



Black soldier fly larvae as a sustainable animal feed ingredient in Kenya

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Black soldier fly larvae as a sustainable animal feed ingredient in Kenya

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To my beloved family

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Chapter 1

General introduction

In low-income countries where the poor rely on agriculture for livelihood, intensification of agricultural production to develop profitable and inclusive fish and livestock enterprises is one way to increase food production and reduce poverty (FAO et al., 2012). Poultry, pig and fish farming are among the fastest growing agribusinesses in Kenya, offering employment and sources of livelihood to the growing population (Bizna, 2019). The poultry sub-sector contributes about 55% to the livestock sector and accounts for about 30% of the agricultural gross domestic product (GDP) of Kenya. The sub-sector employs two to three million Kenyans (Omiti & Okuthe, 2009). Pig keeping is another lucrative business with approximately 350,000 pigs and 7000 pig farmers in Kenya, of which 70% consists of smallholder farmers (Mburugu-Mosoti, 2014). In addition, fish farming in Kenya employs 67,883 farmers with 69,194 stocked fish ponds. By the end of 2014, the national aquacultural fish production in Kenya was estimated at 24,100 metric tonnes, up from 12,200 metric tonnes in 2010 (FAO, 2016). Therefore, the contribution of these sub-sectors to rural and urban livelihoods as sources of food, income, nutrition and insurance against emergencies are of paramount importance.

Human population growth, rising incomes and urbanization are major drivers of the surge in global demand for highly nutritious protein products such as meat, fish, eggs and milk (Steinfeld et al., 1997). The annual global feed production is estimated at one billion tonnes, representing an annual turnover value of over US\$ 400 billion. The FAO projects that feed production will have to increase by 70% to be able to feed the world in 2050, as meat and fish demands are expected to double (IFIF, 2017; Van Huis et al., 2013). Much of the demand for meat in Kenya can be met through increased poultry, pig and fish production. For example, the rapidly growing urban Kenyan population with higher earnings increasingly spent more on animal products such as dairy products, meat and eggs (FAO, 2017). This growing demand is expected to continue following increased urbanization and income earnings across the population, causing farmers to adopt productivity-enhancing technologies (FAO, 2017). The demand-driven transformation of animal production provides opportunities for the development of the Kenyan economy. Animal producers could improve their livelihoods and the development of the poultry, pig and fish sub-sectors could generate substantial socio-economic benefits, such as job creation and employment for both rural and urban populations (FAO, 2017). However, this transformation in the animal production industry will result in novel interactions between humans, animals, land and the environment (Crist et al., 2017). In Kenya, for instance, 1.8 million urban and peri-urban households currently keep poultry, pigs and dairy cows, but waste management practices remain underdeveloped or inappropriate causing environmental and health problems. This situation is likely to continue and over 6 million households are expected to be involved in livestock production by 2050, representing an unprecedented increase with implications for feed supply, health and the environment (FAO, 2017).

The challenge in animal nutrition is the availability of sustainable feeds that enable farmers to meet the rising demand for highly nutritious animal products. Ingredients for animal feed include soybeans, fish oil, and several grains, with fishmeal being the major protein source. However, a major constraint to a sustainable production of the increasingly demanded fish and meat products is that land for soybean cultivation is diminishing globally, while the marine overexploitation has continued to reduce the abundance of the small pelagic forage fish, from which fishmeal and fish oil are derived (Masuda & Goldsmith, 2009; Tacon & Metian, 2008). The growing demand and scarcity of resources to produce fishmeal and soybean meal have led to increased prices of these ingredients, while feed cost, representing 60-70% of animal production costs is already prohibitive and cannot be afforded by resource-poor famers (Hilali et al., 2011; Kumar et al., 2017; Van Huis et al., 2013; Villasante et al., 2013). This situation is also affecting the livelihoods of producers in Kenya, especially the smallholder producers. It will therefore, not be a sustainable option to continue to rely on fishmeal and soybean meal as protein sources in feed production (Van Huis et al., 2013). The Kenyan industry is searching for alternative protein sources for its growing poultry, pig and aquaculture sub-sectors and there is much interest in possible replacements for these expensive ingredients (Githigia et al., 2012). Hence there is a need for viable and sustainable alternatives.

Insects are traditional food sources for humans, poultry, pigs and fish and their use as a feed component has long been proposed (DeFoliart, 1989; Farina et al., 1991; Johnson & Boyce, 1990; Newton et al., 1977; Whitmore et al., 1986). Recently, scientists, academic institutions and policy makers including the FAO have reviewed and recognized the role of insects as food and feed (Van Huis et al., 2013). Most insect amino acid contents are comparable to fishmeal and soybean (Rumpold & Schluter, 2013). Black soldier fly (BSF) larvae, housefly larvae, and yellow mealworms are promising for industrial animal feed production, among which BSF larvae have superior properties for mass rearing for feed (Van Huis et al., 2013).

The black soldier fly *Hermetia illucens*

The black soldier fly *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae) occurs worldwide in tropical and temperate regions (Cickova et al., 2015; Sheppard et al., 1994). The adult is a black, wasp-like fly, ranging between 13-20 mm in size (May, 1961). There are two translucent cuticular “windows” located on the first abdominal segment and the adult black soldier fly possesses bright, white tarsi. The last abdominal segment of males ends in a plate-like structure while in female BSF the ovipositor protrudes at the last abdominal segment. Adults do not approach or enter human habitats and houses and do not constitute a nuisance. The adult flies only ingest water and flower nectar and this substantially reduces the chances to spread pathogens to humans. The adults are

pre-occupied with mating and egg-laying (Sheppard et al., 2002). They do not sting and are not considered to be disease vectors (Cickova et al., 2015; Sheppard et al., 2002).

The BSF develops through five developmental stages including: egg, larval, pre-pupal, pupal and adult stages. The BSF larval phase is divided into six instars (Figure 1). An instar refers to the period between two moulting events that involve renewing of the exoskeleton. During the larval period fat reserves are accumulated to meet the nutritional requirements of adult flies (Myers et al., 2008). Kept at 28 °C, the development time from egg to adult typically ranges between 40-43 days (Tomberlin et al., 2002). The female BSF deposits about 500 eggs in cracks and crevices near or in decaying matter (Diclaro & Kaufman, 2009). The larvae hatch in 3 - 4 days. They feed on organic matter and in about 14 days, they develop to pre-pupae. The pre-pupae disperse from the feeding substrate to sites that are suitable for pupation. The exoskeleton darkens and a pupa develops, from which an adult fly ecloses within approximately 14 days. Approximately two days after adult emergence, the male and female flies are ready to mate (Hall & Gerhardt, 2002).

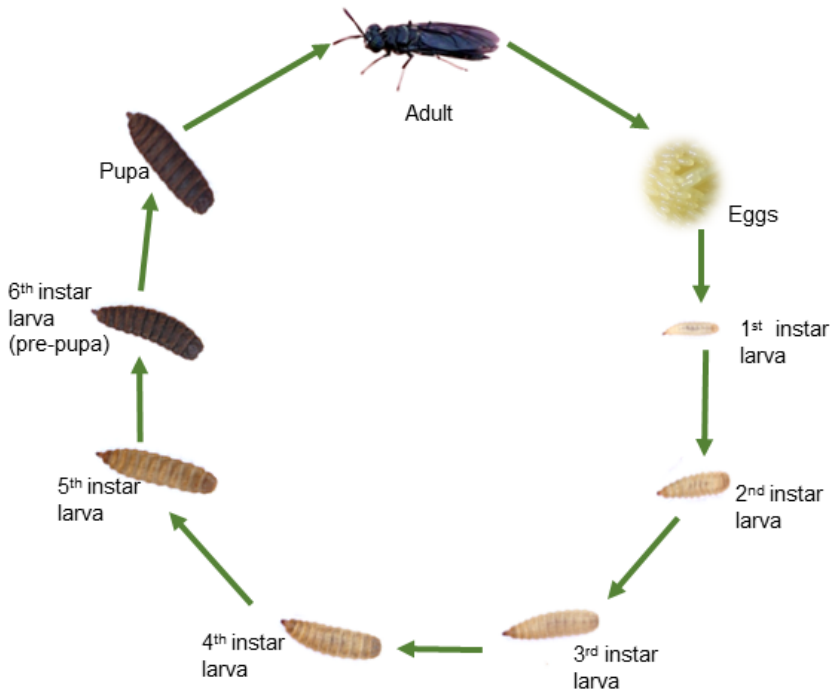


Figure 1. Life cycle of the black soldier fly *Hermetia illucens*. Sizes of different developmental stages depicted are not proportional.

Environmental conditions

Temperature is the most important abiotic factor that influences insect development and overall performance. Insects are particularly sensitive to abiotic conditions due to their small size and proportionately large surface area that increases water loss (Scharf et al., 2015). The BSF has a temperature-dependent pattern of activity. For instance, in tropical regions, the BSF is active throughout the year, while in temperate regions, the insect is only active during the warmer periods of the year (Holmes, 2010). Therefore, modeling its development as function of temperature allows for optimizing their use in waste management and confined animal feeding operations (Holmes et al., 2012). The survival, feeding, development and reproduction of insects are affected by the ambient temperatures. It has been demonstrated that over 95% of BSF larvae reared on a grain-based diet at 27 – 30 °C survive to adults while at 36 °C, less than 1% of the larvae survive (Tomberlin et al., 2009). In another study, where larvae of BSF were reared on three diets at three different temperatures, i.e. 24.9 °C, 27.6 °C and 32.2 °C, temperature and diet significantly affected all immature stages and the number of degree hours required to complete a particular growth stage as well as the final larval weight varied across diets and temperature regimes. At about 28 and 32 °C, larvae required less degree hours and had approximately 30% higher final larval weight than at 25 °C (Harnden & Tomberlin, 2016). The lower temperature range for BSF egg hatching is reported to be 12 - 16 °C with egg hatch in 15 days, while the lower range for larval development is 16 - 19 °C with eggs hatching in about eight days at 19 °C. Mean development time from egg to adult at 19 °C is 72 days (Holmes et al., 2016). This information is important for understanding growth performance, yet limited to only a few temperature regimes and various diets. Therefore, investigating a wider range of temperatures to determine the optimum as well as minimum and maximum threshold temperatures is crucial for optimal larval production to meet the growing demand for a high-quality protein source and innovative waste management strategies.

Demand for insect-based feed

An important consideration for research, policy and commercial production is whether the target users of the proposed novel insect products are aware of and willing to pay for them. A proper understanding of the characteristics that influence the consumers' demand for a product will help in the development of a product that targets those most likely to accept and benefit from it (Channa et al., 2019). In this regard, the success of adoption and use of insect-based feed for animal production as well as use of resulting animal products will largely depend on farmers' knowledge and their willingness to pay (Verbeke et al., 2015). However, information on these attitudes is limited, yet necessary for commercial animal production in Africa.

Research objectives

The main objective of this thesis is to assess the potential of BSF larvae as a feed component and to investigate farmers' willingness to accept insect-based feed for animal production in Kenya. The specific primary objectives of this thesis are:

1. To assess farmers' knowledge, attitudes towards the use of insects and their willingness to pay for insect-based feed in poultry, pig and fish production in Kenya (Chapter 3)
2. To evaluate the effect of agro-industrial by-products on the performance of BSF (Chapter 4)
3. To determine the threshold temperatures for the development of BSF reared on agro-industrial by-products (Chapter 5)
4. To assess the nutritional quality of BSF larvae reared on agro-industrial by-products (Chapter 6)
5. To evaluate the growth performance, health and carcass yield of growing-finishing pigs fed insect-based feeds (Chapters 7 & 8)

Outline of this thesis

Chapter 2 presents an overview of the potential of insects as feed and their contribution to sustainably reshaping global food systems into efficient, climate resilient and nutrition-driven models. It also assesses how insects, with a focus on the BSF, can improve livelihoods of smallholder farmers, while mitigating environmental pollution and emphasizes how and why inclusive business involving edible insects aligns with the Sustainable Development Goals (SDGs).

Chapter 3 assesses farmers' knowledge and attitude towards insects as feed, their acceptance of insect species and willingness to pay for insect-based feed by a contingent valuation method using household level data. This chapter provides the first insight into farmers' knowledge of and attitudes to insects as feed and the potential demand for insect-based feed for fish and livestock production in Kenya. Beer and sugar production result in various by-products which typically end up underutilized.

In **Chapter 4**, a combination of different agro-industrial by-products composed of brewers' spent grains, brewer's yeast and cane molasses was used to evaluate life-history parameters of the BSF such as developmental time and survival of the different life stages. The larval, pre-pupal, pupal, adult biomass, pre-oviposition time, adult fecundity and longevity of starved, water-provided and sugar-fed adults are also presented. The findings reveal that agro-industrial by-products, especially brewers' spent grains, a by-product of beer in Kenya, are suitable for rearing black soldier flies. This has implications for commercial production of high-quality BSF larvae as a suitable alternative to fishmeal in aquaculture and livestock feeds.

These findings present a new interest in valorizing these side streams due their year-round availability, low competitiveness for food and feed as well as the need for sustainable waste management.

Chapter 5 assesses the development rates of BSF reared on two brewers' spent grain substrates, one supplemented with brewer's yeast and the other not supplemented, at nine constant temperatures using temperature-dependent linear and non-linear day-degree models. The minimum, optimum and maximum threshold temperatures, thermal constants as well as the lifetable parameters for BSF development are presented. The results of mean weight of larvae, pre-pupae, pupae and adult BSF are presented. The results are valuable for optimizing commercial mass rearing procedures of BSF under various environmental conditions.

Chapter 6 assesses the effect of the different substrates described in Chapter 4 on the nutritional quality of BSF larvae. The results of proximate, amino acid, fatty acid and mineral compositions of BSF larvae reared on these substrates are presented. High levels of crude protein and predominant saturated fatty acids in larval samples are also presented.

Chapter 7 assesses the potential of substituting fishmeal with BSF larval meal on feed intake, body weight, blood parameters and health of growing pigs as well as economic implications. A conventional level of fishmeal is compared with four dietary inclusion levels of replacement of fishmeal by the BSF larval meal. The findings demonstrate that BSF larval meal is a suitable and cost-effective alternative to fishmeal and present new market opportunities for commercialization of insect-based feed.

Chapter 8 assesses the effect of BSF-based feeds on growth performance and carcass yield of finishing pigs as well as the nutritional value of organ and muscle tissues of pigs. The relationship between live body weight and linear body measurements of finishing pigs and the implication in smallholder pig production are discussed. As indicated in chapter 7, these findings do not only provide valuable insights into commercial production of insect-based feeds, but also provide important nutritional information for pork consumers.

In **Chapter 9**, I integrate the findings presented in the different chapters in this thesis and discuss how farmers' knowledge and attitudes influence willingness to pay and how this knowledge can affect the adoption of and demand for insect-based feed in Kenya. I discuss how a combination of different agro-industrial by-products and temperature affect performance of BSF and the implication of this knowledge in mass rearing BSF and waste management operations. I also highlight the need to nutritionally manipulate brewers' spent grains as rearing substrates for BSF larvae in order to improve fatty acid quality of the resulting larvae for feed. Furthermore, I discuss how BSF larval meal in pig feed supports growth and health as well as the economic and nutritional implications

of this knowledge in commercial production of animal feed and pork consumption in Kenya.

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Chapter 2

Insects for sustainable animal feed: inclusive business models involving smallholder farmers

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Abstract

Global population growth, an increasing demand for animal products and scarcity of conventional feed ingredients drive the search for alternative protein sources for animal feed. Extensive research indicates that insects provide good opportunities as a sustainable, high quality and low-cost component of animal feed. Here, we discuss how insect farming can promote inclusive business for smallholder farmers in the agribusiness value chain. Inclusive business models involving insects as ingredients in feed may contribute to solving socio-economic and environmental problems in developing countries, aligning with the United Nations' Sustainable Development Goals. With low initial capital investments, smallholder insect farmers have good opportunities to increase productivity, improve their livelihood and contribute to food security and a circular economy.

Introduction

While smallholder farmers are responsible for the basis of global food production (Poole, 2017), those in low income communities do not necessarily benefit by gaining access to commercial value chains. Integrating smallholder farmers in a circular economy and thus making them stakeholders in the agribusiness value chain can help to improve their quality of life in a sustainable way. Developing this through inclusive business (IB) models (Golja & Požega, 2012; Kelly et al., 2015) will empower smallholder farmers, and promote their financial viability as well as environmental sustainability.

In the developing world, particularly in Africa where most of the human population increase is expected to occur, economic growth and changing dietary patterns will account for a 70% increase in the demand for livestock products by 2050 (IFPRI, 2017; United Nations, 2017). Feed costs represent 60-70% of total costs of livestock production (Van Huis et al., 2013). Important protein sources in feed are soybean meal whose use competes with food production, and fishmeal whose availability is increasingly limited because of marine overexploitation (Masuda & Goldsmith, 2009; Tacon & Metian, 2008). Costs of these feed ingredients rapidly increase, especially affecting resource-poor farmers. The search for sustainable alternatives has led to a growing interest in insects as feed component (Van Huis et al., 2013).

Insects contain high levels of protein and their production has a small ecological footprint (Van Huis, 2013). Among the insect species that are mass reared, the black soldier fly (BSF) *Hermetia illucens*, house fly (HF) *Musca domestica* and yellow mealworm *Tenebrio molitor*, have received considerable attention because they can feed on different substrates including organic waste streams (Chia et al., 2018a; Chia et al., 2018b). The ability of these insects to convert organic waste into high-quality nutrients has rapidly opened innovative economic prospects. These include insect-based protein as an alternative to fishmeal or soybean meal for pig (Biasato et al., 2019), poultry (Onsongo et al., 2018) and fish (Iaconisi et al., 2017) feeds. BSF and HF larvae are currently reared exclusively as feed ingredients (van der Fels-Klerx et al., 2018; Van Huis et al., 2013).

This review focusses on how the value of insects as feed component can contribute to improving livelihood of smallholder farmers through IB models and reshaping food systems into efficient, climate resilient and nutrition-driven elements of a circular economy. In doing so, we will emphasize how and why IB models in this area align with the Sustainable Development Goals (SDGs) (Figure 1).

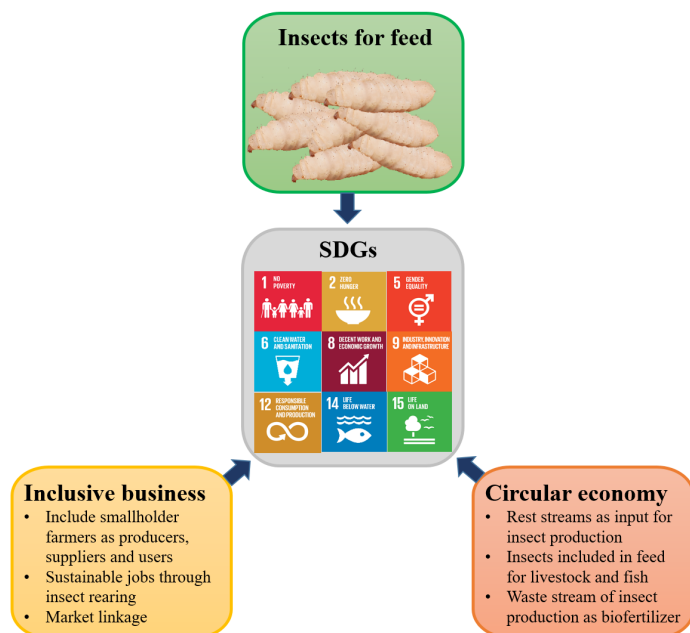


Figure 1. Sustainable production of insects, their use in animal feed, contribution to a circular economy and applying inclusive business models involving smallholder farmers contribute to achieving the Sustainable Development Goals (SDGs) 1, 2, 5, 6, 8, 9, 12, 14 and 15.

Insects as feed

Recent studies indicate that insect meal can be an excellent replacement of fishmeal or soybean meal in animal feed (Biasato et al., 2019; Iaconisi et al., 2017; Onsongo et al., 2018; van der Fels-Klerx et al., 2018; Van Huis et al., 2013). Insects are rich sources of macro- and micronutrients (Finke & Oonincx, 2017; Van Huis, 2013). BSF larvae, for example, contain high levels of protein (37-63%) and fat (20-40 %) that have well-balanced amino acid and fatty acid profiles, respectively (Roos, 2018; Schiavone et al., 2017). Insects are good sources of minerals, such as calcium, iron, potassium, magnesium, phosphorus and zinc as well as vitamins including niacin, vitamin B12, thiamine and riboflavin (Akhtar & Isman, 2018; Spranghers et al., 2017).

When BSF larval meal replaced soybean and fishmeal in proportions of 10 to 56%, broiler quails and chickens had satisfactory taste, aroma and nutritional composition of the meat, confirming that BSF larval meal is suitable for inclusion in poultry diets (Cullere et al., 2018; Onsongo et al., 2018). Insects have also been implemented as fish feed: nursing Nile tilapia fingerlings with different levels of fishmeal replacement by BSF meal resulted in similar growth performance and feed conversion (Devic et al., 2018). Piglets fed diets with 5-10% levels of BSF larval meal exhibited satisfactory

growth performance, with minimal effects on blood profiles (Biasato et al., 2019). Also, at higher inclusion levels, performance similar to the use of conventional feed has been recorded (Chia et al., 2019). Overall, research indicates that BSF larval meal is a suitable component of animal feed (Biasato et al., 2019; Iaconisi et al., 2017; Onsongo et al., 2018; van der Fels-Klerx et al., 2018).

Organic waste reduction and environmental sanitation

The production of insects as feed has interesting characteristics. Insects can efficiently convert low-grade organic substrates into high-quality protein (Van Huis et al., 2013). BSF and HF larvae can be reared on organic waste, which would otherwise end up in dumpsites, causing environmental pollution. BSF (Box 1) is most commonly utilized. For instance, in one day, BSF larvae can reduce 30 metric tons of food waste to *ca.* 10 metric tons (waste reduction 66%), while producing 930 kg of dry biomass (Salomone et al., 2017). Waste reduction of 51-80 % by BSF larvae was recorded on pig, chicken and kitchen waste (Nana et al., 2018). Fly larvae can also be used in environmental sanitation programs to improve human health conditions. In Africa, private companies currently convert human waste from slums into organic fertilizer and fly larvae (Dicke, 2018). These initiatives help to sanitize the environment for poor communities. In conclusion, the production of fly larvae as feed component provides high quality feed ingredients while contributing to a circular economy.

Box 1: Black soldier fly (*Hermetia illucens*)

The BSF is present in tropical and subtropical regions of the world (Sheppard et al., 1994). Larvae can develop on several waste streams including vegetable and fruit waste, animal manure and human excrements resulting in significant waste reduction and high nutritional quality insect biomass (Chia et al., 2018a; Chia et al., 2018b; Nana et al., 2018; Sprangers et al., 2017). Larvae reach highest biomass after *ca.* two weeks under optimal diet and temperature conditions (Chia et al., 2018a; Chia et al., 2018b). Content of individual amino acids as percentage of crude protein is high (1.3-12.8%), which is comparable with fishmeal (2.1-13.1%) and soybean meal (1.3-20.7%) (Liland et al., 2017). BSF is not considered a pest and is not known as a vector of diseases. Adult BSF are not attracted to human habitats and do not constitute a nuisance. These characteristics make BSF an attractive insect species for animal feed.

Environmental sustainability

Implementing insects in nutrient cycling for feed production is innovative and currently receives ample attention (Chaalala et al., 2018). The production of one metric ton of HF larval meal to replace 0.5 metric ton of fishmeal and 0.5 metric ton of soybean meal, for example, resulted in reduced land use and increased energy use (van Zanten

et al., 2015). Similar data were reported for mealworm meal production (Ooninx & de Boer, 2012). Land use is globally under pressure and, thus, production of insects for feed alleviates this. The higher energy consumption was mainly needed to maintain optimal temperatures for larval production (van Zanten et al., 2015). Thus, exploiting environments that match optimal temperatures, such as in tropical regions, may reduce energy use.

Insects convert their feed more efficiently than pigs and cattle (Bosch et al., 2019; Dobermann et al., 2017; Halloran et al., 2018; Rumpold & Schlüter, 2013), which contributes to their importance for valorizing organic waste streams (Chia et al., 2018b). Sustainability of insect production is highest if the insects are fed with organic resources that are currently not suitable as feed for livestock. Current protein sources in feed are soybean meal and fishmeal whose use is under pressure because of environmental aspects (Dicke, 2018). Therefore, rearing BSF as feed may mitigate environmental impact of feed production (Bosch et al., 2019).

2

Inclusive business

For small-scale farmers, the most important costs of livestock production are represented by the costs of feed which amount up to 70% of all costs and this is especially due to the costs of protein components. Soybean meal and particularly fishmeal prices are rapidly increasing (Dicke, 2018). As a consequence, farmers in low and middle-income countries are in need of alternatives that are both effective and affordable.

A survey among smallholder poultry farmers in four counties in Kenya showed that they are aware of the opportunities of insects as feed component (Figure 2). Female farmers appear to be more informed of the opportunities than male farmers. However, these farmers usually depend on external sources for feed, including national and international feed companies (Figure 2). This makes them economically dependent on imported feeds that are commonly based on fishmeal and soybean meal. Yet, fly larvae may be produced locally by smallholder farmers providing opportunities to become feed suppliers in addition to or instead of being feed buyers. They may rear fly larvae as feed component either to be included in feeds that they formulate themselves or to sell to feed millers (knowledge4food.net/research-project/gcp2-insect-products-feed-africa). This will provide farmers with opportunities to actively engage in the emerging insect agribusiness value chain (Pomalégni et al., 2017). Becoming less dependent on international feed producers and simultaneously gaining income from producing fly larvae as feed component contributes to improving livelihood and food security of smallholder farmers (Pomalégni et al., 2017).


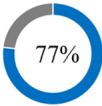


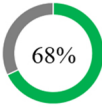


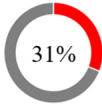


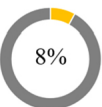
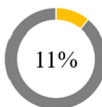
	Farmers		Statistical significance
	Female, n = 243	Male, n = 166	
Aware that insects can be used as feed 	 77%	 60%	***
Aware that insects are a good source of feed 	 68%	 55%	***
Ever used insects as feed 	 31%	 31%	ns
Make their own feed 	 8%	 11%	ns

Figure 2. Farmers’ perception and use of insects as feed for poultry in Kenya. The blue, green, red and yellow colours represent farmers’ responses. Asterisks indicate significant differences between male and female farmers (z-test): ***, P < 0.001, ns: not significant.

Economic aspects

The economics of adopting insects as feed ingredient include the production of the insects as well as incorporating them in feed for livestock. The production costs of fly larvae involve investments in infrastructure such as space and containers. These costs are minor (Roffeis et al., 2018). Additional costs involve resources such as water, electricity and feed substrates for the fly larvae, and labour (Roffeis et al., 2018). Comparing break-even sales prices of feeds that include HF or BSF meal as protein source with prices of conventional feeds in West Africa indicates that insect meals are competitive to feeds based on fishmeal as protein source (Roffeis et al., 2018). Costs of protein ingredients for chicken feed in Kenya are 1.20 and 0.85 US\$/kg for fishmeal and BSF meal respectively (Onsongo et al., 2018). Dietary replacement of soybean and fish meal by BSF meal in broiler feed resulted in an improved feed conversion rate leading to higher yield with less feed input. In combination with lower costs for BSF meal than for fishmeal and soybean meal, this resulted in a 25% higher return on investment when using BSF meal (Onsongo et al., 2018).

The production of fly larvae is based on waste streams as input. Many of these waste streams are currently not valorized and end up in landfills. This leads to environmental damage in terms of e.g. contribution to global warming and limiting of resource recovery (Oyoo et al., 2014). However, the increasing importance of waste streams as input in

the production of a valuable feed component may result in an increase in costs of this resource of insect production. Reducing labour costs will be important to limit the production costs of BSF and HF for feed (Roffeis et al., 2018). Moreover, an additional benefit of producing insects for feed is that the waste stream remaining after harvesting the fly larvae can be valorized as fertilizer, thus providing an additional financial benefit (Roffeis et al., 2018; Xiao et al., 2018). In conclusion, producing HF or BSF as feed component is competitive with the use of fishmeal and although the price of substrates is likely to rise, other benefits of fly production are likely to outweigh this.

2

Insect farming in the context of a circular economy

Insect farming by smallholder farmers can increase local supply of insects as animal feed in an integrated livestock–fish farming system. Farmers may use on-farm waste streams such as crop leftovers as input for BSF production and add the resulting fly larvae to the feed for their livestock. This results in a circular approach that closes the nutrient cycles on farm (Figure 3). With limited space, resource-poor farmers that engage in insect farming may increase their productivity while contributing to waste management (Pomalégni et al., 2017). Smallholders can start up innovative businesses with limited inputs to generate insect meal for animal feed and the waste stream of insect production can be used as organic fertilizer for crop production (Chaalala et al., 2018). Sales from resulting animal products (fish, meat and eggs, insect meal) and crop yields can supplement household income or provide food. Insects can thus effectively close nutrient cycles (Figure 3), avoiding food wasting because waste becomes a resource. One key aspect to consider in closing the loop is the legislative constraints of using insect meal as (ingredient of) livestock feed (van Raamsdonk et al., 2017). Inclusion of insect meal in livestock and fish diets is currently allowed in Kenya and Uganda (Dicke, 2018) and under development in the EU (<http://ipiff.org/insects-eu-legislation/>). Thus, this promising model is now in the process of being accepted by regulators as well.

Sustainable Development Goals (SDGs)

IB models enable individuals, households, entrepreneurs, micro-, small-, and medium-sized enterprises to secure access to affordable goods and services relevant to sustainable livelihoods and engaging in value chains in beneficial and sustainable ways (Asia Development Bank, 2016; Beckwith, 2018; Kelly et al., 2015; Likoko & Kini, 2017). Inclusive business models are sustainable business solutions that expand access to goods, services, and livelihood opportunities for low-income communities in commercially viable ways (Marangu & Adoyo, 2018). Insect production by smallholder farmers may both disconnect them from expensive external inputs such as fishmeal-based feed as well as connect them to local economies by selling the insects to local livestock farmers as well as feed millers (Figure 3).

