF}3WL_{Veg}$ and $2WH_{CF}2WL_{Veg}$. Overall these findings lend support to the previous theory that early nutrition has an influence on later survival.

Diet	N	End weight (g)	Biomass change (%)
H_{CF}	6	223.95 ± 54.73[a]	6387.37 ± 1498.98[a]
L_{BW}	6	4.56 ± 0.61[b]	125.66 ± 13.71[b]
L_{Veg}	5	4.71 ± 3.78[b, c]	131.51 ± 104.92[b, c]
$1WH_{CF}\ 3WL_{BW}$	9	27.32 ± 13.41[b, d]	778.95 ± 380.67[b, d]
$2WH_{CF}\ 2WL_{BW}$	9	47.39 ± 13.93[c, d, e]	1298.92 ± 383.36[c, d, e]
$1WH_{CF}3WL_{Veg}$	6	11.58 ± 1.18[b, e]	341.54 ± 41.01[b, e]
$2WH_{CF}2WL_{Veg}$	6	60.64 ± 5.86[d]	1791.93 ± 255.46[d]

Table 2. Average ending colony weight and biomass accumulation across treatment groups, mean ± SD. Significant differences indicated with letters ($p < .05$).

Data for the cricket end weight yield and biomass accumulation support the findings of the survival data. The H_{CF} populations showed an average 6387% gain in biomass (fresh weight) significantly more than all other treatments ($p < .001$) and in line with previous work (Lundy and Parrella, 2015). $2WH_{CF}\ 2WL_{BW}$ and $2WH_{CF}2WL_{Veg}$ both accumulated over 1200% in biomass over the four weeks, however there was no significant difference in biomass accumulation except for between $2WH_{CF}2WL_{Veg}$ and the two bio-waste only treatments, L_{BW} and L_{Veg} ($p < .05$).

To better understand the performance of the cricket colonies, individual insect sizes were measured. This is important for insects bred for food or feed as they need to be a reasonable size for harvesting. Figure 2 shows the average individual size of crickets from the treatment groups.

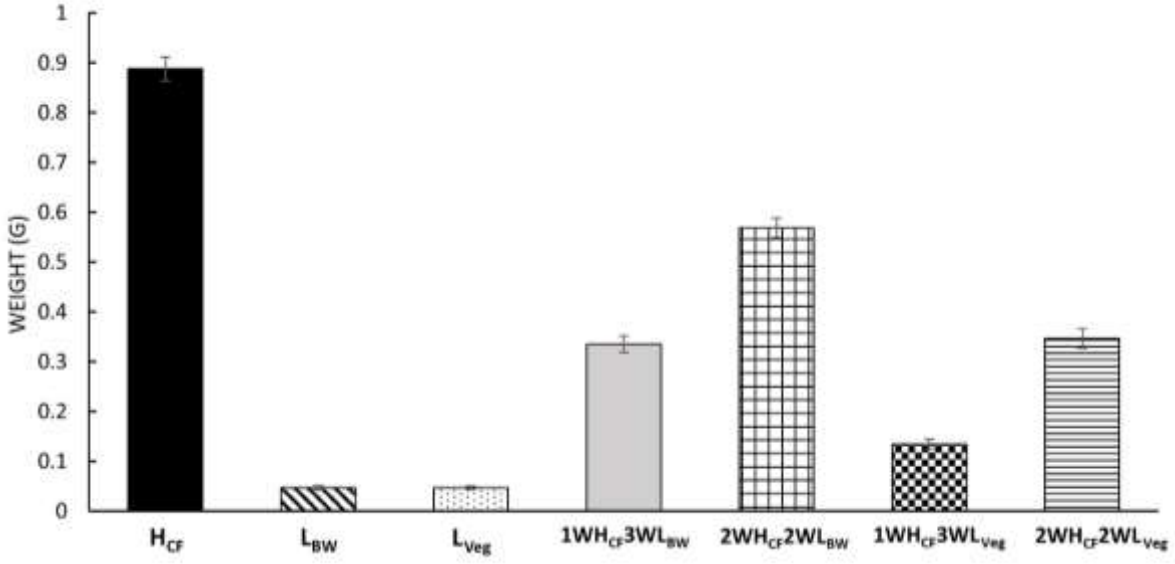

Figure 2. Average size of individual crickets across treatments.

There was an overall significant impact ($p < .001$) of diet on the average individual size of crickets. Crickets fed on the high-quality chicken feed diet (H_{CF}) were significantly ($p <.001$) larger, weighing on average 0.64 (SE ± 0.084) grams more than the crickets from the other diet treatments. The two bio-waste only diets (L_{BW} & L_{Veg}) produced significantly ($p < .001$) smaller crickets than all the other diets, but there was no difference in individual size between them.

The size differences in crickets becomes more complex when looking at the high to low-quality switching regimes. From the survival data it would be expected that the longer crickets spent on the high-quality diet the better they would perform subsequently on low-quality feed. However, the crickets which were feed for 2 weeks on high-quality feed and then switched to 2 weeks of low-quality feed in the form of vegetable waste ($2WH_{CF}2WL_{Veg}$) had an average individual size which was not significantly different ($p = 1.00$) from crickets which were feed for 1 week on high-quality feed and then switched to 3 weeks of low quality feed in the form of beer waste ($1WH_{CF}$ $3WL_{BW}$). Furthermore, crickets fed for two weeks on high-quality feed and then switched to two weeks of low-quality feed in the form of beer waste ($2WH_{CF}$ $2WL_{BW}$) had a significantly larger ($p < .001$) average individual size than all the other high-low treatment groups.

A possible confounding factor to consider is which instar the crickets were in. Prior to becoming adults, crickets in the nymph stage develop through several instars (Masaki and Walker, 1987). Based on expected industrial timeframes, within four weeks crickets should have been in the final instar showing early signs of sexual development (in the form of wing and ovipositor growth). Crickets in the H_{CF} group were the only ones to consistently show these signs. This suggests that the lower nutritional quality of the other diets has stunted the development of the crickets resulting in smaller individual sizes at four weeks.

This highlights that perhaps the low-quality feed at any stage plays a larger role than previously thought and needs to be examined in more detail to ensure that a positive growth start is continued across the live span.

The differences between which feeding treatment groups performed best on overall survival vs. individual weight is interesting. Aside from those on the H_{CF} feed, the groups with the highest survival were not always the ones with the largest individuals. An example is

$2WH_{CF}2WL_{Veg}$ which had the highest survival rate (behind H_{CF}) but was equal to $1WH_{CF}3WL_{BW}$ with regard to average individual cricket size. Whether it is better to have fewer larger insects or a larger quantity of smaller insects depends on the market. If a producer is selling insects by weight their size is irrelevant, as long as the entire colony weighs the most, however if a producer needs to sell a certain size of insects (e.g. as specialist food for lizards of certain sizes) it is of more importance what the characteristics of individuals in the colony are.

It is also worth noting that cannibalism can be a significant problem in mass-rearing of crickets (Harris and Svec, 1964). It is possible that in some groups, such as $2WH_{CF}2WL_{BW}$, which had a lower survival number but higher average individual size, the crickets within the colony ate each other rather than the feed provided. Although cannibalism was not directly observed in these colonies (as crickets were only observed for an hour each day); cannibalism has been previously observed in colonies which were subjected to stress due to lack of appropriate food.

The Efficiency of Conversion of Ingested food (ECI) and the Feed Conversion Ratios (FCR and P-FCR) are shown in Table 3.

Diet	N	ECI	FCR	P-FCR
H_{CF}	6	33.15 ± 4.92[a]	3.07 ± 0.46[a]	1.24 ± 0.18[a]
L_{BW}	6	8.65 ± 0.74[b]	11.63 ± 1.02[a]	5.24 ± 0.46[a, c]
L_{Veg}	5	4.66 ± 4.50[b]	45.09 ± 45.63[b]	8.42 ± 8.52[b, c, d]
$1WH_{CF}\ 3WL_{BW}$	9	17.62 ± 8.21[b, c]	6.77 ± 2.77[a]	3.00 ± 1.23[a, d]
$2WH_{CF}\ 2WL_{BW}$	9	25.02 ± 10.01[a, c]	4.78 ± 2.32[a]	2.03 ± 0.82[a]
$1WH_{CF}3WL_{Veg}$	6	10.13 ± 2.68[b, d]	10.43 ± 2.54[a]	2.84 ± 0.88[a, d]
$2WH_{CF}2WL_{Veg}$	6	23.59 ± 6.41[a, c, d]	4.51 ± 1.20[a]	1.30 ± 0.26[a]

Table 3. Average FCR and P-FCR across treatment groups, mean ± SD, FCR of 1 and ECI of 100 indicates perfect feed conversion. Significant differences indicated with letters ($p < .05$).

Overall, there was a significant effect of feeding regime on ECI, FCR, and P-FCR ($p < .001$). The crickets on the H_{CF} diet where the most efficient, converting 33% of feed into body mass. This was followed closely by the $2WH_{CF}2WL_{BW}$ (25% conversion) and $2WH_{CF}2WL_{Veg}$ (23.6% conversion) groups. These all had significantly higher ECI rates than the other groups, $p < .01$. The ECI rates are also higher than reported for *Acheta domesticus* but roughly on a par with rates for *Blaptica dubia* (Oonincx et al. 2015a). The FCRs and P-FCRs for these three groups also show the same responses. H_{CF} still has the best FCR, but, given

the differing protein content of the diets, the P-FCR is a more accurate measure of conversion and this shows that H_{CF} and $2WH_{CF}2WL_{Veg}$ are almost equal, indicating that crickets on these diets are equally able to convert protein directly into weight gain. The $2WH_{CF}2WL_{BW}$ group is not far behind with its P-FCR. It is worth noting that one of the diets (L_{Veg}) gave a high degree of variability in FCR and P-FCR (as seen by the large SD), likely due to the low survival numbers overall.

Crickets fed on the single bio-waste feeds, L_{BW} and L_{Veg}, had the lowest ECIs. Being at best 1/3rd as efficient at converting feed as the H_{CF} group and at worst only 1/7th as efficient. This rate is, however, not significantly worse than the $1WH_{CF}3WL_{BW}$ and $1WH_{CF}3WL_{Veg}$ groups. Demonstrating that once 2 weeks of a high-quality feed, prior to bio-waste are introduced, there is an improvement in overall ECI. This lends support to previous findings that different cricket life-stages metabolise food differently, as the crickets in the $2WH_{CF}2WL_{BW}$ and $2WH_{CF}2WL_{Veg}$ groups were one week older prior to being introduced to bio-waste (Woodring et al., 1979).

4. Conclusions

Although it is frequently suggested that crickets can be reared as a sustainable alternative to other protein sources, it is becoming increasingly evident that the question of what to feed crickets to ensure their sustainability is not straight forward. Research has shown that crickets struggle to survive at an industry scale on diets composed entirely of bio-waste (Lundy and Parrella, 2015; Oonincx et al., 2015a) and to date chicken feed remains the best option for large scale production. However, in the present study it has been shown that incremental quality feeding regimes are a plausible alternative and could reduce the reliance on chicken feed in commercial scale cricket production. Thus, when crickets were provided with one week of a high-quality feed prior to being switched to a low-quality feed their survival and growth improved and this improved further when it was two weeks of high-quality feed. This could ultimately translate to a 50-75% reduction in the use of chicken feed.

The survival and growth of crickets on the incremental quality feeding regimes was still somewhat lower than crickets fed entirely on chicken feed, signifying that this is not yet the ideal solution. It is evident that a vital nutritional component necessary for cricket survival

and growth is still missing from the low-quality bio-waste feeds. It is most likely that this is due to a lack of protein, since previous work with similar species has identified protein content of feed as a significant driver of survival and growth (Oonincx et al., 2015a). Future research should try to identify sustainable, ideally waste-based, feed sources which are sufficiently high in protein to improve cricket survival. One option would be meat wastes and offal from local abattoirs, although an additional processing step would likely be needed.

Alternatively, other species of insects, which have lower nutritional requirements should be considered. *Hermetia illucens*, for example, has been demonstrated to thrive on a myriad of feed sources from food waste to manure (Oonincx et al., 2015b; Banks et al., 2014). While crickets may be more desirable, future research should focus on species with the most sustainable production potential to avoid placing further stress on global resources. Ultimately, any intended insect production system will require a complete sustainability evaluation to examine potential social, economic and environmental impacts.

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Using *Hermetia illucens* to process Ugandan waragi waste

Abstract

Waragi, a form of homemade gin, is produced throughout Uganda in both legal and illegal breweries. Waste produced during the illegal brewing process is predominantly disposed of via indiscriminate dumping into surrounding environments and reports from local communities have indicated this to be harmful to crops and livestock. The larvae of *Hermetia illucens* are documented to consume a wide range of otherwise unappealing waste products. In addition to this, the prepupal stages of the larvae can serve as a high-quality protein feed for animal livestock. Therefore, the feasibility of the larvae of *H. illucens* to digest waragi waste was evaluated. A dietary toxicity trial was run to establish an LC_{50} value for waragi inclusion in larval diets. This was followed by a larger scale trial utilising waragi waste in combination with various in situ available feed stuffs to further assess the viability of processing waragi waste using *H. illucens.* Larvae were able to eat diets composed of up to 85% waragi waste without any significant impact ($p > .01$) on survival or growth. When combined with locally available feed sources, e.g. chicken offal, cottonseed cake, sunflower meal or groundnut cake, larvae showed high survival (> 95%) and growth rates on diets including 25% waragi waste. Results indicate that *H. illucens* larvae may be a useful tool in processing waragi waste.

Keywords: Hermetia illucens, waste management, waragi, waste reduction, sustainable feed

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1. Introduction

Adequately managing hazardous waste by-products is vital to protecting public health and preventing environmental damage. This is particularly true in Sub-Saharan Africa where 28% of the population relies on water from unprotected wells or surface water (e.g. rivers, lakes, canals) (UNICEF and WHO, 2017). With nearly 10% of the global burden of disease attributed to lack of access to clean water ensuring that water stays as safe as possible is paramount (Prüss-Üstün et al., 2008). Additionally, water for agricultural land, if its irrigated, is drawn or diverted from rivers and lakes resulting in an increased risk of crop damage from contaminated water.

One such contaminant risk is the unregulated dumping of waste by-products from the illegal waragi home-brewing industry in Uganda (Republic of Uganda, n.d.). Waragi is a local gin spirit brewed from molasses and cassava/millet flour and its unregulated production accounts for 80% of the liquor produced in Uganda (Gatsiounis, 2010; Tumwine and Munguti, 2002). There is no research published on the impact of waragi waste dumping on the environment in Uganda or on why it is hazardous. However, there is ample anecdotal evidence from local newspapers and radio shows to support that the dumping has significantly polluted drinking water, killed fish, damaged agricultural land and crops, and caused health issues in individuals who have come in contact with contaminated water (Elunya, 2009; Kasamba and Mugo, 2007; Ssebuyira, 2014; Ssekika, 2015).