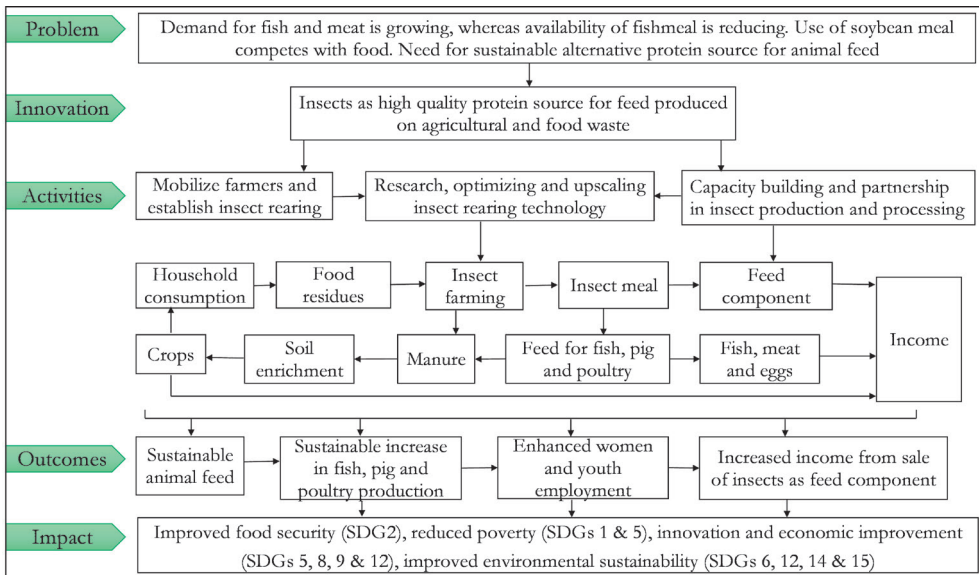


Figure 3. Insect farming for feed in the context of a circular economy can generate income and create employment: innovation, activities, outcome and impact.

In January 2016, the United Nations officially adopted the SDGs and called for a universal action to end all forms of poverty, fight inequalities and tackle climate change within a package of 17 SDGs. Specific for this call is the inclusion of poor, middle- and high-income people. However, efforts towards achieving the SDGs are felt differently among low- and high-income people, men and women, developed and developing countries (Asia Development Bank, 2016). Implementing innovative and sustainable food production strategies such as insect farming for animal feed involving smallholder farmers may contribute substantially to several of the SDGs, which are interconnected (Figure 1) (Dicke, 2018; Poole, 2017; Veglio & Fiedler, 2016). Access to and control over natural resources globally impact how rural people secure decent livelihoods, escape hunger, participate in decision making and overcome social and economic exclusion (Franco & Monsalve Suárez, 2018). With minimal inputs, resource-poor people can set up small insect farms to produce for themselves or the local market (Figure 3) hence reducing poverty (SDG 1) and hunger (SDG 2) (Chaalala et al., 2018).

Water scarcity, poor water quality and inadequate sanitation negatively impact food production and livelihoods, thus worsening malnutrition. In South Africa, preliminary trials show that BSF larvae can be effectively used in urine diversion dehydrating toilets to manage human faeces, while conserving water to alleviate sanitation problems faced mainly by the rural poor (SDG 6) (Mutsakatira et al., 2018). Commercial insect farming is becoming a new sector for economic growth and employment opportunities (SDG 8).

Inclusive insect farming may promote sustainable industrialization, increase employment and local technology development in low-income communities (SDG 9) as well as improve gender equality (SDG 5). Insect bio-conversion can ensure sustainable use and reduction of food waste (SDG 12). Increased insect production as feed may provide a sustainable alternative to fishmeal, thus reducing effects on biodiversity due to overfishing and conversion of forests to agricultural land (SDGs 14 and 15).

In conclusion, IB models align with the SDGs by strengthening stakeholder engagement in agricultural value chains, while mitigating the effects of food production on the environment.

2

Future prospects

Changes in feed systems are dependent on several potential drivers, including technological, environmental, political, economic, cultural and demographic drivers (De Brauw et al., 2019). Technological drivers are clearly present for insects as feed (Biasato et al., 2019; Iaconisi et al., 2017; Onsongo et al., 2018; Van Huis et al., 2013). Fly larvae have great prospects for animal feed and waste management (Van Huis et al., 2013). Environmental drivers include the valorization of waste streams as well as mitigation of biodiversity loss and climate change (Dicke, 2018; Van Huis et al., 2013). Producing insect meal requires limited land and water. Insects can sustainably close nutrient cycles while providing animal proteins and useful by-products, creating employment, increasing local productivity and connecting smallholder farmers to the agribusiness value chain. As long as the fly larvae can be reared on substrates that are a true waste stream, the production of fly larvae as feed will not interfere with food production and will provide a sustainable alternative for fishmeal. Future research should focus on the suitability of a diversity of waste streams that effectively support the growth of the fly larvae while not competing with other use. The remaining drivers, including political, economic, cultural and demographic drivers, are institutional and require a multi-stakeholder involvement (De Brauw et al., 2019). The production of fly larvae for on-farm and local use may make smallholder farmers less dependent on feed millers that provide feed based on expensive and unsustainable fishmeal or soybean meal. For smallholder farmers to be able to effectively connect to the agribusiness value chain and supply fly larvae to national feed millers, it is important that they can supply sufficient volume. To do so, farmers likely need to organize in cooperatives. This will not only improve supply volume but can also empower them within the value chain. The novel production of insects as feed ingredient by smallholder farmers aligns with various SDGs: smallholder farmers can benefit from new markets while generating meaningful profits and increasing economic resilience in low-income communities. Developing the institutional drivers will be vital for successfully implementing the use of insects for feed via inclusive business models.

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Chapter 3

Knowledge and willingness of smallholder farmers in Kenya to pay for insect-based feeds

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Marcel Dicke

Submitted

Abstract

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Edible insects are increasingly considered as sustainable alternatives to fish and soybean meals in animal feed because of their high nutritional quality and environmental benefits. However, the successful introduction of a new product to the market depends on the target user's acceptance. Thus, evaluating the potential demand of insect-based feeds would provide relevant information for policy development. The present study assessed farmers' knowledge of insects as feed, their acceptance of insect utilization as ingredient in animal feeds and willingness to pay (WTP) for insect-based feed (IBF) using a contingent valuation method. A household survey was conducted among 957 randomly selected farmers including: 409 poultry, 241 fish and 307 pig farmers in four counties in Kenya. Results of the study reveal that over 70 and 80% of poultry and fish farmers, respectively, are aware that insects can be used as a feed ingredient. In addition, over 60 and 75% of poultry and fish farmers, respectively, consider insects as a good component of feed. Poultry, pig and fish farmers interviewed accepted and showed willingness to pay for IBF. Regression analysis indicated that age, gender, education, marital status, distance to feed trader, awareness of insects as feed, attitude towards insects, acceptance of insect species, availability of agricultural inputs, use of commercial feeds, availability of training and market information had a significant influence on the WTP for IBF. Therefore, increased extension services to educate farmers on the nutritional benefits of insect meals in animal feeds and existing market opportunities are expected to improve farmers' attitude towards utilization and consequently enhance WTP for IBF, which in return would significantly reduce the existing pressure on conventional fishmeal feed resources. Our findings provide the first insights into the market opportunities of including insect meals in the animal feed value chain in Kenya.

Keywords: edible insects, alternative protein source, animal feed, willingness to pay, Kenya

Introduction

In livestock and aquaculture production, feed is the most important input, representing 60-70% and 40-80% of total cost of production, respectively (Kumar et al., 2017; Makkar, 2018; Mosig, 2018). Feed production requires high resource inputs and the current food-feed competition as well as overfishing represent major sustainability issues that need viable solutions. Global demand of feed is increasing and projection by 2050 revealed that over a billion tonnes of cereals will be required in animal feed as opposed to about eight hundred million tonnes currently used. Developing countries will likely experience most of the increase in demand of animal feed (Makkar, 2016; Makkar, 2018). Livestock and aquaculture production provide employment, income generation and food security opportunities especially in vulnerable communities (FAO, 2009; Rajee & Mun, 2017; Shava & Gunhidzirai, 2017; Thornton, 2010).

The livestock sector, including poultry and pig production among other livestock species contributes about 42% of the Kenya's agricultural Gross Domestic Product (GDP), 12% of the national GDP, 30% of total marketed agricultural products and employs about 50% of the agricultural sector labour force (Republic of Kenya Ministry of Agriculture Livestock Fisheries and Irrigation, 2019; Shibia et al., 2017). Kenya's poultry population is estimated at 31 billion birds, 75% of which are indigenous chicken, 22% are broilers and layers (Vernooij et al., 2018). The sector produces about 605,000 metric tonnes of meat annually. In pig production, smallholder farms keep 5-100 pigs and make up 70% of the total pig producers. Feed costs alone represent up to 80% of total costs of production (Githigia et al., 2012). The Kenyan fisheries and aquaculture sector employs about 20,000 people (FAO, 2016; Kenyan National Bureau of standards (KNBS), 2017). Kenya is the fourth largest producer of freshwater fish in Africa. However, several factors including lack of market information, low levels of extension services and inadequate availability of quality and affordable feeds prevent the sector from realizing its full potential (Kenya Marine and Fisheries Research Institute (KMFRI); Nyandat & Owiti, 2013).

In Kenya, major poultry feed categories include chick mash, growers' mash, layers' mash, broilers' mash and Kienyeji mash. Pigs are fed with pig starter, creep pellet, sow and weaner and pig finisher feeds. Fish feeds include floating pellets and mash feed. In these feeds, fishmeal and soybean meal are the major protein ingredients. However, reduced availability, high cost and environmental implications of exploiting these resources represent major constraints to achieving optimal production, especially for smallholder producers in the developing countries (Abiodun, 2019; Gordon & Maurice, 2015; Katende, 2017; Nwokocho & Nwokocho, 2013; Ssepunya et al., 2017). In view of the above concerns, researchers, policy makers, private and public institutions including the Food and Agricultural Organization (FAO) have called for diversification and

innovation towards sustainable feed protein sources such as edible insects (Makkar & Ankers, 2014; Van Huis et al., 2015; Van Huis et al., 2013).

Edible insects have traditionally been part of livestock diets especially in the tropics and may provide an alternative source of protein and other nutrients in livestock and aquaculture feeds (Henry et al., 2015; Makkar et al., 2014; Van Huis et al., 2015). Insects contain valuable proteins with well-balanced amino acid profiles, fats with rich fatty acid contents as well as micronutrients. The use of insects as an alternative protein source is advantageous because they can be sustainably mass reared on organic side streams and agro-industrial by-products (Rumpold & Schlüter, 2013; Rumpold & Schlüter, 2013). The black soldier fly (BSF) *Hermetia illucens* L. (1758) (Diptera: Stratiomyidae) and the synanthropic housefly *Musca domestica* L. (Diptera: Muscidae) for example, feed on organic side streams and produce nutrient-rich larvae that could be used as ingredients in animal feeds while helping to reduce waste on which the larvae are reared (Chia et al., 2018; Van Huis et al., 2013). Insects contain 40-60% protein on a dry matter basis and have been found to be a suitable alternative to fishmeal and soybean meal in animal feed. Furthermore, insects release smaller amounts of greenhouse gases per unit of protein produced than cattle, pigs and chickens (Makkar et al., 2014; Mosig, 2018; Ooninx et al., 2010; van Huis, 2013).

Feed manufacturers are willing to include insects in their feed formulation, given favourable legislation and marketplace acceptance (AllaboutFeed, 2015). However, little is known about farmers' perception towards the use of insects in animal feed. Such perception may affect the success of introducing insect-based feed (IBF), as well as the consumer acceptance of products resulting from animals fed IBF (Verbeke et al., 2015). So far, only a few studies have documented consumer acceptance of insects as feed, all in European countries including: Belgium (Verbeke et al., 2015), France (Bazoche & Poret, 2016), Germany (Ankamah-Yeboah et al., 2018), Poland (Kostecka et al., 2017), Italy (Laureati et al., 2016; Mancuso et al., 2016) and the United Kingdom (Popoff et al., 2017). Overall, these studies found a favourable attitude and willingness to accept insects in animal feed and resulting products from animals fed with IBF among respondents. Furthermore, consumer willingness to pay (WTP) for insects and insect-based products for human consumption has been assessed and results show that consumers who are familiar with the idea of insects as food are more likely to accept insect-based foods. In addition, consumers are willing to accept insect-based products with high nutritional quality. Therefore, information campaigns and identifying suitable target markets are crucial for promoting a new product (Alemu et al., 2016; Lombardi et al., 2019; Tan et al., 2015; Verbeke, 2015).

While the findings from these previous studies are useful, such information is limited or lacking for Africa, particularly for smallholder farmers who make up 70% of all the producers. The present study aims to provide the first insights into farmers' knowledge

of insects as feed and the potential demand for IBF for fish and livestock using household level data in major poultry, fish and pig producing counties of Kenya. It is worth noting that the successful introduction of a new product in the market depends on the product's marketplace acceptance by the target users, which ultimately will affect the WTP for the product (Etim & Benson, 2016; Henson, 1996; Martinez-Carrasco et al., 2015; Rumpold & Schluter, 2013). Therefore, we evaluated knowledge, attitudes, practices and WTP for IBF among poultry, fish and pig farmers in four counties in Kenya across different agro-ecological zones.

Theoretical framework

Producers are in a constant search for new technologies or inputs with novel attributes to reduce production costs and increase revenues. However, these products do not have an existing market, making it hard to estimate their demand potential. As a result, the producer demand estimation relies on stated acceptance. One of the stated-preference methods used to elicit demand is known as the contingent valuation method (CVM) (Heinzen & Bridges, 2008). The CVM is a non-market valuation method used to find the economic value of non-market commodities. It uses hypothetical survey questions in order to elicit peoples' acceptance of public goods. It is used to find out what the people are willing to pay for specified improvements in the goods. In CVM, absence of markets is circumvented by presenting the consumers/producers with hypothetical markets where they can be provided with information about the products and then asked how much they are willing to pay to obtain the good described. There are four commonly utilized elicitation formats in CVM: open-ended, dichotomous choice, payment card, and bidding game (Heinzen & Bridges, 2008). The bidding game was used in this study. It involves a series of yes/no questions aimed at finding the maximum willingness to pay. The repeated nature of this technique allows a greater amount of time for the respondent to scrutinize their response, and thus gives results that have greater construct validity. Elicitation of contingent valuation employs either of two methods: single or double bounded contingent valuation method.

In the single bound model, the respondents are faced with a single bid value to which their response is either a "yes" or "no" (Heinzen & Bridges, 2008). "Yes" denotes WTP the proposed amount while "no" denotes refusal to pay the proposed amount. Alternatively, they can be assessed on the likelihood of paying for the product without attaching any price to it. The probability of obtaining either a "yes" or "no" response can be written as follows:

$$Prob (no) = \pi^n = G (BID; \theta), Prob (yes) = \pi^y = 1 - G (BID; \theta)$$

Where $G (BID; \theta)$ is the statistical distribution function with parameter θ , which can be estimated using a logit or probit model, a qualitative choice model. Logit or probit

model for a single bid value can be expressed in two forms; log-logistic or the logistic cumulative distribution.

The log-logistic cumulative distribution is expressed as follows:

$$G(\text{Bid}) = 1 / ([1 + e^{(a-b(\ln \text{Bid}))}])$$

The logistic cumulative distribution is expressed as follows:

$$G(\text{Bid}) = 1 / ([1 + e^{(a-b(\text{Bid}))}])$$

where $\theta = (a, b)$, a and b are the intercept and slope coefficients to be estimated. The statistical model can be interpreted to mean that an individual whose aim is to maximize utility within a random utility context will say “yes” to a *BID* only if the *BID* is less than or equal to his maximum WTP and will say “no” if the *BID* is greater. Alternatively, for a case that has no bid value attached to the model, the probability of obtaining either a “yes” or “no” response can be written as follows:

$$\text{Prob}(\text{no}) = \pi^n = G(X; \theta), \text{Prob}(\text{yes}) = \pi^y = 1 - G(X; \theta)$$

where X represents the control, variables used in the model (Hanemann et al., 1991).

In a double-bound model, the respondents are faced with a two-sequence-bid offer. In the first offer, they are asked whether they will accept or reject the bid, then the second bid is offered depending on the respondent's first bid response, a higher bid if the response was yes and a lower bid if the response was no. This results in four possible responses: (1) both answers are “yes”, (2) both answers are “no”, (3) a “yes” followed by a “no” and 4) a “no” followed by a “yes” (Herriges, 1999).

This two-sequence-bid provides a bound of the respondent's WTP. The WTP is right censored if the answer to the initial and higher bids is “yes” and left censored if the response to the first and second bids is “no”. If both answers are alternate of yes and no, then their WTP is intermediate with the second bid acting as an upper or lower bid. The likelihood of these outcomes is as shown in Fig. 1.

It is assumed that a respondent's maximum WTP is lower than or equal to the lowest bid ($\max\text{WTP} \leq \text{Bid}_i^L$) if he or she rejects the first and second (lower) bid offers. It is assumed that the respondent's maximum WTP lies between the lower and the first bid offer ($\text{Bid}_i^L \leq \max\text{WTP} < \text{Bid}_i^H$) if the respondent rejects the first bid but accepts the second lower bid offer. If the respondent is willing to accept the first bid but rejects the second higher bid offer, it is assumed that the respondent's maximum WTP lies between the second higher and the first bid offers ($\text{Bid}_i^H > \max\text{WTP} > \text{Bid}_i^L$). Finally, if the respondent accepts the first and second higher bids, then it is assumed that the respondent's maximum WTP is greater than or equal to the second higher bid offer ($\max\text{WTP} \geq \text{Bid}_i^H$).

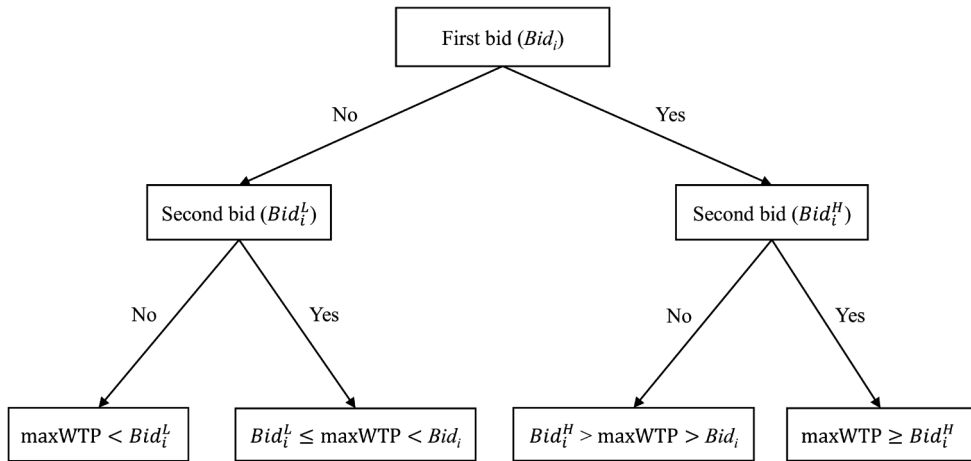


Fig. 1. Double-bound dichotomous choice contingent valuation methods bid sequence. Bid_i^H = the second bid, which is an amount greater than the first bid (Bid_i); Bid_i^L = the second bid, which is an amount smaller than the first bid if the individual response is “no” to the first bid.

The double bound dichotomous choice model improves on the single bound dichotomous choice model by providing a two-level bidding process (Hanemann et al., 1991). In this study a double-bound logit model was used to estimate WTP and the factors that influence WTP for IBF among the farmers. A positive correlation between a variable and WTP means that an increase in the variable leads to an increase in the probability of WTP for IBF. Furthermore, a negative correlation with WTP means that an increase in the variable leads to a decrease in the probability of WTP for IBF.

Materials and methods

Study area and data collection

This study was conducted in four counties in Kenya, including Kiambu, Nyeri, Kakamega and Uasin Gishu (Fig. 2). A purposive sampling method was employed to select sub-counties in each of four counties, based on the production statistics of the three animal types including pig, poultry and fish. Respondents within each sub-county were randomly selected. The sample frame composed of a census of active smallholder pig farmers, poultry farmers and fish farmers in the survey sites compiled by the respective sub-county agricultural officers for these. In total, 409 poultry famers were interviewed, distributed as follows: Kiambu (79), Nyeri (89), Kakamega (98) and Uasin Gishu (143). A total of 307 pig farmers were interviewed: Kiambu (102), Nyeri (63), Kakamega (96) and Uasin Gishu (46). A total of 241 fish farmers were interviewed: Kiambu (29), Nyeri (68), Kakamega (75) and Uasin Gishu (69). Data were collected at the household level

by trained enumerators using CSPro version 7.0, data collection software addressing the following aspects: socioeconomic characteristics of the respondents, their knowledge, attitudes, practices and acceptance of different insect species, availability of agricultural support services, feed use and distance to feed market (trader). Farmers' WTP for IBF and prices they are willing to offer per unit of IBF, availability of market and financial institutions were also assessed.

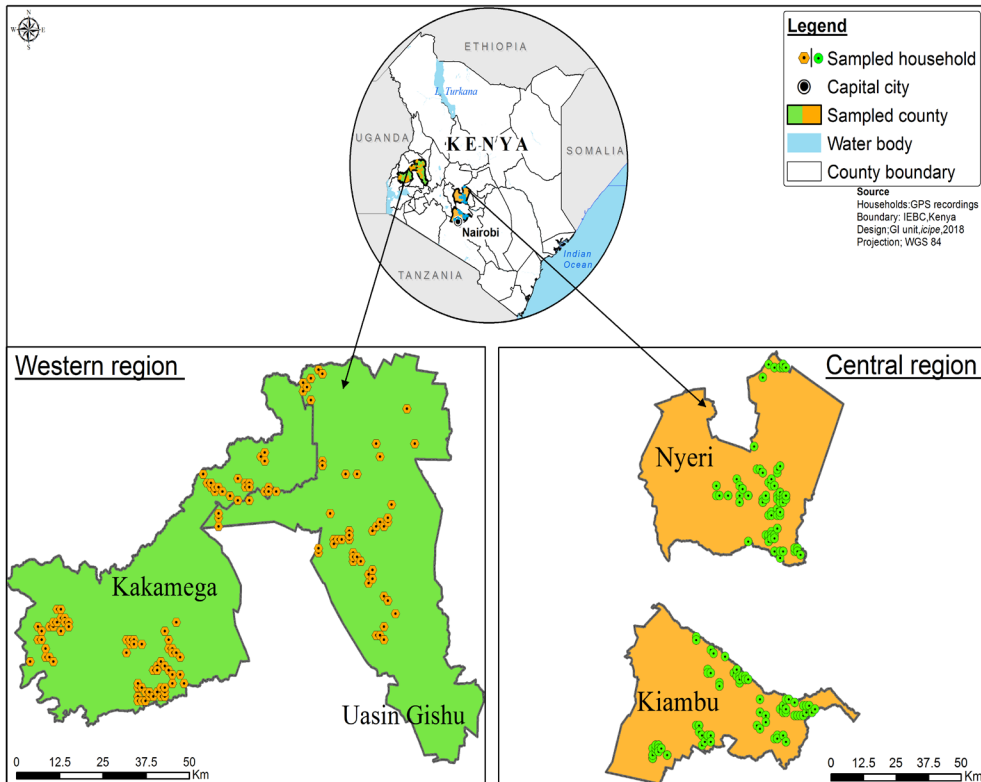


Fig. 2. Map representing the four study areas (counties) in two geographical regions of Kenya. Green colour represents counties sampled in the Western region, orange represents counties sampled in the Central region of Kenya.

Empirical model

We model a farmer's WTP for IBF using a single-equation logit model of the form:

$$Y_i = \beta_0 + \beta_i X_i + \varepsilon_j$$

where Y is a binary variable for farmers' WTP (having value of one if farmers are willing to pay for IBF and zero otherwise), i indexes individual farmer's WTP, β_0 is the intercept, β_i is the regression coefficient, X is a vector of explanatory variables that affect farmers' WTP, ε_j is an error term, which assumes a normal distribution (mean = 0,

variance = 1). Selection of explanatory variables was guided by a review of theoretical and empirical studies on the determinants of WTP for agricultural products (Alemu et al., 2016; Mancuso et al., 2016; Tan et al., 2015; Verbeke, 2015).

We estimate the empirical model for several categories of poultry, pig, and fish farmers based on the expectation that factors associated with WTP for insect-based feed will vary across farmer type. Poultry farmers were categorized into those rearing Kienyeji (an indigenous type of chicken), Layers (laying chickens aged 19 – 76 weeks), Growers (chickens aged 8 – 18 weeks), and Chicks (young birds aged 0 – 8 weeks). Fish farmers were grouped according to type of feed currently used: floating pellets (finely ground feed that has been compressed and molded into pellets in a pellet mill and float on the surface of water when served to grower and finisher fish stages) and feed mash (a finely ground feed formulated and used in moist form for farmed juvenile fish). Pig farmers included those raising finisher (pigs weighing over 55 kg) or sows and weaners (pigs up to 55 kg) and adult breeding pigs.

Results

Demographic characteristics of the study population

A total of 957 farmers participated in this study. Fifty nine percent (59%) of poultry, 34% of fish and 49% of pig farmers were females (Table 1). Mean age varied significantly for male and female respondents in poultry, fish and pig production. Fish farmers had the highest (52.5 and 49.2 years) mean age while pig farmers had the lowest (48.5 and 45.7 years) mean age for male and female respondents, respectively. For all categories (poultry, fish and pig), mean number of years of education differed significantly for male and female respondents; male farmers were more educated than the females (Table 1). Household size and distance to feed trader were similar for male- and female-headed households for poultry, fish and pig farmers. There were on average, five members per household (Table 1) across the study locations.

Table 1. Demographic characteristics of the farmer populations

Parameter	Range	Poultry (N = 409)			Fish (N = 241)			Pig (N = 307)		
		Male N (%)	Female N (%)	t-value	Male N (%)	Female N (%)	t-value	Male N (%)	Female N (%)	t-value
Age (years)	18-30	13 (3.2)	21 (5.1)		14 (5.8)	7 (2.9)		21 (6.8)	21 (6.8)	
	31-40	20 (4.9)	57 (13.9)		19 (7.9)	12 (5.0)		29 (9.5)	38 (12.4)	
	41-50	42 (10.3)	67 (16.4)		38 (15.8)	26 (10.8)		34 (11.1)	38 (12.4)	
	>50	91 (22.3)	98 (24.0)		89 (36.9)	36 (14.9)		72 (23.5)	54 (17.6)	
	Sub-total	166 (40.6)	243 (59.4)		160 (66.4)	81 (33.6)		156 (50.8)	151 (49.2)	
Mean age	51.24	47.23	-3.12**	52.47	49.23	-1.70	48.48	45.74	-1.68	
Education (years)	No formal	2 (0.5)	3 (0.7)		2 (0.8)	3 (1.2)		1 (0.3)	6 (2.0)	
	Primary	45 (11)	98 (24.0)		60 (24.9)	32 (13.3)		47 (15.3)	68 (22.2)	
	Secondary	62 (15.2)	93 (22.7)		50 (20.8)	34 (14.1)		68 (22.2)	57 (18.6)	
	Tertiary	57 (13.9)	49 (12.0)		48 (19.9)	12 (5.0)		40 (13.0)	20 (6.5)	
	Mean duration	11.38	10.09	-3.64**	10.83	9.70	-2.24*	10.96	9.46	-3.75**
Household size	1-4	77 (18.8)	93 (22.7)		73 (30.1)	32 (13.3)		79 (25.7)	54 (17.6)	
	5-8	73 (17.9)	127 (31.1)		73 (30.1)	42 (17.4)		65 (21.2)	83 (27.0)	
	>8	16 (3.9)	23 (5.6)		14 (5.8)	7 (2.9)		12 (3.9)	12 (3.9)	
	Mean	5.27	5.40	0.51	5.19	5.26	0.19	4.94	5.19	0.92
Distance to feed trader (Kkm)	0.01-15	28 (6.9)	80 (19.6)		26 (10.8)	11 (4.6)		21 (6.8)	16 (5.2)	
	16-30	4 (1.0)	7 (1.7)		4 (1.7)	2 (0.8)		13 (4.2)	8 (2.6)	
	31-45	22 (5.4)	12 (2.9)		2 (0.8)	2 (0.8)		8 (2.6)	5 (1.6)	
	>45	19 (4.7)	26 (6.4)		6 (2.5)	3 (1.2)		4 (1.3)	5 (1.6)	
Mean	29.54	35.5	0.57	41.81	53.47	0.35	23.67	32.35	1.09	

Significance levels: ** P < 0.01, * P < 0.05, t-test. Values in parentheses represent percentage of male or female within a given age range.

Farmer knowledge, attitudes and practices towards insects as an alternative source of feed for poultry, fish and pigs

The proportion of female farmers who were aware that insects can be used as feed for poultry was significantly higher than for the males. Male and female fish farmers were similarly aware that insects can be used as feed for fish (Table 2). A significantly higher proportion of female poultry farmers had a positive attitude towards insects as feed than the males. There was no significant difference between proportions of male and female fish farmers with regards to their attitude toward the use of insects in animal feeds. However, only a small proportion of both poultry and fish farmers demonstrated that they had previously used insects to feed their animals (25-38% of respondents).

The proportion of male farmers who previously used insects as feed for their fish was significantly higher than for female fish farmers. It was common to find poultry, fish and pig farmers engaged in the practice of making their own feed as well as using commercial feeds. Feed items (conventional feed) frequently used by the smallholder farmers included: vegetables, grains, food remains. On average, less than 20% of male and female respondents made their own feed in all animal categories (Table 2). More male than female pig farmers made their own feed while more female poultry farmers than males used conventional feeds (Table 2).

Acceptance of insect species and availability of agricultural support services and inputs

Similar proportions of male and female farmers accepted cockroaches, housefly, BSF larvae, crickets, termites and grasshoppers as alternative feed components for poultry and fish production (Fig. 3). The proportion of male pig farmers who accepted housefly and BSF larvae was significantly higher than for female pig farmers. However, similar proportions of male and female pig farmers accepted the other insect species investigated (Fig. 3). The acceptance of termites was significantly higher compared to other insect species.