One prospective solution for processing the waragi waste is the larvae of *Hermetia illucens*, known commonly as the black soldier fly. *H. illucens* larvae are detrivores and significant research has demonstrated that the larvae are capable of processing livestock manure, human faecal waste, and food waste without difficulty (Banks et al., 2014; Newton et al., 2005; Oonincx et al., 2015; Sheppard et al., 1994). In fact, research demonstrated the larvae were able to reduce *the Escherichia coli* and *Salmonella enterica servor enteriditis* load within livestock manure and human faecal waste, making it safer to subsequently dispose of (Erickson et al., 2004; Lalander et al., 2013; Liu et al., 2008). After 10 days, the larvae also showed no further traces of *E. coli* or *Salmonella* within their own digestive systems, making them safe to utilise further.

H. illucens larvae are predominantly utilised as an animal feed stuff due to their valuable nutritional profile. On average, larvae are reported as consisting of 40% protein and 35% fat (Newton et al., 2005; Sheppard et al., 1994), though these values can range from 37 to 67% protein and 7 to 39% fat depending on the feeding substrate of the larvae (Barragan-Fonseca et al., 2017; Tschirner and Simon, 2015). They also have an amino acid profile closely resembling fish meal (Elwert et al., 2010; Tschirner and Simon, 2015) making them an ideal alternative to expensive fish meal in omnivorous fish farming. Studies feeding *H. illucens* larvae as part of other feed stuffs to chicken, pigs, catfish and tilapia have demonstrated predominantly positive results (Barragan-Fonseca et al., 2017; Bondari and Sheppard, 1987; Elwert et al., 2010; Hale, 1973). The cost of livestock feed, both economically and environmentally, is exerting increasing pressure on food systems, particularly for small-holder farmers(McDermott et al., 2010). Finding sustainable, affordable alternatives is paramount and *H. illucens* is one option.

Lastly, the adult flies are not a nuisance species or a mechanical disease vector. Owing to the fact that the adult flies do not feed, living off fat stores, and female flies only ovipost on the edges of larval food sources avoiding contact with harmful pathogens (Copello, 1926; Furman et al., 1959). The presence of *H. illucens* larvae in waste also serves to deter oviposition of other species such as *Musca domestica* which do act as a vector to spread disease (Furman et al., 1959; Sheppard, 1983).

These factors combined make *H. illucens* larvae the ideal candidate species for potentially processing harmful waragi brewing waste and preventing it from contaminating the environment. This trial examines the viability of this solution by examining at which, if any, level of inclusion in the diet waragi waste becomes toxic to *H. illucens* larvae and testing the impact of experimental diets which include waragi waste and other feed ingredients locally available in Uganda on the growth, survival and development of larvae.

2. Materials and methods

2.1 Feed sources

The waragi brewing waste was sourced directly from a waragi distiller in the Mbale district, Uganda. Due to the illegal nature of waragi brewing its production is not strictly standardised. However, it is generally produced using a combination of molasses, pure

ethanol and other unidentified chemicals which are fermented in old oil drums and then distilled resulting in a black, sticky waste by-product (Heath, 2015).

Organic millet flakes were purchased from Tree of Life UK Ltd., product is sourced from various countries and packaged in the UK. Groundnut cake, sunflower seed meal and cotton seed cake were sourced from local feed producers in Uganda. Chicken offal was sourced from a butcher in the United Kingdom. If necessary, feed was ground to attain homogeneity. All feeds were made fresh every 2-3 days and stored at 4℃.

2.2 Dietary toxicity trial

A dietary toxicity trial was set-up to initially establish at which level, if any, of inclusion in the diet waragi waste becomes harmful to *H. illucens* larvae. This trial was modelled after standard Organisation for Economic Co-Operation and Development Guidelines for Testing of Chemicals in both insects and poultry (OECD, 2012, 1995, 1984). Table 1 displays the nine dosage levels and the feed composition used for the larvae. Chicken mash (Fancy Feed Layers' Mash) was used as the base feed for all diets and all diets were formulated to a minimum of 60% moisture content (Diener et al., 2009). Waragi waste was used in its natural liquid form as methods for drying it would not be available in situ, due to this any dosage above 65% waragi had a moisture content also above 65%.

	% Waragi	*% Chicken mash*	*% Water*	*% Moisture*
Dosage level				
Control	0	35	65	65
D1	25	35	40	65
D2	35	35	30	65
D3	45	35	20	65
D4	55	35	10	65
D5	65	35	0	65
D6	75	25	0	75
D7	85	15	0	85
D8	95	5	0	95

Table 1. Dietary formulation of dosage levels in dietary toxicity trial
Diets were made up fresh every two days and stored at 4°C in between feeding. Food was available *ad libitum* to the larvae and larvae were checked twice a day to ensure food was available.

Each dosage level contained 5 replicates with 30 larvae per replicate. Seven-day old larvae of *H. illucens* were obtained from Entomics Biosystems Ltd., they were previously fed on a

starter diet comprised of vitamins, egg yolk, protein powder, bran and semolina (exact recipe is withheld to protect company). Larvae were placed in plastic rearing pots with mesh lids, no other substrate was provided; pots were kept in a climate control chamber at 30°C with 70-75% humidity and 14-hour days.

Survival was measured daily for each replicate for 72 hours and then again at the first signs of pupation, indicated by a change in colour from light to dark (Tomberlin et al., 2009). Individual larvae were also weighed daily for 72 hours to monitor growth and again at the first signs of pupation, larvae were not washed prior to weighing as previous research has indicated it does not significantly impact weight (Banks et al., 2014). Lethal concentration (LC_{50}) was defined as the percent inclusion of waragi which resulted in 50% mortality of the larvae.

2.3 Pilot feeding trial

A pilot trial was conducted to identify which locally available feed ingredients in Uganda could be used in combination with the waragi waste to ensure the end diet was nutritionally suitable for growth in situ. Initial diets were composed predominantly of millet flakes, however impaired larval growth (significantly smaller larvae, $p < .05$) was observed over a two-week period compared to the groups on chicken feed. Given the nutritional composition of millet flakes at approximately 75% carbohydrates (USDA, n.d.) it was concluded this growth restriction was likely due to low protein levels in the feed, which has been previously demonstrated to significantly impact growth (Oonincx et al., 2015). As such four feed sources (chicken offal, groundnut cake, sunflower seed meal, and cottonseed cake) higher in protein were identified and incorporated into the diets.

2.4 Feeding Trial

The feeding trial was designed to examine the impact of including waragi waste in the diets of *H. illucens* larvae on growth, survival and development. Four experimental and one control diet were examined in triplicate. The amount of waragi waste in the experimental diets was held constant at 25%, as this was an amount which would be reasonably available in situ and was well in line with results from the dietary toxicity portion of the study.

All diets were formulated to a protein level of 15% ($\pm$ 0.04) with a moisture content of 60%. All ingredients were dry excluding the chicken offal, moisture content of the offal was

established and accounted for in composition calculations. The main variation between diets was the primary source of protein. The protein came from either chicken mash (Control), chicken offal (CP), groundnut cake (GN), sunflower seed meal (SSM) or cottonseed cake (CSC). Table 1 lists detailed diet compositions.

	Control	CP	GN	SSM	CSC
Ingredient					
Chicken Mash	36	-	-	-	-
Wheat Bran	4	-	-	-	-
Millet Flakes	-	36	26.2	18.4	31.2
Chicken Offal	-	15.2	-	-	-
Groundnut Cake	-	-	13.8	-	-
Sunflower Seed Meal	-	-	-	21.6	-
Cottonseed Cake	-	-	-	-	8.8
Waragi	-	25	25	25	25
Water	60	24	35	35	35

Table 2. Diet composition, by percent inclusion of ingredients

Seven-day old larvae of *H. illucens* were obtained from Entomics Biosystems Ltd again. 300 larvae were placed in plastic containers with a volume of 650 ml (17 cm L x 11.5 cm W x 5.5 cm D) of which the bottom had been replaced with geotextile material. Each container contained 1 cm of coconut coir in the bottom to provide larvae with enough substrate to burrow in and maintain adequate moisture levels; previous trials demonstrated the larvae would not consume coconut coir. Larvae containers were housed at 30˚C with 14-hour day conditions, and 60% humidity, substrate was misted with water throughout trial as needed to maintain moisture levels.

Fresh feed was provided daily to larvae to avoid fungal growth in the cages, sufficient feed was provided to ensure *ad libitum* feeding. A subset of 15 individuals was weighed from each replicate every other day to monitor growth. Feeding and weighing continued until first signs of pupation.

At pupation, larvae numbers for each replicate were counted to assess survival. Pupae were placed in moist clean coconut coir and returned to climate chamber to incubate. Adult *H. illucens* from each group were counted to establish emergence rates and ensure diet did not negatively impact development; previous work has demonstrated sub-optimal diets to

impact adult development (Gobbi et al., 2013). Development time was defined as the time from larval eclosion to the signs of the first prepupae.

2.5 Statistical analysis

Data were analysed using IBM SPSS Statistics 22 (IBM Corporation, New York) and Microsoft Excel (Microsoft, Washington) to look for differences between groups using one-way between subjects analysis of variance (ANOVA). Average daily weight gain was calculated by taking the starting weight of individual larvae minus the end weight divided by the length of the trial (in days).

3. Results and Discussion

3.1 Dietary toxicity

Figure 1 shows the average larval survival percent over the initial 72 hours of the trial and at the end of the trial, when prepupae were observed, which occurred 10 days after the start of the experiment.

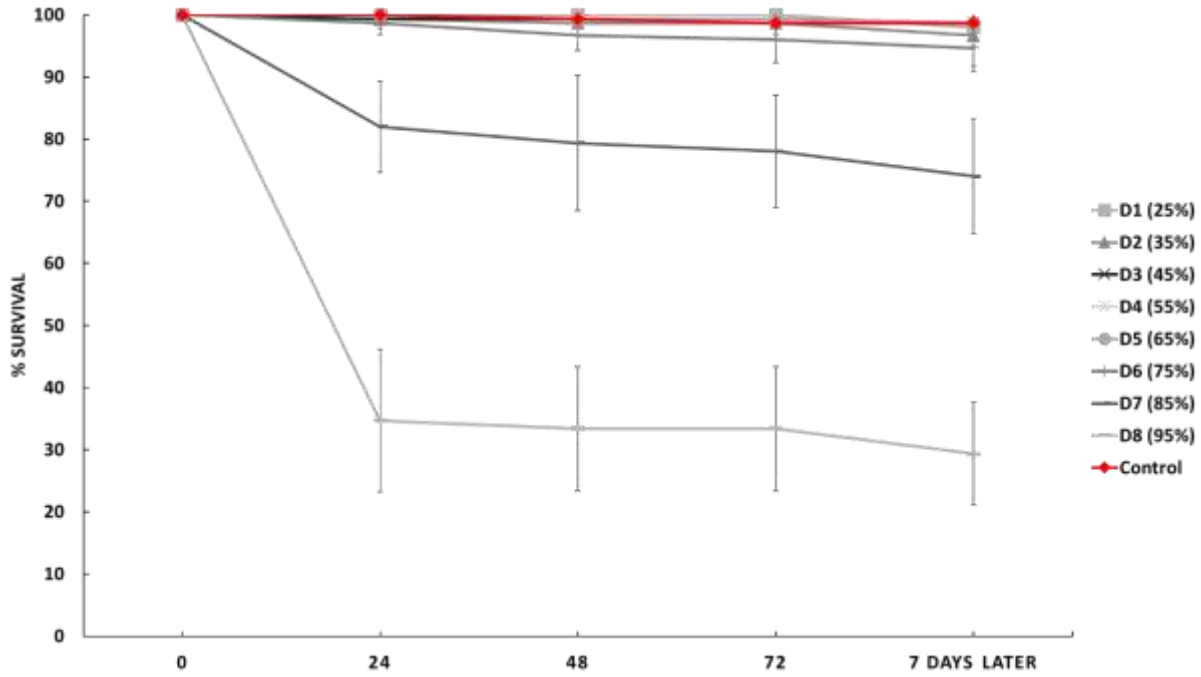

Figure 1. Average larval survival percentage on Control and D1 to D8 (waragi content) with SD

Of the eight dosage levels only two (D7 and D8) showed a significant effect on larval survival ($p < .001$). While D7 reduced survival by approximately 25% over the course of the trial, LC_{50} was only seen at D8, 95% inclusion of waragi waste. Within 24 hours of exposure D8 had

killed nearly 65% of the larvae, while D7 had killed less than 20% and survival stayed well over 95% for all other dosage groups. This result must be considered in light of the fact that the D8 diet was 95% waragi waste. Given the liquid state of the waragi waste and thus the high moisture content of the D8 and D7 diets, drowning cannot be excluded as the potential cause of death rather than toxicity from the waragi waste itself. Previous research has demonstrated the *H. illucens* eggs do not mature in feed with a moisture content above 80%, and similar observations of failure to thrive have been seen in larvae kept in high moisture feeds (Cheng et al., 2017; Fatchurochim et al., 1989). Further to this, waragi waste likely lacks sufficient nutrients to support larval development.

Studies have established that in addition to sufficient moisture in feed, protein and fat content are the next two largest determinants of larvae thriving (Oonincx et al., 2015). Thus, the lack of these two components may be the reason larvae fed on higher concentrations of waragi waste failed to thrive or died in greater numbers.

This failure to thrive is also seen in the results of the individual larval growth rates in Figure 2. Overall, a significant effect of dosage on end larval weight and average daily gain ($p <$ .001) was seen.

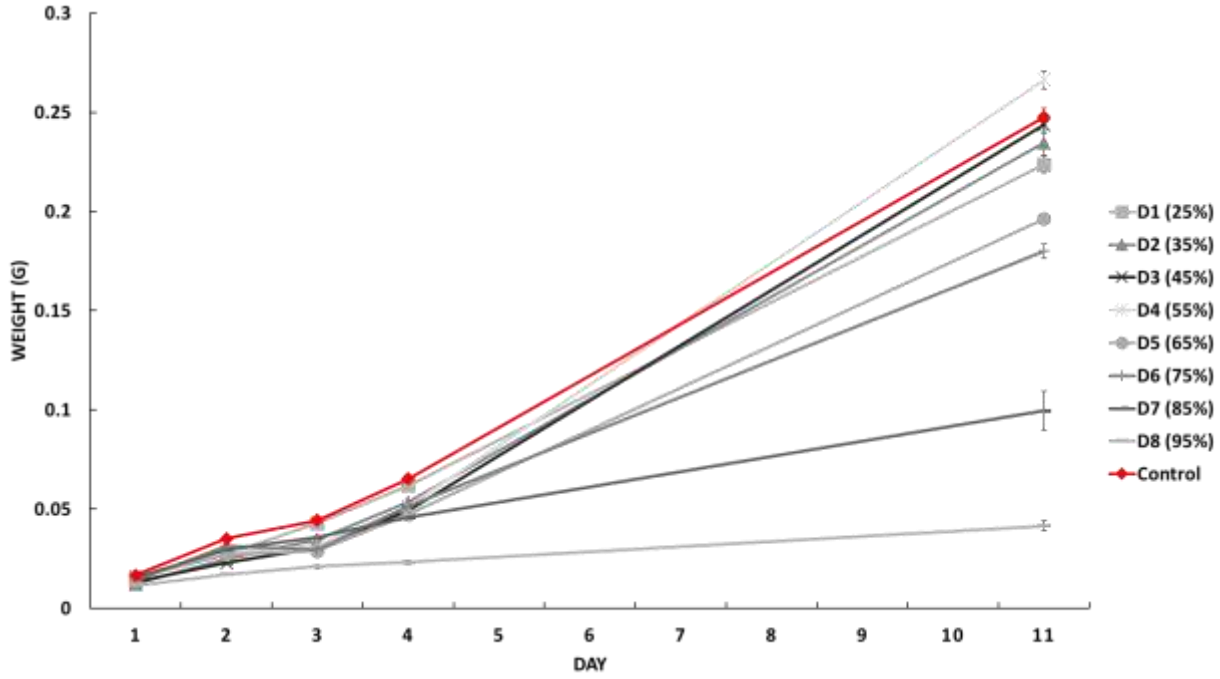

Figure 2. Average individual larval weight and growth rate on Control and D1 to D8 (waragi content) with SE

Larvae on D5, D6, D7 and D8 had significantly lower end weights ($p < .001$) than the larvae on the control diet. D1, D2, and D3 all had significantly higher end weights than D4 to D8 ($p < .001$). The initial weight gain was also lower in all of the dosages when compared with the control, although some of the dosage groups (D1, D2, D3 & D4) did catch up and attained similar or greater end weights than the control group, though this difference was not significant. D7 and D8 displayed particularly low growth, with D7 larvae attaining only at most half of the final weight of the control group and D8 larvae weighing 5 times less than the control. In addition to this, D7 and D8 larvae showed no signs of pupation at the end of the trial, while all other dosages did.

There are two additional trends within the results which lend support to the waragi waste playing at least some part in the larvaes' failure to thrive and death, even if it is not entirely responsible. Larvae in the D6 group showed significantly decreased survival rates and impaired individual growth ($p < .001$). However, the moisture content (75%) of the D6 diet was well within established limits for successfully rearing larvae (Cheng et al., 2017; Diener et al., 2009). This was seen again when examining the individual weights. D5 preformed significantly ($p < .001$) worse than the lower dose groups and the control although they have an equivalent moisture content. This difference in performance is likely due to the increased exposure to and consumption of the waragi waste, either due to toxicity or nutritional deficiency suggesting that the low survival in D7 and D8 may not be entirely accounted for by drowning. This is further supported by the death trend seen in D7 and D8. There is an initial steep drop off in survival for both D7 and D8 and then it levels out again, with no further significant death in the groups. If the death was entirely caused by drowning it would be expected that the entire group would ultimately die, however as this was not the case some portion of the death is presumably down to exposure to the waragi waste and some larvae may be more resistant to the toxic aspects of it than others. Future trials should establish definitively if the waragi waste is the cause by drying it and incorporating it into the diet on a dry basis thus allowing the moisture content to be held consistent across dosages. However, care would need to be taken to ensure that no properties of the waragi waste which may contribute to toxicity are lost in this process.

In insect production both survival and end weight are critical parameters for determining success of a feed ingredient. As such, although larvae can survive on high doses of waragi

waste within their diet, they only grow at equivalent or better rates to the control on diets which include ≤ 55% waragi waste.

3.2 Feeding trial

Table 3 displays the average end larval size, the calculated average daily weight gain per larvae, the percent of larvae which survived to pupation and the percent of pupae which successfully emerged as adults.

Diet	End larval size (g)	Average daily weight gain (g)	Survival %	Adult Emergence %
N (per diet)*	45	45	3	3
Control	0.24 (0.03)[b]	0.025 (0.003)[b]	98.56 (1.02)	98.32 (1.47)
CP	0.26 (0.04)[a]	0.029 (0.005)[a]	99.44 (0.51)	97.54 (1.03)
GN	0.24 (0.03)[a]	0.027 (0.005)[a]	98.67 (0.67)	95.72 (5.08)
SSM	0.23 (0.03)[b]	0.025 (0.003)[b]	98.56 (1.39)	87.62 (10.40)
CSC	0.25 (0.04)[a]	0.028 (0.004)[a]	99.78 (0.38)	95.33 (2.30)

*N refers to the number of individual larvae in the case of end larval size and average daily weight gain, and the number of biological replicates in the case of survival and adult emergence

Table 3. Average end larval size, daily weight gain, colony survival percentage, and adult emergence percentage (± SD)

There was no significant effect of diet on survival percent. Larvae on the CP diet and CSC diet had the highest survival, though only by approximately 1%. However, there was a significant effect of diet on the end larval size and average daily weight gain ($p < .001$). Larvae fed on the CP diet were, on average, larger at the end of the trial and gained more weight daily, though they were only significantly ($p < .001$) larger than larvae on the control or SSM diet. This difference is potentially due to that fact that they received the highest quality protein, chicken offal, of all the groups. Research has previously shown that larvae perform exceedingly well on animal-based protein sources (St-Hilaire et al., 2007).

There was also no significant effect of diet on adult emergence rates. The CP and Control diets showed the highest adult fly emergence rates, achieving close to 100% emergence. On the SSM diet, 10% fewer adult flies emerged than all other diets. Diet is known to play a role in larval and adult survival and development, significant changes have previously been seen in diets which were not nutritionally adequate (Gobbi et al., 2013)**.** However, these differences have generally been linked to survival and growth rather than adult emergence rates. As survival and growth were not impaired in the SSM group it is not clear at this stage what may have impacted the adult emergence rates.

All diets had a larval development time of between 18 and 22 days, which is quicker than has been reported for some previous research utilising other waste sources were development times have been reported to take up to 120 days at times (Sealey et al., 2011). This difference in development times is most likely accounted for by the age of the larvae at the start of the trial. In this study larvae were used after first being reared for seven days on a starter diet, as has been standard in other research to facilitate accurate counting and weighing (Li et al., 2011; Myers et al., 2008). Trials in which long development times (> 40 days) have been noted, larvae were introduced to the waste diet from hatching (Sealey et al., 2011). It is established that larval age at transfer to a waste source and the diet quality in early development play a role in the subsequent development time of the larvae, as such future work should determine if the above diets are subtle to be used from hatching with similar positive results or if a specialisted starter diet is still needed for the larvae (Oonincx et al., 2015).

Although these differences exist it is evident that the inclusion of 25% waragi did not impact the overall survival and development of the larvae, but rather the individual feed compositions likely account for variations in growth and weight. The results on individual larvae growth in Figure 3 support this.

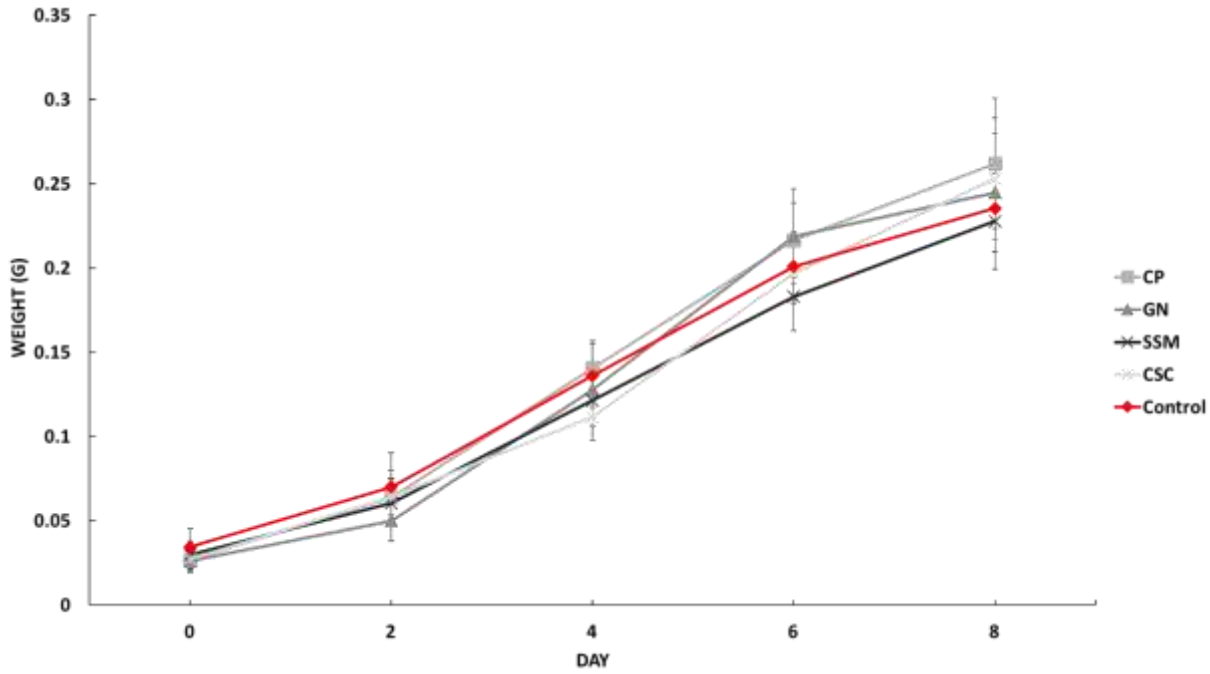

Figure 3. Average individual larval growth over time with SD

Two of the four trial diets, CP and GN, out preformed the control diet at Day 6, with individual larvae weighing on average 0.01 grams more at this point, although only the CP and CSC fed larvae had a significantly ($p < .01$) larger average daily weight gain. This growth difference does even out as larvae move closer to pupation but is a notable difference if the aim is to produce a certain weight of larvae quickly. All larvae showed signs of pupation within 24 hours of each other, indicating the diets and specifically the presence of the waragi waste did not impede development.

4. Conclusions

The dietary toxicity trial provides clear indications that *H. illucens* larvae can readily survive on waragi waste at an inclusion percentage up to 85% without significant impact on survival or growth. It is also plausible that at higher inclusion percentages (> 75%) larval death is due to drowning or nutrient deficiency rather than toxicity, although individual growth data does suggest at least some stunting caused by the inclusion of waragi waste at high percentages. The further feeding trial with a 25% inclusion of waragi waste in the diet demonstrated no differences in survival between trial and control diets, however there was an effect on end weight and average daily gain; however, this is likely explained by the slight differences in feed composition rather than due to the waragi as inclusion was kept constant across diets.

Overall, the data demonstrates that the larve of *H. illucens* can successfully be used to process waragi waste in combination with other locally available feed stuffs or wastes; as demonstrated by survival rates higher than reported in previous research of larvae on waste sources (Gobbi et al., 2013; Myers et al., 2008; Oonincx et al., 2015). This finding presents a unique opportunity for communities in Uganda to make use of an otherwise useless substance.

It is paramount to note that these finding should not encourage increased production of waragi waste to feed larvae. Although the larvae can process the waragi waste it is not essential that they do; they are able to thrive equally well on diets without waragi waste. Feeding the waragi waste to the larvae should only be used as a short-term environmental protection mechanism, not a long-term breeding protocol, while solutions can be found to reduce waragi brewing. One such solution may be to divert molasses from waragi brewing

to *H. illucens* rearing to then sell on into the animal feed system, providing the income that the waragi brewing otherwise would.

Thus, future research should focus strongly on strategies to combat illegal waragi brewing first and foremost, while also identifying methods to provide stable income for communities. Rearing *H. illucens* larvae as animal feed may be one solution to providing income. Continued research should endeavour to develop production systems most appropriate in situ and to continue to refine the ideal feed source for rearing larvae in an economically and environmentally sustainable way. Research should also ensure that the waragi waste composed diet does not impact the overall safety of the larvae as an animal feed.

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Impact of heat-processing on the nutritional content of *Gryllus bimaculatus* (black cricket)

Abstract

Insects are increasingly suggested as a potential novel solution to global nutrition challenges. However, limited research is available on the impact of processing methods on the nutritional content of edible insects. This trial examines the effect of heat processing on the nutritional profile of the black cricket, *Gryllus bimaculatus*. Adult black crickets were killed by freezing and then dried at either a low (45°C) or high (120°C) temperature followed by nutritional analysis of protein and micronutrient. An additional set of samples was either freeze-dried or dried at 32°C, 45°C, 72°C, or 120°C followed by nutritional analysis of lipid content. Analysis showed that protein content was significantly higher in crickets dried at 45°C, a difference of roughly 1%. Similarly, calcium content was also significantly higher in crickets dried at 45°C, although no other measured micronutrients were affected. Additionally, the fatty acid content was significantly influenced by higher temperature processing. Freeze-drying black crickets conserved significantly more of the long-chain polyunsaturated fatty acids than drying at 120°C. Insects hold potential as a source of essential nutrients and fatty acids; however, consideration must be given to heat processing at high temperatures as this may affect the nutritional profile.

Keywords: edible insects, nutrition, heat- processing, black crickets

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1. Introduction

One of the suggested solutions to malnutrition globally is an increase in the use of edible insects, specifically to combat micronutrient deficiencies (van Huis *et al.* 2013; Nadeau *et al.* 2014). Insects are a valuable source of protein and contain vital lipids, micronutrients and amino acids (Bukkens 1997; Ramos-Elorduy *et al.* 1997; Rumpold & Schlüter 2013) and a regular part of the traditional diet for at least 2 billion people in 113 countries (Rumpold & Schlüter 2013; van Huis *et al.* 2013). The use of insects to treat malnutrition could potentially offer a cheaper solution more in line with existing food preferences (Horton 2006; Moreira-Araújo *et al.* 2008).

A small number of trials have formulated insect-based intervention foods and analysed these for nutritional properties, storage capability and/or sensory acceptability. One such trial used termites (species not specified) in combination with *Rastrineobola argentea* (silver cyprinid fish) and found the termite-supplemented food provided higher levels of energy, protein, fat and zinc than the same complementary food without termites; it was also found to have a shelf life of 6-months before harmful microbial activity occurred (Kinyuru *et al.* 2015). Similarly, cookies made with 10% flour made from *Rhychophorus phoenicis* (palm weevil) larvae had 85% more protein and a higher energy value than those made with standard wheat flour (Adeboye *et al.* 2016). In another study, termite-based foods were just as acceptable to infants and mothers as corn-soy blend foods (Konyole *et al.* 2012) and there were no significant differences in aroma and taste acceptability scores of wheat flour buns enriched with between 0% and 20% of termite flour (Kinyuru *et al.* 2009). Another trial developed a caterpillar-based cereal product for children, comprising dried caterpillars (species not specified), ground corn, sugar, salt and palm oil. Thirty grams portions of the product, which met the World Health Organization nutritional requirement guidelines for protein and fat levels of a complementary food for 6-11-month-old infants, were provided to 20 mother-child dyads for a week and the acceptability assessed. Overall, the product was found to be biologically safe and highly acceptable to the mothers and infants (Bauserman *et al.* 2015a). The trial was extended to 175 6month-old infants, who were either provided with the caterpillar-based cereal product as complementary food or not on a daily basis for 12 months, after which anthropometric and biological samples were collected. No difference in stunting prevalence between the two test groups was found;

however, the group fed on the caterpillar-based cereal product showed fewer cases of anaemia, suggesting it offered micronutrient benefits (Bauserman *et al.* 2015b). To date, this is the only trial to have directly utilised an insect-based food in a nutrition intervention.