Table 2. Farmers' knowledge, attitude and practices towards insects as an alternative source of feed for poultry, fish and pigs

	Poultry (n = 409)		Fish (n = 241)		Pig (n = 307)		z-value	z-value	z-value
	Male (n = 166)	Female (n = 243)	Male (n = 160)	Female (n = 81)	Male (n = 156)	Female (n = 151)			
Knowledge									
Aware that insects can be used as feed (%)	60	77	83	74	-	-	3.75**	-1.54	-
Attitudes									
Insects are a good source of feed (%)	55	68	79	75	-	-	2.77**	-0.72	-
Practices									
Make their own feed (%)	11	8	20	16	21	9	-1.24	-0.74	-2.95**
Ever used insects as feed (%)	31	31	38	25	-	-	0.12	-2.09*	-
Used commercial feeds (%)	81	80	74	69	69	67	-0.22	-0.76	-0.32
Used conventional feeds (%)	67	85	68	67	85	82	4.37**	-0.23	-0.74

Significance levels: ** P < 0.01, * P < 0.05, z-test. (-) Not evaluated. Conventional feed = vegetables, grains and food remains used as feed.

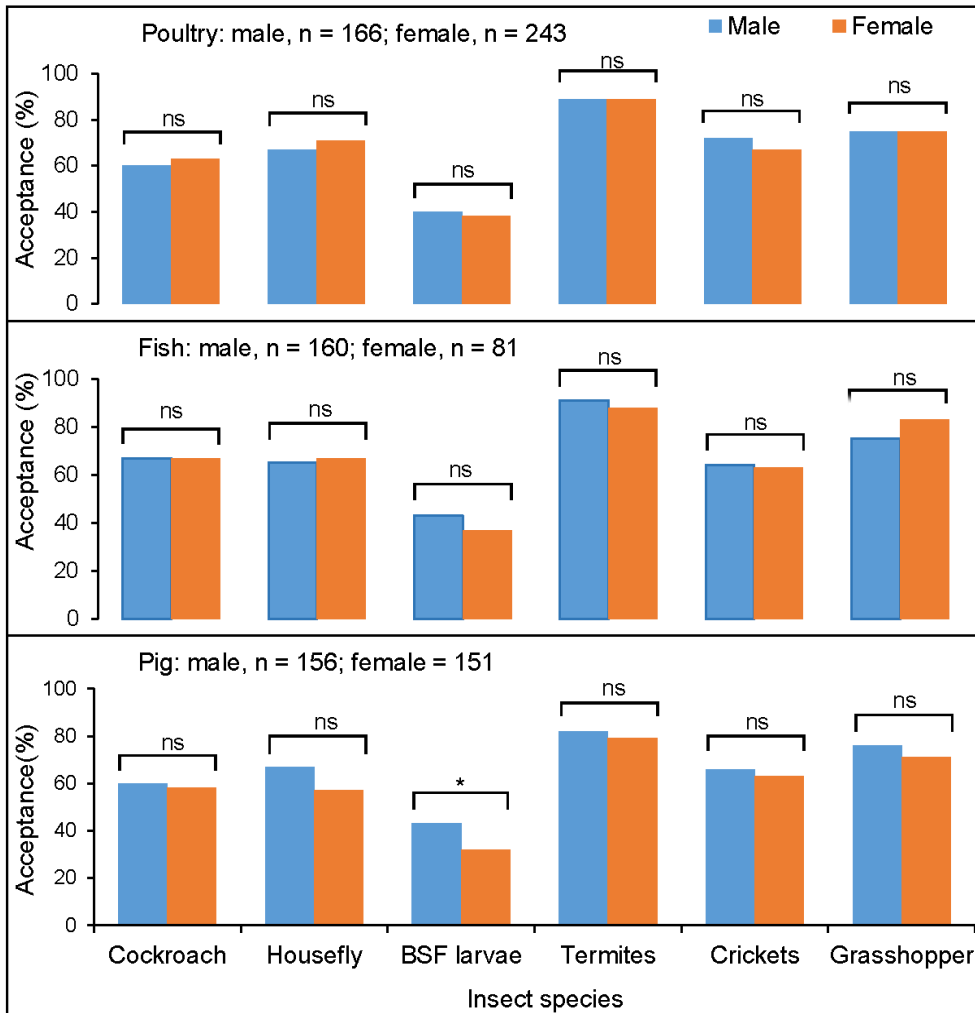


Fig. 3. Percentage farmers that accept insects as feed ingredients among poultry, fish and pig farmers. Bars with asterisk are significantly different for male and female respondents, $P < 0.05$, two-proportion z-test. Bars with “ns” are not significantly different for male and female respondents, $P < 0.05$, two-proportion z-test. BSF = black soldier fly. For additional statistical analyses see Table 3.

The BSF larvae had the lowest acceptance, which differed significantly from other insect species for both male and female poultry, fish and pig farmers (Table 3). Cockroaches, houseflies and crickets were similarly accepted as alternative feed ingredients for poultry fish and pig feed (Table 3). Among support services and inputs, new technologies were the least available: ca 25-35% of farmers had access to this input (Fig. 4).

Table 3. Comparison of farmers' acceptance of different insect species as feed ingredients

Comparison	Poultry farmers				Fish farmers				Pig farmers			
	Male		Female		Male		Female		Male		Female	
	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.
Cockroach vs Housefly	1.75	ns	3.51	ns	0.14	ns	0	ns	1.64	ns	0.03	ns
Cockroach vs BSF	13.24	***	30.32	***	18.56	***	14.51	***	9.0	**	20.55	***
Cockroach vs Termites	36.63	***	44.94	***	27.69	***	10.18	**	18.28	***	15.38	***
Cockroach vs Crickets	5.31	*	0.85	ns	0.32	ns	0.28	ns	1.20	ns	0.79	ns
Cockroach vs Grasshopper	8.49	**	8.16	**	2.48	ns	5.50	*	9.15	**	5.55	*
Housefly vs BSF	24.25	***	53.25	***	15.54	***	14.51	***	18.10	***	19.04	***
Housefly vs Termites	23.34	***	24.55	***	31.42	***	10.18	**	9.21	**	16.74	***
Housefly vs Crickets	0.98	ns	0.91	ns	0.04	ns	0.28	ns	0.04	ns	1.13	ns
Housefly vs Grasshopper	2.57	ns	0.98	ns	3.80	ns	5.50	*	3.09	ns	6.40	*
BSF vs Termites	86.77	***	136.1	***	83.10	***	44.67	***	50.46	***	67.31	***
BSF vs Crickets	34.39	***	40.89	***	14.14	***	10.88	**	16.59	***	29.0	***
BSF vs Grasshopper	41.48	***	67.54	***	33.76	***	35.49	***	35.14	***	45.82	***
Termites vs Crickets	15.24	***	34.2	***	33.34	***	13.60	***	10.35	**	9.36	**
Termites vs Grasshopper	10.99	**	16.1	***	14.47	***	0.81	ns	1.69	ns	2.57	ns
Crickets vs Grasshopper	0.38	ns	3.77	ns	4.55	*	8.17	**	3.78	ns	2.18	ns

Significance (sig.) level: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns = not significant; chi-squared test.

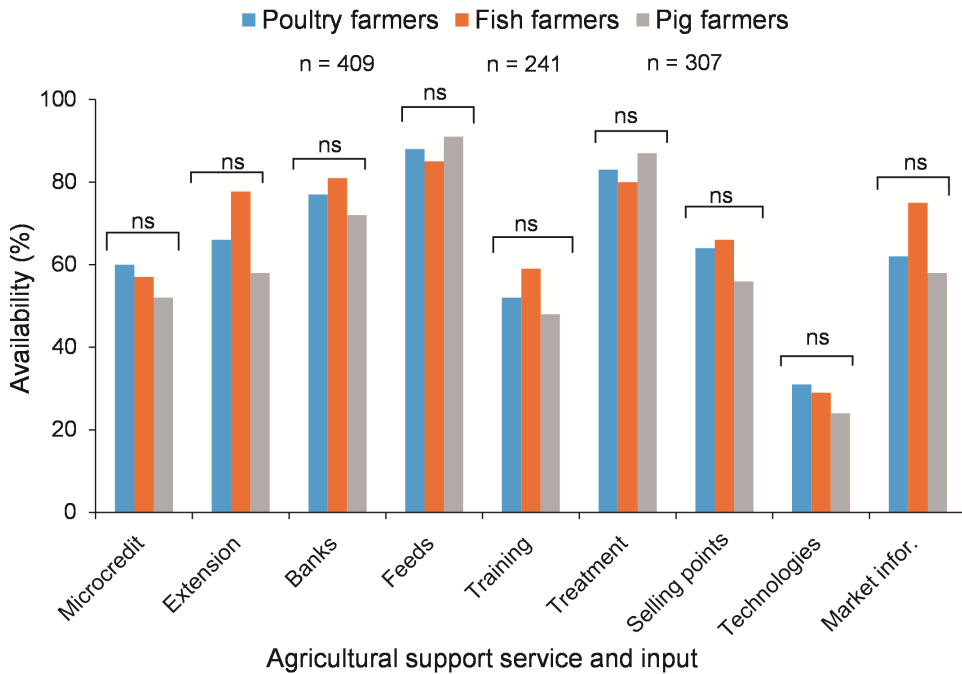


Fig. 4. Percentage availability of agricultural support services and inputs to poultry, fish and pig farmers. Bars with “ns” are not significantly different, $P < 0.05$, Chi-squared test. Microcredit = availability of savings and credit cooperatives that provide saving and credit facilities at low interest rates; Extension = availability of agricultural extension services; Banks = availability of main stream banking services; Feeds = availability of commercial feeds and feed ingredients for the different livestock types; Training = availability of production education programs; Treatment = availability of vaccines and general disease control facilities; Selling points = selling points for poultry, fish and pig products; Technologies = availability of improved feeds, feeding, housing and general production methods; Market infor. = availability of information regarding demand and supply of farm inputs and outputs.

Willingness to pay for insect-based feeds (IBF) among poultry, fish and pig farmers

A total of 899 respondents were willing to pay for IBF, whereas 58 respondents were not, accounting for 94% and 6%, respectively. When asked if they would buy IBF (before the introduction of any bidding process), more than 90% of male and female poultry, fish and pig farmers responded positively (Fig 5).

For each animal category (poultry, fish and pig), more than 70% of the farmers were willing to buy the different feed types at the market price (Fig. 6, 7 and 8). WTP was high and ranged from 65-88% (Fig. 6, 7 and 8). Furthermore, 82-100%, 75-88% and 100% of all poultry, fish and pig farmers, respectively, were willing to buy at a discounted price (Fig. 6, 7 and 8).

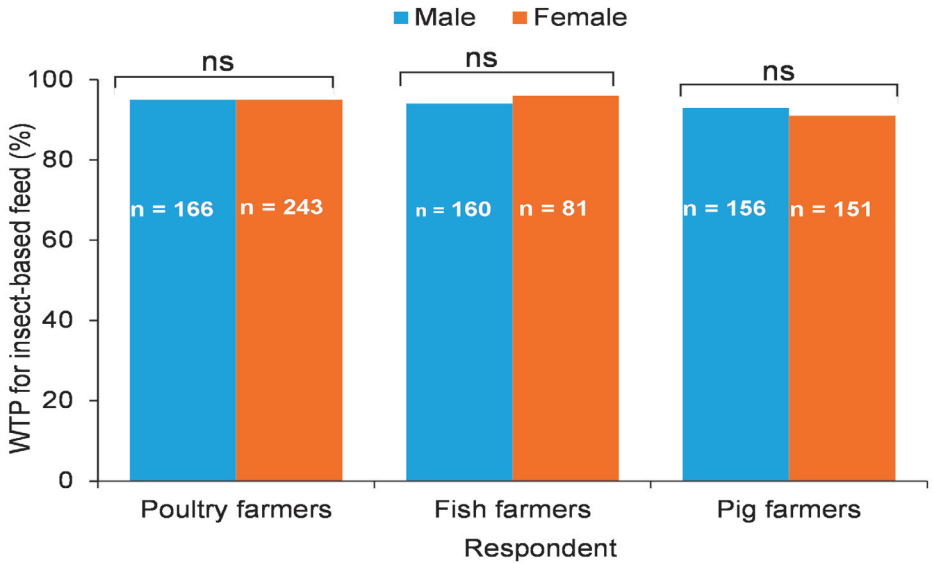


Fig. 5. Percentage farmers willing to pay for insect-based feeds among male and female poultry, fish and pig farmers. Bars with “ns” are not significantly different for male and female respondents, $P < 0.05$, two-proportion z-test.

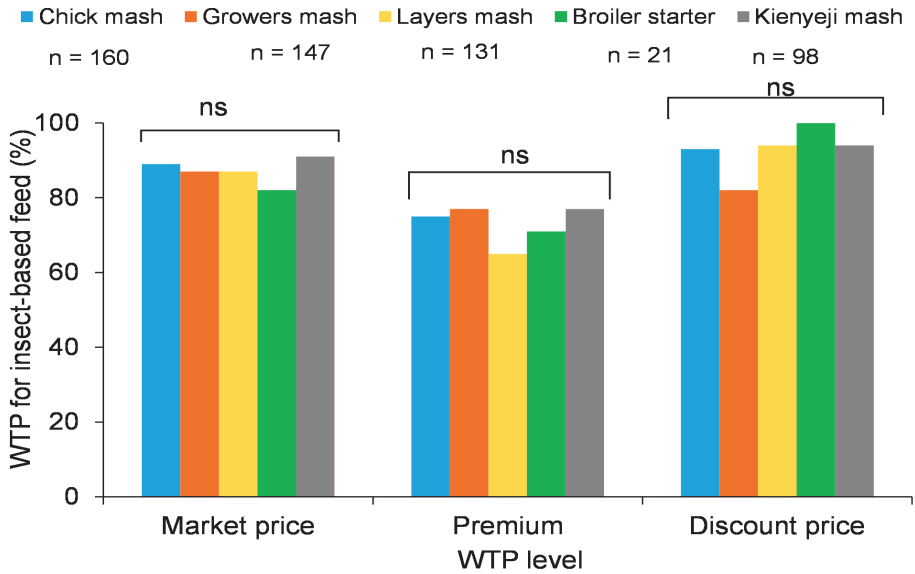


Fig. 6. Percentage farmers willing to pay for insect-based poultry feeds at market price, discount price and premium price. Bars with “ns” are not significantly different, $P < 0.05$, Chi-squared test. Chick mash = a ground form of feed fed to chicks aged 0-8 weeks. Growers mash = ground form of feed for birds aged 8-18 weeks. Layers mash = ground form of feed for laying birds aged 19-76 weeks. Broiler starter = a protein-dense feed formulated to meet the dietary requirements of young broilers aged approximately 1-21 days and are raised purposely for meat. Kienyeji mash = a ground form of feed for indigenous type of chicken commonly known as “Kienyeji”.

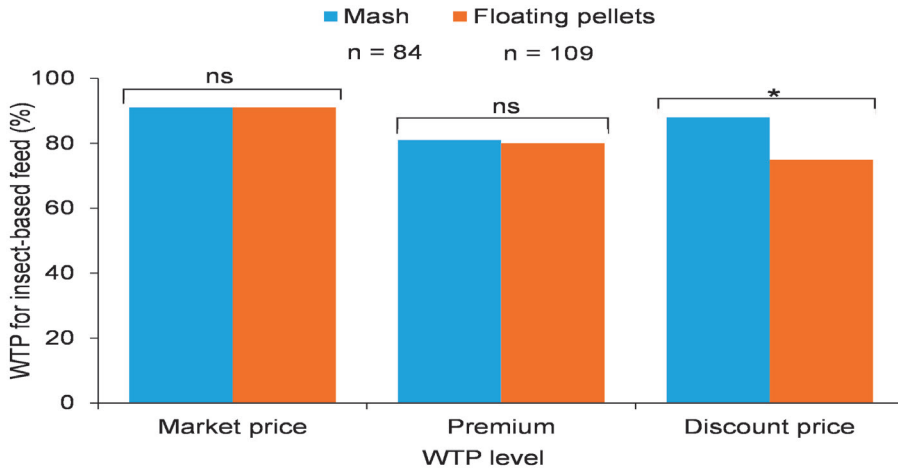


Fig. 7. Percentage farmers willing to pay for insect-based fish feed at market, discount and premium price. Bars with an asterisk are significantly different for mash and floating pellets, $P < 0.05$, z-test. Bars with “ns” are not significantly different, $P < 0.05$, two-proportion z-test. Mash = a finely ground feed formulated and used in moist form for farmed juvenile fish. Floating pellets = finely ground feed that has been compressed and molded into pellets in a pellet mill and float on the surface of water when served to grower and finisher fish stages.

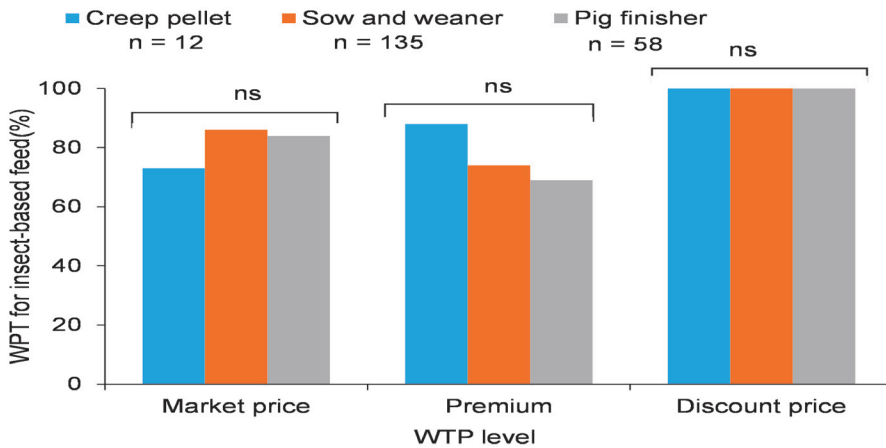


Fig. 8. Farmers’ willingness to pay (WTP) for insect-based pig feed at market, discount and premium price. Bars with “ns” are not significantly different, $P < 0.05$, Chi-squared test. Sow and weaner = Feed type for growing pigs up to 55 kg live body weight and adult breeding pigs. Pig finisher = Feed for pigs weighing over 55 kg live body weight.

When the market price of feeds was reduced by 5-15%, most (96-100%) poultry farmers were willing to buy, whereas an increase in the market price resulted in a decrease in the percentage (50-74%) of poultry farmers willing to buy the different poultry feed types (Fig 9). The majority (94-99%) of the fish farmers were willing to buy floating pellets at a reduced price, but an increase in the market price resulted in a decrease in the percentage of farmers willing to buy at a premium price (Fig 10). Similarly, reducing

the market price of sow and weaner feed by 5-15% resulted in all pig farmers willing to buy at the reduced (discount) price (Fig 10). Furthermore, an increase in the price of feed reduced farmers' WTP and less than 60% of the farmers accepted to buy sow and weaner feed at a premium price (Fig 10). At 15% increase, less than 50% of the pig farmers accepted to buy (Fig 10).

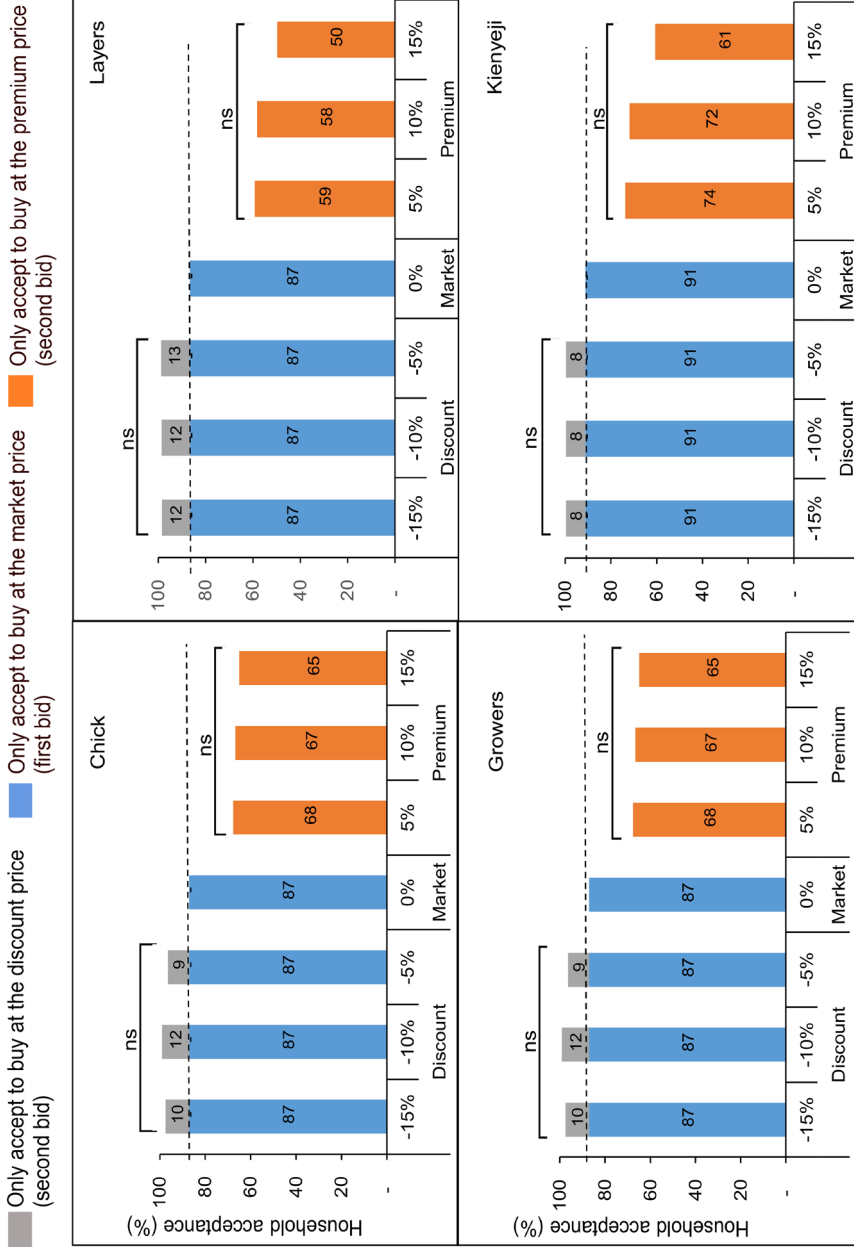


Fig 9. Effect of bid offers on willingness to pay (WTP) level among poultry farmers. The broken line indicates the market price. Bars with “ns” are not significantly different, $P < 0.05$, Chi-squared test. Chick mash = a ground form of feed fed to chicks aged 0-8 weeks. Growers = feed for birds aged 8-18 weeks. Layers = feed formulated for laying birds aged 19-76 weeks. Kienyeji = a ground form of feed for indigenous type of chicken commonly known as “Kienyeji”.

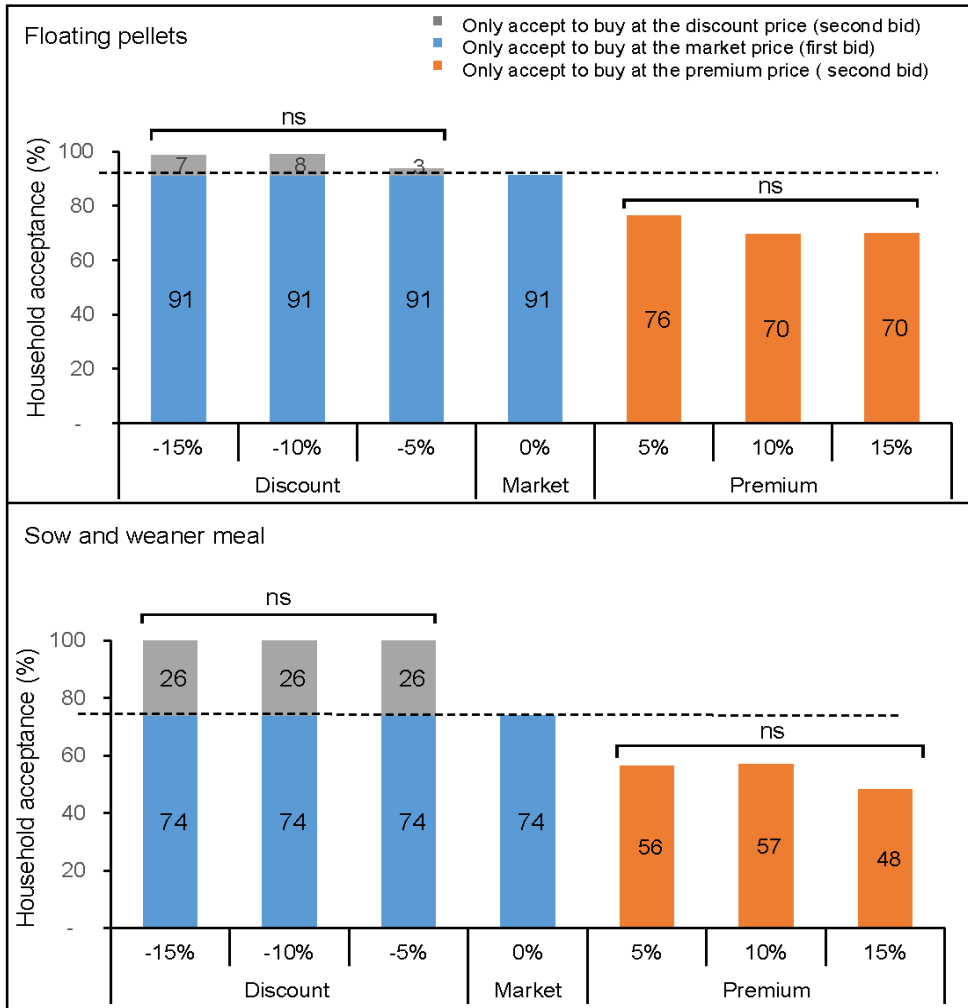


Fig. 10. Effect of bid offers on willingness to pay (WTP) level among fish and pig farmers. The broken line indicates the market price. Bars with “ns” are not significantly different, $P < 0.05$, Chi-squared test. Floating pellets = feed that has been compressed and molded into pellets in a pellet mill and float on the surface of water when served to grower and finisher fish stages. Sow and weaner = Feed type for growing pigs up to 55 kg live body weight and adult breeding pigs. Pig finisher = Feed for pigs weighing over 55 kg live body weight.

Farmers were willing to pay a premium price for IBF (Table 4). Poultry farmers were willing to pay Ksh 60-70 per kilogram of IBF, representing 16-57% increase from the benchmark price (market price) for the different poultry feed types. Kienyeji mash and broiler starter feeds had the highest and lowest percentage change, respectively for poultry farmers. Fish farmers had the lowest percentage change (12-28%) compared to the other farmers in the study. Pig farmers accepted to pay 30-70% higher prices for IBF (Table 4).

Table 4. Farmer's willingness to pay (WTP) level and mean price premium (% change) for insect-based feed

Feed type	WTP price (Ksh/kg)	Standard error	Market price (Ksh/kg)	Standard error	Premium (%)
Poultry					
Chick mash	70.05	2.54	48.92	1.04	43.19
Growers mash	63.76	2.70	46.06	1.12	38.43
Layers mash	57.92	2.12	44.04	1.19	31.52
Kienyeji mash	58.47	3.54	37.34	1.21	56.59
Broiler starter	71.11	2.10	61.32	1.91	15.97
Broiler finisher	62.41	8.18	48.62	3.70	28.36
Fish					
Mash	101.92	8.84	91.12	9.98	11.85
Pellets	179.44	17.81	139.82	12.59	28.34
Pig					
Creep feed	84.19	30.49	49.38	10.04	70.49
Sow and weaner	53.47	3.06	35.69	1.42	49.82
Pig finisher	52.83	3.73	40.65	3.48	29.96

Premium (%) = ((WTP price – Market price)/Market price)*100, Ksh: Kenyan shillings. WTP = willingness to pay. Chick mash = a ground form of feed fed to chicks aged 0 - 8 weeks, Growers mash = feed for birds aged 8 - 18 weeks, Layers mash = feed for laying birds aged 19 - 76 weeks, Kienyeji mash = feed for indigenous type of chicken commonly known as “Kienyeji”. WTP = willingness to pay. Broiler starter and finisher = a protein-dense feed formulated to meet the dietary requirements of young broilers aged approximately 1- 21 days and raised purposely for meat. Broiler finisher = feed formulated to meet the dietary requirements of broilers aged above 21 days. Mash = a finely ground feed formulated and used in moist form for farmed juvenile fish (fry), Floating pellets = finely ground feed that has been compressed and molded into pellets in a pellet mill and float on the surface of water when served to grower and finisher fish stages, Creep feed = high-nutrient feed designed to supplement nursing animals, Sow and weaner = Feed type for growing pigs up to 55 kilograms live body weight and adult breeding pigs, Pig finisher = Feed for pigs weighing over 55 kilograms live body weight.

Factors influencing farmers' WTP for insect-based feed

Explanatory variables were regressed with WTP for IBF among farmers for the different feed types within the animal categories (Table 5). The regression results showed that awareness that insects can be used as feed, acceptance of BSF larvae as feed, availability of extension services and market information positively influenced WTP, whereas variables such as age, making of their own feed and attitude that insects are a good feed source negatively influenced WTP among farmers using Kienyeji feed (Table 5). For farmers using layers feed, availability of training positively influenced WTP, whereas

marital status, household size, availability of microcredit and market information negatively influenced WTP (Table 5). For farmers using grower feed, acceptance of crickets and the availability of banks positively influenced WTP (Table 5).

For farmers using chick feeds, WTP for IBF was not affected by the variables (Table 5).

In fish production, WTP for IBF also varied for the different feed types (Table 5). The use of commercial feeds and acceptance of BSF larvae as feed ingredient positively influenced WTP for farmers using floating pellets, whereas distance to feed trader, acceptance of housefly and availability of treatment negatively influenced WTP. For farmers using mash feed, educational level positively influenced WTP (Table 5).

In pig production, WTP was positively influenced by age, distance to feed trader and availability of new technologies, whereas factors such as making of their own feed, acceptance of cockroaches as feed and market information negatively influenced WTP among farmers using pig finisher feeds (Table 5). For farmers using sow and weaner feeds, WTP was positively influenced by acceptance of BSF larvae as feed whereas the acceptance of housefly maggots negatively influenced WTP (Table 5).