One potential limiting factor in the use of insects as a nutritional intervention food is the high degree of variability seen in the nutritional content of insects, even within the same species (Bukkens 1997; Ramos-Elorduy *et al.* 2002). Reviews of the nutritional composition of edible insects have identified clear within-species variation in the proportions of macro- and micro-nutrients (Payne *et al.* 2016; Dobermann *et al.* 2017). For example, the reported standard deviation of the manganese content of *Acheta domesticus* (house cricket), 1.66 mg ± 0.906, exceeds 50% of the mean (Payne *et al.* 2016) and for *Tenebrio molitor* (yellow mealworm) larval calcium content has been reported to range from 13 to 472 mg/100 g (Nowak *et al.* 2016).

It is well established that food processing, particularly using heat, can cause nutritional degradation of many foods (Lund 1988) and this is also true for insects. For example, preparation of *Imbrasia epimethea* (African moth) via evisceration and cooking, or drying and evisceration, reduces the amount of carbohydrates and heating reduces the amount of monosaturated fatty acids (Lautenschläger *et al.* 2017). A similar trial using *Imbrasia belina* (mopane worm) larvae found that the cooking method affected the ash, calcium, phosphorous, zinc, manganese and crude protein content (Madibela *et al.* 2007). However, in this trial all samples, regardless of initial processing, were ultimately dried at 105˚C for 24 hours prior to processing. This step introduces a significant confounding variable, making it difficult to establish if the nutritional changes were due to the first processing steps or the subsequent heating. There have been no trials where insects were dried at multiple temperatures and then analysed for the impact of drying.

These findings highlight that processing may influence the nutritional content of insects. This is important because insects bred for food are currently not processed in a uniform manner. The trial reported here examined the effect of heat processing on the nutritional profile of the insect, *Gryllus bimaculatus* (black cricket). This species was selected because of the wide popularity of crickets both as a traditional food and as a ‘gateway’ insect commonly eaten in the form of flour or protein bars in countries which do not traditionally consume insects.

2. Materials and Methods

This study was designed to examine the impact of heat processing on the nutritional content of the edible black cricket. Outcomes measures were protein content, micronutrient content (arsenic, calcium, cadmium, cobalt, iron, potassium, magnesium, manganese, sodium, lead, selenium, zinc), and fatty acid profile [total fatty acids and the individual fatty acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and α-linolenic acid (18:3).

2.1 Gryllus bimaculatus (black cricket)

Adult black crickets were sourced from Monkfield Nutrition Ltd, Royston, UK where they had been reared on a standard commercial chicken feed diet for 4-5 weeks.

2.2 Analyses

All black crickets were killed by freezing at -20°C for 24 hours. Following this, one set of black crickets was dried at either 45°C or 120°C for 36 hours for protein and micronutrient analysis. For fatty acid analysis, black crickets were dried at either 32°C, 45°C, 75°C, or 120°C for 36 hours, and one set of black crickets was freeze-dried. Black crickets were dried over a larger range of temperatures for fatty analysis as fatty acids are known to be particularly sensitive to heat processing (Lautenschläger *et al.* 2017).

2.2.1 Protein content

Three hundred mg of each black cricket sample was placed in the auto-sampler of a Leco TruMac Combustion Analyser (Dumas method) to determine total nitrogen and carbon, using five replicates per temperature. Protein content was determined by multiplying the nitrogen content by 6.25 (Jones 1941; Sosulski & Imafidon 1990). Although recent research suggests that the 6.25 nitrogen to protein conversion factor, which has been standard until now, potentially overestimates protein content (Janssen *et al.* 2017), any overestimation would affect all of the treatments equally and therefore the comparisons between treatments remain valid.

2.2.2 Micronutrient content

Five hundred mg of each black cricket sample was placed in 25 ml graduated digestion test tubes, using five replicates per temperature, with one sample out of every three repeated to check analysis accuracy. Two wheat flour samples were included as a certified standard, and

two blank control tubes to establish any potential contamination in the solvents. Samples were pre-digested in 5 ml of 15:85 nitric:perchloric acid (HNO_3:$HClO_4$) for 5 hours then heated overnight to 175°C to evaporate the acid. Five ml of 25% HNO_3 was added and the tubes heated to 80°C for 60 minutes. Samples were made up to a final volume of 25 ml with ultra-pure water, decanted and analysed using Optima Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) (Zhao *et al.* 1994). All Nitric acid was Aristar grade unless otherwise stated.

2.2.3 Fatty acid content

Twenty µg of either black cricket sample was analysed, using five replicates per temperature. Samples were placed in 4.5 ml glass vials and 5 µl methyl heptadecanoate (C17:0) was added as an internal standard. Glass vials and pipettes were used throughout the procedures. Extraction/methylation mix (1.5 ml), comprising 85% MeOH, 10% HCl, 5% 2-2, dimethoxypropane, was added and the vials were capped and heated to 80-85°C for 1 hour. Vials were then cooled and 1 ml of 1% NaCl added. The samples were mixed, and the fatty acids extracted into 1 ml hexane. Following methylation, the hexane fraction was concentrated under nitrogen and re-suspended in 300 µl solvent prior to injection of 1 µl of a 1 in 10 dilution, onto the gas chromatography (GC) column. Methyl ester derivatives of the total fatty acids extracted were analysed by GC (Agilent 7890A) using an Agilent DB-225 column (size: 30 m × 0.32 mm × 0.3 µm). The inlet and detector temperature were set to 250°C and 2 µl of each sample (4 µl for the cow manure feed due to its low fatty acid content) was analysed using splitless injection and a constant flow rate of 2 ml/min. Chromatograms were analysed using Agilent ChemStation software. The retention time and identity of each FAME peak was calibrated using the FAME mix rapeseed oil standard (Supelco™, Sigma-Aldrich Ltd., Poole, UK).

2.2.5 Data analyses

Data were analysed using IBM SPSS Statistics 22 and Microsoft Excel. Differences between temperature groups (45°C or 120°C) for protein and micronutrient content were analysed using independent samples T-Tests. Differences between temperature groups (32°C, 45°C, 75°C, 120°C or freeze dried) for fatty acid were analysed using one-way between subjects analysis of variance (ANOVA). ANOVA with Welch's F test was used when Levene's test of homogeneity of variance was violated. Scheffe post hoc tests were used to explore

differences between specific temperatures. Significance was set at the $p > 0.05$ level. Where appropriate, standard errors or standard deviations are reported.

3. Results

3.1 Protein content

There was a significant difference in protein content between black crickets dried at 45˚C and 120˚C [t (8) = -10.467, p = .000]. Figure 1 displays the average protein content in black crickets dried at the two temperatures, there is roughly a 1% average difference between the two drying temperatures.

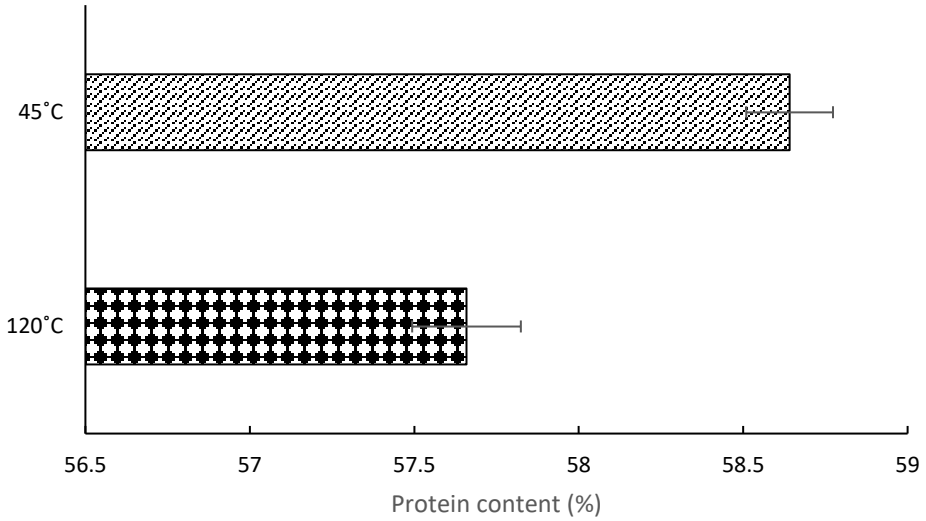


Figure 1. Average protein content (%) in black crickets dried at 120˚C and 45˚C with ± standard deviation.

3.2 Micronutrient content

Arsenic, Cadmium, Cobalt, Lead, and Selenium were not present within ICP-OES detection limits. Of the other micronutrients displayed in Figure 2 only calcium showed a significant difference in content between the two temperatures [t (8) = -3.181, p = .013] with black crickets dried at the 45˚C containing the most calcium. There were no significant differences between the high and low processing temperature for magnesium [t (8) = -2.291, p = .051] sodium [t (8) = 1.201, p = .264], potassium[t (8) = .583, p = .576], iron [t (8) = 1.165, p = .278], manganese [t (8) = -1.106, p = .301], or zinc [t (8) = -1.645, p = .139].

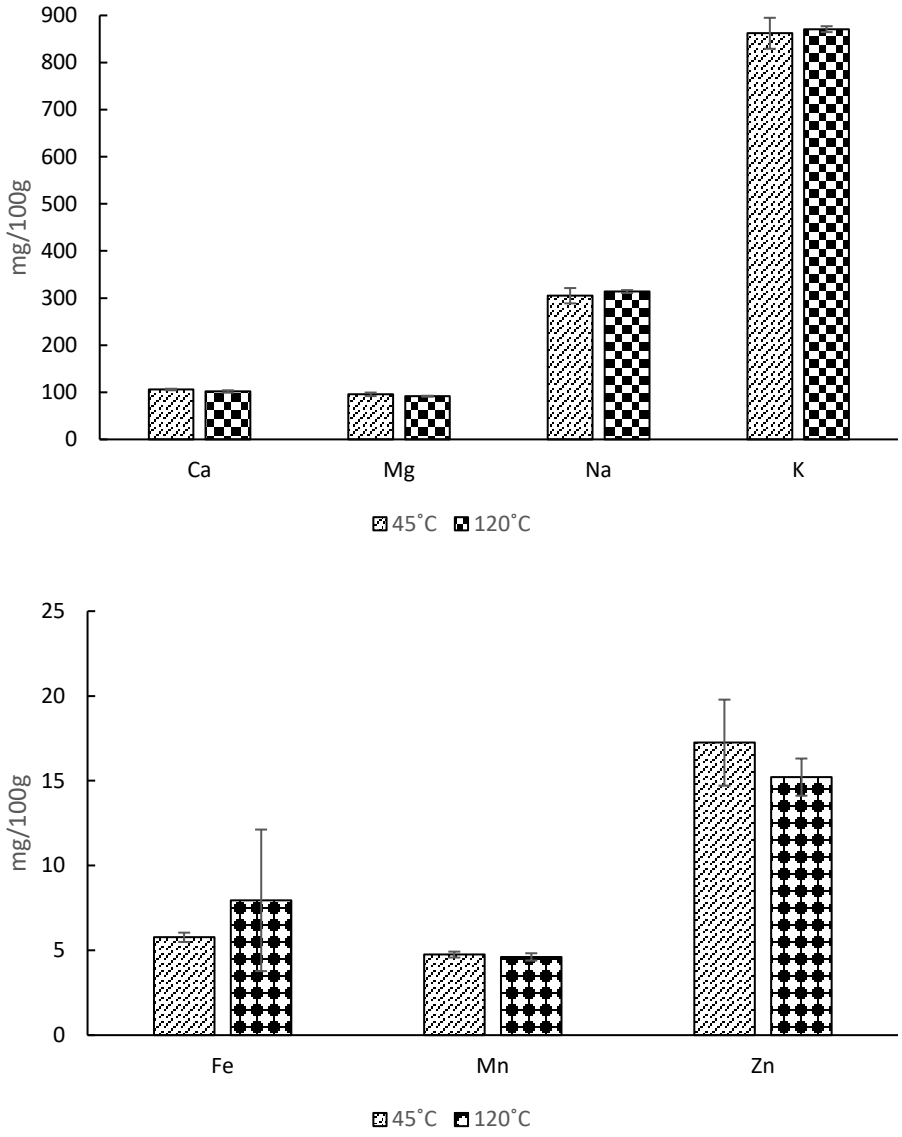
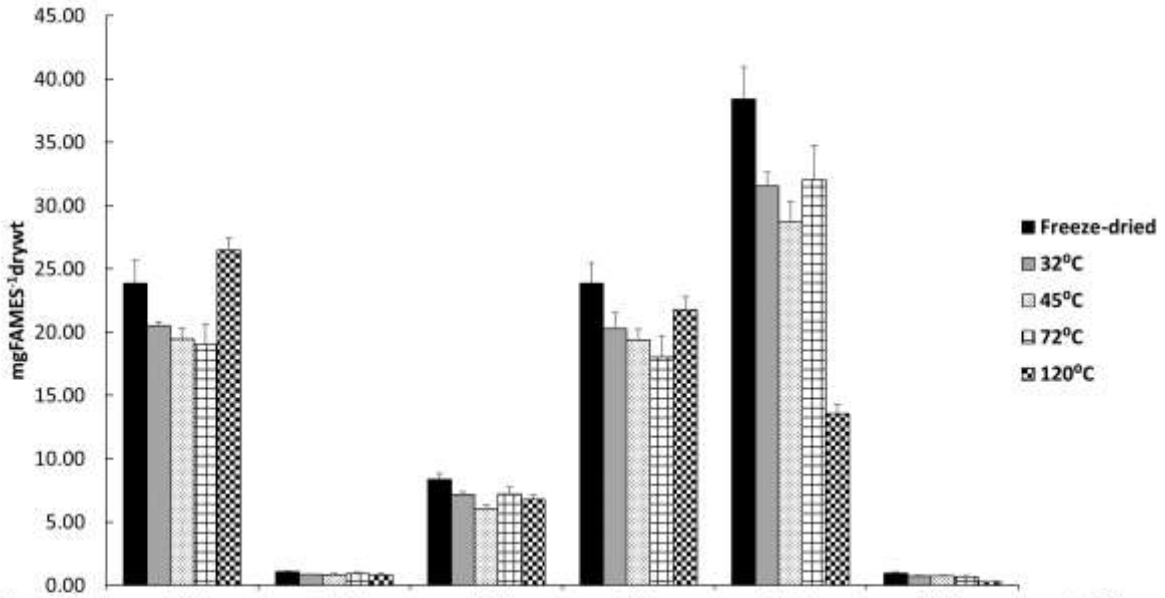


Figure 2a and 2b. Average content of selected micronutrients (mg/100 g) in black crickets dried at 120˚C and 45˚C with standard deviation. Ca, calcium; Mg; magnesium; Na, sodium; K, potassium; Fe, iron; Mn, manganese; Zn, zinc.