Table 5: Regression results for factors influencing willingness to pay (WTP) for insect-based feeds among fish, pig and poultry farmers

Variable	Fish farmers		Pig farmers		Poultry farmers			
	Pellets	Mash	Finisher	Sow	Kienyeji	Layer	Grower	Chick
Gender	-	-37.36	-	-	-	-	-	-
Age	-1.85	-	0.59**	0.13	-0.65*	0.04	0.28	0.22
Education level	-1.63	5.25*	-	-	-	-	-	-
Marital status	50.74	-17.98	13.35	-	-3.56	-4.83*	-	-
Household size	-	-	-	-	-1.55	-2.69**	-1.31	-
Commercial feed	293.34**	26.13	-	-	-	-	-	-
Distance	-0.27*	-	0.68**	-	-	-	-	-
Make own feed	-	-	-21.77*	-9.09	-16.42*	-	-	-
Aware that poultry feed on insect	-	-	-	-	35.92**	-10.97	-	2.43
Insect are a good source of poultry feed	-	-	-	-	-30.79**	9.38	-	-
Number of growers owned	-	-	-	-	-0.16*	0.02	0.05	-
Number of chicks owned	-	-	-	-	0.27	0.32	0.35*	-
Ever used insect as feed	-44.50	-	-	-	-	-	-	-
Housefly maggots-acceptance	-57.29*	-	-6.76	-13.58*	-13.03	-	-	-3.83
Crickets-acceptance	-	-	-	-	-7.91	-	12.87*	3.18
Black soldier fly-acceptance	127.54**	-	-	14.23**	19.39**	5.28	3.10	9.04
Cockroach-acceptance	-	-	-14.58*	-	-	-	-	-
Availability of microcredits	-	-	-	-	-5.40	-12.39**	-	7.08
Availability of banks	-	-	-	-	12.60	-	11.02*	2.01
Availability of extension services	65.08*	17.58	-	-	24.59**	-	-	-
Availability of training	-	-	-	-	-8.36	13.86**	-4.72	-
Availability of agricultural inputs	-112.78	-	-11.85	16.55	-	-	-	-
Availability of new technologies	-	-	21.26**	6.12	-	-	-	-
Availability of treatment	-99.83*	20.81	-	-	12.58	-	6.44	-
Availability of market information	62.19	-	-14.10**	-	13.28*	-10.29*	-0.84	-
Constant	2.49	49.64	-2.53	71.02	48.82	79.74	42.86	54.31

Significance levels: ** $P < 0.01$, * $P < 0.05$, logistic regression. (–) variable not included in the model. Floating pellets = finely ground feed that has been compressed and molded into pellets in a pellet mill and float on the surface of water when served to grower and finisher fish stages. Mash = a finely ground feed formulated and used in moist form for farmed juvenile fish. For pig finisher = Feed for pigs weighing over 55 kilograms live body weight. Sow and weaner = Feed type for growing pigs up to 55 kilograms live body weight and adult breeding pigs. For Kienyeji = feed for indigenous type of chicken commonly known as “Kienyeji”. Layers = feed for laying birds aged 19 – 76 weeks. Growers = feed for birds aged 8 – 18 weeks. Chick = a ground form of feed fed to chicks aged between 0 – 8 weeks. WTP = willingness to pay.

Discussion

In most developing countries, agribusiness is an important component of the economy. Central to its sustainability are consumers' attitudes and market acceptance of the products (Alemu et al., 2016; Etim & Benson, 2016). One way of ensuring this, is by assessing the hypothetical WTP prior to production (Alemu et al., 2016; Martinez-Carrasco et al., 2015). The present study explored farmers' knowledge of and attitudes towards insects as an alternative feed ingredient, the practice and utilization of local feed formulations, and their WTP for IBF. Socio-demographic characteristics of a household present the ability of the household to produce and consume goods. They affect the household's access to and WTP for farm inputs (Manja et al., 2015). The present study shows that farmers in Kenya are sufficiently educated and thus exposed to information which is important in decision making. In most peasant economies in developing countries, household labour is used to produce either for their own use or for the market (Asaminew, 2014). In the present study, we recorded a mean household size of five, which indicates that family labour is available for production. In addition, farmers generally had a medium to high access to agricultural inputs and support services such as microcredit, extension services, banking services and agricultural inputs such as: feed, training, selling points for livestock products, new technologies and market information.

Information regarding farmers' knowledge, attitude towards insects as feed and the practice of making and using their own feeds may be used in strategies geared towards introducing IBF. Our results show that Kenyan livestock producers are well aware of the potential of insects being used as a feed ingredient. This awareness probably provided farmers with the opportunity to develop a positive attitude. This positive attitude would promote the decision maker taking a risk regarding a novel input while a negative attitude discourages the decision maker from taking risk. This result is similar to previous reports that socio-economic characteristics and farmers' knowledge affect interests in insect as feed (Mancuso et al., 2016). The finding that farmers are well aware of the use of insects as feed ingredient with a positive attitude towards insects as a feed ingredient as well as the finding that farmers have already used insects to feed their animals, provides an enabling environment for implementing IBF.

A key finding in the present study is that farmers are willing to pay more for IBF than for major commercial feeds used in poultry, fish and pig production. This is consistent with the farmers' high knowledge level and positive attitude towards insects as an alternative feed ingredient in this study and agrees with observations from studies in other countries (Mancuso et al., 2016). This provides an indication of a potential market acceptance of IBF among farmers. The high percentage of farmers' WTP recorded in this study is not surprising considering that insects have been part of the natural diet of

poultry, fish and pigs in their natural environment (Laureati et al., 2016). The socio-demographic variables represent differential influences on farmers' WTP in this study. The variable 'age' for example, negatively correlated with WTP for farmers using Kienyeji and floating pellets for poultry and fish, respectively, but showed a positive correlation with WTP for pig finisher feed. These results are in agreement with previous reports that the variable 'age' could either negatively or positively influence a farmer's WTP for a new product (Etim & Benson, 2016; Manja et al., 2015; Oladele, 2008). Similar findings were obtained for variables such as 'aware that insects can be used as feed', which positively and negatively correlated with WTP for Kienyeji and layers feed, respectively. It is worth noting that for poultry and pig, farmer age and acceptance of insects showed a significant correlation with WTP, in at least one of the feed types for each animal category. A negative correlation of 'farmer age' with WTP indicates that the younger the farmers, the more willing they are to pay for IBF for poultry and fish and vice versa (Laureati et al., 2016). This also agrees with other reports that younger people are more willing to try new products than older people (Lombardi et al., 2019). This also suggests a greater potential adoption of insects as feed ingredient among young farmers compared to older farmers, except for pig farmers where older farmers appear to be more willing to adopt IBF. Furthermore, acceptance of insects as feed ingredients generally showed a significant correlation with WTP, with BSF showing a strong positive correlation with WTP. This means that an increased availability of the BSF would greatly influence farmers' WTP for IBF.

We used household-based data and adopted the contingent valuation method (Etim & Benson, 2016) and the double bound-logit model to assess farmers' WTP, WTP level and the factors that influence WTP, using market price as a benchmark price for IBF for poultry, fish and pig farmers in Kenya. Our results show that above 90% of the surveyed male and female farmers are willing to pay for IBF. The farmers are willing to pay at least 16%, 12% and 30% extra for IBF for poultry, fish and pigs, respectively. Female poultry farmers are significantly more aware that insects can be used as feed ingredients than males. More female poultry farmers have a positive attitude towards insects as feed than males. Furthermore, the significantly negative correlation between WTP and the variables: "insects are a good feed source" for farmers using Kienyeji feed and "acceptance of housefly maggots" for fish and pig farmers in the present study, may have been due to lack of information on the negative impact of total reliance on the traditional feed ingredients. In one study for example, respondents expressed a strong negative attitude towards fish raised on IBF. When informed of the impacts of overfishing for farmed fish, the result showed a strong positive correlation, indicating that respondents' knowledge can strongly affect acceptance and willingness to accept alternatives (Ankamah-Yeboah et al., 2018). The present study is one of only few studies to assess consumer opinion on insects as feed, and the first to document farmers' knowledge and

attitude towards insects as feed and their intentions to purchase IBF in sub-Saharan Africa. We conclude that farmers are willing to pay for IBF and understand the benefits of using IBF in animal production. Farmers' WTP for IBF is a function of several socio-demographic factors, especially: age, gender, awareness of insects as feed, acceptance of insect species, availability of agricultural extension services and market information. Therefore, such factors are crucial for designing policy strategies to ensure effective adoption of IBF in Kenya.

The implication of our study is that feed companies can replace fishmeal and soybean meal with insect meal in poultry, fish and pig feeds without major impacts on market demand because most of the farmers are willing to pay for IBF. This, therefore, presents an excellent opportunity for innovative and sustainable use of resources through insect rearing and minimizes the pressure on the agricultural land and marine resources. Our findings provide the first insight into the market opportunities of including insects in the animal feed value chain in Kenya, particularly following the recent authorization of the use of insects in animal feed by the Kenyan government (Kenya Final Approved Standard KS 2711_2017).

To enhance farmer uptake of the innovative technology and WTP for more sustainable and readily available alternatives such as IBF, improvements in extension support services are of paramount importance (Makkar, 2016; Popoff et al., 2017). We therefore recommend the following: First, there is a need to increase farmer's knowledge on the nutritional value of insects, especially the BSF, as well as their use as alternative feed ingredients. From the empirical results of this study, awareness and acceptance of insect species significantly influence farmers' WTP. Developing local insect production systems can increase availability of insect meal and, thus promote local IBF production. In the present study for instance, "distance to feed trader" had a significant influence on WTP. This means that the closer the feed trader is to the farmer, the higher the probability that the farmer will be willing to buy the feed and vice versa. Second, our results indicate that the use of commercial feeds and acceptance of BSF larvae as feed positively influence WTP among fish farmers for floating pellets. This means that an increase in these variables leads to an increase in WTP. So, engaging feed millers and traders and training them with regard to the advantages of insect meal in animal feed, can promote inclusion of insects in commercial feed without affecting demand. Finally, creating linkages between farmers and the markets will further enhance utilization of innovative and locally available feed resources such as insect meal among fish and livestock farmers in Kenya.

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3

Chapter 4

Effects of waste stream combinations from brewing industry on performance of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae)

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Abstract

In recent years, there has been a rapidly growing demand for readily accessible substrates for mass production of black soldier fly, *Hermetia illucens* Linnaeus. Beer production results in various by-products that typically end up in uncontrolled dumpsites, constituting pollution problems, which merits urgent attention. The present study investigated whether the 12 formulated diets composed of brewers' spent grains (BSGs), brewers' yeast and cane molasses can serve as substrates for *H. illucens* production. Four different BSGs were selected and formulated into 12 diets, aiming at varying protein and net energy levels. The diets were offered to newly hatched (~1 h old) *H. illucens* larvae and the influence on developmental duration, survival, wet weight, pre-oviposition time, fecundity, and longevity were compared. Developmental duration of the larvae (16–21 days) and pre-pupae (8–11 days) differed significantly across the different diets. The developmental duration of the pupae (8.7–9.1 days) was not affected by diet. The larval (86–99.2%), pre-pupal (71–95%), and pupal (65–91%) survival rates varied significantly between flies reared on the different diets. The pre-oviposition time was similar for flies provided with water (7–11 days) and 10% sugar solution (8–14 days) or across the different diets. The mean fecundity per female ranged from 324–787 eggs and did not differ between females provided with water or sugar solution. However, the number of eggs laid per female varied significantly across the different diets when provided with water. The longevity of starved *H. illucens* adults was significantly lower (5 days) compared to those provided with water (11–14 days) or sugar solution (14–15 days). The implications of these findings as part of a quality control procedure for commercial production of high-quality *H. illucens* larvae as an alternative protein ingredient in livestock and aquaculture feed are discussed.

Keywords: *Hermetia illucens*, protein, mass rearing, quality control parameters, agro-industrial by-products

Introduction

The United Nations figures project global human population growth of almost 50% since 2000 to 9.5 billion by 2050 (United Nations, 2015). The increase in human population has resulted in an increase in the demand for protein and, consequently, an increase in the production of livestock, which is constrained by the availability of protein-rich feedstuffs (Herrero et al., 2015; Mottet et al., 2017; Tallentire et al., 2018). Commonly used protein sources in livestock and aquaculture feeds include fish-derived and plant-derived protein sources, which are directly and indirectly competing with human nutrition (Shewry & Halford, 2002; Van der Spiegel et al., 2013), creating an unsustainable pressure on the food value chain (Evans, 2009). Therefore, the development of innovative, cost-effective, and environmentally friendly options such as farming of insects on organic waste streams as alternative protein sources becomes important because they are increasingly considered an attractive, viable, and sustainable alternative to animal and plant protein sources (Henry et al., 2015; Makkar et al., 2014; Van Huis, 2013). Insects are rich in crude protein (35–77%), carbohydrate, fat, vitamins, and minerals (Ganguly et al., 2013; Henry et al., 2015; Makkar et al., 2014; Van Huis, 2013).

Insects like the black soldier fly *Hermetia illucens* Linnaeus, offer promising alternatives of nutrient recovery while accumulating high-quality nutrient body biomass with an average of 42–43% crude protein, 33% fat and micronutrients such as iron and zinc (Barragán-Fonseca, 2018; Makkar et al., 2014; Oonincx et al., 2015; Rumpold & Schluter, 2013; Spranghers et al., 2017). However, the nutritional status of insects varies depending on the species and rearing substrates (Liland et al., 2017; Meneguz et al., 2018; Tschirner & Simon, 2015). The use of *H. illucens* larvae as an alternative to fishmeal or soybean meal in poultry, pig, and fish feeds has been advocated worldwide (Gasco et al., 2016; Ji et al., 2016; Lock et al., 2016; Makkar et al., 2014; Renna et al., 2017; Schiavone et al., 2017; Veldkamp & Bosch, 2015) and provides opportunities for income generation (Dobermann et al., 2017; Kelemu et al., 2015; Van Huis et al., 2013). To meet the increasing demand for high-quality *H. illucens*-based protein ingredients, mass production of *H. illucens* on readily available organic waste streams is important (Sanchez-Muros et al., 2014).

Organic waste management is a major challenge in Kenya, especially in Nairobi, the rapidly growing capital. In Nairobi, over 2,400 tons of waste are generated every day (Kasozi & von Blottnitz, 2010), of which only 38% is collected and less than 10% recycled (Japan International Cooperation Agency (JICA), 2010). The remaining 62% being organic waste largely from households, restaurants, hotels, markets, and agro-industrial manufacturing processes (Hoornweg & Bhada-Tata, 2012; UN-HABITAT, 2010a). For agro-industrial manufacturing processes in Kenya, Kenya Breweries Limited (KBL) and Mumias Sugar Company Limited generate huge amounts of waste. Only a small proportion of these massive waste streams has occasionally been used as supplements in

livestock feed, since the advent of beer production in many countries in the world (Aliyu & Bala, 2011; Calvert, 1991; Farhat et al., 2001; Liguori et al., 2015; McDonald et al., 2002), but this is not the optimal use, as the spent grains are difficult for animals to digest (Newman & Jennings, 2008).

The use of *H. illucens* larvae to digest a wide range of organic waste streams, including animal manure (Xiao et al., 2018) fruit remains (Nguyen et al., 2013), and vegetable remains (Meneguz et al., 2018), or even some indigestible food such as coffee pulp (Diener et al., 2009) has been well documented. Larvae of *H. illucens* can convert these organic waste streams to useful nutrients, maintaining a balance between high larval weight and reduction of organic solid matter up to about 42–56% (Diener et al., 2011; Diener et al., 2009; Li et al., 2011; Nguyen et al., 2015). Interest in the use of these waste streams as a source of value-added products is increasing rapidly due to their availability, year-round accessibility, affordability, low competitiveness for food or feed and the need for sustainable waste management procedures. According to Van Huis et al. (2013), bioconversion of these waste streams using *H. illucens* will be more sustainable than other waste conversion and handling techniques as the insects are able to utilize massive amounts of organic waste and reduce the unpleasant smells emanating from the waste (Lardé, 1990), reduce efficiently the accumulation of polluting elements (nitrogen, phosphorous) from manure and compost (Beskin et al., 2018; Sanchez-Muros et al., 2014; Van Huis, 2013; Xiao et al., 2018). Larvae of *H. illucens* also modify the microflora in organic waste thereby reducing the occurrence or abundance of undesirable bacteria (Erickson et al., 2004; Yu et al., 2011). Larvae of *H. illucens*, thus, add value to the waste (bio-fertilizers) and are efficient converters as they produce a protein and lipid-rich biomass from substrates that can be poorly used by monogastric animals (Gobbi et al., 2013; Tomberlin & Sheppard, 2002; Tomberlin et al., 2002; Xiao et al., 2018). These characteristics, linked to a short production cycle, make *H. illucens* larvae very good candidates for intensive production. Therefore, waste that would otherwise contaminate the environment and put human and animal health at risk could be a source of income generation and employment creation through well-established recycling and resource recovery (Diener et al., 2011; Diener et al., 2009; Liguori et al., 2015; Nguyen et al., 2013; UN-HABITAT, 2010b).

Although, the economic importance of this fly as a potential candidate for mass rearing is well established, knowledge on important aspects of the reproductive biology of *H. illucens* on agro-industrial waste streams (mixed diets of brewer's spent grains (BSGs), brewers' yeast, and cane molasses) as suitable substrates for mass production remains largely unknown. The process of beer and sugar manufacturing generates various by-products, typically BSGs, brewers' yeast, and molasses. These by-products are produced in large quantities daily, readily available and highly accessible throughout the year and easy to handle. Here, we investigate the suitability of these waste streams as

substrate for *H. illucens*.

It is well known that the quality of larval diet significantly affects mass rearing of insects, especially growth, survival, and biological traits of adult flies because large females have large ovaries and lay more eggs than small females (Blackmore & Lord, 2000; Churchill-Stanland et al., 1986; Gobbi et al., 2013; Roper et al., 1996). Thus, larval diet quality and feeding are crucial to overall fitness (Moreau et al., 2006; Tikkanen et al., 2000; Tomberlin et al., 2002). In this study, we combined different agro-industrial waste streams and determined the life-history parameters of *H. illucens* by focusing on the following research questions: how does the quality of the larval diet affect (a) developmental duration of immature life stages, (b) their survival, (c) larval, pre-pupal, pupal, and adult biomass, (d) pre-oviposition duration, (e) adult fecundity, and (f) longevity of starved, water-provided and sugar-fed adults.

Materials and Methods

Insect culture

This study was carried out at the Animal Rearing and Containment Unit of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. *H. illucens* colony was established in 2016 from eggs of wild-trapped *H. illucens* populations in Kasarani, Nairobi County (S 01°13'14.6"; E 036°53'44.5", 1,612 m a.s.l.) following the method described by Booth & Sheppard (1984) and Sripontan et al. (2017) with slight modifications. The egg clusters were transferred to metal trays (76 × 27.5 × 10 cm) containing 2,000 g of BSG diluted in 3,200 ml of water. The diet was hydrated to approximately 70 ± 2% moisture by weight and confirmed using a moisture sensor with two 12 cm long probes (HydroSense™ CS620; Campbell Scientific, Inc., Logan, UT, USA). The culture was monitored daily for larval development. The pre-pupal stages after self-dispersal from the substrate were kept in four litre transparent rectangular plastic containers (21 × 14 × 15 cm) (Kenpoly Manufacturer Ltd., Nairobi, Kenya) containing moist wood shavings (sawdust) as pupation substrate according to Holmes et al. (2013). An opening (14.5 × 8.3 cm) was made in the lid of each container and covered with fine netting organza material capable of retaining emerging adult flies. Conditions in the rearing room were maintained at 28 ± 1 °C, 70 ± 2% relative humidity (RH) and a photoperiod of L12:D12. Adults were transferred to outdoor cages where water and sugar solution were provided ad libitum to the flies. When the adult flies in the cage were 7-days-old (Nakamura et al., 2016), moist chicken manure (500 g diluted in 800 ml of water) was provided in plastic containers (30 × 15 cm) with the surface covered with wire mesh. Strips of cardboard with flutes along the edges were placed on top of the wire mesh, which provided the flies with sites for laying eggs. The containers were checked daily to collect egg clusters deposited by the flies. The cardboard strips with egg clusters were

transferred to plastic containers and placed in climate-controlled chambers. The newly hatched larvae were fed ad libitum BSG until full development into pre-pupal stages. The pre-pupal stages were transferred into two l transparent rectangular plastic containers containing a 2.5 cm layer of moist wood shavings and monitored daily for pupal formation. The pupae collected were regularly transferred into four-litre transparent plastic rectangular containers containing a 2.5 cm layer of moist wood shavings until emergence. The emerged flies were transferred to the outdoor rearing cages designed specifically to hold the adult fly stock populations. The *H. illucens* colony has been in culture for ~2 years and once every 6 months, wild-caught flies are added to the colony to prevent inbreeding depression. In addition, adult *H. illucens* populations in the cages were maintained in low numbers (approximately 2,000 adult flies in a 1 × 1.2 × 1.8 m cage) to avoid stressful crowding effects, which is very common in insect mass production (Sørensen & Loeschcke, 2001).

Experimental substrates and diet formulation

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The BSGs used were sourced once from the KBL, Nairobi, Kenya; main producer of major beer brands in the country: Tusker (malt and corn starch); Guinness (malt and barley); Senator (sorghum and barley), and Pilsner (barley). The liquid form of brewer's yeast from the processing of each of the beer brands was also collected as part of waste streams to be used during the experiments. The fresh BSGs were placed on plastic sheets with moving dry air at ambient temperature (28.0 ± 1 °C) for 48 h using an Xpelair® heater (WH30, 3 KW Wall Fan Heater; Peterborough, UK). Possible fermentation of the BSGs at this temperature was avoided by turning the substrates twice daily to ensure proper aeration and to prevent molding within the substrates. Thereafter, the semi-dried products were oven-dried at 60 °C for 72 h to approximately 90% dry matter (DM) (~10% moisture). The dried BSGs were later passed through a three mm sieve in a Munch hammer mill (Munch, Wuppertal, Germany) to obtain particle size suitable for incorporation into *H. illucens* diet. Molasses was obtained in liquid form from Mumias Sugar Company Limited.

Dried BSGs from the four main beer brands were formulated into 12 different diets as follows: The first group was the “control” for which 50 g of each BSG was mixed with 80 ml of water only: malt/corn-starch/water; malt/barley/water; sorghum/barley/water, and barley/water. Each diet was hydrated to approximately $70 \pm 2\%$ moisture content and confirmed using a moisture sensor with two 12 cm long probes (HydroSense™ CS620; Campbell Scientific, Inc., Logan, UT, USA). In the second group, each of four BSGs was supplemented with waste brewer's yeast. Fifty grams of each BSG was mixed with 90 ml of brewer's yeast to generate the following treatments: malt/corn-starch/brewer's yeast; malt/barley/brewer's yeast; sorghum/barley/brewer's yeast, and barley/brewer's yeast. In the third group, 50 g of each BSG was supplemented with 45 ml of

waste brewers' yeast + 45 ml of molasses: malt/corn-starch/brewer's yeast/molasses; malt/barley/brewer's yeast/molasses; sorghum/barley/brewer's yeast/molasses, and barley/brewer's yeast/molasses.

Chemical analysis of experimental diets

Prior to conducting proximate analysis of the various diets using the method described by AOAC (1990), weighed samples were oven-dried at 60 °C for 72 h. DM content of each sample was measured by oven drying at 105 °C for 48 h until constant weight was achieved (AOAC, 1990; Okedi, 1992; Pen et al., 2013). Moisture content was determined using the oven set at 105 °C for 24 h (AOAC, 1990). Nitrogen content was determined using the Kjeldahl method (AOAC, 1990) and later converted to crude protein content by multiplying with factor 6.25 (Finke, 2007). The ash content was determined using a muffle furnace and samples heated at 550 °C overnight according to the method described by (AOAC, 1990). Velp solvent extractor (SER 148/6) was used to determine fat content (crude fat) with ethyl ether as extractant (AOAC, 1990). All parameters discussed above were determined in triplicate per sample and expressed as a percentage.

Experimental design

Before the start of the experiment, the rearing room was maintained at 28.0 ± 1 °C using an Xpelair heater (WH30, 3 KW Wall Fan Heater; Peterborough, UK). The RH in the experimental room was adjusted and maintained at $70 \pm 2\%$ using an adiabatic atomizer humidifier (Condair ABS3; Hornsby, Australia), while maintaining 12:12 L:D photoperiod. The condition of the room was monitored daily using a WiFi Sensor (Wi-Fi-TH Corintech Ltd., Fordingbridge, UK; Firmware version 5.1.7/13.3.3G/R4.11). Thereafter, 120 egg batches (~3 h old) collected from the adult stock culture maintained in the outdoor cages described above were distributed equally in 12 sterilized disposable 100×15 mm petri dishes and monitored at 6 h intervals daily for egg eclosion. According to the method described by Gobbi et al. (2013), 300 neonate larvae (~1 h old) were individually counted with the aid of entomological tweezers and a moist fine camel hair brush under a stereomicroscope (Leica MZ 125 Microscope; Leica Microsystems Switzerland Limited, Heerbrugg, Switzerland), fitted with a Toshiba 3CCD camera using the Auto-Montage software (Syncrosopy; Synoptics Group, Cambridge, UK) at magnification of $\times 25$. The larvae were carefully lined on moistened pieces of sterilized black cloth, which were thereafter placed on top of the experimental diet in each of the 12 transparent plastic containers (12×4.5 cm). The lid of each container was designed with an opening (8×4 cm) fitted with fine netting material of 1.3×1.3 mm mesh size for ventilation. Each experimental setup was then maintained in the climate-controlled

rearing room described above. The larvae in each treatment were provided ample feeding substrate to carry them throughout the larval developmental phase to pre-pupae (non-feeding phase). The larvae generally have a cream-like color but at the fifth instar stage there is a recognizable on-set of exoskeletons (skin) color change to beige (dark brown) before they undergo the last molt to the charcoal-grey colored pre-pupal stage (Dortmans et al., 2017). Once the larvae turned into pre-pupae, they were transferred individually into plastic containers ($3 \times 4 \times 3$ cm) with 2.5 cm layer of moist wood shavings (sawdust). Each container had an opening (2.5 cm diameter) covered with fine netting organza material for ventilation. The containers were checked daily, and pupae formed were recorded. The pupae were collected and maintained individually in similar plastic containers until emergence. Stage-specific developmental time, survival and wet weight were calculated for each treatment. Weight measurements of the different life stages were carried out using a Kern-PCB 350-3 precision balance (0.001–350 g). The experiments were replicated five times for each experimental diet.

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Pre-oviposition period, oviposition period, fecundity, and longevity when starved or provided with water or sugar solution

To determine the effect of each of the 12 experimental larval diets on life-history parameters, ninety paired newly emerged (< 24 h old) adult flies were randomly selected by collecting fully winged male and female flies that emerged from each dietary treatment. The paired adult flies were subdivided into three groups of 30 each. Individual pairs of flies from each group were kept in transparent rectangular Perspex cages ($30 \times 16 \times 16$ cm) with openings covered with breathable material. Two strips of cardboard with holes along the edges were provided for laying eggs. The first group of paired flies from each diet was starved (unfed) throughout the experiment, while the second and third groups were provided with water and 10% sugar solution on soaked cotton wool, respectively. Each experimental set-up was observed daily to record the number of eggs laid. The pre-oviposition period was calculated from the first day of emergence of an adult female to the first day of oviposition. Eggs laid on each day were collected with the aid of a fine wet black camel hair brush. Each egg clutch collected was physically separated by spreading it on the surface of an electrically powered light box (2×15 W 6,500 K, model 44077 B.S.4533; Sascos, London, England) and counted with the help of a tally counter. The light box allowed for easy identification of individual eggs during counting. The experiment was terminated when the female and male flies died. Both longevity and fecundity were calculated for each diet.

Statistical analysis

Larval weight, pre-pupal weight, pupal weight, adult weight, development duration, adult longevity, number of eggs (female fecundity), and pre-oviposition period data were subjected to analysis of variance (ANOVA) to evaluate the effect of the waste streams on these variables. Number of eggs was log-transformed prior to ANOVA to stabilize variance. Tukey's honestly significant difference test was used to separate means. A t-test was used to compare the pre-oviposition period between treatments with sugar solution and water within each experimental diet. Further, orthogonal contrasts were created and evaluated using the `glht` function in the `multcomp` package (Hothorn, Bretz & Westfall, 2008) to explore the structure in the treatments, the agro-industrial wastes. The differences among treatment means were considered statistically significant at $\alpha = 0.05$. All statistical analyses were implemented using R version 3.3.3 (R Core Team, 2017).

Results

Nutrient composition of experimental diets

Marked variation was observed on the nutritional composition (on DM basis) of the diets used in this study (Table 1). There was a significant difference in crude protein ($F = 194.90$; $df = 11, 24$; $P < 0.0001$), crude fat ($F = 45.09$; $df = 11, 24$; $P < 0.0001$), ash ($F = 8.48$; $df = 11, 24$; $P < 0.0001$) and moisture ($F = 261.90$; $df = 11, 24$; $P < 0.0001$) contents among the experimental diets. Diets supplemented with brewers' yeast only had higher crude protein levels compared to the other diets. The inclusion of brewers' yeast plus molasses in diets (spent grains) resulted in lower crude protein contents compared to diets mixed with water only.

Table 1. Nutrient composition (on dry matter basis) of experimental diets

Larval diet	Crude protein (%)	Crude fat (%)	Ash (%)	Moisture (%)
BW	30.33 ± 0.24 ^{bc}	6.38 ± 0.17 ^c	4.15 ± 0.18 ^{bcd}	11.57 ± 0.10 ^{cd}
MBW	28.89 ± 0.23 ^{cd}	6.78 ± 0.25 ^c	3.80 ± 0.19 ^{cd}	12.38 ± 0.13 ^c
MCW	27.38 ± 0.11 ^d	6.46 ± 0.41 ^c	3.03 ± 0.18 ^d	12.10 ± 0.15 ^c
SBW	29.43 ± 0.30 ^c	11.8 ± 1.07 ^a	3.72 ± 0.18 ^{cd}	10.38 ± 0.13 ^{def}
BY	31.99 ± 0.08 ^a	5.39 ± 0.03 ^{cd}	4.74 ± 0.28 ^{abc}	11.53 ± 0.12 ^{cd}
MBY	30.22 ± 0.10 ^{bc}	6.96 ± 0.13 ^c	4.41 ± 0.34 ^{bc}	10.76 ± 0.26 ^{dc}
MCY	27.72 ± 0.23 ^d	6.04 ± 0.15 ^c	5.14 ± 0.2 ^{ab}	9.91 ± 0.11 ^{ef}
SBY	31.39 ± 0.17 ^{ab}	9.48 ± 0.15 ^b	4.32 ± 0.25 ^{bc}	9.17 ± 0.20 ^f
BYMo	22.14 ± 0.48 ^e	3.95 ± 0.31 ^{def}	5.8 ± 0.19 ^a	19.51 ± 0.23 ^a
MBYMo	22.32 ± 0.71 ^e	3.23 ± 0.17 ^f	4.08 ± 0.22 ^{bcd}	18.24 ± 0.20 ^b
MCYMo	19.10 ± 0.35 ^f	3.42 ± 0.02 ^{ef}	4.31 ± 0.21 ^{bc}	18.79 ± 0.62 ^{ab}
SBYMo	21.69 ± 0.14 ^e	5.18 ± 0.15 ^{cde}	4.71 ± 0.20 ^{abc}	18.66 ± 0.22 ^{ab}

Means in a column followed by different lower-case letter are significantly different ($P < 0.05$, ANOVA plus HSD). BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

Effect of rearing diet on development of immature stages of *H. illucens*

There were significant differences in larval ($F = 14.16$; $df = 11, 48$; $P < 0.001$) and prepupal ($F = 12.45$; $df = 11, 48$; $P < 0.001$) developmental time among experimental diets (Table 2). Pupal development time did not differ significantly between diets ($F = 0.89$; $df = 11, 48$; $P = 0.55$). Total developmental time (larva-adult) was significantly different among diets ($F = 40.57$; $df = 11, 96$; $P < 0.001$). Development time was similar for males and females from the same diet (Table 2) and there was no significant interaction ($F = 0.09$; $df = 11, 96$; $P = 1.0$) between diet and sex. Larval developmental time did not differ significantly between non-supplemented diets vs. diets supplemented with brewers' yeast only.