3.3 Fatty acid content

For fatty acids there was a significant main effect of heat treatment on the total fatty acid content [F (4, 20) = 16.21, p = 0.000], with total fatty acid content decreasing as temperature increased (see Figure 3). There was also an overall significant effect of heat treatment on the amount of palmitic acid [16:0; Welch's F (4, 12.55) = 38.13, p < 0.0001], stearic acid [18:0; Welch's F (4, 12.77) = 12.29, p < 0.0001], oleic acid]18:1; Welch's F (4, 11.86) = 10.96, p < .001], linoleic acid [18:2; Welch's F (4, 10.26) = 365.4, p < .0001] and α-

linolenic acid [18:3; Welch's F (4, 10.28) = 394.39, $p < .0001$] found in the black crickets, Figure 3.



Fatty Acids	16:0	16:1	18:0	18:1	18:2	18:3	Total
Freeze-dried	23.84^{a}	1.07^{a}	8.36^{a}	23.84^{a}	38.41^{a}	0.94^{a}	96.47 (6.67)^{a}
32°C	20.52^{b}	0.87$^{a, b}$	7.18$^{a, b}$	20.27$^{b, c}$	31.54^{b}	0.74^{b}	81.11 (2.26)^{b}
45°C	19.45^{b}	0.82$^{a, b}$	6.04^{b}	19.35^{c}	28.68^{b}	0.75^{b}	80.53 (3.95)^{b}
72°C	19.02^{b}	0.93$^{a, b}$	7.2$^{a, b}$	18.01^{c}	32.03^{b}	0.68^{b}	77.87 (6.37)^{b}
120°C	26.46^{c}	0.79^{b}	6.78^{b}	21.76$^{a, b}$	13.54^{c}	0.27^{c}	73.93 (2.80)^{b}

Figure 3. Average amount [mg of fatty acid methyl esters (FAMEs) per g of dry weight] of specific fatty acids in black cricket processed at various temperatures, n = 5. Error bars ± standard deviation (SD). Different letters within a column indicate significant differences based on Scheffe post-hoc tests, p <0.05. Palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), α-linolenic acid (18:3)

Post hoc tests (see Figure 3) showed that freeze-drying the black crickets conserved significantly more of both the longer chain fatty acids (linoleic acid (18:2) and α-linolenic acid (18:3)) and those with more than one desaturation than any of the other drying temperatures (p <0.05). Although, interestingly there was no significant effect on the amount of oleic acid (18:1) between black crickets which were freeze dried and those which were dried at 120˚C (p >0.05). The largest difference was seen in the long chain polyunsaturated fatty acids (PUFAs), linoleic acid (18:2) and α-linolenic acid, with drying at 120˚C resulting in significantly higher amounts of 16:0 and lower amounts of linoleic acid (18:2) and α-linolenic acid (18:3) than any of the other drying temperatures (p <0.05). There

was no difference in fatty acid content between black crickets dried at 32, 45 or 72˚C (all p >0.05).

4. Discussion

To date only a limited number of studies have examined the impact of processing temperature on the nutritional profile of edible insects, and predominantly within the context of how the insects are traditionally prepared rather than systematically (Mutungi *et al.* 2017). This study examined the impact of various drying temperatures on the nutritional content of black crickets and found that while some effect is seen on protein and micronutrient content, the most marked change is seen in the fatty acid profile which black crickets dried at higher temperatures showing lower levels of PUFAs.

The finding that the protein content was slightly higher in the black crickets which were dried at 45°C, compared to 120°C, is in direct agreement with a previous paper which found that oven dried housefly larvae had lower protein levels than sun-dried larvae (Aniebo & Owen 2010).

The finding that temperature treatment predominantly does not affect the micronutrient components of black crickets is in line with previous studies (Madibela *et al.* 2007; Mutungi *et al.* 2017), although neither of these studies compared a distinct set of temperatures but rather distinct processing methods such as boiling versus frying. The finding that micronutrients in black crickets are largely not affected by processing temperature is important as it suggests that insects can be processed into foods such as buns, biscuits, flour, and porridge without reducing their micronutrient value.

The results of the present study show that at 120˚C, there is a sharp drop in the linoleic acid content of the crickets. This is in line with findings that PUFAs are sensitive to oxidation especially in combination with increasing temperatures above 60˚C (Jacobsen & Bruni Let 2008). Further, it is possible that the PUFAs originally present in the black crickets were broken down via oxidation into the shorter chained palmitic acid (16:0) accounting for the increasing 16:0 content seen at higher drying temperatures. Black crickets dried at 32, 45, 72 or 120˚C did not show marked differences in total fatty acid content, although their content was significantly lower than black crickets which were freeze-dried. Given the caloric value of fats (Youdim 2016) the implications of this finding are that due to the lower

total fatty acid content of black crickets dried at higher temperatures they will also be of lower caloric value. Although fatty acids are often not considered in malnutrition, their role particularly in children, should not be disregarded. The brain requires high levels of PUFAs for optimal growth and development, specifically eicosatetraenoic acid (20:4) and docosahexaenoic acid (22:6), which are synthesized from their respective essential fatty acid (EFA) precursors 18:2 (linoleic acid) and 18:3 (α-linolenic acid) (Wainwright 1992). Essential fatty acid deficits have been identified in malnourished children and are linked to a number of clinical problems such as increased risk of infection, impaired wound healing, fatty liver and psychomotor changes coupled with growth retardation (Aaes-Jørgensen 1977; Hansen *et al.* 1963; Koletzko *et al.* 1986; Rivers & Frankel 1981). Linoleic acid has also been found to be vital in infant nutrition, with symptoms of deficiency appearing quickly, although this can be resolved when diets are changed to include at least 1% of their calories as linoleic acid (Hansen *et al.* 1963).

The European Food Safety Authority (2009) recommends, for adults, a daily intake of 2 g of α-linolenic acid and 10 g of linoleic acid. In the present trial, samples which were dried at 120˚C had, on average, less than 15 mg of linoleic acid per gram of dry cricket, while all other drying temperatures had approximately twice as much linoleic acid. Similarly, the samples dried at 120˚C had less than 0.3 mg of α-linolenic acid, while other temperatures had closer to 1 mg per gram of dry crickets. Although this may not appear to be a large amount of these acids it can make a difference within diets and thus this is a strong argument for taking steps to avoid processing insect-based foods aimed at children above 120˚C.

It is important to note that if heating during processing is kept below 120˚C, microbial contamination can still be controlled by immersing in boiling water for 120 seconds prior to further processing. Previous work has also demonstrated that a short heat step is sufficient to reduce microbial load to safe levels (Klunder *et al.* 2012). The drying temperature made a significant difference in the fatty acid profiles of the crickets and this is likely due to the extended nature of the heat exposure as drying occurs over several hours or days. At present, most insects consumed in rural developing communities are wild harvested and cooked directly or sun-dried at temperatures that would not exceed approximately 50˚C;

meaning current practices in entomophagous communities already preserve the full fatty acid profile of insects making them more nutritionally valuable as a food source.

5. Limitations and Future Directions

There are two key limitations to this study. First, only two temperatures were used to test the effects of heat processing on micronutrient and protein contents. This was done as there was no previous work which suggested that a significant effect of drying temperature on protein or micronutrient content should be anticipated. However, given the above results it is evident that protein and perhaps some micronutrients are sensitive to heat, and a wider range of temperatures would have been useful in elucidating at which temperature the changes in content occur. Future research should explore the effects of a wider range of temperatures. Second, there is some evidence to suggest gut content of insects has an impact on their nutrient content (Mutungi *et al.* 2017) and this was not controlled for in the study. While the crickets had all been given free access to food prior to being dispatched it is not outside the realm of plausibility that some of the crickets had fuller guts than others. This could account for the significant group differences observed for in calcium content, as the chicken feed used to rear the crickets is high in calcium. Future trials could control for this 'gut load' effect by degutting the insects or introducing a fasting period to ensure the digestive systems of the insects have emptied.

Lastly, as it has been shown in this trial that fatty acids are sensitive to processing temperature a future trial should work to establish at which temperature the above-mentioned fatty acid content changes occur. There are a range of temperatures between 75°C and 120°C, knowing at which temperature the changes in fatty acids occur is particularly important to producers who are trying to balance nutritional content with microbial safety considerations. In addition, future nutrient work must be coupled with food safety work to ensure that if a lower drying temperature is recommended to preserve nutritional content it is not in opposition to the temperature required to reduce microbial risk to a safe level (Rumpold & Schlüter 2013).

As edible insects are increasingly considered for nutrition interventions, it is important that researchers and food manufactures are aware that heat processing has an impact on the

nutrients they deliver. Insects have the potential to be a good source of essential nutrients and fatty acids, but only if processed correctly.

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Growth of Nile tilapia, *Oreochromis niloticus* (Abbassa strain) fed on insect meal, fish meal or plant-based diets in Egypt

Abstract

Identifying safe, sustainable and affordable fish feed stuffs is a priority within aquaculture. Edible insect meal has been presented as a suitable alternative to fish meal and other protein sources for omnivorous fish. Insect meal would be of use within rural small-holder communities as farmers could rear the insects themselves. However, only few trials have examined the use of insect meal within fish diets and always within the context of partial substitution of the fish meal component of the diet. As such a preliminary study was conducted to evaluate the impact of complete replacement of fishmeal with insect meal in tilapia diets. Juvenile Egyptian Nile Tilapia (Oreochromis niloticus) of the Abbassa strain were feed one of four diets and monitored over 10 weeks. One diet was a control diet utilizing fishmeal while the other three utilized either plant only protein sources, black soldier fly (*Hermetia illucens*) larvae meal, or black cricket (*Gryllus bimaculatus*) meal. All diets were formulated to be nutritionally balanced. Overall, there was no significant effect of using insect meal within tilapia diets on feed consumption and conversion efficiency, fish survival, general health or growth. These findings suggest that insect meal can be used as a viable alternative to fish meal in Nile tilapia feeds.

Keywords: Nile tilapia, insect meal, growth, survival

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1. Introduction

In Egypt aquaculture production has more than doubled in the last decade, increasing from around 500 thousand tonnes in 2002 to over a million tonnes in 2013 and placing it as the 8th largest global producer of fish and the largest in Africa (FAO, 2013; Shaalan et al., 2017; Shaheen, 2013). Egypt is the world's second largest producer, after China, of tilapia species, which accounts for over 65% of fish production in Egypt (Ibrahim et al., 2013; Norman-López and Bjørndal, 2009; Soliman and Yacout, 2016). Tilapia are a common species of choice for aquaculture because of their fast growth rate, prolific breeding and resilience in less than ideal environments (Lim, 1998). They are also popular with consumers as bones are not contained within the tissue sarcomas making direct consumption easy and they accumulate limited organic pollutants due to the low fat content of their musculature (Shaheen, 2013).

The increase in fish production in Egypt has been accompanied by a shift from low-input culture systems to larger, intensive and feed-dependent systems, placing pressure on feed supply chains within Egypt (El-Sayed et al., 2015; Shaalan et al., 2017). While the number of local feed mills has increased almost fifteen-fold to meet the demand, there is still a significant reliance on imported raw feed materials; with 60 to 99% of materials being imported (El-Sayed et al., 2015; Shaalan et al., 2017; Soliman and Yacout, 2016). Feed accounts for 75 to 85% of the running costs and this, combined with the cost of importing materials, restricts Egypt's ability to expand to meet production demands in a sustainable and affordable manner (Dickson et al., 2016; Kleih et al., 2013). Efforts to identify feed components which are available locally are needed to reduce this reliance on imported materials and thus create a more secure production system with increased opportunities for local companies.

Of particular interest is fish meal, which next to soybean meal, is the main protein source used in fish feeds and included at rates up to 22% in feeds produced in Egypt (El-Sayed et al., 2015). Fish meal is consistently the most expensive feed ingredient due to its finite supply and the ever increasing demand from the booming aquaculture industry (Cummins et al., 2017). Cost aside, with global wild fish catches, where fish meal is sourced from, at or exceeding limits of what aquatic ecosystems can sustainably provide the limited supply of fishmeal is a growing concern within aquaculture production (Deutsch et al., 2007; FAO, 2012). While measures, such as stricter production quotas and the increased use of would-

be fishmeal fish for direct human consumption, have been taken to reduce the production of fishmeal the demand for it within aquaculture sectors as a feed material has only increased with production rises and suitable alternatives must be found (Jackson, 2009; Naylor et al., 2000; Tacon and Metian, 2015).

A majority of trials aimed at finding fishmeal replacements, have focused on an increased use of plant-based protein sources, such as soybean, or turned to alternate marine sources such as algae. Trials with plant proteins have given mixed results (Gómez-Requeni et al., 2004; Kaushik et al., 2004; Olvera-Novoa et al., 1998), with the main limitations being the presence of phytic acids (which impact the overall protein digestibility), the micronutrient availability and the limited amino acid profiles(Gatlin et al., 2007; Hardy, 2010), especially methionine (García-Ortega et al., 2016).

Recently, increasing attention has been focused on the potential of insect-meal as a replacement for fishmeal in aquaculture feeds (Rumpold and Schlüter, 2013; van Huis et al., 2013). Several insect species have been trialled, with outcomes largely dependent on the protein content of the insect-meal and the ratio of lipids to other nutrients (Dobermann et al., 2017). While research is still in its infancy results to date have been predominantly positive, with the majority of trials, which have used omnivorous fish, finding that the partial replacement of fishmeal with insect-meal had no adverse effects on survival, growth or feed conversion efficiency (Makkar et al., 2014; Taufek et al., 2016). Trials using carnivorous fish species have encountered more hurdles in using insect-meal, although in some cases replacement rates between 20 to 50% have been successful (Iaconisi et al., 2017; Magalhães et al., 2017; Piccolo et al., 2017).

Overall, although insect-meal has been found to be easily digestible by fish and to give a high protein efficiency ratio (Ssepuuya et al., 2017), to date few trials have replaced 100% fishmeal in with insect-meal. One trial using Nile tilapia, found that the complete replacement of fishmeal resulted in lower survival, weight gain and poor feed conversion, while another found no significant impact on growth at 100% replacement (Ali et al., 2015; Wang et al., 2017). Given the conflicting results the trial reported here aimed to examine the impact on tilapia of the complete replacement of fishmeal in their diet, with insect meal, either *Hermetia illucens* (black soldier fly) or *Gryllus bimaculatus* (black cricket).