Table 2. Development time (mean number of days \pm SE) of *H. illucens* stages and comparison between treatment (diet) groups using orthogonal contrasts

Diet	Larva	Pre-pupa	Pupa	Larva-adult	
				Male	Female
BW	17.2 \pm 0.5 ^{cd}	8.8 \pm 0.1 ^{bed}	9.1 \pm 0.1 ^a	35.0 \pm 0.5 ^{cdA}	35.2 \pm 0.5 ^{cdA}
MBW	16.4 \pm 0.2 ^a	9.5 \pm 0.1 ^{abc}	8.7 \pm 0.2 ^a	34.5 \pm 0.4 ^{dA}	34.7 \pm 0.3 ^{dA}
MCW	18.3 \pm 0.4 ^{bed}	8.3 \pm 0.1 ^{ed}	8.8 \pm 0.1 ^a	35.5 \pm 0.6 ^{cdA}	35.5 \pm 0.6 ^{cdA}
SBW	20.6 \pm 0.3 ^a	8.8 \pm 0.3 ^{bed}	9.0 \pm 0.0 ^a	38.3 \pm 0.5 ^{abA}	38.4 \pm 0.4 ^{abA}
BY	17.9 \pm 0.5 ^{bcde}	10.2 \pm 0.3 ^a	8.9 \pm 0.1 ^a	37.0 \pm 0.3 ^{bcA}	37.0 \pm 0.3 ^{bcA}
MBY	19.1 \pm 0.4 ^{ab}	10.3 \pm 0.3 ^a	8.8 \pm 0.1 ^a	38.2 \pm 0.6 ^{abA}	38.3 \pm 0.4 ^{abA}
MCY	19.0 \pm 0.3 ^{ab}	10.5 \pm 0.4 ^a	9.0 \pm 0.1 ^a	38.5 \pm 0.7 ^{abA}	38.5 \pm 0.5 ^{abA}
SBY	16.6 \pm 0.4 ^{de}	8.5 \pm 0.2 ^{cd}	8.8 \pm 0.1 ^a	34.1 \pm 0.4 ^{dA}	33.7 \pm 0.4 ^{dA}
BYMo	19.4 \pm 0.3 ^{ab}	10.9 \pm 0.4 ^a	9.0 \pm 0.1 ^a	39.3 \pm 0.4 ^{AA}	39.3 \pm 0.5 ^{AA}
MBYMo	19.5 \pm 0.3 ^{ab}	10.0 \pm 0.3 ^{ab}	8.8 \pm 0.1 ^a	38.3 \pm 0.4 ^{abA}	38.2 \pm 0.4 ^{abA}
MCYMo	20.2 \pm 0.2 ^a	10.4 \pm 0.4 ^a	9.0 \pm 0.2 ^a	39.4 \pm 0.3 ^{AA}	39.8 \pm 0.2 ^{AA}
SBYMo	18.5 \pm 0.2 ^{bc}	8.1 \pm 0.1 ^d	8.9 \pm 0.1 ^a	35.4 \pm 0.3 ^{cdA}	35.4 \pm 0.2 ^{cdA}

Comparison	Contrasts					
	Larva	Pre-pupa	Pupa	Larva-adult		
				Male	Female	
	df = 1, 48	df = 1, 48		df = 1, 48	df = 1, 48	
NonSup. vs YM-Yeast	F = 8.768, P = 0.044	F = 33.919, P < 0.0001	ns	F = 35.83, P < 0.0001	F = 38.53, P < 0.0001	
NonSup vs Yeast only	F = 0.008, P = 1.000	F = 27.974, P < 0.0001	ns	F = 11.83, P = 0.012	F = 10.06, P = 0.025	
Yeast vs Molasses/yeast	F = 24.552, P < 0.0001	F = 0.241, P = 1.000	ns	F = 12.17, P = 0.010	F = 19.42, P < 0.0001	
Barley vs Corn-starch	F = 12.624, P = 0.008	F = 0.762, P = 0.98	ns	F = 3.24, P = 0.49	F = 5.11, P = 0.22	
Barley vs Sorghum	F = 1.836, P = 0.80	F = 44.449, P < 0.0001	ns	F = 9.97, P = 0.026	F = 15.77, P = 0.003	

Means in a column followed by the same lower-case letter are not significantly different ($P < 0.05$, ANOVA plus HSD). Means for both sexes within a treatment followed by the same upper-case letter are not significantly different. ns, not significantly different ($P < 0.05$). BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses. Non-supplemented diets = BW, MBW, MCW, and SBW; Yeast/Molasses + yeast-supplemented diets = BY, MBY, MCY, SBY, BYMo, MBYMo, MCYMo, and SBYMo; Yeast-based diets = BY, MBY, MCY, and SBY; Molasses-based diets = BYMo, MBYMo, MCYMo, and SBYMo; Barley-based diets = BW, BY, and BYMo; Corn-starch-based diets = MCW, MCY, and MCYMo; Sorghum-based diets = SBW, SBY, and SBYMo. NonSup. = Non-supplemented diets; YM-Yeast = diets supplemented with a mixture of yeast and molasses + diets supplemented with yeast only.

Effect of diet on the larval survival, pre-pupal survival, and pupal survival of *H. illucens*

Larval survival ($F = 2.13$; $df = 11, 48$; $P = 0.036$), pre-pupal survival ($F = 3.67$; $df = 11, 48$; $P = 0.001$), and pupal survival ($F = 2.54$; $df = 11, 48$; $P = 0.013$) were significantly affected by diet type (Fig. 1). Orthogonal contrasts between treatment groups showed no significant differences in larval survival, pre-pupal survival, and pupal survival between diets supplemented with brewers' yeast and the non-supplemented diets (Table 3).

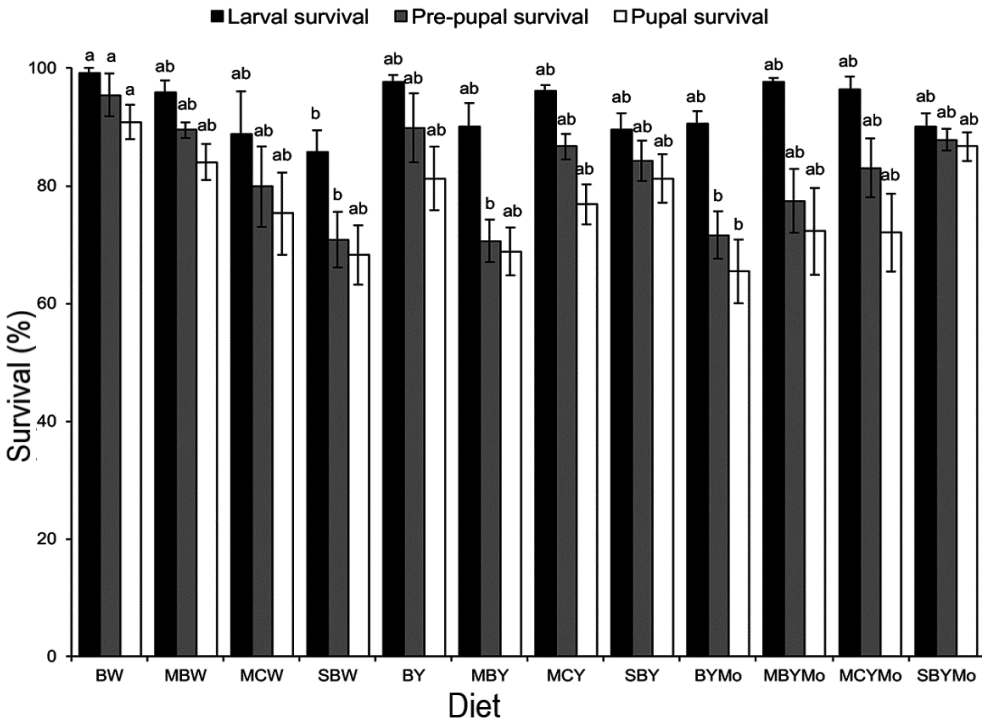


Fig. 1. Stage-specific survival of *H. illucens* reared on various larval diets. Means (\pm SE) followed by the same lower-case letter for a given life stage are not significantly different across diets ($P < 0.05$, ANOVA, HSD). BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

Table 3. Comparison of stage-specific survival between treatment groups using orthogonal contrasts for *H. illucens*

Contrast	Larva	Pre-pupa	Pupa
	df = 1, 48	df = 1, 48	df = 1, 48
Non-supplemented vs YM-Yeast	F = 0.58, P = 0.99	F = 0.88, P = 0.97	F = 1.73, P = 0.82
Non-supplemented vs. Yeast	F = 0.36, P = 1.0	F = 0.12, P = 1.0	F = 0.52, P = 0.99
Yeast vs. molasses + yeast	F = 0.01, P = 1.0	F = 0.88, P = 0.97	F = 0.70, P = 0.99
Barley vs. Corn-starch	F = 0.69, P = 0.99	F = 0.49, P = 1.0	F = 1.20, P = 0.92
Barley vs. Sorghum	F = 7.46, P = 0.078	F = 1.77, P = 0.81	F = 0.01, P = 1.0

Non-supplemented diets = BW, MBW, MCW, and SBW; YM-Yeast (all diets supplemented with a mixture of yeast and molasses or yeast only) = BY, MBY, MCY, SBY, BYMo, MBYMo, MCYMo, and SBYMo; Yeast-based diets = BY, MBY, MCY, and SBY; Molasses/yeast diets = BYMo, MBYMo, MCYMo, and SBYMo; Barley-based diets = BW, BY, and BYMo; Corn-starch = MCW, MCY, and MCYMo; Sorghum-based diets = SBW, SBY, and SBYMo.

Effect of larval diet on pre-oviposition period, fecundity, and longevity of *H. illucens*

Pre-oviposition time of adult *H. illucens* provided with a 10% sugar solution (F = 2.36; df = 1, 75; P = 0.08) or water (F = 0.38; df = 1, 75; P = 0.46) was not significantly affected by larval diet (Table 4). On all diets, starved adult female flies failed to reach oviposition age as the female could only survive for a maximum of 6 days.

Table 4. Pre-oviposition period (mean \pm SE) of female *H. illucens* fed on sugar solution or water

Larval diet	Pre-oviposition period (days)	
	Sugar solution	Water
BW	9.5 \pm 0.5 ^{aA}	9.2 \pm 1.1 ^{aA}
MBW	8.8 \pm 0.5 ^{aA}	9.6 \pm 0.9 ^{aA}
MCW	10.0 \pm 1.5 ^{aA}	11.0 \pm 2.0 ^{aA}
SBW	8.0 \pm 0.0 ^{aA}	7.3 \pm 0.8 ^{aA}
BY	13.5 \pm 3.5 ^{aA}	8.2 \pm 0.7 ^{aB}
MBY	9.8 \pm 0.3 ^{aA}	10.2 \pm 2.4 ^{aA}
MCY	9.0 \pm 0.6 ^{aA}	7.8 \pm 0.5 ^{aA}
BYMo	10.5 \pm 0.5 ^{aA}	7.5 \pm 0.5 ^{aA}
MBYMo	10.2 \pm 1.1 ^{aA}	8.0 \pm 1.1 ^{aA}
MCYMo	10.2 \pm 1.0 ^{aA}	8.4 \pm 0.4 ^{aA}
SBYMo	10.7 \pm 3.7 ^{aA}	7.0 \pm 0.3 ^{aA}

Means within a column followed by the same lower-case superscript are not significantly different (P < 0.05, HSD). Means within a row followed by the same upper-case superscript are not significantly different between sugar- and water-fed female *H. illucens* for each diet (P < 0.05, t-test).

The fecundity of female flies provided with sugar solution was similar ($F = 0.73$; $df = 10, 27$; $P = 0.69$) across dietary treatments but varied significantly from diets provided with water ($F = 2.89$; $df = 10, 38$; $P = 0.010$) (Fig. 2). The fecundity of female flies was higher for almost all the diets supplemented with brewers' yeast or molasses/brewers' yeast than for the non-supplemented diets (Fig. 2). Egg production was similar ($F = 0.88$; $df = 1, 85$; $P = 0.35$) for female flies provided with water or sugar solution (Fig. 2). There was a significant interaction between adult food and sex of *H. illucens* ($F = 5.99$; $df = 2, 806$; $P = 0.004$). However, no significant interaction was observed between larval diet and sex on adult fly longevity ($F = 0.80$; $df = 11, 806$, $P = 0.64$). The longevity of both starved (unfed) male and female *H. illucens* was significantly lower ($F = 208.79$; $df = 2, 806$; $P < 0.001$) compared to flies that were provided with sugar solution or water (Fig. 3).

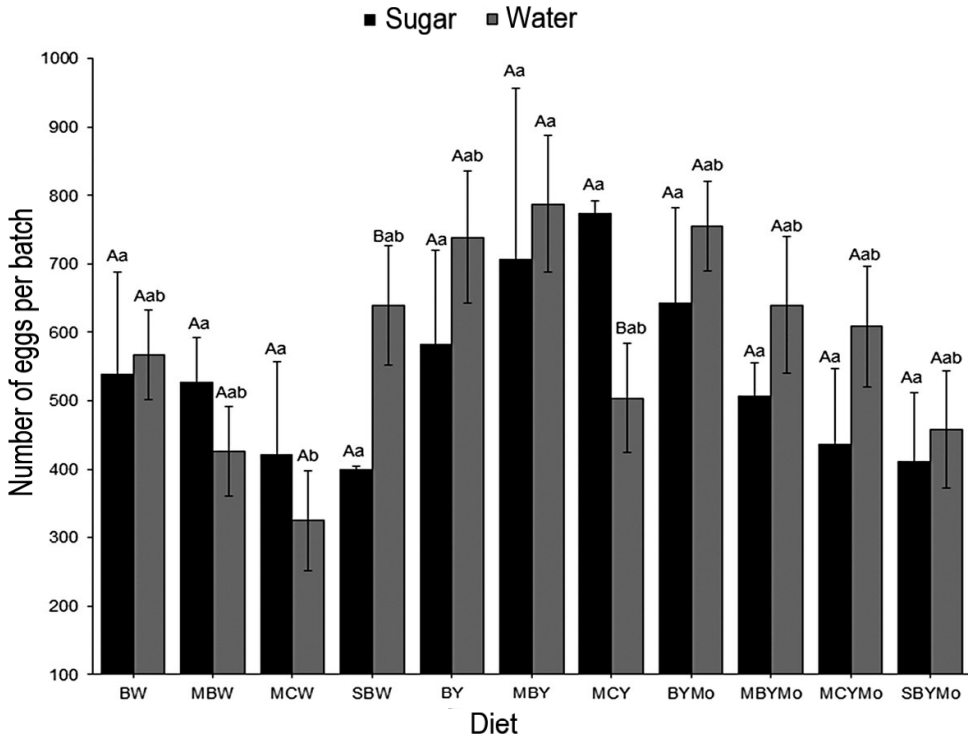


Fig. 2. Mean number of eggs laid per adult female *H. illucens* reared on different larval diets. Bars (\pm SE) followed by different upper-case letters are significantly different between sugar- and water-fed flies for each diet. Bars followed by different lower-case letters are significantly different among diets ($P < 0.05$, HSD). BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

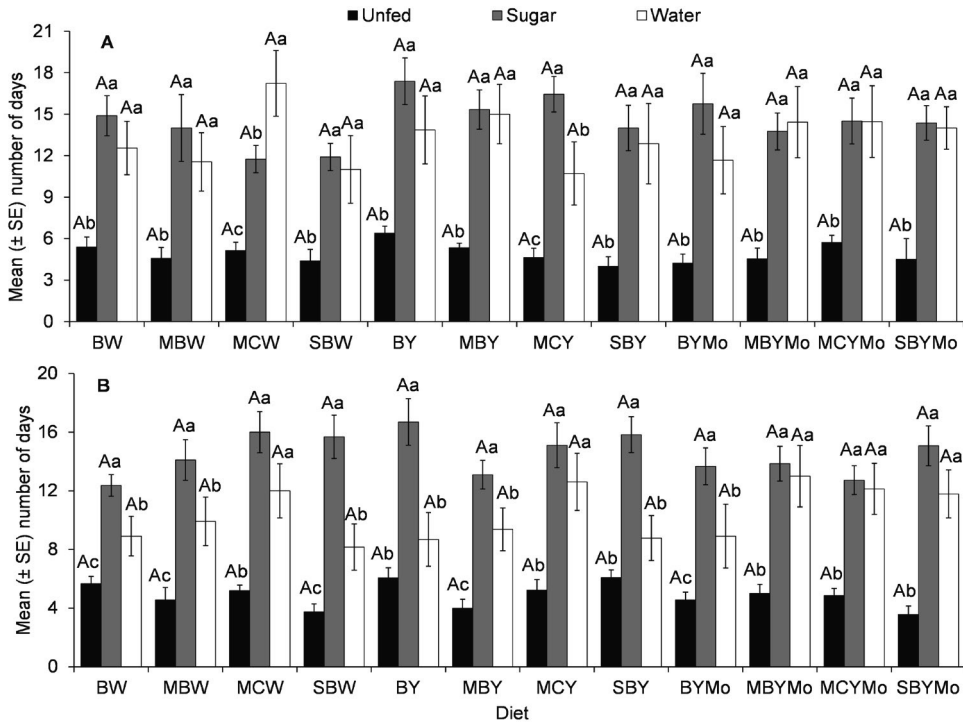


Fig. 3. Longevity (mean \pm SE) of adult male (A) and female (B) *H. illucens* fed on different diets as larvae and provided with sugar solution or water or remaining unfed as adults. Bars followed by the same upper-case letter are not significantly different among diets ($P < 0.05$, HSD). Bars followed by different lower-case letters are significantly different among unfed, sugar-fed, and water-fed flies for each diet ($P < 0.05$, HSD). BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

Effect of larval diet on wet weight of *H. illucens* life stages

Fifth instar larval weight of *H. illucens* was significantly different ($F = 5.46$; $df = 11, 48$; $P < 0.001$) among diets tested. Larval diet significantly affected weight of pre-pupa ($F = 8.004$; $df = 11, 48$; $P < 0.001$), pupa ($F = 9.08$; $df = 11, 48$; $P < 0.001$), adult male ($F = 39.40$; $df = 1, 96$; $P < 0.001$), and female ($F = 89.40$; $df = 1, 96$; $P < 0.001$) (Figs. 4 and 5). Larvae fed on non-supplemented diets weighed significantly ($F = 106.3$; $df = 1, 57$; $P < 0.001$) less than those fed on diets supplemented with brewers' yeast (Table 5). The weight of larvae fed on diets supplemented with brewer's yeast or molasses/brewers' yeast was not significantly different, whereas weight of pre-pupae differed significantly between non-supplemented diets and diets supplemented with either brewer's yeast or molasses/brewers' yeast (Table 5).

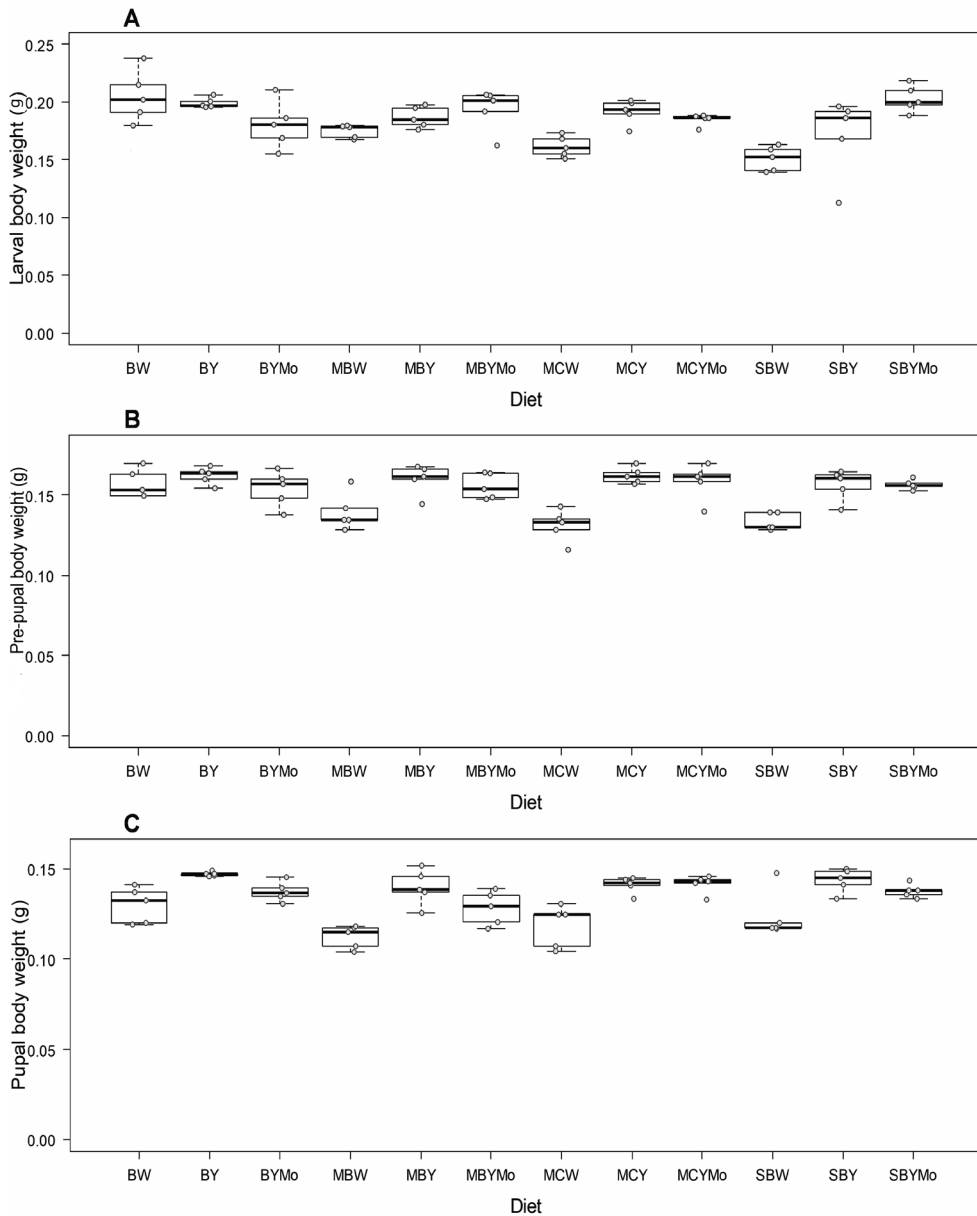


Fig. 4. Boxplots showing wet weight (g) of larval (A), pre-pupal (B), and pupal (C) stages of *H. illucens* reared on different diets. The middle quartile or median (the line that divides the box into two parts) marks the midpoint of the data. The middle box (inter-quartile range) represents 50% of the data for each diet. BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

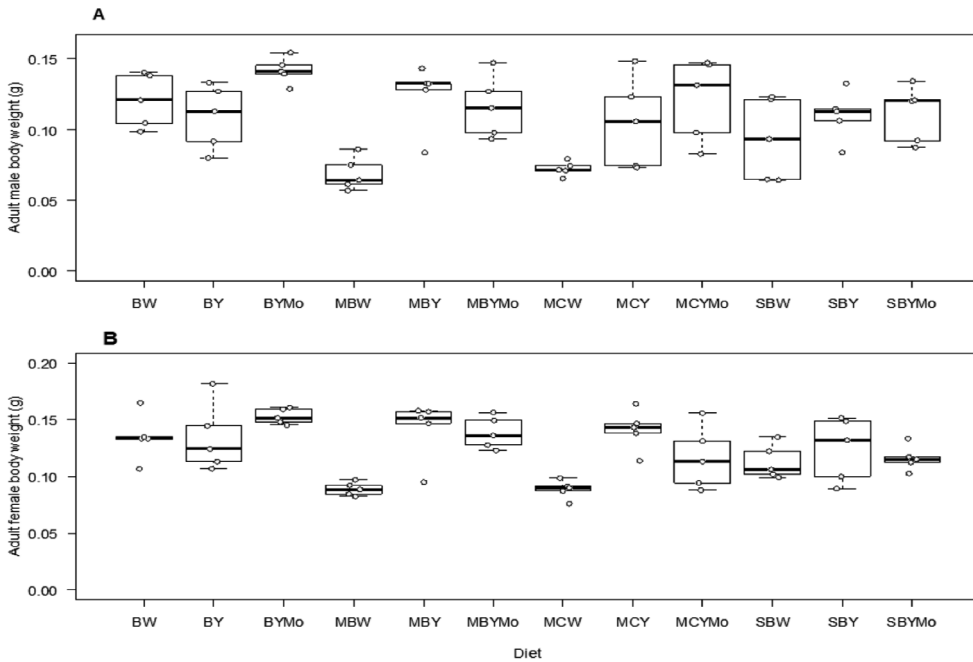


Fig. 5. Boxplots showing wet weight (g) of adult male (A) and female (B) *H. illucens* reared on various diets. The middle quartile or median (the line that divides the box into two parts) marks the midpoint of the data. The middle box (inter-quartile range) represents 50% of the data for each diet. BW, Barley/water; MBW, Malt/Barley/water; MCW, Malt/Corn-starch/water; SBW, Sorghum/Barley/water; BY, Barley/brewer's yeast; MBY, Malt/Barley/brewer's yeast; MCY, Malt/Corn-starch/brewer's yeast; SBY, Sorghum/Barley/brewer's yeast; BYMo, Barley/brewer's yeast/Molasses; MBYMo, Malt/Barley/brewer's yeast/Molasses; MCYMo, Malt/Corn-starch/brewer's yeast/Molasses and SBYMo, Sorghum/Barley/Molasses.

Table 5. Comparison of mean wet weight for different life stages of *H. illucens* between treatment groups using orthogonal contrasts

Contrast	Larva	Pre-pupa	Pupa	Male adult	Female adult
	df = 1, 48	df = 1, 48	df = 1, 48	df = 1, 48	df = 1, 48
Non-supplemented vs YM-Yeast	F = 12.80, P = 0.001	F = 56.19, P < 0.0001	F = 69.92, P < 0.0001	F = 23.33, P < 0.0001	F = 24.88, P < 0.0001
Non-supplemented vs Yeast only	F = 7.78, P = 0.07	F = 52.33, P < 0.0001	F = 72.92, P < 0.0001	F = 11.66, P = 0.013	F = 21.76, P < 0.0001
Yeast vs Molasses/yeast	F = 0.38, P = 1.0	F = 2.20, P = 0.71	F = 6.73, P = 0.10	F = 2.36, P = 0.68	F = 0.48, P = 1.0
Barley vs Corn-starch	F = 7.07, P = 0.09	F = 5.03, P = 0.23	F = 2.37, P = 0.67	F = 9.47, P = 0.038	F = 12.11, P = 0.012
Barley vs Sorghum	F = 11.83, P = 0.013	F = 7.77, P = 0.07	F = 1.12, P = 0.94	F = 5.75, P = 0.17	F = 9.99, P = 0.022

Non-supplemented diets = BW, MBW, MCW and SBW; YM-Yeast (All diets supplemented with a mixture of yeast and molasses or yeast only) = BY, MBY, MCY, SBY, BYMo, MBYMo, MCYMo, and SBYMo; Yeast-based diets = BY, MBY, MCY, and SBY; Molasses/yeast diets = BYMo, MBYMo, MCYMo, and SBYMo; Barley = BW, BY, and BYMo; Corn-starch-based diets = MCW, MCY, and MCYMo; Sorghum-based diets = SBW, SBY, and SBYMo.

Discussion

This study provides insight into the effects of mixing different waste types on *H. illucens* growth performance. We observed a shorter larval developmental duration in *H. illucens* reared on the 12 diet types compared to that documented in the literature (Cammack & Tomberlin, 2017; Myers et al., 2008; Nguyen et al., 2013; Oonincx et al., 2015). The larval developmental time in our study was 5–25 days shorter (17–21 days) than recorded in the studies mentioned above (21–46 days), a record similar to that reported by (Barragán-Fonseca, 2018). Differences in developmental time between studies may have been due to variation in the quantity and/or quality of the larval diet. A reduction in larval food supply could delay *H. illucens* larval development up to 4 months (Furman et al., 1959). Other factors that affect larval development include larval density, larval feeding rate, and pH of the feeding medium (Barragán-Fonseca, 2018; Morrison & King, 1977; Paz et al., 2015) as well as the physical texture of the feeding medium (Gobbi et al., 2013). Pre-pupal recovery, pupal recovery, and adult emergence of *H. illucens* reared on diets supplemented with brewers' yeast or molasses/brewery yeast compared favorably with, and sometimes exceeded, those obtained on the non-supplemented BSG diets. Percentage pupal recovery obtained for *H. illucens* was well within the range reported by several authors on a variety of rearing substrates (Myers et al., 2008; Nguyen et al., 2013; Oonincx et al., 2015). Percentage emergence of adults for the different diet types in our study was high, which agrees with previous studies (Cammack & Tomberlin, 2017; Tomberlin et al., 2002). Considerably variable patterns have been reported for other dipterans like *Bactrocera dorsalis* (Hendel) and *Zengodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) that successfully completed development in diets containing the local waste stream, brewers' yeast (Chang et al., 2007; Chang et al., 2004). In our study, supplementation of BSGs with either brewers' yeast or molasses/brewers' yeast outperformed the spent grain diets mixed with water only in terms of increased larval, pre-pupal, pupal, and adult weight. The weight measurements of the different life stages observed in our study were comparable to those reported in previous research (Nguyen et al., 2013, 2015; Tomberlin et al., 2009; Tomberlin et al., 2002). These studies report means of larval weight ranging from 0.11 to 0.23 g, pre-pupal weight of 0.07 – 0.22 g and adult weight of 0.04 – 0.11g, which are all similar to our findings. The stage-specific weight increase observed in our study has been viewed as a useful quality control criterion in insect mass-rearing since it is correlated with mating success. This result may be useful in the management of waste in a traditional brewing system which often generates very little (if any) value and may have negative impacts if the brewery must pay to get rid of waste water and spent grains. Adult flies from heavy pupae experience higher mating success than those from lower-weight pupae (Churchill-Stanland et al., 1986). Large flies exhibit greater flight ability than small flies (Sharp et al., 1983).