2. Methods

2.1 Nutritional analysis of Hermetia illucens and Gryllus bimaculatus meal

Samples of black soldier flies and black crickets were obtained from Monkfield Nutrition Ltd. and Entomics Biosystems Ltd. They were killed by freezing at -20°C, then dried at 80°C, ground and analysed for protein, micronutrient, lipid and amino acid content prior to feed formulation.

Total nitrogen was determined using the Leco TruMac Combustion Analyser (Dumas method) and protein content was determined by multiplyingby the standard factor of 6.25 (Sosulski & Imafidon, 1990; Jones, 1941).

Standard analysis of protein hydrolysates was done using an ion-exchange analyser (Biochrom 30) and amino acid content determined by the University of Cambridge Protein and Nucleic Acid Chemistry Facility.

For micronutrients, each sample was digested using the standard nitric:perchloric acid ($HClO_4$:HNO_3) digestion matrix and analysed using Optima Inductively Couples Plasma – Optical Emission Spectrometer (ICP-OES) (Zhao et al., 1994).

For fatty acids, these were extracted and transmethylated with heptadecanoic acid (17:0) as the internal standard; methyl ester derivatives were then analysed by Gas Chromatogrpahy (Agilent 7890A) using an Agilent DB-225 column (30 m×0.32 mm×0.3 µm). The retention time and identity of each fatty acid methyl ester (FAME) peak was calibrated using the FAME mix rapeseed oil standard (Supelco™, Sigma-Aldrich Ltd., Poole, UK).

2.2 Tilapia feeding trial design

Mixed sex, juvenille Nile tilapia from the Abbassa selection line (Khaw et al., 2009; Rezk et al., 2009), were sourced from the WorldFish centre farm, Abbassa, Egypt. All fish were deemed healthy, based on visual inspection at the start of the trial and had not previously been exposed to vaccination or antibiotic treatment.

Fifty fish (50 g initial weight each) were stocked in each 15 m^3 (3 x 5 x 1 metre) outdoor floating net cage, 'hapa', within a larger earthen pond filled with fresh ground water and aerated using a 1.5 HP paddle wheel (pH 8.0-9.0, >5 mg/l O^2). Fresh water was added daily for four hours to compensate for any evaporation; mean water temperature was 28°C ± 4°C

with a 12 to 14- hour photoperiod. The fish were fed daily by hand at 9.30 and 15.30 for 15 minutes and feed intake was recorded, any dead fish were removed and recorded.

Fish were fed four different diets, with four replicates of each diet and the diets were allocated randomly across the 16 hapas to account for any pond location effects.

The trial was split into three 23-day phases. At the start and end of each phase, surviving fish in each hapa were counted and the group weighed. Fish were fed at 6% body weight for the first phase of the trial and 3% body weight for the last two phases. All feeds were produced as sinking pelleted feed. Due to the short water stability of pelleted feeds and high loss rates, feeding trays were inserted into the bottom of each hapa to catch and retain feed for the fish, the trays also allowed fish to feed continuously outside of feeding times (Lim, 1998; Mcginty et al., 1989).

2.3 Feed formulation

Four isonitrogenous (35% crude protein) feeds, Veg, Fish, BSF (black soldier fly), and GB (black cricket), containing 6% crude fat were formulated using standard available ingredients; there was no significant caloric difference between feeds. Table 1 provides the detailed composition of the feeds used in the trial.

	Veg	**Fish**	**BSF**	**GB**
Ingredients (g/kg dry)				
Maize gluten meal	100	--	--	--
Fish meal	--	100	--	--
Black soldier fly meal	--	--	100	--
Black cricket meal	--	--	--	100
Soybean meal	472	476	553	500
Rice bran	176	185	121	153
Maize distillers grains	100	100	100	100
Maize	100	100	100	100
Vitamin premix	52	39	26	47

Table 1. Composition of feeds in g/kg dry weight

2. 4 Statistical analyses

All data were analysed using Microsoft Office Excel 2016 and IBM SPSS Statistics 23 and are shown as means with standard error. Comparison of means was done with one-way analysis of variance (ANOVA), significant differences were considered at $p < 0.05$. Feed conversion rate, survival rate and estimated average daily gain were calculated as follows:

$$\text{Feed Conversion Rate} = \frac{\text{Total feed amount given (g)}}{(\text{Final biomass} - \text{initial biomass})\ (\text{g})}$$

$$\text{Survival rate} = \frac{\text{number of fish at end of trial}}{\text{number of fish at start of trial}} * 100\%$$

$$\text{Estimated Average Daily Gain} = \frac{(\text{Estimated individual end weight} - \text{estimated individual start weight})}{\text{Phase duration (days)}}$$

Estimated individual weight was obtained by weighing all the fish in one hapa and dividing by the number of fish present in the hapa. Fish were not weighed individually as this type of handling causes significant stress to the fish and can result in subsequent infection, impaired growth or death; using nets and handling fish as a group has shown significantly lower stress impact (Barton and Iwama, 1991; Foo and Lam, 1993; Pankhurst and van der Kraak, 1997).

3. Results & Discussion

3.1 Nutritional potential of black soldier fly larvae and black crickets as fish feed

Table 2 shows the nutritional profile for the black soldier flies and black crickets used in the feeding trials, it also compares these values, where possible, to reported average content values for fishmeal.

Protein % dry weight											
BSF	35.51										
GB	54.53										
FM	70.6										
Micronutrients (mg/g dry weight)*											
	Ca	Fe	K	Mg	Mn	Na	P	S	Zn		
BSF	11.60	0.06	8.85	2.54	0.10	1.14	6.67	2.37	0.05		
GB	2.03	0.08	8.05	0.76	0.04	3.11	6.32	3.78	0.12		
FM	43.4	0.37	8.7	2.3	0.02	11.3	27.9	--	0.10		
Amino Acids (% of protein)											
	Thr	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	
BSF	8.12	15.36	3.10	9.48	14.50	13.14	8.47	6.72	9.97	11.13	
GB	3.95	6.47	1.81	4.57	8.20	5.70	7.67	2.64	6.03	6.75	
FM	4.1	4.9	2.7	4.2	7.2	3.1	3.9	2.4	7.5	6.2	
Fatty Acids (% total)											
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:5	22:6
BSF	49.34	6.96	13.13	4.33	1.55	13.30	9.69	1.50	0.19	--	--
GB	--	--	24.36	0.92	7.83	28.12	36.53	1.51	0.74	--	--
FM	0.09	5.40	20.30	5.24	4.32	13.11	1.81	1.13	0.11	7.86	18.32

*Unremarkable levels of: Al, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Se, Ti
(Data for FM from: Turan and Kaya, 2007; Heuze, Tran and Kaushik, 2015)

Table 2. Nutritional profile of meals of black soldier fly (BSF), black cricket (GB) and average reported values for fishmeal (FM)

From the data in Table 2 it is apparent that both insect meals have a lower crude protein content than does fishmeal. This means that diets that replace fish meal with insect meal will need more insect meal or supplementation with another protein source, such as soybean meal, to make up the difference. There is also a large difference in the crude protein content between the two insect meals with meal from black crickets containing twice as much as the black soldier fly meal. This is probably due to the difference in the life stages of the insects. Black soldier flies are used in the larval form, when they are biologically storing more fat to prepare for future pupation, whereas black crickets are used in the fully mature adult form (Aguila et al., 2007).

It should be noted that the protein content was calculated as described in Methods, using a factor of 6.25 to convert nitrogen values to protein content but recent research shows that for insects as this potentially overestimates protein content while other findings say the factor is appropriate (Finke, 2007, 2015; Janssen and Vincken, 2017). New nitrogen to protein factors have been suggested for some insect species and if this is confirmed protein content of the insect meals used here may be over estimates.

The amount of calcium present in the black crickets and black soldier fly larvae meals was twenty and four times lower, respectively, than that found in fishmeal. This is a large difference but is probably not relevant to the tilapia as there is no evidence that they require dietary calcium; rather they attain it from the water (Dato-Cajegas and Yakupitiyage, 1996). Iron and sodium levels were both lower in the insect meal, magnesium was equivalent in black soldier fly larvae but lower in the crickets with the opposite being true for zinc. Potassium content was roughly equal across the three meal types, while manganese was higher in the insect meals.

There is limited research on the specific dietary micronutrient requirements for tilapia but they are likely to need the same micronutrients as warm-blooded animals for tissue formation and metabolism, with the most important being phosphorus, magnesium, zinc, selenium, manganese, copper and iron (Lim, 1998). In addition, high dietary calcium has been demonstrated to cause deficiency in phosphorus, zinc, iron and manganese (Mjoun et al., 2010). Mineral content in diets is generally not focused on for tilapia as they can also absorb micronutrients through the water and thus deficiencies are of limited concern (Lim, 1998; Mjoun et al., 2010). However, phosphorus has been shown to have the most impact on tilapia growth rates (Dato-Cajegas and Yakupitiyage, 1996). While the phosphorus content of the insect meals is considerably lower than that in the fishmeal it still equates to roughly 0.6% phosphorus in the diet and generally dietary values of 0.3 to 0.9% are recommended in tilapia diets, and although in soybean meal there is enough phosphorous to meet dietary needs, roughly two-thirds is phytate bound and poorly available to fish (Dato-Cajegas and Yakupitiyage, 1996).

It is well established that fish require essential amino acids, and it is the lack of one or more which often impedes the successful substitution of ingredients in feed formulations (Nguyen et al., 2009). In this study the amino acid profile of the crickets is roughly the same as that of fish meal. The black soldier fly larvae contain higher levels of all the amino acids than the fish meal (Table 2). This suggests that as far as amino acids are concerned substituting insect meal for fish meal should not pose a problem. Given that tilapia are omnivorous fish their fatty acid requirements tend to be more flexible than those of their carnivorous counterparts. While previous research has shown that diets with n-3 and n-6 fatty acids were vital for attaining maximal growth rates in juvenile tilapia (Chou and Shiau, 1999), the

majority of work has demonstrated that diets high in 18:2 n-6 have produced better growth results than diets high in 20:5 n-3 (fish oils) (Lim, 1998; Ng et al., 2003). With this in mind, the fatty acid profile of the two insect meals analysed should be favourable to tilapia diets.

3.2 Growth and survival of fish in the feeding trials

Table 3 shows the average survival rates, the estimated average daily gain and the feed conversion rate of tilapia fed on the four diets. Overall there were no significant differences ($p > .05$) between any of them.

Table 3. Total survival rate, estimated average daily weight gain and feed conversion ratio of tilapia over the entire trial (standard deviation)

Diet	Survival %	Estimated average daily gain (g)	Feed conversion rate
*Veg**	90.7 (4.2)	1.6 (0.1)	1.9 (0.2)
FM	91 (3.5)	1.4 (0.2)	2.2 (0.3)
BSF	86 (13.5)	1.5 (0.1)	2.3 (0.5)
GB	78 (22.3)	1.5 (0.2)	3.5 (2.7)

*N = 3, one replicate was lost due to cage failure resulting in fish escaping

Although not significant there does appear to be some difference between the GB diet and the other three, particularly with regard to survival percent and FCR. Fish on the GB diet tended to have more variable survival rates and a high FCR, suggesting the diet is not nutritionally ideal. While fish on the BSF diet trended towards displaying rates very similar to the more standard diets. This difference between the two insect-based diets may be due to varying amounts of chitin present. Chitin is a primary component of the exoskeleton of insects; hard-bodied insects such as crickets tend to have higher chitin contents than the soft-bodied insects such as larvae. Research on chitin content in the specific insects is sparse but data suggests *H. illucens* larvae contain approximately 57g/kg whereas crickets (*Gryllus testaceus*) contain approximately 87g/kg chitin by dry matter (Spranghers et al., 2017; Wang et al., 2005). Previous research with tilapia and other farmed fish has found significantly depressed growth rates and dietary digestibility of feeds in tilapia fed chitin and chitosan (Koprucu and Ozdemir, 2005; Lindsay et al., 1984; Shiau and Yu, 1999). It is therefore plausible that this difference in chitin is what is resulting in the differences seen here in survival and FCR.

Figures 1 and 2 show the average number of fish alive in each treatment over the three phases of the trial and the estimated individual weight of those fish, respectively. Again, there was no significant difference ($p > .05$) between any of the treatments.

Figure 1. Average number of fish alive over trial period by diet with standard deviation

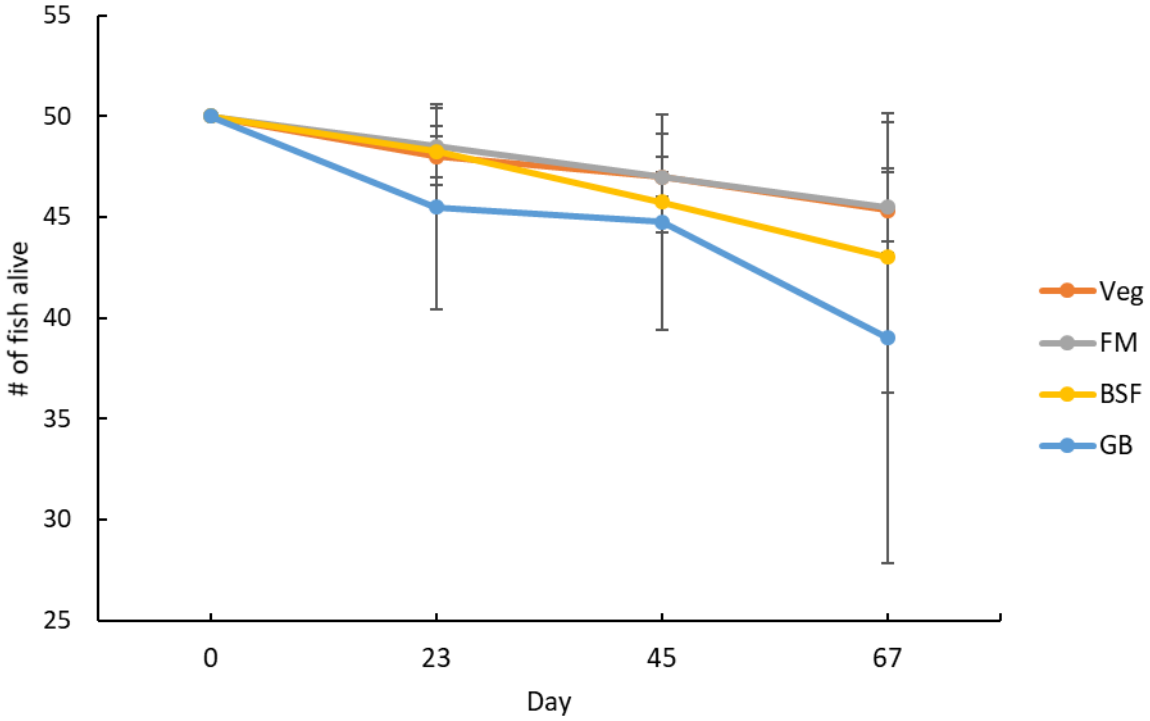
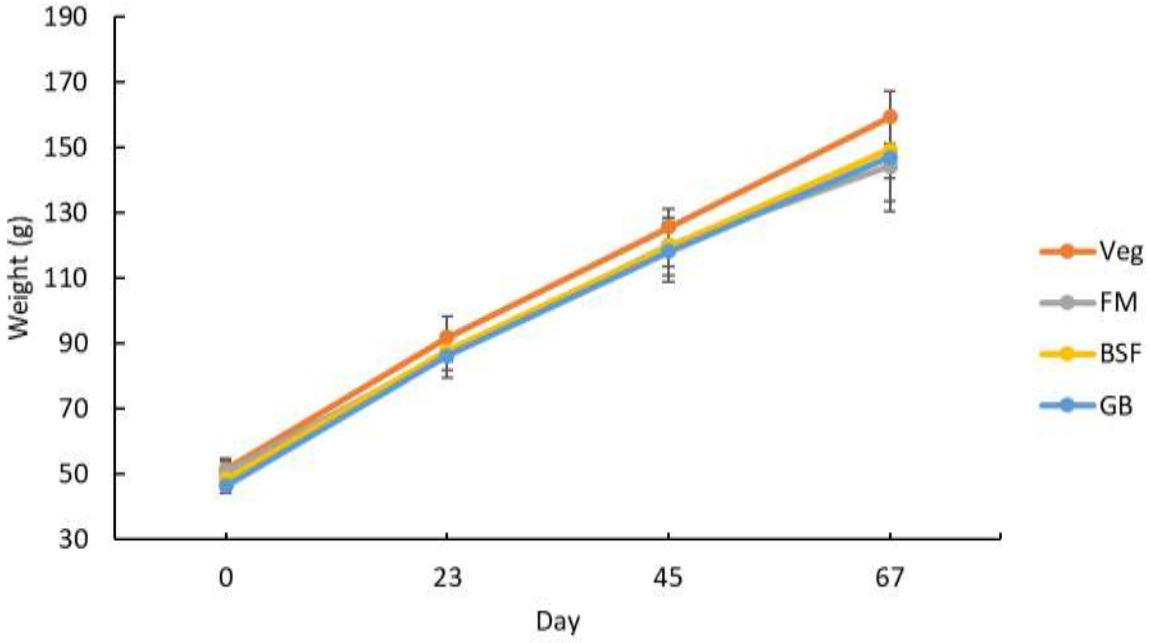


Figure 2. Calculated estimated average individual weight of fish over trial period by diet with standard deviation



When examining the estimated individual weight of the fish there is less visible disparity than seen in the survival percentage and FCR values. End weights for the fish are only slightly

higher in the Veg diet group, the other three diets displayed almost equivalent ending weights, with the insect-based diets showing marginally higher weights than the FM diet.

The weights of the tilapia in this trial cannot be compared to other research trials as the Abbassa tilapia selection line has been breed as a large fast-growing strain, growing generally 28% faster than traditional tilapia strains (Ibrahim et al., 2013; Khaw et al., 2009; Rezk et al., 2009). However, given the FM diet was formulated to an industry standard, the growth of the tilapia in the FM diet treatment serves as a comparative control. The insect-based diets result in estimated end weights at or above the weight produced by the FM diet, indicating these diets are likely suitable alternatives from an individual weight perspective.

4. Conclusion

The results from this trial demonstrate that fish-meal can successfully be replaced by insect-meal in tilapia diets, and that growth rates and FCRs will be preserved and will be comparable to the already developed vegetable-based diets. This is an encouraging result for the development of insect-based locally sourced diets for fish rearing. However, future trials should focus on seeing if diets can be improved by optimising inclusion rates of insects in feed formulations, it is possible that a higher rate or combination of multiple insect species might create more successful and sustainable formulations. The more feed ingredients can be produced or sourced locally the greater the positive impact on the aquaculture industry and socio-economic status of farmers. The significant costs of importing fish feed are also the major hurdle to increasing aquaculture production in Africa, particularly sub-Saharan Africa – where identifying locally available feed materials provides an opportunity for aquaculture to reach its full potential and provide a vital nutrition source while reducing the negative impacts of relying on mass grain production of maize and soybean (Dickson and Henriksson, 2016; Gabriel et al., 2007).

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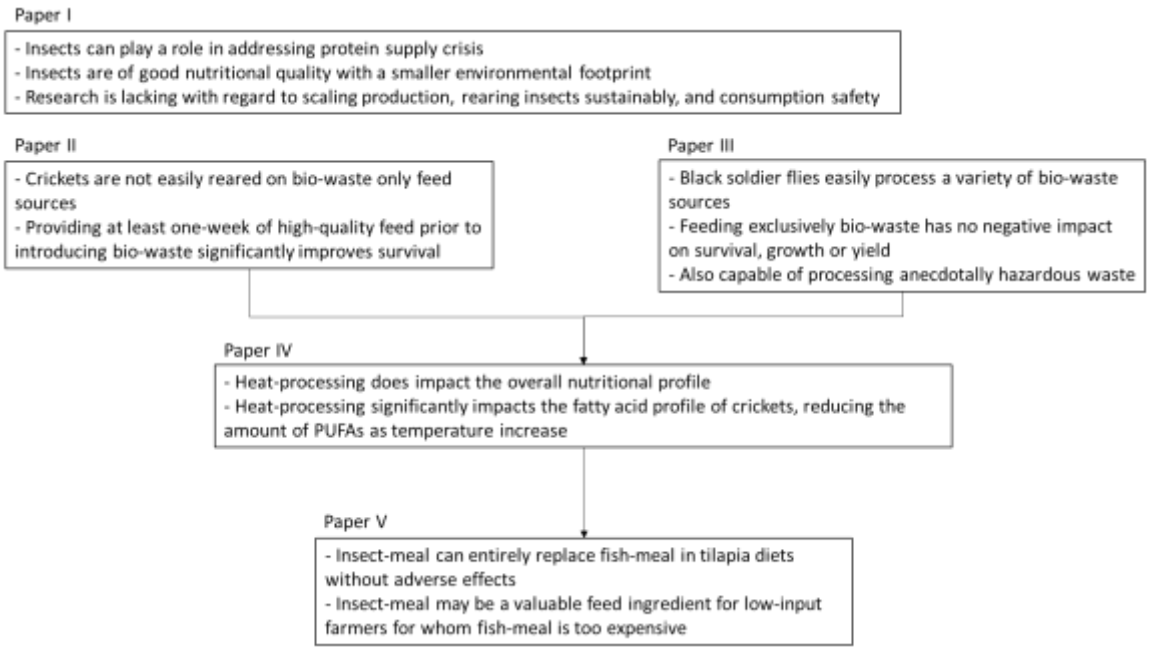
Discussion

General findings

The overall aim of the study reported in this thesis was to evaluate the viability of insects as food and feed. The studies are presented as a series of papers, and a summary of the key findings is given in Figure 1.

Figure 1. Summary of main findings from each paper.



The results presented in Paper II initially showed that *Gryllus bimaculatus* (black crickets), a species which is widely farmed and consumed (Halloran et al., 2018, 2017; Hanboonsong et al., 2013), demonstrated significantly lower survival when reared on beer waste and vegetable waste food sources. However, it was further demonstrated that this high mortality can be partially mitigated by a feeding regime that starts with high quality feed prior to switching to one of the lower quality bio-waste feeds. While survival rates did not match the control groups, they were improved significantly when even one-week of high quality feed was incorporated.

Paper III reports that when *Hermetia illucens* larvae were fed on diets composed of several bio-waste sources (brewing waste, chicken offal, cottonseed cake, sunflower seed meal and groundnut cake) their survival and growth was not significantly impacted. In fact, the larvae were even able to process the environmentally hazardous Ugandan waragi brewing waste

(Elunya, 2009; Kasamba and Mugo, 2007; Ssekika, 2015) up to inclusion levels of 85%. Using waragi waste as a feed source would both produce livestock feed and recycle a waste material that is otherwise difficult to get rid of. The findings from papers II and III show that there is promise for *G. bimaculatus* and *H. illucens* production but emphasise the need for further research to achieve sustainable, industrial scale production systems.

Paper IV reports the impact of heat processing on the nutritional quality of *G. bimaculatus* and found that whilst it did not alter protein or micronutrient levels it did significantly impact fatty acids. Generally, the higher the temperature used for the insect processing, the fewer beneficial polyunsaturated fatty acids were present, making them potentially less nutritious and the lower totally fatty acid content reducing their energy content. Overall the work shows that producing insects as feed is complex and both quantitative and qualitative outcomes need to be optimised and that some assumptions about the nutritional value of edible insects may need to be re-assessed.

Following on from this, paper V reports a study that looked at the practicalities of replacing fish-meal with two different insect-meals, *Hermetia illucens* and *Gryllus bimaculatus*, in the rearing of tilapia as a food source for human consumption. No differences in survival or growth rates were found between fish fed on fish-meal and fish fed on insect-meal based diets, raising the exciting prospect of using these two meals as potentially more sustainable, cheaper alternatives to fish-meal. This could offer a much more sustainable method of food for human consumption in many countries where protein sources are limited.

The overall hypothesis of the work presented here was that insects have the potential to play a vital nutritional role within existing food systems. However, the results have been mixed, and in some cases contradictive to pre-existing assumptions (in survival on bio-waste, in nutritional manipulation) making it difficult to formulate sustainable protocols for increasing the use of insects. Yet, within these findings there are several key lessons which can be taken away to guide future work.

Insect Production

One of the key selling points for the increased integration of insects into existing food systems has been the assumption that they are easy to rear and can offer a use for waste materials (DeFoliart, 1997; Meyer-Rochow, 1975; van Huis et al., 2013). It is an assumption

which has been repeated frequently, with increasingly innovative ideas for sourcing bio-waste feeds but with limited data regarding viability outside of *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, and *Acheta domesticus* (Li et al., 2013; Meijer et al., 2017; Murugan et al., 2016; Pastor et al., 2015; Ramos-Elorduy et al., 2002; van Zanten et al., 2015; Varelas and Langton, 2017; Zhang et al., 2012). Before the present study, the only other large scale cricket trial found that crickets were not capable of being reared successfully on singular vegetarian organic waste sources (Lundy and Parrella, 2015). These findings are strongly supported by the results in Paper II, where survival was low when crickets were reared on a vegetarian pre-consumer bio-waste source from hatching. Trials to date which found success of rearing crickets on bio-waste sources, similar to Paper II, have only introduced bio-waste feed after a certain number of days, or made use of a more omnivorously focused diet, extensively processed on a lab-scale crickets have never been successfully reared side-stream feed sources from hatching on a larger scale and this has notable repercussions (Collavo et al., 2005; Miech et al., 2016; Nakagaki and Defoliart, 1991).

However, it is acknowledged that it would be naïve to expect a single feed source, bio-waste or otherwise, to be nutritionally adequate from start to finish, just as providing a person with only oranges for their entire life would not result in positive outcomes or constitute an appropriate diet. Although many crickets are predominantly herbivores, they are still omnivorous and have displayed large variability in dietary choice, even when herbivorous they will eat the blossom, fruits and leaves of the plants, rather than just a single component (Walker and Masaki, 1989). A study has demonstrated the benefit of dietary diversity to development in grasshoppers, (Malinga et al., 2018a).

However, even when fed diets with variety, cricket survival was still rather low (Paper II). There are two possibilities to explain this. Firstly, the natural survival rate of insects in the wild is low, as such they are biological wired, with high fecundity, to produce a high number of offspring to ensure that enough make it to adulthood to reproduce (Berger et al., 2008; Southwood et al., 1974; Thornhill and Alcock, 1983). So just because we are seeing what is perceived as a low survival rate, it does not necessarily mean that the feed is unsuccessful, in fact it may be reflecting wild population survival rates. Furthermore, the main reason these survival rates are considered to be low is because of the benchmark used for

comparison, i.e. crickets in industrial production. Crickets in industrial production are fed predominantly on chicken feed, which, given its high protein and micronutrient content, is arguably the cricket equivalent of a body-builders diet and therefore likely to artificially elevate survival rates compared to wild populations. Other studies have already highlighted the importance of protein within insect diets and this is potentially the largest limiting factor of bio-waste diets (Oonincx et al., 2015).

A final consideration that arises from Paper II is the feed conversion rates of crickets. Coupled with the claim that insects can be fed on bio-wastes, is the claim that they have very efficient feed conversion ratios (FCR) (DeFoliart, 1992; Nakagaki and Defoliart, 1991; van Huis, 2011; van Huis et al., 2013). Again, this has previously been refuted by empirical evidence with crickets having feed conversion ratios not substantially better than larger livestock, e.g. beef cattle, and only showing conversion rates on a par or better than all livestock when calculations were controlled for the estimated edible portion (Oonincx et al., 2015). Broiler chickens average a FCR less than two (Best, 2011), and the present study found that crickets on high-quality feed, high in protein, consistently had an FCR just above two, which is in line with previous trials. This strongly suggests that previous claims of insect FCRs are an overestimate, at least within the current feeding practices (Collavo et al., 2005; Nakagaki and Defoliart, 1991; van Huis et al., 2013). It is worth noting that the feed presently considered to be a high-quality control feed for crickets is chicken feed (Lundy and Parrella, 2015; Oonincx et al., 2015), however this is and always will be a feed designed for the nutritional requirements of chickens, not crickets. If you provided pig feed to poultry or vice versa the FCRs would also not be what is anticipated for the species the feed was designed for. The counter argument to this is that a higher percentage of each insect is edible, generally upwards of 80%, whereas less than 60% of most other livestock is consumed (van Huis, 2011), meaning a slightly higher feed conversion ratio does not necessarily make insects inefficient, rather more relevant measures such as protein efficiency of food conversion should also be considered.

It is evident from these findings and previous studies that the process of rearing crickets on bio-wastes is not as simple as anticipated by proponents of edible insects and increasingly research gives the impression that bio-waste feeds are not nutritionally adequate to sustain cricket populations. However, this is a somewhat inaccurate impression. To date the

majority of researchers have made their own feed mixes based on some available data and tested these, rather than taking a more systematic approach. It is clear these mixes are lacking in some nutritive or sensory way but due to a lack of systematic testing it is unclear what exactly is missing. Additionally, data from Paper II suggests that, as with other conventional production livestock, crickets may have differing nutritional requirements at different life stages and that earlier stage nutrition is the most critical to survival and growth. Without first creating a full nutrient requirement profile for crickets, or the desired production species, and using this to design and match feeds using novel bio-wastes will always be a guessing game.

The data presented in Paper III demonstrate that other insect species do not necessarily have the same problems with utilising bio-waste as do crickets. *H. illucens* larvae have repeatedly, in this trial and previous work, been shown to be capable of processing a variety of materials generally without any negative repercussions to their survival or growth as long as key protein and moisture parameters are maintained (Banks et al., 2014; Diener et al., 2009; St-Hilaire et al., 2007; Tomberlin et al., 2002). However, when insects are considered for human consumption species such as *H. illucens* are ruled-out due to a perceived lack of acceptability , even though research supports the view that they can easily be incorporated into existing food products without compromising quality (González et al., 2018; Wang and Shelomi, 2017).