The difference in body weight observed in our study may be explained by the quality of diet and the resulting critical weight, one of the physiological factors that regulate variation in body size. The critical weight has been defined as the minimal mass at which further growth is not necessary for a normal time course to pupation (Davidowitz et al., 2003). Previous studies show that insects reared on low quality diets have low critical weight values (Davidowitz et al., 2003; Davidowitz & Nijhout, 2004).

Unlike the development of immature life stages, adult parameters (fecundity and adult longevity) were clearly affected by diet type on which the larvae were reared except for pre-oviposition duration. Previous studies have shown that in females, the nutritional quality of the diet (especially higher protein-based diets) ingested during the immature phase improves adult performance and affects ovarian development leading to higher fecundity rates (Cangussu & Zucoloto, 1993, 1997; Cangussu & Zucoloto, 1995) (Fernandes-da-Silva & Zucoloto, 1997). Large protein-fed insect males are more likely to have their sperm stored in the females (Taylor & Yuval, 1999).

Dietary effects on body size could be mediated through alterations in the quantity of nutrients stored as lipids and as proteins prior to pupariation (Nestel & Nemny-Lavy, 2008; Nestel et al., 2004). Nutrient composition of the brewers' yeast (*Saccharomyces cerevisiae*) is about 45% crude protein (Raven & Walker, 1980) with an excellent lysine (amino acid) profile (Huige, 2006). BSGs contain 21–31% crude protein, and approximately 2,080 kcal/kg metabolizable energy on DM basis (National Research Council, 1994; Westendorf & Wohlt, 2002), but it is a poor source of other minerals (Hussain et al., 2010; Westendorf & Wohlt, 2002). Molasses is a source of readily available dietary energy (Van Niekerk, 1981), niacin, and pantothenic acid (Cleasby, 1963). We observed that diets supplemented with brewers' yeast or molasses/brewers' yeast resulted in slightly higher number of eggs produced than the non-supplemented diets, a trend similar to that observed by (Barragán-Fonseca, 2018) who recorded heavier *H. illucens* adults and higher egg production for diets with higher dietary protein contents.

Adult longevity of male and female flies was similar across diets when flies were starved. This implies that the nutritional quality of the larval diets appears to have had minimal effects on life span of the flies and significant impact on egg production (no eggs were laid). Water, unlike food, is essential for adult *H. illucens* to reproduce (Sheppard et al., 2002), which might explain why less vigorous and dehydrated adults were unable to lay eggs. In our experiment, adult longevity increased with 10% sugar solution or water supply as food in separate treatments. The longevity of male and female flies varied on each diet type when adult flies were subjected to water only or 10% sugar solution treatments. Although we did not evaluate the effects of protein and carbohydrate on adult *H. illucens* longevity, previous research has indicated that dietary protein and carbohydrate contents are important and affect both larval and adult performance of *H. illucens* (Barragán-Fonseca, 2018).

Conclusion

The successful development of *H. illucens* on all 12 diet types clearly indicates the high nutrient quality of the brewery by-products. Based on quality control parameters of *H. illucens* reared on the combination of these agro-industrial waste streams, our values are comparable to, and sometimes higher than, those documented in literature on other organic wastes. Thus, the study at hand confirms the application potential of the black soldier fly in industrial solid waste management and the importance to investigate future large-scale mass rearing possibilities. The combination of waste treatment capacity together with generation of a valuable product, that is, a high-quality cheap alternative protein source for animal feeds, instead of discarding the waste into open plots, makes the black soldier fly technology a highly promising tool for waste management and feed production. The conversion of organic waste into high nutritional biomass has now opened new economic opportunities for municipalities and offers small entrepreneurs the possibility of income generation without high investment costs. Concurrently, this reduces the environmental impact of organic waste stream considered as one of the serious environmental problems confronting urban governments in low- and middle-income countries in Sub-Saharan Africa. Hence, composting by utilizing black soldier fly larvae should be recommended in Kenya and other African countries as a sustainable method of dealing with organic municipal waste that embraces the concept of a circular economy. Being a financially more attractive option for municipal waste management, private sectors, with stronger focus in business opportunities and marketing approaches should be in the center of attention (Wang et al., 2008).

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4

Chapter 5

Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production

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Abstract

Efforts to recycle organic wastes using black soldier fly (BSF) *Hermetia illucens* into high nutrient biomass that constitutes a sustainable fat and high-quality protein ingredient in animal feeds have recently gained momentum worldwide. However, there is little information on the most suitable rearing conditions for growth, development and survivorship of these flies, which is a prerequisite for mass production technologies. We evaluated the physiological requirements for growth and reproduction of *H. illucens* on two diets [spent grains supplemented with brewers' yeast (D1) and un-supplemented (D2)]. Development rates at nine constant temperatures (10–42°C) were fitted to temperature-dependent linear and non-linear day-degree models. Thereafter, life history parameters were determined within a range of favourable temperatures. The thermal maximum (TM) estimates for larval, pre-pupal and pupal development using non-linear model ranged between 37.2 ± 0.3 and 44.0 ± 2.3 °C. The non-linear and linear day-degree model estimates of lower developmental temperature threshold for larvae were 11.7 ± 0.9 and 12.3 ± 1.4 °C for D1, and 10.4 ± 1.7 and 11.7 ± 3.0 °C for D2, respectively. The estimated thermal constant of immature life stages of development of BSF was higher for the larval stage (250 ± 25 DD for D1 and 333 ± 51 for D2) than the other stages evaluated. Final larval wet weight was higher on D1 compared to D2. The population growth rate was most favourable at 30-degree Celsius (°C) with higher intrinsic rate of natural increase ($r_m = 0.127$ for D1 and 0.122 for D2) and shorter doubling time (5.5 days for D1 and 5.7 days for D2) compared to the other temperatures. These results are valuable for the optimization of commercial mass rearing procedures of BSF under various environmental conditions and prediction of population dynamics using computer simulation models.

Introduction

The black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) (Fig. 1) is an indigenous saprophagous fly of the Neotropics. However, the distributional range of these fly has widely changed over time to include the warmer parts of the world (Marshall et al., 2015). There has been substantial interest in the last decades to use these flies in organic waste management, given that their larvae are voracious eaters of organic waste (detritivores in compost heaps) (Diener et al., 2009; Lalander et al., 2015; Newton et al., 2005; Nguyen et al., 2013; Sheppard et al., 1994). The ability of BSF to convert waste into high-quality nutrient biomass has rapidly opened innovative economic prospects for municipal solid waste management. Also, the larvae of BSF after waste management are nutrient-rich consisting of an average of 42.1–43.2% crude protein, 33% fat, and micronutrients (Bonso, 2013; Makkar et al., 2014; Newton et al., 2005; Rumpold & Schluter, 2013; St-Hilaire et al., 2007), thus advocated as an appropriate alternative to fishmeal or soybean meal in poultry, pig and fish feeds (Barragan-Fonseca et al., 2017; Makkar et al., 2014; Van Huis et al., 2013), and provides opportunities for income generation (Newton et al., 2005; Sheppard et al., 1994). Therefore, there has been an increasing interest in developing novel methods of mass producing BSF as an agent of organic waste management and composting as well as sustainable novel protein-rich ingredients in animal feeds. However, the paucity of scientific data and the reluctance of commercial producers to share detailed information impairs up-scaling BSF production technology among smallholder farmers (Sanchez-Muros et al., 2014). As such many important aspects related to mass production technologies of BSF remains poorly studied, especially temperature. Among these factors, temperature remains one of the most important factors (Goulson et al., 2005; Saska et al., 2013) that considerably impacts behaviour, distribution, development rate, immature survival and reproduction, thus hampering the establishment of successful rearing systems across the world (Bale et al., 2002). To understand the dynamics and ecological system-specific BSF mass production strategies, data on temperature-driven population growth parameters of this insect become crucial, especially when dealing with small to medium-scale enterprises. Although information on development time, egg eclosion and adult emergence of BSF is reported for limited sets of temperature (Harnden & Tomberlin, 2016; Holmes et al., 2016; Tomberlin et al., 2009), no information is available on temperature-driven effects on the life history parameters including mortality and reproduction at an extensive range of temperatures. Several nonlinear models have been used (Briere & Pracros, 1998; Briere et al., 1999) to define the developmental and survivorship rates of different insects (Baek et al., 2014; Fand et al., 2014; Tanga et al., 2018; Tanga et al., 2015) over a varied array of temperatures but this has never been applied to BSF. Studies have shown that temperature significantly impacts the development of life stages, thus directly impacting the quality and quantity of insects produced in rearing facilities

(Brevault & Quilici, 2000; Duyck & Quilici, 2002; Liu & Ye, 2009; Rwomushana et al., 2008; Salum et al., 2014; Trudgill et al., 2005; Vargas et al., 2000; Vayssieres et al., 2008). Although studies have shown that below and above the optimal temperature range of insect development, which is commonly limited by upper and lower developmental thresholds, survival does not occur but there are indications that this can vary depending on the insect-specific life stage or geographical origin of the insect species (Honěk & Kocourek, 1990). Thus, knowledge on developmental thermal requirements of insects provides strong basis for estimation of optimal response (Dixon et al., 2009). There is a deficiency of information on temperature-driven development of BSF which is needed to maximize production. Thus, to enhance mass production technologies, additional studies are warranted on temperature requirements of BSF. In the present study, we evaluated the stage-specific developmental time, survival, wet weight, pre-oviposition time, fecundity and adult longevity at nine constant temperatures (10 - 42°C) for BSF larvae reared on spent grains with or without supplementation with brewer's yeast. The present work has implications for the mass production of BSF larvae as high-quality protein ingredient for animal feed.



Fig. 1. Illustration of black soldier fly (BSF) *Hermetia illucens* (Diptera: Stratiomyidae) and high-quality larvae. Sizes of larvae and adult fly are not proportional.

Materials and methods

Origin of *H. illucens* population and colony maintenance

A stock culture of the wild-caught fly population was established following the methodology described by (Booth & Sheppard, 1984) and (Sripontan et al., 2017) with slight modifications. Egg-trapping of wild BSF was carried out in Kasarani, Nairobi County, Kenya (S 01° 13' 14.6"; E 036° 53' 44.5", 1612 m above sea level). Chicken manure, rabbit

manure, mixed fruit wastes and household food wastes were used as baiting materials for adult flies. The baiting materials were placed separately inside 6-L plastic buckets (22 cm high by 18 cm bottom diameter by 23 cm top diameter) designed with six openings (10 cm diameter each), 5 cm from the lid to facilitate entry of adult flies. The bait inside each container was maintained at approximately 70% moisture level. In cases where household food waste or mixed fruit waste were used, four–five holes were made at the bottom of the containers to allow excess fluid to drain out during the decomposition process. Three–five corrugated cardboard flutes (~10 cm length by 5 cm width) were attached vertically to the walls of the containers over the bait to serve as oviposition sites for adult flies. The lids of the containers were fastened to prevent desiccation or interference from heavy rainfall. Traps were labelled with date, location, time and GPS coordinates. Thereafter, the traps were hung on wooden or metallic stands (1 m above the ground) under shades around homesteads or close to garbage dump sites. We ensured that the wooden or metallic stands holding the traps were smeared with Tanglefoot1 (Tangle-trap) (Tanglefoot1 Company, Grand Rapids, MI) insect paste, which served as a barrier to stop intrusion by predators (ants, reptiles, etc). These traps were checked regularly, and available egg clusters deposited by BSF in the cardboard flutes were harvested. The egg clusters were immediately transferred into other rearing containers holding a diet specifically formulated for the newly hatched neonates, hatching took approximately 4 days at $28 \pm 1^\circ\text{C}$. Ten days after hatching the young larvae were transferred to bigger metal rearing trays containing wet brewer's spent grains sourced from Kenya Breweries Limited, Nairobi, Kenya. The top surface of the rearing trays measured 76 cm length by 27.5 cm width, 10 cm height while the bottom of the tray measured 52 cm length by 27.5 cm width. In the rearing facility, conditions were maintained at $28 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH and L12:D12 photoperiod. User-friendly thermo-hygrometers (TH-812E) were maintained in each rearing room to allow us track slight changes in temperature and RH. Pre-pupal stages harvested from the rearing trays were kept in 4-L transparent rectangular plastic containers (21 x 14 x 15 cm) (Kenpoly Manufacturer Ltd., Nairobi, Kenya) containing moist wood shavings (sawdust) until eclosion. On the lid of each container, an opening (14.5 x 8.3 cm) was introduced and screened with fine netting organza material. Emerging flies were transferred to an outdoor cage (1 x 1 x 1.8 m). The flies were provided ad libitum 60% sugar solution on cotton wool or soaked pumice granules in 2-L rectangular transparent plastic containers (21 x 14 x 7 cm) (Kenpoly Manufacturers Ltd., Nairobi, Kenya). Some of the flies (15 males and 15 females) were rendered inactive by freezing them (-20°C) for 20 min and later preserved in 90% alcohol for taxonomic identification based on morphological features (Oliveira et al., 2016; Roy et al., 2016). Thereafter, the preserved specimens were identified at the Biosystematics unit of *icipe*, which also holds reference materials of the specimens. BSF has been in culture for ~2 years. In addition, BSF was kept at high numbers (2000–2500

adults per cage) to avoid inbreeding depression while also avoiding stressful crowding effects (Sørensen & Loeschcke, 2001).

Sources of experimental substrates and diet formulation

Before the commencement of the experiment, fresh brewer's spent grains (BSGs) (malt/corn starch) and brewer's yeast, both by-products of brewing beer were sourced from the Kenya Breweries Limited, Nairobi, Kenya. The fresh BSGs were subsequently dried using moving dry air at 28.0 ± 2 °C (using Xpelair1 heater: WH30, 3KW Wall Fan Heater, United Kingdom) for two days (48 h). Afterwards, the semi-dried products were maintained in the oven for 3-days (72 h) at 60 °C to dry properly to achieve approximately 90% DM (~10% moisture). The dried BSGs were later passed through the Munch hammer mill (Munch, Wuppertal, Germany) to reduce the materials to a 3 mm particle size, suitable for incorporation into BSF diet when needed. Two experimental diets were then formulated for the BSF larvae, which consisted of diet one (D1) (50 g BSGs mixed in 90 ml of brewer's yeast) and diet two (D2) (50 g BSGs mixed in 80 ml of water (Control)). Each diet was hydrated to approximately $70.0 \pm 2\%$ moisture by weight according to the protocol described by Cammack & Tomberlin (2017) and confirmed using a moisture sensor with two 12-cm-long probes (HydroSense™ CS620, Campbell Scientific, Inc., Logan, UT, USA). Five replicates were conducted for each experimental diet fed ad libitum until the late larval stage.

Temperature effect on the development and survivorship rate of *H. illucens* life stages

Thermostatically controlled incubators (MIR-554-PE, Sanyo/Panasonic cooled incubators, Japan) were used to conduct the experiments set at one of the nine constant temperatures conditions [(10, 15, 20, 25, 30, 35, 37, 40 or 42 °C (± 0.03 °C)]. The relative humidity in each incubator was maintained at $70 \pm 2.0\%$ and photoperiod of 12:12 L:D. In each incubator, the effect of temperature on the developmental time and survival of the different life stages were assessed. EasyLog USB data loggers (EL-USB-2, RH/Temp data logger; MicroDAQ.com, Ltd. USA, 603-746-5524) were placed inside each incubator, which recorded the inside temperature at 15 minutes interval. Eggs of BSF were obtained by providing the adult flies with oviposition media that consisted of corrugated cardboard with flutes (cut into sizes as described above). The cardboards were attached to the wall of cages near the baiting materials and on top of the substrates (baiting material). Freshly laid egg clusters were obtained from the stock colony ~1 h after the eggs were laid.

Egg. Camel hair brush with a fine tip was used to collect 300 eggs (~1 h old), which were carefully transferred unto sterilized Petri dishes (150 x 25 x 20 mm). The Petri

dishes containing eggs were transferred to the nine incubators described above. The experimental setup with the eggs were monitored at regular interval of 6 h daily until they hatched. All egg eclosion at each temperature regime was recorded with the help of entomological tweezers under the microscope (Leica MZ 125 Microscope; Leica Microsystems Switzerland Limited). The stereomicroscope used for counting the emerged neonates was fitted with Toshiba 3CCD camera and an auto-montage software (Syn-croscopy, Synoptics Group, Cambridge, UK) at 25X magnification to ensure no damage was observed. Time until hatching of larvae and percentage egg hatch was determined. The experiments were replicated five times per temperature treatment.

Larva. Per temperature treatment, 300 newly hatched larval BSF (~1 h old) were randomly obtained from the clusters of hatched eggs maintained at each experimental temperature and transferred onto square (5 cm²) sterile filter papers. The square filter papers with the young BSF larvae were transferred onto a 50 g formulated diets; D1 or D2 placed in 2-L rectangular plastic rearing containers. The plastic containers containing the neonates were then maintained in respective thermostatically controlled incubators. The opening made on the lids of the containers were covered with a netting of 1.3 x 1.3 mm mesh size to allow for sufficient ventilation. The larvae were then fed ad libitum, until pre-pupal stage. Fifty 5th instar larvae were randomly selected from each diet in the different temperature treatments and separated into ten replicate groups of five and weighed to determine the wet weight. Stage-specific developmental time and survival was recorded for each temperature treatment. For each experiment in the different temperature regimes, five replications were achieved.

Pre-pupa. A total of 300 newly formed pre-pupae were selected randomly from the experimental culture maintained on each diet at each temperature treatment. The pre-pupae used for the experiment were maintained individually in small plastic containers with a top and bottom diameter of 5 and 4 cm, respectively and a height of 4 cm. The containers were provided with breathable lids. Each container had a 2.5 cm-deep moist sawdust, which served as pupation substrates. The containers used for the experiment were monitored daily for the development and survival of the pre-pupal stage. Each experiment was replicated five times.

Pupa. From experimental cultures maintained on each diet at each temperature, 300 pupae were randomly selected and transferred individually into plastic containers with a 2.5 cm layer of moist sterile sawdust for emergence. Pupae that failed to emerge within the anticipated period were allowed for an addition 1-month period before pronouncing them dead. Emerged adults were recorded by sex, and their adult weight (50 individuals) recorded. Each experiment was replicated five times for each diet and temperature treatment.

Temperature effect on adult *H. illucens* fecundity, oviposition and longevity

Upon emergence, one female and one male adult *H. illucens* (~1 h old) were paired. The individual BSF adult pairs were placed in well ventilated Perspex cages (15 x 15 x 10 cm). In each cage, the paired flies were provided sugar water on soaked cotton balls. Thereafter, the flies were provided with two corrugated cardboard flutes stocked on the walls of the container. The corrugated cardboards were maintained in the cages throughout the lifespan of the flies. Egg clusters produced daily were checked and counted, while the longevity of individual flies was also recorded. In each egg cluster collected, total number of eggs laid per female was counted and recorded. Thirty pairs of *H. illucens* were monitored for each temperature treatment and diet. Pre-oviposition duration was calculated based on the days required for a newly emerged female BSF to start ovipositing. Adult longevity was calculated based on the length of time lived by an adult fly from the date of emergence until death. Fecundity was considered as the number of eggs laid per female.

Life history parameters

The net reproductive rate (R_o), intrinsic rate of natural increase (r_m), generation time (G), and doubling time (DT) were estimated using the method described by Carey (1993) using the modified spreadsheet of (Portilla et al., 2014). R_o , which is an indication of the number of offspring that an individual female fly can produce during its life span was calculated as:

$$R_o = \sum_{x=0}^w l_x m_x$$

The maximum population growth, r_m was assessed using the iterative bisection approach from the Euler-Lotka equation with age indexed starting from zero. Life table with data on the r_m at different temperature regimes provide insight into the characteristic life patterns of BSF:

$$\sum_{x=0}^w e^{-r(x+1)} l_x m_x = 1$$

The finite rate of increase (λ), which represents overall female offspring per female per day, was calculated using the formula:

$$\lambda = e^{r_m}$$

The mean generation time (G), which is defined as the length of time that a population requires to increase to R_0 -fold of its population size at the stable age-stage distribution was estimated using the formula:

$$G = \frac{\ln R_0}{r_m}$$

The gross reproductive rate (GRR) was calculated as:

$$GRR = \sum_{x=0}^w m_x$$

The doubling time (DT) is defined as the number of days required by a population to double and was calculated as shown below:

$$DT = \frac{\ln(2)}{r_m}$$

where l_x is the female survival rate from egg to age x , w is the oldest surviving age, x is the age class in days and m_x is mean female progeny occurring during age x .

Temperature-dependent models

For temperature dependent models, both linear (Campbell et al., 1974) and nonlinear models were fitted to developmental rate stage-specific data of the insect. The linear model expressed below evaluated the relationship between *H. illucens* developmental rate and temperature:

$$r(T) = a + bT$$

In the model, T is the ambient temperature ($^{\circ}\text{C}$), r is an indication of the development rate [1/ developmental duration presented in days], while the intercept (a) and slope (b) are model parameters. The minimum temperature (T_{min}) and standard error were calculated using the following equations (Campbell et al., 1974):

$$T_{min} = \frac{-a}{b}$$

$$SE_{T_{min}} = \frac{y_m}{b} \sqrt{\frac{S^2}{N \times y_m^2} + \left[\frac{SE_b}{b}\right]^2}$$

where y_m is the average value of the developmental rate, b is estimated slope of the fitted line, S^2 is the residual mean square of the linear model, and N is the sample size (Andreadis et al., 2013). The total thermal energy (heat units) necessary to complete development, which is considered to be above the low temperature development threshold (LTDT) and referred to as the thermal constant K , is represented in degree-days (DDs). The value of K , and its standard error were calculated using the following equations (Campbell et al., 1974):

$$K = \frac{1}{b}$$

$$SE_K = \frac{SE_b}{b^2}$$

Many empirical non-linear models fitted to developmental rate stage-specific data have been used to determine minimum temperature thresholds (T_{min}), optimal temperatures (T_{opt}) and upper temperature thresholds (T_{max}). Optimum temperature (T_{opt}) is defined as the temperature when developmental rate is observed to be maximal, while T_{max} is referred to as threshold temperatures above which temperatures are lethal. Between the different non-linear models evaluated, Brière 1 model provided a better explanation of the temperature effect on the development of *H. illucens* life stages in comparison to other models tested. Brière 1 model is expressed as below:

$$r(T) = n * T * (T - T_{min}) * \sqrt{T_{max} - T}$$

Here, r is considered as the developmental rate, derived as a function of temperature T , n being an empirical constant, T_{min} the lower development temperature threshold, and T_{max} the upper temperature threshold. The optimal temperature of *H. illucens* development rate was estimated using the following equation (Briere et al., 1999):

$$T_{opt} = \frac{2mT_{max} + (m + 1)T_{min} + \sqrt{(4m^2T_{max}^2 + (m + 1)^2T_{min}^2 - 4m^2T_{min}T_{max})}}{4m + 2}$$

where $m = 2$ (Briere et al., 1999).

Statistical analysis

A two-way analysis of variance (ANOVA) was used to analyze the data on development time, survival of immature life stages, adult longevity, fecundity and pre-oviposition period (dependent variables) to evaluate the effect of temperature and rearing substrate (independent factors) and their interaction. Percentage of survival and average wet weight for the different life stages of BSF at different temperatures and rearing substrates were also analysed using a two-way ANOVA. In the event of a significant F test ($P < 0.05$), the Student Newman Keuls (SNK) test was used to compare means. Prior to ANOVA, all proportion data (percentage survival and adult emergence) were transformed using angular transformation to stabilize variance. All statistical analyses were performed using R version 3.4.1 (R Core Team, 2017).

Results

Temperature effect on developmental and survival rate of *H. illucens* immature life stages and adults

The time to egg eclosion differed significantly ($F = 117.3$; $df = 6, 19$; $P < 0.0001$) among the different temperatures (Fig. 2). The eclosion time of the eggs incubated at 15°C was 14-days, compared to those incubated at 35°C, which eclosed in 2.60 days. After monitoring the egg experimental set-up for over 40 days, eggs at 10 and 42°C were observed to have completely collapsed and deemed not viable. Larval development time differed significantly among temperatures ($F = 843.1$; $df = 6, 28$; $P < 0.0001$ for D1 and $F = 153.5$; $df = 6, 28$; $P < 0.0001$ for D2) and between rearing substrates ($F = 114.1$; $DF = 1, 56$; $P < 0.0001$). There was a significant interaction ($F = 20.2$; $df = 6, 56$; $P < 0.0001$) between the effect of temperature and rearing substrate on larval developmental time.

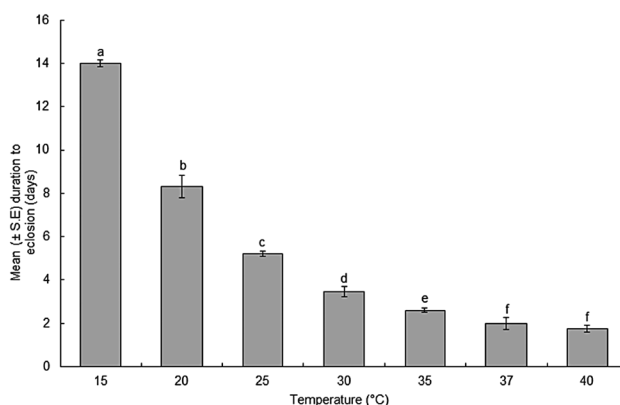


Fig. 2. Duration of *H. illucens* egg eclosion at different temperatures. Bars with different letters are significantly different ($P < 0.05$, SNK test).

Larval developmental time on D1 ranged between 12.8 ± 0.34 days at 30°C and 61.6 ± 0.91 days at 15°C (Fig. 3). The longest larval developmental time was recorded at 15°C (65 days), while the shortest developmental time was at 30°C (13 days) and 35°C (16 days) when reared on D1 and D2, respectively (Fig. 3). Larvae reared on D1 and D2 had significantly different developmental time at 20, 25 and 30°C but similar developmental time at 15, 35, 37 and 40°C (Fig. 3). The pre-pupae from larvae fed on D1 and D2 failed to complete development to the pupa at 40°C . The pre-pupal development time differed significantly among temperatures tested ($F = 61.22$; $df = 5, 22$; $P < 0.0001$ for D1 and $F = 349.3$; $df = 5, 22$; $P < 0.0001$ for D2) and between the different diets ($F = 4.743$; $df = 1, 44$; $P = 0.0350$). The interaction ($F = 12.35$; $df = 5, 44$; $P < 0.0001$) between the effect of temperature and diet was significant. Pre-pupae from larvae reared on D1, showed significant variation in developmental times at 15, 20, 25, 30 and 35°C , but similar values at 20 and 37°C (Fig. 3). The longest pre-pupal developmental durations were 86 days and 83 days for D1 and D2, respectively at 15°C , while the developmental time was shortest at 30°C (10 and 8 days for D1 and D2, respectively) (Fig. 3). The development time of pupae obtained from larvae fed on D1 and D2, displayed significant variation between the different temperatures ($F = 175.4$; $df = 5, 22$; $P < 0.0001$ and $F = 304.5$; $df = 5, 22$; $P < 0.0001$, respectively). The effect of larval diets (D1 and D2) on pupal developmental time did not differ significantly ($F = 2.002$; $df = 1, 44$; $P = 0.1640$) across the different temperature. No significant interaction was observed between the effect of temperature and diet on pupal development ($F = 2.272$; $df = 5, 44$; $P = 0.0642$). Overall developmental duration from larva to adult flies showed a significant difference among the temperature regimes ($F = 209.3$; $df = 5, 22$; $P < 0.0001$ and $F = 885.4$; $df = 5, 22$; $P < 0.0001$ when reared on D1 and D2, respectively) and between diets ($F = 5.25$; $df = 1, 44$; $P = 0.0274$). The effect of temperature and diet on developmental time showed a significant interaction ($F = 26.54$; $df = 5, 44$; $P < 0.0001$). For D1, the developmental time from larva to adult ranged between 28 days at 30°C to 184 days at 15°C , whereas those reared on D2, completed development to adult stage in 31 days at 30°C and 181 days at 15°C (Fig. 3).