This raises a very important dilemma. If we are going to try to put insects directly into human food do we choose a species which is seen as more appealing by 'Western' standards, but is difficult to rear, or one which may be less acceptable but is proving repeatedly to be more sustainable and easier to produce. This issue of acceptances is complex with 'Western', high-income countries often having very different attitudes towards consuming insects than do the majority of countries which presently consume insects; to the point where it has at times encouraged the spread of negative attitudes towards insects as food and thus discouraged their continued consumption in a country which previously accepted them (Caparros Megido and Sablon, 2014; Defoliart, 1999; Evans et al., 2015; Yen, 2009).

Excessive focus, positive or negative, on the 'Western' preference of how to use or rear insects as food or feed, runs the risk of impacting attitudes and consumption behaviours in

other countries (Wilderspin and Halloran, 2018). This focus comes foremost through the language surrounding insects as food. A distinct term, 'entomophagy', adapted from the field of entomology, had previously only been used to describe insects who ate other insects, but has been adopted by 'Western' researchers to distinguish eating insects from other food sources, setting a precedent of 'otherness' throughout the field (Looy et al., 2014). The term was originally used specifically to denote an eating habit considered out of the 'ordinary' by the researchers who encountered it in an anthropological setting (Evans et al., 2015). A term for the eating of insects is missing from the majority of cultures where insects are eaten frequently and accepted as the norm, much in the way Europeans eat crustaceans and have no distinct term for this (Evans et al., 2015). The language and descriptions used around a food item, novel or familiar, have been shown to strongly influence the appeal of the food itself and attaching the word 'entomophagy' to insects as a food source may continue to be detrimental, unless the negative associations can be shifted (Martins and Pliner, 2005; Mielby and Frøst, 2010; Rozin and Fallon, 1987; Tuorila et al., 1998; Wansink et al., 2005).

Further evidence suggests that the current approach to integrating insects into food systems, where they are not current is perpetuating existing power relations (Müller et al., 2016). The global distribution of research authors working on edible insects is in stark contrast to the global distribution of insect consumption cultures; with the research field being dominated by authors from non-insect eating cultures in high-income countries, able to access and compete in English-language publications, thus elevating their 'voice' to a higher level than their counterparts in middle to low income countries (Müller et al., 2016). If this trend continues, the field runs the risk of inadvertently forming internal power groups (Crane, 1969; Jonathan et al., 2004) resulting in the research being guided by individuals from non-insect eating cultures who pose questions out of line with what insect eating cultures are truly interested in and could benefit from (Payne and Kinyuru, 2018).

Future Directions

Whilst studies to date have shed light on some of the challenges of domestic insect husbandry there is a clear need for more work. If there is a determination to continue pushing for industrial scale farming of species such as crickets, research should refocus on fundamental building blocks. Future research needs to return to the basics of feed

development and start from the bottom up, by looking at what the natural diet of the insect would be, rather than attempting to work top down from the current practise of using chicken feed. By examining what a natural diet would be efforts can then focus on identifying what the specific nutritional requirements of each insect species are, as has been done with conventional production animals. Systematic approaches to diet determination for insects have been used in the past, studies should return to these foundations and build on them (Fraenkel, 1955; Fraenkel and Leclercq, 1956; Ito and Fraenkel, 1966; Patton, 1978, 1967; Tyree et al., 1976). Research has shown that no-choice and multi-choice dietary acceptance bio-assays can be used with insect species to establish preferred feeds (Malinga et al., 2018b) and this can be easily and efficiently applied to several species to form a starting point for feed development.

Comparing survival results from feed trials to industrial survival rates for crickets is not technically a fair comparison when aiming to identify diets which work. Current industrial practice is focused on producing the most crickets, rather than the most efficient crickets, meaning they use diets, such as chicken feed, which are ultimately not sustainable if production is to be scaled up. Although meeting or exceeding current industrial survival and growth rates is the end goal, steps must first be taken to identify what improves cricket survival at a basic level compared to wild survival rates. Continued work should aim to identify which nutritional manipulations have which impacts on survival and growth outcomes. Onwards from this, continued efforts must be made to establish what the critical life stage nutritional requirements are, much as has been done with other livestock, and some insect species (Fraenkel and Leclercq, 1956).

Next to this, research needs to be rigorous in quantifying and illustrating the mechanisms of producing and processing individual insect species to facilitate others in making educated assessments of what the ‘ideal’ insect species is for their given situation, be that large scale production or subsistence farming; rather than just using species deemed to be the most desirable. Traditional knowledge from insect-eating communities (Hanboonsong and Durst, 2014; Niassy et al., 2016) should be strongly integrated into this work to ensure that outcomes provide maximum benefit across that food system and not just in one environment. Both researchers and funders need to give platforms to researchers and individuals from existing insect-eating communities to mitigate the perpetuation of existing

power relations and to avoid selfishly appropriating edible insects(Müller et al., 2016; Payne and Kinyuru, 2018).

Insect Use

Human Consumption

Besides the issue of producing insects on a sustainable global scale there is a much more basic question of whether or not insects should be consumed as a food, particularly in regions which do not presently consume them. This question has three components, firstly the nutritional and/or health appropriateness, secondly the safety of consumption and third cultural and sociological acceptance. The work presented on this thesis has made a contribution to the understanding of the first of these components.

Nutritional Aspects

The nutritional composition of edible insects has been discussed frequently, with the general consensus that they hold potential to positively contribute within diets (Bukkens, 1997; Rumpold and Schlüter, 2013). In fact, studies have suggested a use in enriching otherwise nutritionally poor diets (Banjo et al., 2006; Bodenheimer, 1951; Kinyuru et al., 2009; Konyole et al., 2012; Ramos-Elorduy et al., 1997; Santos Oliveira et al., 1976; Tao and Li, 2018). If insects are to be used as a supplementary food, particularly in situations of nutritional vulnerability there must be sound information on their nutritional profile, yet a great deal of the data available reports large margins of variability which warrant further exploration (Dobermann et al., 2017; Payne et al., 2016). To our knowledge, the study in Paper IV is the first concerted effort to examine the potential implications of subsequent heat processing on the nutritional profile of edible insects. This shows that a large portion of the variability can potentially be accounted for by how insects are processed post-harvest. This is in line with previous work which demonstrated that low-level heat processing is needed to preserve fatty acid profiles (Fombong et al., 2017). This has a key implication for the future utilisation of insects as food or feed. If insects are to be used as a food source within populations which are already nutritionally vulnerable as a nutritional intervention food, particular attention must be paid to how the insects are processed to ensure there is no notable disparity between batches of food. The production of insect for food needs to be as stringently regulated as the production of any other animal-based protein source

presently being used to ensure the nutritional claims being made to consumers are being met.

The reality is that insects cannot, and should not, be introduced as a food source in a time of crisis unless we are sure they will make a consistent nutritional contribution to the diet. There are myriad of claims surrounding potential health benefits of edible insects, yet the reality is that few trials have been conducted in humans or using standardised assays accepted by clinical health research (Roos and van Huis, 2017).

Previous work to examine the economic and social benefits of introducing insect farming in vulnerable populations (Halloran et al., 2018; Naukkarinen, 2016; Weigel et al., 2018), found hurdles associated with the high input costs and difficulty in tracking benefits but did not look at the nutritional aspects of the insects used in the studies or how these related to the feed used. It is irresponsible to recommend insects as a food item to counter micronutrient nutritional challenges (Tao and Li, 2018) if clearer answers are not available with regard to how exactly processing or other even rearing methods impact their nutritional profile.

Safe Consumption

It is usually assumed that because an estimated two billion people traditionally consume insects (van Huis et al., 2013), without any obvious ill effects, they must be safe to eat by all populations. However, there is evidence to suggest that environmental factors, dietary patterns, and even exposure to infection may play a role in the ability of individuals to safely consume certain foods and/or the development of allergies (Cochrane et al., 2009; Sicherer and York, 2011). In addition, increasingly evidence is showing that early childhood exposure to foods shapes subsequent allergies; consistently it is demonstrated that the later a food is introduced into the diet the higher the risk of an allergy (Du Toit et al., 2008; Katz et al., 2010; Koplin et al., 2010). Which raises a double-edged issue, it suggests that individuals exposed to insects for the first time later in life are more likely to have an allergic reaction but simultaneously suggests that if we want to incorporate insects into our diet we should perhaps expose children to them earlier to mitigate potential allergies. This coupled with the results from Paper VI, which demonstrated a significant in-vitro cytotoxic effect of insects, suggests that whether or not insects are safe to eat across global populations may not be straightforward.

Whilst one trial showed that feeding insects to rats gave no adverse effects (Han et al., 2016), there have been documented negative reactions following insect consumption, though it was unclear if the reaction were directly linked to the insects or general food spoilage (Chomchai and Chomchai, 2018). At present there is no robust epidemiological data available to identify consistent risk factors for adverse reactions to consuming insects. Trials have made progress in identifying allergens (Ribeiro et al., 2018; Srinroch et al., 2015) however allergy is only one aspect of safe consumption.

It was assumed for years that humans were incapable of digesting chitin, however a digestive enzyme, acidic mammalian chitinase (AMCase), has been identified which breaks down chitin (Paoletti et al., 2007). It was established that individual humans display differing levels of activity of AMCase and that it is considerably less active in humans than is the corresponding enzyme in other primates; where insects form a larger more regular part of the diet. The argument that primates can safely consume insects is often used to argue for human insect consumption. However, chitin studies, examining the *CHIA* genes which encode AMCase, have identified species differences in *CHIA* functionality, namely that the more insectivorous a species the more functional copies of *CHIA* genes are present (Janiak et al., 2018; Krykbaev et al., 2010; Paoletti et al., 2007); demonstrating that direct inferences shouldn't be drawn between different primates. Also given the differences seen in the activity levels of the chitin digestion enzyme within a human cohort it is reasonable to assume that these differences continue across populations and are perhaps geographically influenced, such as has been seen with lactose intolerance (Cavalli-Sforza et al., 1987; Simoons, 1978; Yatsunenko et al., 2012). The question that arises from this is whether it is a matter of upregulation or not, meaning, if an individual consumes more insects would this result in higher more active levels of AMCase. If this is the case then we arrive at a bit of a chicken-egg dilemma, where we potentially have to tolerate ill effects in order to ultimately be able to consume insects.

Future Directions

Insects cannot be introduced as a food with the assumption that a given species will continually have the same nutritional profile regardless of what it is fed on and how it is subsequently processed. While it is reasonable to assume that new introductions of insects are likely to come from production systems it is the consistency of production method of

these systems which is vital. For example, if a production system endeavours to make use of pre- or post-consumer food waste there is bound to be variation in waste composition which could translate into nutritional variation between insect production batches. For this reason, research needs to focus on if and how nutritional quality changes occur in specific insect species in order to offer appropriate production guidelines. A series of systematic feeding trials, in which diets are manipulated in detail, and which analyse the subsequent nutritional profile of the insects would serve best to elucidate the relationship between feed and insect.

If insects are intended to significantly improve household nutritional status of small-holder farmers either rearing them on farm or in low-middle income communities, then research must follow through with studies to establish if this will actually work. To date, trials have introduced interventions with nutritional status in mind (Weigel et al., 2018), however due to a lack of comprehensive follow through and a thorough understanding of insect nutritional profiles these have shed little light on the impacts insects can have.

There is increasing evidence that traditional post-harvest processing methods serve to preserve the nutritional profile of insects and protect against microbial risk (Lautenschläger et al., 2017; Mutungi et al., 2017; Raheem et al., 2018). However, while information is available on traditional preparation methods and recipes, it is sparse in comparison to the number of communities preparing and consuming insects (Acuña et al., 2011; Costa Neto, 2013; Raheem et al., 2018; Santos Oliveira et al., 1976; Srivastava et al., 2009; Yen, 2009). Continued work should aim to collect and aggregate data on traditional methods of processing and research should draw from established methods rather than attempt to reinvent the wheel. These traditional methods also need to be more consistently combined with further analysis, such as nutritional or microbial safety, to provide results which reflect reality rather than lab conditions (Lautenschläger et al., 2017; Madibela et al., 2007).

Furthermore, it is becoming increasingly evident that assuming everyone, allergies aside, can safely consume insects may be an irresponsible assumption, as seen by the increasing identification of novel allergens, cytotoxicity and negative reactions to insect ingestions (Barre et al., 2014; Chomchai and Chomchai, 2018; Lee et al., 2015; Srinroch et al., 2015). There is a significant lack of data on safe consumption and what the risk factors are, particularly in more vulnerable populations such as children. Several trials have forged

ahead and used insects as a food ingredient in vulnerable populations (Bauserman et al., 2015; Homann, 2015), generally focusing on acceptability of food items and not what happens when consumption continues over time. It is paramount that future trials focus on identifying risk factors and mechanisms of any negative reactions. Two approaches could be taken: 1) trials which have previously focused on acceptability of insect food items could be extended to follow cohorts for longer periods of time and compare potential effects 2) there are already populations in place which have consumed insects for an extended period of time, and there is likely potential within these populations to conduct cohort studies which control for confounding factors, such as being rural or urban based. Additionally, with the appropriate, extensive controls in place it may be possible to compare these populations to others which have not been consuming insects for an extended time.

These could provide a way of identifying individual characteristics, particularly the gut microbiome and digestive enzymes, to better understand where potential problems there may be. This type of data collection requires a large-scale collaborative effort by researchers but would be invaluable in progressing the field.

Conclusions

Assuming the need for caution in introducing insects into human diets, it would perhaps be prudent to suggest that the ideal immediate place for insects in our food system is as an animal feed, as most livestock already consumes insects as a part of their natural diet. Data in Paper V, just as in previous trials, demonstrated that insects can easily be used in fish feed without any negative repercussions (Henry et al., 2015; Tschirner and Kloas, 2017). Whilst the nutritional value of insects is also vital in the formulation of animal feed the possible repercussions of any unforeseen variation in nutritional profile, are likely to be less profound than in vulnerable human populations such as children.

Using insects as an animal feed, as opposed to a direct food, carries several benefits supported by research. Foremost it introduces a step of protection for people while the research catches up with the health claims and it ensures that insects can be safely used within food systems. Second, insects can provide just as much benefit being used as an animal feed, in the right production system, since feed for livestock is one of the big prohibitive costs small-holder farmers face, particularly in low-middle income countries

(Chakeredza et al., 2008; Devendra and Sevilla, 2002; Mcdermott et al., 2010; Salem and Smith, 2008). Using insects such as *H. illucens* to process and recycle waste provides farmers with access to a high value protein feed stuff for their livestock which they may not otherwise have. This could take the form of: 1) specialised insect production systems where the output is purchased by farmers or feed companies 2) farmers adopting insect production technology individually to farm insects on-site and use back into their own farm systems. Both models have the potential to contribute positively to the security of global food systems while empowering farmers and local communities if the above key concerns regarding the appropriation of insects is kept in mind.

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