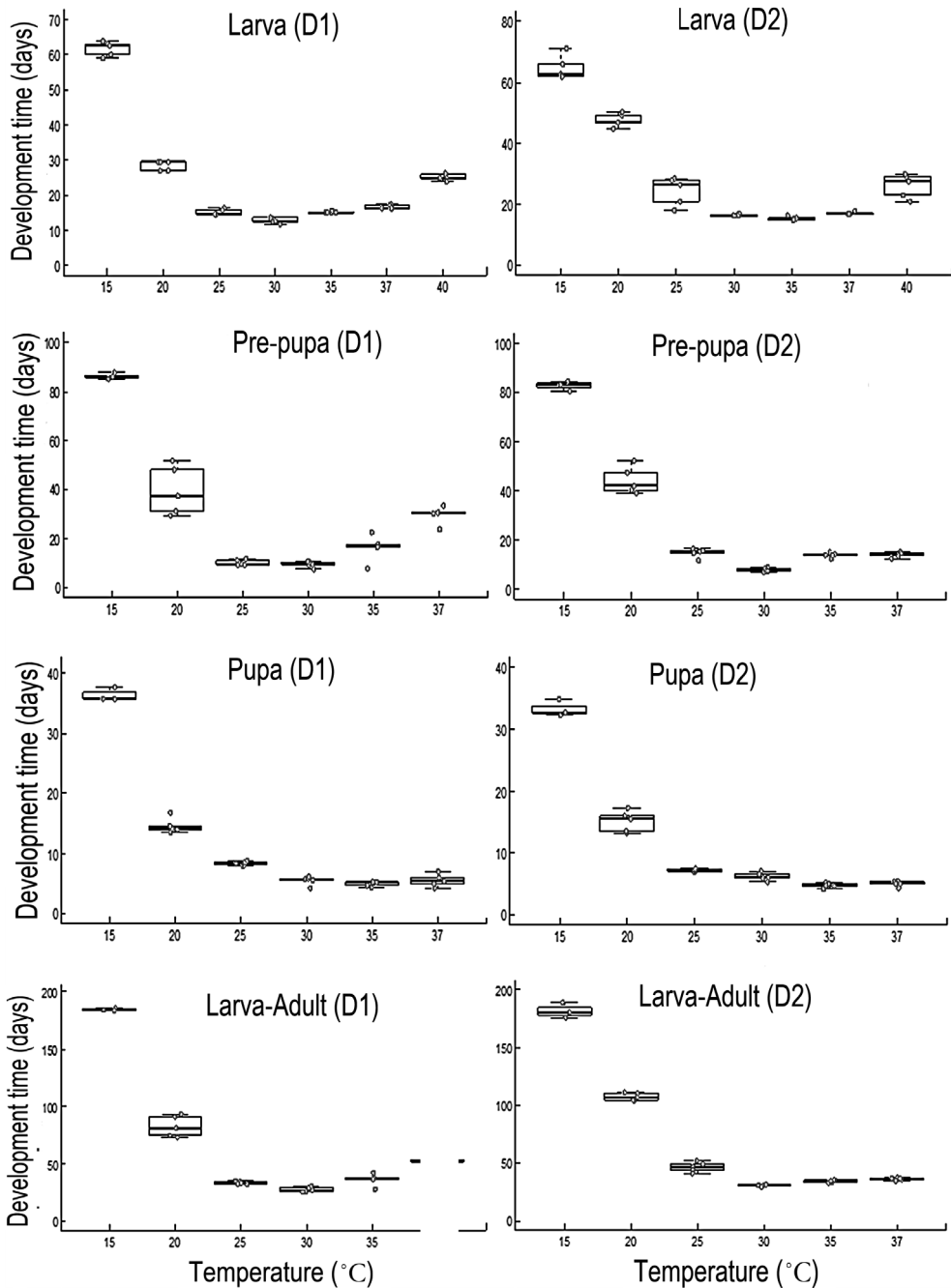


Fig. 3. Development time of different life stages of *H. illucens* fed on two diets at constant temperatures. The middle quartile or the median (line that divides the box into two parts) marks midpoint of the data. Middle box represents 50% of the scores for each treatment and the middle 50% values fall within the inter-quartile range. D1 = diet 1; D2 = diet 2.

Most favourable temperature range of *H. illucens*

The stage-specific survival of BSF at nine constant temperatures is presented in Table 1. Egg viability was extremely low (< 11%) at 15, 37 and 40 °C compared to the other temperatures evaluated. The highest percentage of egg eclosion was recorded at 30 °C (80%) and 35 °C (75%) (Table 1). Percentage larval survival differed significantly ($F = 7.68$; $df = 6, 28$; $P < 0.0001$ for D1 and $F = 19.79$; $df = 6, 28$; $P < 0.0001$ for D2) among the different temperature treatments. The interaction between the effects of temperature and diet was significant ($F = 2.60$; $df = 6, 56$; $P = 0.0217$). However, survival rate was comparable at 15, 20, 25, 30, 35 and 37 °C, whereas it was lower at 40 °C (Table 1). For D2, larval survival rate was high at 35 °C (92%) and 30 °C (90%) and low at 40 °C (28%). Pre-pupal survival was significantly influenced by temperature when larvae were reared on D1 ($F = 26.58$; $df = 5, 22$; $P < 0.0001$) and D2 ($F = 4.08$; $df = 5, 22$; $P = 0.0090$). There was a significant interaction ($F = 11.91$; $df = 5, 44$; $P < 0.0001$) between temperature and diet on pre-pupal survival. For D1, the highest pre-pupal survival rate was recorded at 25 °C (83%) and 30 °C (82%), while the lowest value was recorded at 37 °C (24%). For D2, the highest percentage of survival was observed at 35 °C (79%) and 30 °C (77%), whereas at 25 °C the survival rate was the lowest (54%). However, the survival rate across the different temperatures was similar, except at 25 °C. The pupal survival rate differed significantly between temperatures when the larvae were reared on D1 ($F = 42.28$; $df = 5, 22$; $P < 0.0001$) or D2 ($F = 25.96$; $df = 5, 22$; $P < 0.0001$). The interaction between the effect of rearing diet and temperature on pupal survival was significant ($F = 6.67$; $df = 5, 44$; $P < 0.0001$). The percentage of survival recorded for the pupal stage was observed to decrease from 77% at 30 °C to 5% at 37 °C for D1 and from 75% at 30 °C to 20% at 37 °C for D2 (Table 1).

Table 1. Mean percentage survival of immature life stages (egg, larval, pre-pupal and pupal) of *H. illucens* at constant temperatures

Temperature (°C)	Egg	Larva		Pre-pupa		Pupa	
		D1	D2	D1	D2	D1	D2
10	-	-	-	-	-	-	-
15	10.6 ± 2.1c	82.8 ± 8.8aA	87.0 ± 3.5aA	58.7 ± 1.3bA	65.3 ± 6.4abA	49.3 ± 7.1bA	62.0 ± 3.1bA
20	59 ± 10.6b	74.6 ± 5.7aA	82.4 ± 5.4aA	68.4 ± 5.2abA	61.0 ± 2.7abA	61.6 ± 4.5abA	59.4 ± 1.9bcA
25	59.8 ± 8.5b	93.0 ± 1.4aB	58.0 ± 5.0bA	83.1 ± 2.9aB	54.4 ± 5.0bA	67.4 ± 4.0abB	46.3 ± 2.5cA
30	80.0 ± 5.6a	92.6 ± 2.4aA	90.4 ± 0.7aA	82.2 ± 4.2aA	77.2 ± 4.0aA	77.1 ± 5.9aA	74.8 ± 4.2aA
35	74.8 ± 14.7a	90.8 ± 1.7aA	92.2 ± 3.5aA	75.2 ± 3.1aA	79.0 ± 4.5aA	65.6 ± 3.3abA	54.1 ± 5.9bcA
37	9.8 ± 3.1c	89.4 ± 6.3aA	84.0 ± 1.5aA	24.1 ± 3.0cB	63.6 ± 6.8abA	5.4 ± 1.0cB	19.6 ± 2.4dA
40	9.4 ± 2.1c	34 ± 15.0bA	27.6 ± 7.7cA	-	-	-	-
42	-	-	-	-	-	-	-

D1 = diet 1, D2 = diet 2. For the egg, larval, pre-pupal and pupal stages, means within the same column followed by different lower-case letter are significantly different ($P < 0.05$, SNK Test) for D1 or D2. For the larval, pre-pupal and pupal stages, means within the same row for each life stage followed by the same upper-case letter are not significantly different ($P < 0.05$, t-test) for D1 or D2. (-) no survival was observed.

Longevity and reproduction of *H. illucens* at different temperatures

The longevity of adult BSF was significantly affected by temperature for both females ($F = 52.48$; $df = 5, 29$; $P < 0.0001$) and males ($F = 53.27$; $df = 5, 29$; $P < 0.0001$). Fig 4 indicates that adult flies live longer at intermediate temperatures than at upper extreme temperatures as further illustrated by the quadratic model fitted to longevity (Fig. 5) for both sexes when their larvae were reared on D1 or D2. Average pre-oviposition period was observed to vary significantly across the different temperatures, being longest at 20°C (16 days) and shortest at 35 °C (5 days) when larvae were reared on D1 and D2 (Fig. 6). Fecundity was significantly affected by temperature, especially at the lower (15 °C) and upper (37 °C) temperatures evaluated. The highest fecundity of BSF was observed at 30 °C (516 and 475 eggs when flies were reared on D1 and D2, respectively) (Fig. 6).

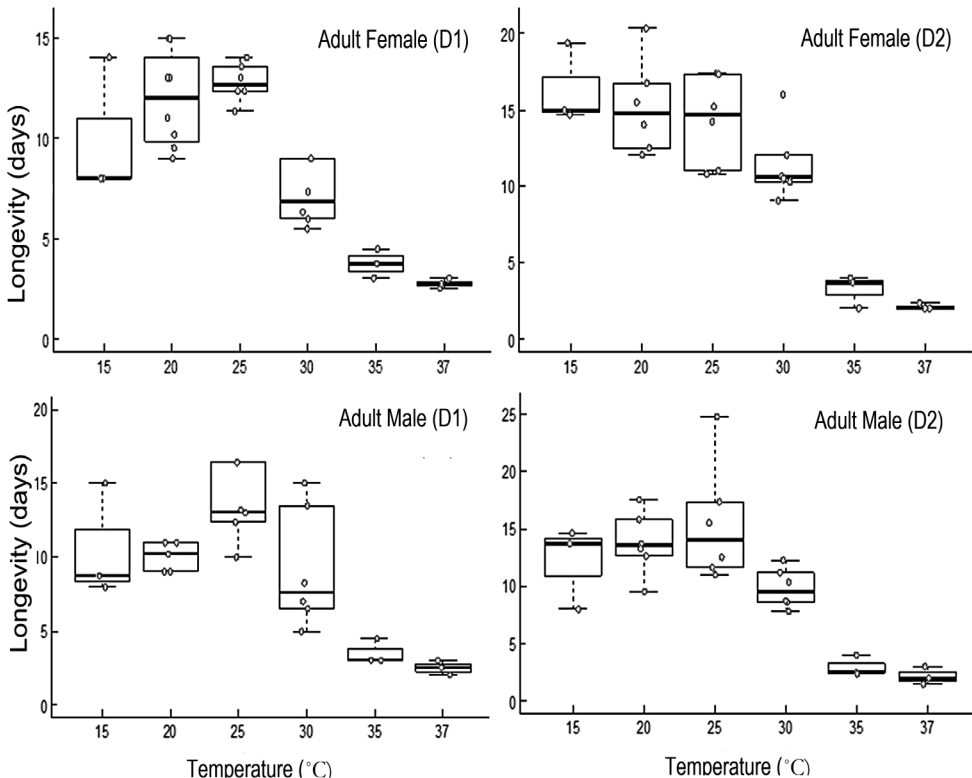


Fig. 4. Boxplots showing longevity of adult female and male *H. illucens* at constant temperatures. Middle quartile (line that divides the box into two parts) shows midpoint of the data. Middle box represents 50% of the scores for each treatment and the middle 50% values fall within the inter-quartile range. D1 = diet 1; D2 = diet 2

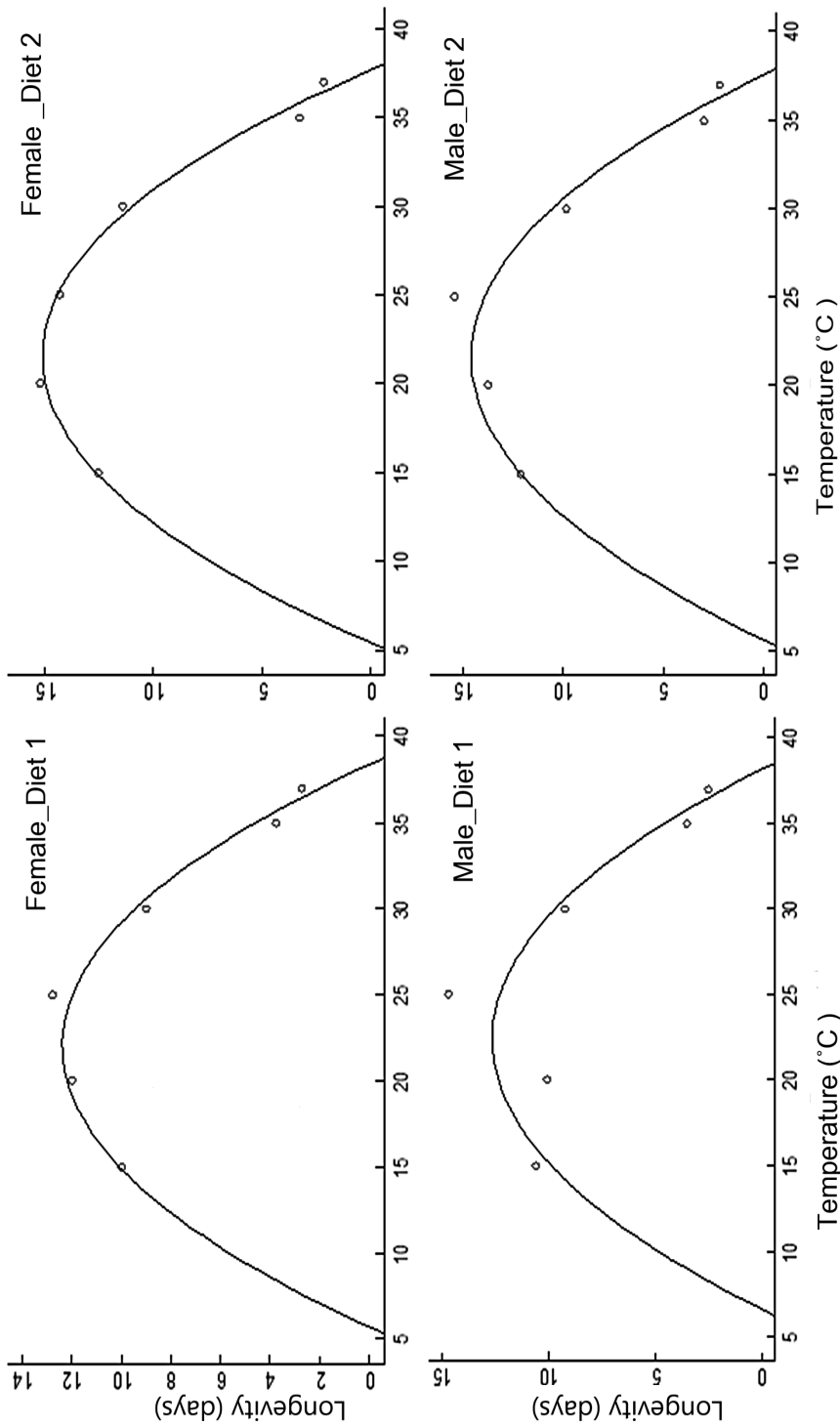


Fig. 5. Quadratic model curves describing longevity of adult female and male *H. illuensis* at constant temperatures. Quadratic: $n(T) = a + bT + cT^2$; $n(T)$ represents the longevity function at temperature T ($^{\circ}\text{C}$); a , b and c are empirical parameters of the model.

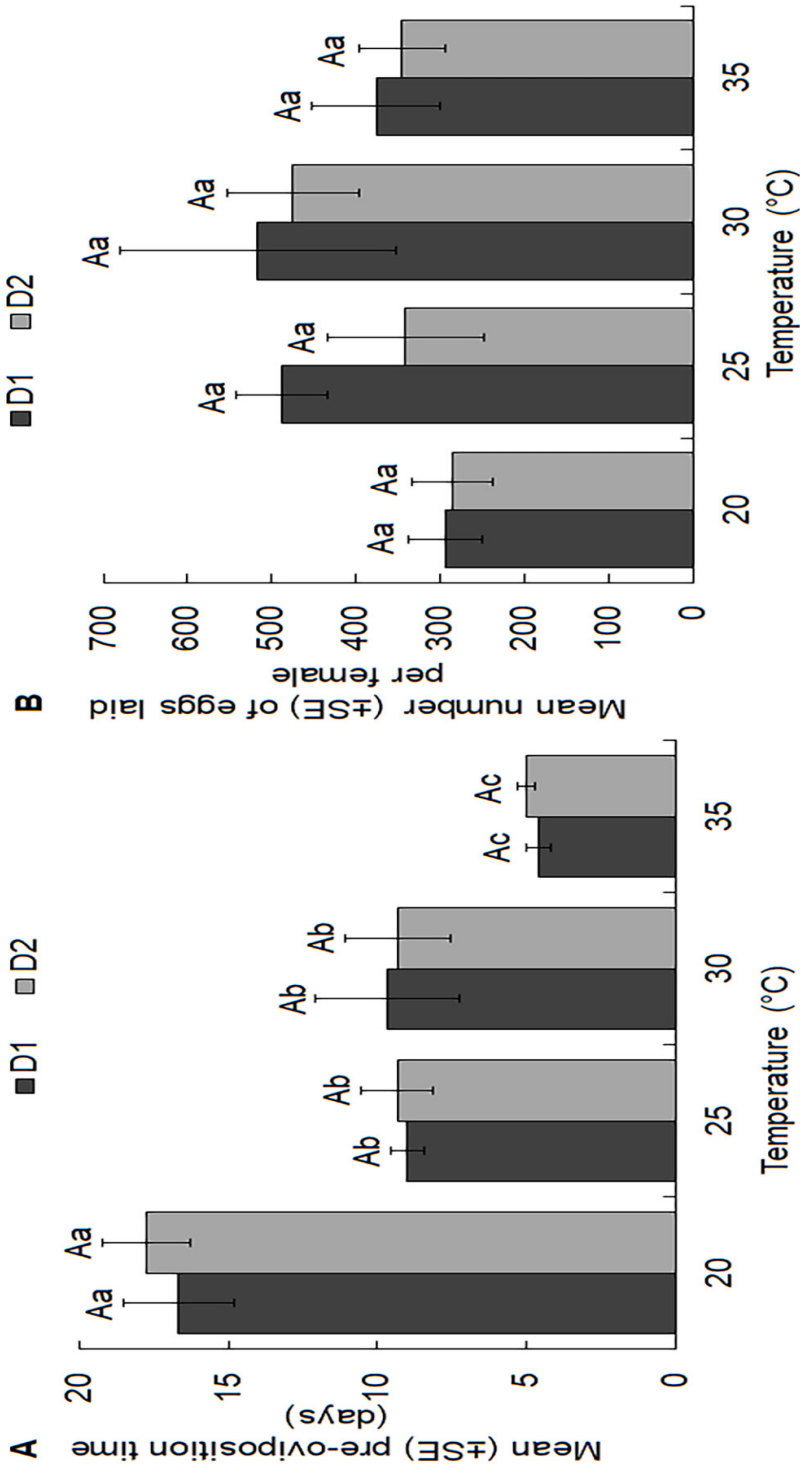


Fig. 6. Mean number of days before oviposition by female *H. illucens* (pre-oviposition period) (A) and mean number of eggs laid per female during her lifespan (B) at constant temperatures. D1 = diet 1; D2 = diet 2

Body weight of *H. illucens* life stages reared on two diets at constant temperatures

The mean wet weight for the different life stages of BSF reared on two diets is presented in Fig. 7. The estimated mean wet weight was higher for all BSF life stages reared on D1 compared to D2 across the different temperature regimes. Body weight of 5th instar larvae varied significantly among the different temperature regimes ($F = 6.26$; $df = 6, 26$; $P < 0.0001$ for D1 and $F = 25.8$; $df = 6, 26$; $P < 0.0001$ for D2) and between rearing diets ($F = 267.81$; $df = 1, 52$; $P < 0.0001$). The interaction between the temperature and diet was found to be significant ($F = 9.52$; $df = 6, 52$; $P < 0.0001$). Mean larval weight was highest (0.216 g) at 37 °C and lowest (0.159 g) at 15 °C when reared on D1 while on D2, mean larval weight was highest (0.168 g) at 35 °C and lowest (0.084 g) at 15 °C. Pre-pupal weight differed significantly across the temperature treatments ($F = 31.59$; $df = 6, 26$; $P < 0.0001$ for D1 and $F = 15.94$; $df = 6, 26$; $P < 0.0001$). A significant interaction was observed ($F = 6.78$; $df = 6, 52$; $P < 0.0001$) between temperature and diet for pre-pupal weight. The highest mean pre-pupal weight when the larvae were fed on D1 (0.186 g) or D2 (0.152 g) was recorded at 35 °C and the lowest at 20 °C (0.121 g) and 15 °C (0.087 g) for D1 and D2, respectively. Pupal weight was significantly different among temperature regimes ($F = 6.63$; $df = 5, 22$; $P < 0.0001$ for D1 and $F = 17.22$; $df = 5, 22$; $P < 0.0001$ for D2) and between the rearing diets ($F = 77.28$; $df = 1, 44$; $P < 0.0001$). There was a significant interaction ($F = 4.56$; $df = 5, 44$; $P = 0.002$) between the effects of temperature and rearing diet on pupal weight. The highest mean pupal weight was recorded at 35 °C for either D1 (0.145 g) or D2 (0.126 g) while the lowest weight was recorded at 15 °C for either D1 (0.122 g) or D2 (0.087 g). Adult weight varied across the temperature treatments for each diet ($F = 113.2$; $df = 6, 26$; $P < 0.0001$ for D1 and $F = 245.2$; $df = 6, 26$; $P < 0.0001$). The highest mean adult body weight was recorded at 35 °C for either D1 (0.078 g) or D2 (0.077 g).

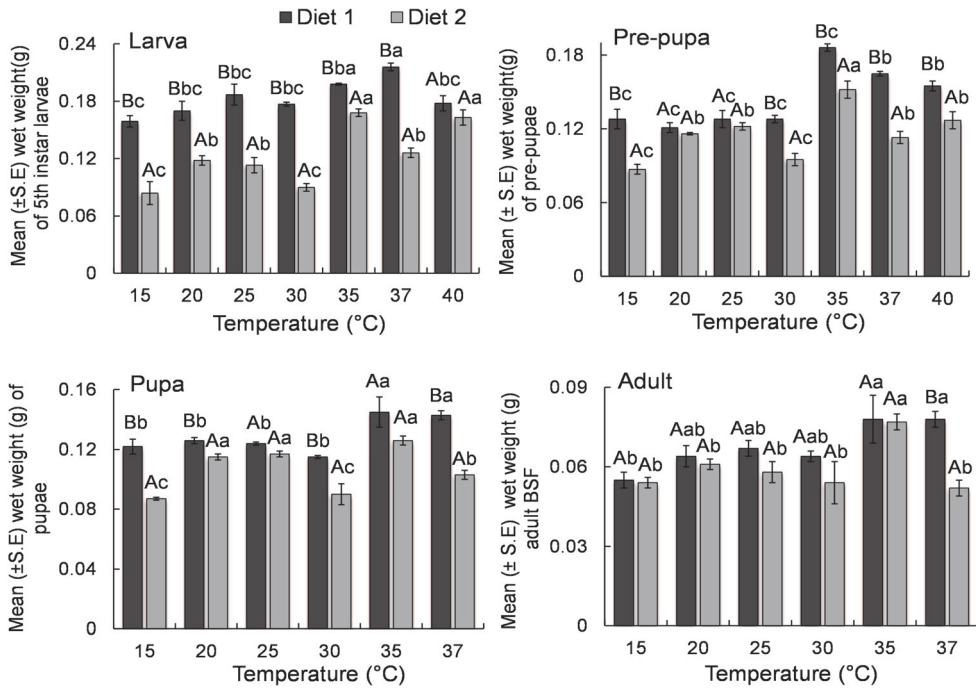


Fig. 7. Mean wet weight of *H. illucens* stages reared on two diets at constant temperatures. Bars within the same dietary treatment with the same lower-case letter are not significantly different. Bars within the same temperature treatment with the same uppercase letter are not significantly different ($P < 0.05$, SNK).

Temperature-dependent developmental models of *H. illucens*

Table 2 and Table 3 present the parameter estimates for BSF eggs and immature life stages, respectively, obtained from the non-linear Brière-1 model and linear models fitted to developmental rate. The fitted models for developmental rates (1/d) versus temperature for all the different life stages are presented in Fig. 8 (egg) and Fig. 9 (larval, pre-pupal and pupal stages). The lower temperature threshold (T_{min}) for larval, pre-pupal and pupal stages estimated using Brière-1 model were lower as compared to estimates of the linear regression model on both diets. Using the linear model, T_{min} of BSF eggs was estimated at 13.6 °C. For the larvae, pre-pupae and pupae, T_{min} was estimated as 12.3, 13.3 and 13.3 °C, respectively, for D1, and 11.7, 14.6 and 12.2 °C, respectively, for D2 (Table 3), which were all similar to that of Brière-1 model estimates. The optimal temperature threshold for larval, pre-pupal and pupal developmental stages reared on D1 were estimated as 31.3–36.0 °C, and 32.3–36.4 °C for D2. The upper temperature thresholds were estimated to range from 37.2–44.0 °C for the different life stages (Table 2 and Table 3). The BSF egg required 50.74 degree-days (DD) for the successful completion of eclosion, whereas the larval, pre-pupal and pupal stages required 250.2, 142.9 and 142.9 DD for D1; and 333.3, 125.0 and 111.1 DD for D2, respectively.

Table 2. Estimates of linear model parameters describing the relationship between temperature and developmental rate of the *H. illucens* eggs

Model parameters	Estimate (\pm SE)
a	-0.269 ± 0.067
b	0.011 ± 0.002
T_{min}	13.647 ± 1.98
k	50.736 ± 5.751
RSS	0.013
R^2	0.940

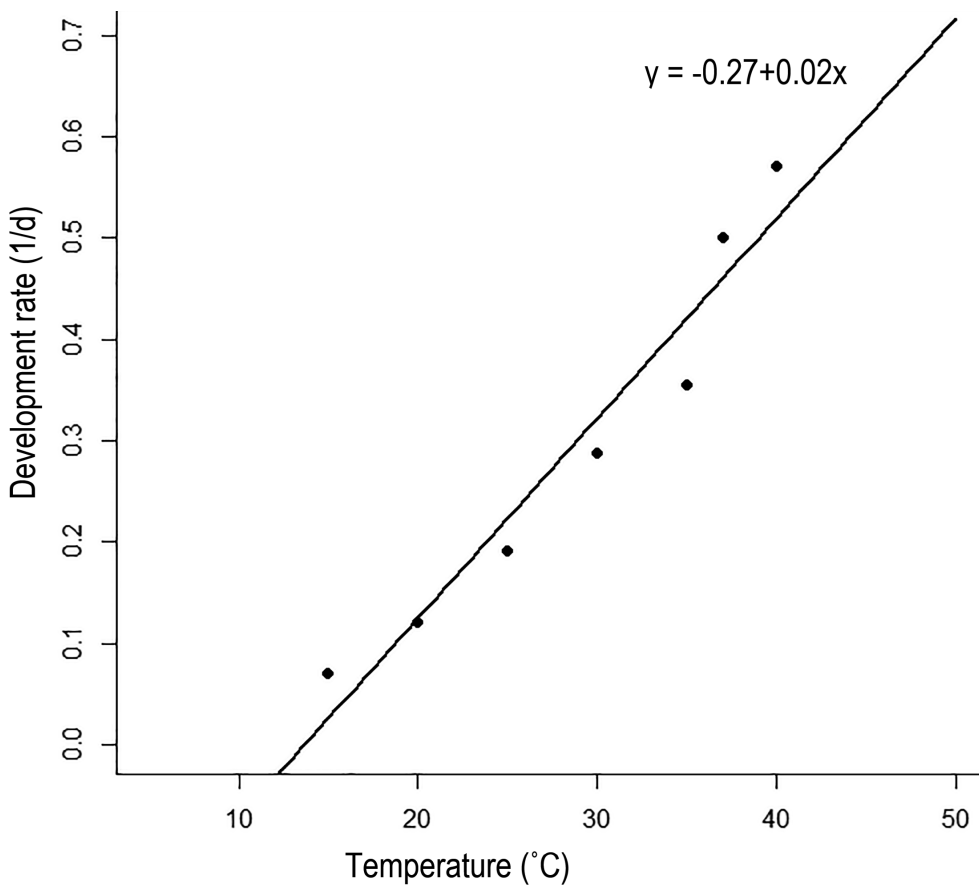


Fig. 8. Linear model fitted to observed values of development rate of *H. illucens* eggs at constant temperatures.

Table 3. Estimates of model parameters describing the relationship between temperature and developmental rate of *H. illucens* life stages on two diets (D1 and D2)

Model	Model parameters	Larva	Pre-pupa	Pupa
D1				
Linear	a	-0.049 ± 0.011	-0.093 ± 0.042	-0.093 ± 0.015
	b	0.004 ± 0.001	0.007 ± 0.002	0.007 ± 0.001
	T_{min}	12.25 ± 1.41	13.29 ± 2.65	13.29 ± 1.92
	k	250.2 ± 25.50	142.86 ± 39.17	142.86 ± 39.17
	RSS	5.83×10^{-5}	8.20×10^{-4}	8.20×10^{-4}
	R ²	0.976	0.876	0.876
Brière-1	n	2.09×10^{-5}	6.77×10^{-5}	9.59×10^{-5}
	T_{min}	11.74 ± 0.94	12.17 ± 3.90	11.75 ± 1.08
	T_{max}	43.23 ± 0.47	37.18 ± 0.29	41.21 ± 0.81
	T_{opt}	35.99	31.26	34.39
D2				
Linear	a	-0.035 ± 0.011	-0.117 ± 0.036	-0.110 ± 0.024
	b	0.003 ± 0.001	0.008 ± 0.002	0.009 ± 0.001
	T_{min}	11.67 ± 3.00	14.63 ± 1.83	12.22 ± 1.67
	k	333.33 ± 50.59	125.00 ± 25.88	111.11 ± 11.90
	RSS	5.80×10^{-5}	5.91×10^{-4}	2.58×10^{-4}
	R ²	0.954	0.926	0.976
Brière-1	n	2.93×10^{-5}	7.50×10^{-5}	7.92×10^{-5}
	T_{min}	10.39 ± 1.68	14.28 ± 3.41	10.66 ± 1.96
	T_{max}	41.22 ± 0.34	38.02 ± 0.97	43.99 ± 2.34
	T_{opt}	34.20	32.25	36.44

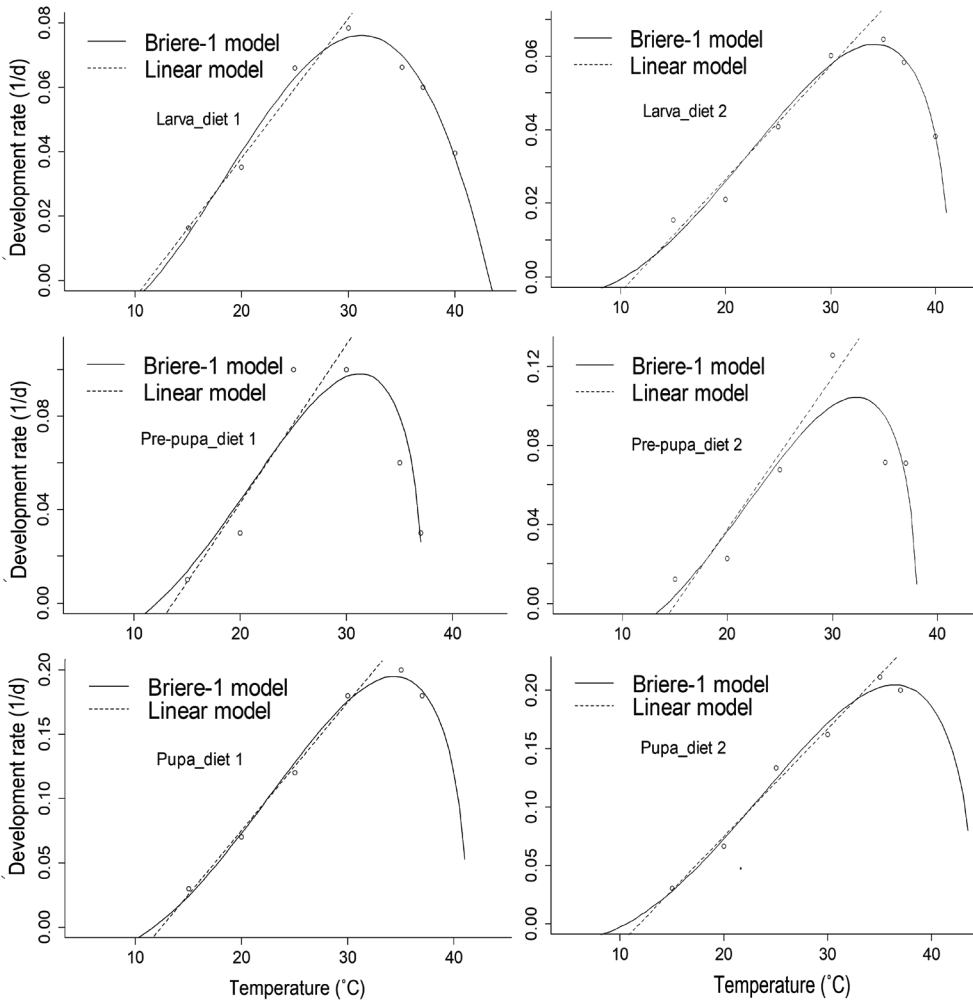


Fig. 9. Linear and non-linear models fitted to observed values of the development rate of *H. illucens* life stages at constant temperatures.

Estimated life table parameters of *H. illucens* reared on two diets at different temperatures

The net reproductive rate (R_0) ($F = 4.57$; $df = 3,13$; $P = 0.021$ for D1 and $F = 5.79$; $df = 3,13$; $P = 0.010$ for D2), intrinsic rate of increase (r_m) ($F = 26.91$; $df = 3,13$; $P < 0.0001$ for D1 and $F = 30.68$; $df = 3,13$; $P < 0.0001$ for D2), gross reproductive rate (GRR) ($F = 3.33$; $df = 3,13$; $P = 0.053$ for D1 and $F = 3.86$; $df = 3,13$; $P = 0.036$ for D2) and finite rate of increase (λ) ($F = 25.36$; $df = 3,13$; $P < 0.0001$ for D1 and $F = 28.95$; $df = 3,13$; $P < 0.0001$ for D2) values were significantly higher at 30°C, when compared to the

other temperature treatments evaluated (Table 4). The doubling time (T_d) ($F = 28.14$; $df = 3, 13$; $P < 0.0001$ for D1 and $F = 29.64$; $df = 3, 13$; $P < 0.0001$ for D2) and mean generation time (G) ($F = 751.60$; $df = 3, 13$; $P = 0.0001$ for D1 and $F = 11.84$; $df = 3, 13$; $P = 0.0001$ for D2) at 30 °C was significantly shorter compared to the other temperature treatments evaluated. The values of R_0 generated for all the temperatures and diets indicated significant growth of *H. illucens* population rather than a decline. In addition, for all temperature treatments and diets, analysis of life table and fecundity parameters yielded positive values of intrinsic rate of increase.

Table 4. Life table parameters of *H. illucens* reared on two different diets at constant temperatures

Parameter	20°C		25°C		30°C		35°C	
	D1	D2	D1	D2	D1	D2	D1	D2
Intrinsic rate of increase (r_m)	0.043 ± 0.002cA	0.036 ± 0.002cA	0.098 ± 0.005bA	0.070 ± 0.004bB	0.127 ± 0.014aA	0.122 ± 0.013aA	0.109 ± 0.013aA	0.108 ± 0.005bA
Doubling time (T_d) (days)	16.25 ± 2.50aA	19.55 ± 3.12aA	7.10 ± 0.63bA	9.86 ± 0.91bB	5.46 ± 0.99bA	5.67 ± 1.00bA	6.39 ± 0.73bA	6.43 ± 0.78bA
Net reproductive ratio (R_0)	116.10 ± 2.96bA	105.80 ± 2.70bA	97.66 ± 5.06bA	66.27 ± 3.43bA	186.93 ± 22.52aA	196.26 ± 23.64aA	118.07 ± 4.26bA	102.33 ± 3.69bA
Gross reproductive ratio (GRR)	190.32 ± 4.86bA	179.32 ± 4.57bA	145.77 ± 7.55abA	144.07 ± 7.46bA	242.76 ± 29.24aA	261.68 ± 31.52aA	178.90 ± 6.45bA	189.50 ± 6.83bA
Mean generation time (G) (days)	111.91 ± 1.24aA	131.46 ± 1.24aB	46.65 ± 1.16bA	59.65 ± 1.16bB	41.18 ± 2.52bA	43.18 ± 2.52cA	43.95 ± 0.71bA	42.95 ± 0.71cA
Finite rate of increase (λ)	1.044 ± 0.002cA	1.036 ± 0.002cA	1.103 ± 0.005bA	1.073 ± 0.004bB	1.135 ± 0.016aA	1.130 ± 0.016aA	1.115 ± 0.015aA	1.114 ± 0.005bA

D1 = diet 1, D2 = diet 2. Means within the same row followed by different lower-case letters are significantly different

($P < 0.05$, SNK Test) for D1 or D2. Means within the same row for each temperature regime followed by the same

upper-case letters are not significantly different ($P < 0.05$, T-test) for D1 and D2.

Discussion

The rate of black soldier fly growth and development was considerably influenced by temperature and diet, which are the two most critical environmental factors. Previous studies have shown that insects including the BSF are sensitive to several environmental factors, especially temperature, which is considered the most important abiotic factor (Ratte, 1984) that can impact not only insect developmental rate, seasonal and daily cycles (Logan et al., 1976) but also, indirectly effect different aspects of the insect biology, such as immature survival, adult life span, growth, fecundity, fertility and population growth parameters (Gabre et al., 2005; Schneider, 2009; Summers et al., 1984). As such, temperature would profoundly influence the behaviour, abundance, colonization, distribution, life table parameters and fitness of the insect species. Thus, knowledge generated on thermal requirements of *H. illucens*' development will have significant implications for production programs. Life history studies would provide successful ways of following up changes in a population's growth and several other important aspects of the insect's life cycle that are temperature-dependent (Golizadeh et al., 2009; Karimi-Malati et al., 2014a; Karimi-Malati et al., 2014b; Khanamani et al., 2013; Park et al., 2014; Schneider, 2009). Here, we used nine different constant temperatures to evaluate the influence that different temperature regimes would have on black soldier fly development. The lower (10 °C) temperature was lethal to the insects given that all the eggs completely failed to hatch at this temperature.

Our results confirmed the sensitivity of BSF to extremely low and high temperatures as indicated in previous studies (Holmes et al., 2016; Tomberlin et al., 2009). Egg viability and hatchability observed in this study occurred between 15–40 °C. Our observation at lower temperature threshold concurs with that presented by Holmes et al. (2016) at 16 °C. The levels of egg viability at 15 and 40 °C were extremely low (< 12%), which is consistent with the report by Holmes et al. (2016) at 16 °C. The present result is consistent with the results obtained by Holmes et al. (2013). Contrary to observations by Holmes et al. (2016), who reported that newly hatched larvae of BSF failed to survive at 16 °C, we found that BSF young larvae at 15 °C did not die after hatching, suggesting that the lower developmental threshold for BSF eggs could be at 15 °C. Thus, hatchability or egg eclosion could be a pointer to colony efficiency in any mass-rearing systems (Vantomme et al., 2012). According to previous studies, nutrient quality of larval food has been shown to have considerable influence on the amount and quality of emerged adult flies, which indirectly affects the developmental duration, survival and growth of the black soldier larvae (Ekesi et al., 2007; Kaspi et al., 2002; Krainacker et al., 1987; Tomberlin et al., 2009; Vargas et al., 1994). The two types of diet tested in this study supported the development of *H. illucens* larvae. All BSF life stages (larval, pre-pupal and pupal) completed development to adults in the range of 15–37 °C. Survivorship rates of BSF

immature life stages differed remarkably at different temperature regimes. Temperature treatments below 15 °C and above 40 °C were unfavourable with complete mortality of *H. illucens* life stages observed. The present study showed that the upper temperature for larval survival was 40 °C, with survival of 34 and 28% on D1 and D2, respectively. Only 5 and 20% of the flies from D1 and D2, respectively, at 37 °C emerged as adults; many of the flies showed signs of crippling malformations (i.e. could neither walk nor fly normally and were unable to feed).

At 40 °C, none of the pre-pupal stages successfully completed development to proceed to pupal stage on the various diets. These findings slightly deviate from those reported by Tomberlin et al. (2009), who showed that although 73% of *H. illucens* larvae were able to develop and survive to pre-pupae (post-feeding stage) at 36 °C, only 0.1% of pupae successfully developed and emerged as adults, thus implying an upper developmental temperature threshold at 36 °C. The reasons for these differences between the two studies are unknown but nutrient content of diet types, geographical strains of the two populations (Kenyan and Texas, USA populations) and adaptations of both populations of *H. illucens* might have contributed to the observed variation. Furthermore, this is supported by other studies in the literature (Diamantidis et al., 2011; Vargas & Carey, 1989), which revealed that insect populations from various geographical landscapes may vary in their reproductive fitness and life table traits (Nedvěd & Honěk, 2012).

The linear relationship between the developmental rates and temperature for rearing BSF life was positive. The estimated lower temperature developmental threshold (LTDT) of BSF eggs according to the linear model was 13.65 °C. Here, we publish the first report on LTDT of BSF immature life stages, which is consistent with reports of other dipteran species like tephritid fruit flies (Duyck & Quilici, 2002; Tanga et al., 2015). The divergence observed between the different reported LTDTs may be attributed to variations in rearing conditions and possible feedstocks utilized as food for the BSF larvae (Xue et al., 2010). The optimum developmental temperature range for *H. illucens* life stages reared on D1 was 31.3–36.0 °C and 32.3–36.4 °C on D2. In literature, there are no studies reported with regards to upper developmental threshold or lethal upper temperature thresholds of BSF, which according to the present study were estimated to range between 37.2–44.0 °C for the various developmental stages. Lethal temperature thresholds established in the current study may be applicable for future mass production technology programs for BSF. The present findings revealed that the eggs required 50.74 degree-days (DD) to complete development to the next stage (larval stage). Total degree days required for BSF larval stages to successfully change to the pre-pupal stage was recorded at developmental temperature threshold of 10.4–14.3 °C, which was higher (250–333 DD) compared to the other BSF life stages investigated (< 150 DD). Several authors have also reported discrepancies in thermal requirements for different insect species and immature life

stages, which can be ascribed to differences in methodological approaches or aspects related to larval food quality and quantity as well as larval density in the rearing facilities (Duyck & Quilici, 2002; Fletcher, 1989; Trudgill et al., 2005; Vargas et al., 1996). It is worth noting that insects require fewer degree days for development when fed on high quality food resources compared to low quality resources (Amalraj et al., 2005), which explains the considerable variation observed when the larvae were provided the two types of diet in the present study. Pronounced quality control parameters observed in this study included high pupal recovery and heavier puparia when the fly larvae fed on D1, which was supplemented with brewers' yeast compared with those fed on D2 (not supplemented). It is important to note that the pupal mass of insects have been demonstrated to be a practical quality control benchmark in insect mass rearing facilities, where size has been observed to show strong correlation with male fly mating successes (Churchill-Stanland et al., 1986). Furthermore, the insect pupal weight has been used as an estimate of size, (Churchill-Stanland et al., 1986) and revealed that adult flies eclosing from heavy pupae showed higher mating success than those resulting from lower weight pupae. Also, large flies have been shown to demonstrate better flight capability than smaller flies (Sharp et al., 1983). Heavy female insects have also been shown to have a proportionately higher lifetime fecundity compared to less heavier females (Karlsson & Wickman, 1990). This implies that increased pupal weights will produce larger individuals (adults). Thus, from a commercial point of view, this highlights the significance of safeguarding maximum pupation while optimizing female-to-male ratio to promote healthy mating behaviour within the insect stock colony (Singh, 1982; Tch-uinkam et al., 2011; Vantomme et al., 2012). Black soldier fly fecundity recorded is an important parameter, which was observed to be directly related to differences in the temperature regimes. This attribute has been documented in different insects, where an increase in rearing temperature frequently results in visible decrease in female fly productivity or complete cessation of egg production (Mehrparvar & Hatami, 2007; Vasicek et al., 2002), as realized fecundity could have been restricted by both temperature dependency of egg maturation and oviposition (Berger et al., 2008). The present study demonstrates that adult female BSF reared on the two diets were capable of reproducing between the temperature range of 20–35 °C. We also observed increased physical inactivity of larvae and reduced feed intake followed by death with increase in temperature (>35 °C). The male and female flies' lifespan gradually decreased with increased temperature from 15 to 37 °C. Thus, at temperatures above 35 °C, the longevity of adult female flies was 4 – 5 times less compared to those at 15 °C. The life time fecundity of black soldier flies was temperature-dependent revealing a curvilinear response curve for fecundity to reach its maximum at 30 °C with a decrease at temperature levels below and above this temperature. This implies that the prevailing optimal temperature recorded in the present study can play a major role in defining climatic fitness for mating and oviposition of adult black soldier flies. The phenomenon of decreased female fecundity has become

noticeable in different insects in the face of temperature increase (Mehrparvar & Hata-mi, 2007; Vasicek et al., 2002). A comparable trend in temperature-dependent fecundity has been observed for different insects with temperatures between 25–30 °C reported as suitable for reproduction, whereas temperatures above 35 °C remained extremely unfavourable (Tanga et al., 2018).

Our study reports for the first time the effect of temperature on *H. illucens*' fecundity with slightly higher number of eggs oviposited per female throughout its lifespan in comparison to that documented in literature (Tomberlin et al., 2002). This could be attributed to the dissimilar ovipositional behavioural response of BSF when fed on various diets (Tomberlin et al., 2002). Oviposition pattern of insects is an important life table component, thus information on temperature-driven, age-specific off-spring production is critical for generating models that forecast BSF population growth (Wagner et al., 1984). However, fecundity is affected by many factors, which include larval and adult density, availability of feedstock, nutrition of immature life stages, and several environmental factors, which need to be studied further to improve BSF mass-production.

Life-table data offer readily available means of tracking population growth as well as other changes (Khanamani et al., 2013), and remain the most powerful tool for analyzing and understanding the impact that abiotic factors have upon the growth, survival, reproduction and rate of increase of an insect population (Bellows et al., 1992). The impact of temperature on BSF life table parameters is reported here for the first time and describes a series of temperature regimes, which are within the ecological niche suitability limits related to BSF development, establishment and colonization. These findings provide answers to the optimum developmental threshold temperature conditions of BSF mass production with 30°C being the most favourable temperature with higher intrinsic rate of natural increase and shorter doubling time. Thus, the implication of using life table information to improve mass rearing conditions has been reported for many insect species by assessing the variations in their reproductive rate and the total number of female offspring produced per female per generation (Birch, 1948). The intrinsic rate of natural increase is extremely essential as it describes the population increase of insects in an unrestricted environment, basically addressing the differences between birth rate and death rate (Birch, 1948) when the insects are exposed to various food sources (Fouly et al., 1995; Hansen et al., 1999; Hodek & Honek, 1996; Richards & Evans, 1998; Souissi & Le Ru, 1997; Valicente & Oneil, 1995).

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Chapter 6

Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products

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Abstract

Black soldier fly (BSF) larvae *Hermetia illucens* (Linnaeus) (Diptera: Stratiomyidae) bio-convert organic side streams into high quality biomass, the composition of which largely depends on the side stream used. In the present study, BSF larvae were reared on twelve substrates, composed of brewers' spent grains supplemented by either water, waste brewer's yeast or a mixture of waste brewer's yeast and cane molasses. The nutritional composition (proximate, minerals, amino acids, and fatty acids) of the BSF larvae fed each feedstock was determined. The effect of substrate, supplementation and their interaction on crude protein, fat and ash contents of BSF larvae was significant. Calcium, phosphorus and potassium were the most abundant macrominerals. Differences in amino acid profiles of the larvae were small. Lysine, histidine and arginine were the most abundant essential amino acids. The larval fatty acid profiles predominantly consisted of saturated fatty acids, of which lauric acid, palmitic acid and stearic acid were generally the most abundant. These findings provide important information to support the use of BSF larval meal as potential new source of highly suitable nutrient-rich and sustainable animal feed ingredients to substitute expensive and scarce protein sources such as fishmeal and soya bean meal. The implications of BSF larval meal in animal nutrition are discussed.

Key words: insect meal, brewers' spent grains, protein, minerals, amino acids, fatty acids, animal feed ingredients

Introduction

Animal feed is composed of major ingredients such as corn meal, wheat, rice, soybean, fishmeal, and fish oil, therefore competing with human food requirements (Rana et al., 2009; Van Huis et al., 2013). The growing human population and demand for food and feed places continuous pressure on the environment (Foley et al., 2011). The increasing demand for fishmeal and soybean meal, as major protein sources in animal feeds has led to their scarcity and increasing market prices. Furthermore, feed costs represent about 70% of total aquaculture and livestock production (Ssepuuya et al., 2017; Van Huis et al., 2013). It is, therefore, a matter of utmost urgency to search for alternative and sustainable sources of protein for aquaculture and livestock. The potential of insect-based protein and other nutrients has attracted much interest from scientists and public organisations (Dicke, 2018; Van Huis, 2013; Van Huis, 2015; Van Huis, 2016; Van Huis et al., 2015; Van Huis et al., 2013; van Huis & Vantomme, 2014). Among several insect species recommended for animal feed, the black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) has the highest potential for large-scale production (Rumpold et al., 2018; Van Huis et al., 2013). Larvae of BSF convert low-grade organic side streams into high-quality protein and provide an innovative strategy for waste valorization (Meneguz et al., 2018; Nguyen et al., 2015; Nyakeri et al., 2017; Spranghers et al., 2017; Surendra et al., 2016). The Food and Agriculture Organization recommends that insect species suitable for feed are those that can be mass-reared on a large industrial scale, with a minimum reach of 1 tonne per day of insect fresh weight (Van Huis et al., 2013). However, meeting this recommendation requires a considerable amount of substrate for insect feeding. Moreover, nutritional composition of BSF larvae is influenced by the substrate used (Barragán-Fonseca et al., 2018; Diener et al., 2009; Liland et al., 2017; Spranghers et al., 2017; Wong et al., 2019).

Availability of sufficient amounts of feedstock is of paramount importance for sustainable insect production. Brewers' spent grain (BSG) is a by-product from beer production and a suitable protein source utilized as feed for livestock. However, the composition of BSG varies greatly depending on the grains used and the industrial processes and conditions such as fermentation, preservation and temperature employed. Moreover, wet BSG contains 75-80% water and deteriorates rapidly because of bacterial and fungal growth restricting their utilization as animal feed to the first few days after collection. Previous studies have demonstrated that feeding pigs on BSG stored for more than one week results in decreased feed intake and body weight loss (Aguilera-Soto et al., 2008; Thomas et al., 2010). Palatability of feed mixed with BSG has also been shown to decrease with storage time and any uneaten feed rapidly deteriorates resulting in feed wasting. Fresh BSG left in open space spoils quickly and constitutes an environmental nuisance (Heuzé et al., 2017). Dehydrating or ensiling wet BSG can help to prevent

spoilage, but these are time- and energy-consuming processes (Heuzé et al., 2017). Beer production results in more brewer's yeast than required for beer brewing. This is the second most common by-product of beer production, which is also used as a protein supplement in animal feed (Huige, 2006). Brewer's yeast has a short shelf life and its use as a feed supplement is limited. For prolonged use, drum-drying is required or it is mixed with BSG and then dried in a steam-tube drier. Again, these processes require machinery, energy and are therefore not economical (Heuzé et al., 2018).

Refining sugarcane results in a black syrup called molasses, which is rich in carbohydrates and minerals. In Kenya, this by-product is mainly used as a supplement in animal feed. It can cause environmental pollution, especially in water bodies if major spills or factory effluents drain into rivers or streams (M'Ndegwa, 2016). In the context of the above concerns on utilizing BSG, brewer's yeast and molasses in animal feeding, it is therefore important to consider alternative ways of utilizing these by-products. There is limited information on the nutritional quality of BSF larvae reared on BSG (Meneguz et al., 2018; www.entocycle.com/process). An in-depth study of the nutritional quality of BSF larvae reared on brewery and sugar by-products can fill this important knowledge gap. Therefore, in the present study, we reared BSF larvae on BSG, supplemented with either water, brewer's yeast or brewer's yeast plus cane molasses, which are generated by the beer brewers and sugar companies in Kenya. In order to assess the nutritional quality for animal diets, the proximate, mineral, amino acid and fatty acid contents of BSF larvae were analyzed.

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Materials and methods

Insect rearing and harvesting

Black soldier fly eggs were collected from the BSF colony at Animal Rearing and Containment Unit of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya.

As substrate for the BSF larvae, four BSGs, resulting from the production of major beer brands such as: Tusker, Guinness, Senator and Pilsner as well as liquid brewer's yeast were obtained from the Kenya Brewery Limited, Nairobi, Kenya. The BSGs obtained were generated from: barley (B), malted barley (MB), malted corn (MC), and sorghum plus barley (SB). Liquid cane molasses was obtained from Mumias Sugar Company Limited, Kakamega, Kenya. Drying and preparation of substrates and their proximate compositions were previously described by Chia et al. (2018). In the present study, substrates for rearing BSF larvae were formulated as follows: Five hundred grams of each BSG (moisture content 10%; Chia et al., 2018) were mixed with 800 mL of water (W) to provide optimal moisture for larval feeding and growth. Another group of substrates consisted of 500 g of each BSG, mixed with 900 mL of liquid brewer's yeast (Y).

The last group of substrates consisted of 500 g of each BSG, mixed with 450 mL of liquid brewer's yeast plus 450 mL of liquid cane molasses (YM).

Twenty batches of freshly laid BSF eggs were placed on the surface of each substrate in a 7 L plastic container in a temperature-controlled room at 28 ± 1 °C and $70 \pm 2\%$ relative humidity, as previously described by Chia et al. (2018). The experimental containers were screened with fine mesh and eggs hatched after 3-4 days. Larvae were provided with 500 g of freshly prepared substrates every three days until the fifth instar larval stage, recognisable by a beige colour of the larvae; this occurred 2-3 weeks after egg hatching. At harvest, larvae were sieved and then manually separated from the substrate using forceps according to Spranghers et al. (2017). Harvested larvae were washed with water and frozen at 0 °C until further analysis. Before analysis, larvae were oven-dried at 60-70 °C, then ground to powder using a blender (Preethi Trio Mixer Grinder 500W, India).

Proximate composition analysis

Larval samples were subjected to proximate analysis to determine their dry matter (DM), crude protein, fat and ash according to AOAC (1990). Dry matter content of larval samples was assessed by oven drying the samples at 105 °C until constant weight and water content was determined as the weight difference before and after oven-drying. For the crude protein content, the nitrogen content was determined following the Kjeldahl method and the value was multiplied by a conversion factor of 4.76 (Janssen et al., 2017) to obtain the crude protein value. Fat content of larvae was determined by diethyl ether extraction in a fat extraction unit (VELP SER 148) following the Randall technique, which involves: immersion of samples in a hot solvent (diethyl ether) to ensure rapid solubility; washing off the solvent after boiling and the recovery by evaporation and condensation of the solvent. Ash content was determined by ignition of samples at 550 °C in a muffle furnace. These analyses were performed in triplicate.

Analysis of minerals

Mineral composition of BSF larvae was assessed by inductively coupled plasma emission spectrometry (ICP-AES). Sample preparation for mineral analysis involved incineration at 450 °C until a grey to reddish brown colour of the ash was observed. The ash was then dissolved in a mixture of nitric acid (65%), hydrochloric acid (37%), and hydrogen peroxide (30%) (Manditsera et al., 2019). For each treatment, three subsamples from one biological replicate were analysed for: iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), sodium (Na), sulphur (S), magnesium (Mg), potassium (K), aluminium (Al), phosphorus (P), and calcium (Ca) contents. The Ca/P ratio was calculated as the ratio of the concentration of Ca to the concentration of P in the BSF larval samples.

Analysis of amino acids

Samples of BSF larvae were analysed for their amino acid composition by liquid chromatography coupled to a mass spectrometer (LC–MS) following acid hydrolysis of the samples. The specification of the LC–MS system and column conditions are given in Appendix A. Approximately 100 mg of each sample were weighed to the nearest 0.01 mg, placed into a Pyrex tube to which 5 mL of 6 N HCl was added carefully. The tubes were capped immediately and placed in boiling water for 10 minutes and then transferred to an oven at 110 °C for 24 hours. After this period, samples were removed from the oven and allowed to cool, and then vortexed for 10 seconds. The hydrolyzed samples were filtered through Whatman No. 1 filter paper into 10 mL falcon tubes, while rinsing the Pyrex tubes with double distilled water. Three microliters of the filtrate for each sample were then injected into an LC–MS for chromatographic separation, identification and quantification of amino acids. For each treatment, two subsamples from one biological replicate were analysed. Amino acids were identified based on molecular weight, mass-to-charge ratio (m/z) and the retention time using a standard mixture of amino acids (see below) and Enhanced ChemStation, Agilent technologies.

A standard mixture of amino acids, which contained Ala, Pro, Lys, Tyr, His, Ser, Arg, Val, Met, Ile, Leu, Gly, Glu, Thr, Phe and Asp in the concentration of 2.5 mmol L⁻¹ and Cys in the concentration of 1.25 mmol L⁻¹ (Sigma-Aldrich, St. Louis, Missouri, USA), were also analysed by LC–MS. Column conditions: as shown in Appendix A. Calibration curves for quantification of the amino acids were constructed through the injection of different volumes of this standard solution (0.1–5 µL) (Appendix A, Table A2).

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Analysis of fatty acids

Fatty acid methyl ester (FAME) composition of BSF larvae was determined on oven-dried, ground samples by gas chromatography–mass spectrometry (GC–MS). The GC machine specifications and column conditions are described in Appendix A. A methyl esterification reaction was performed on each sample according to Musundire et al. (2016). Five hundred microliters of 15 mg/mL of methanolic sodium methoxide were added to 50 mg of dried BSF larval sample and incubated for one hour at 60 °C. After this period, 100 µL of deionised water was added to the sample and vortexed for one minute. The resulting methyl esters were extracted using GC-grade hexane (Sigma–Aldrich, St. Louis, USA) and then centrifuged at 14,000 rpm for 5 minutes and the supernatant was dried over anhydrous Na₂SO₄ (Musundire et al., 2016). One microliter of extract was injected into the GC-MS. For each treatment, two subsamples from one biological replicate were analysed. Fragment ions were generated by McLafferty Rearrangement ion. Fatty acids were identified as methyl esters by comparison of gas chromatographic fragmentation patterns with those of reference spectra published by library–MS databases: National Institute of Standards and Technology 05, 08, and 11.

Statistical analysis

Average crude protein, fat, ash, water and the mineral contents of larvae were compared among substrates and supplements through a two-way analysis of variance (ANOVA) ($\alpha = 0.05$), with least significant difference test (LSD) as post-hoc test. The relationship between the rearing substrates and proximate, and mineral contents of BSF larvae was evaluated using principal component analysis (PCA). All statistical analyses of the data were implemented using R software (version 3.5.1).

Results

Proximate composition

The main effects of substrate and supplementation on protein, fat and ash contents were significant (Table 1). There was a significant interaction between substrate and supplementation (Table 1). Larvae reared on SB supplemented with brewer's yeast had the highest protein content followed by those reared on SB supplemented with brewer's yeast plus molasses, whereas larvae reared on MB supplemented with brewer's yeast plus molasses had the lowest protein content. Overall, larvae reared on substrates supplemented with brewer's yeast had a higher protein content compared to those reared on water- or yeast plus molasses-supplemented substrates. Fat content of larvae reared on B, MB and MC supplemented with brewer's yeast plus molasses was higher than when supplemented with water only or brewer's yeast only. Larvae reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses had the lowest fat content (Table 1). There were no significant effects of substrate and supplementation on larval water content. However, their interaction was significant (Table 1).

Regarding the PCA, the first two principal components (PC) accounted for most (86.2%) of the variance in the data. The first PC accounted for 64.6% while the second PC accounted for 21.6% of the variance in the data (Figure 1). The PCA results revealed a positive correlation between the crude protein and ash contents, whereas the fat content was negatively correlated to the crude protein content (Figure 1). Larvae reared on B, MB and MC and supplemented by a mixture of brewer's yeast and molasses had a higher fat content than for the other substrates, whereas SB supplemented by either brewer's yeast or a mixture of brewer's yeast and molasses resulted in higher values for larval crude protein and ash contents. Overall, larvae grown on brewer's yeast-based substrates had higher crude protein and ash contents, whereas larvae fed on brewer's yeast plus molasses-based substrates had higher fat contents compared to larvae fed on water-based substrates (Figure 1).

Table 1. Proximate composition (% dry matter) of black soldier fly larvae reared on agro-industrial by-products

Variable	Treatment												P value (Two way ANOVA)		
	B			MB			MC			SB			Main effect (Substrate)	Main effect (Supp)	Interaction (Subst x Supp)
	W	Y	YM	W	Y	YM	W	Y	YM	W	Y	YM			
Crude Protein	37.4 ± 0.62f	41.9 ± 1.25c	31.7 ± 0.08g	39.9 ± 0.34e	41.3 ± 0.22cd	29.9 ± 0.75h	40.6 ± 0.07de	39.8 ± 0.40e	31.8 ± 0.60g	40.3 ± 0.17e	45.7 ± 0.07a	44.6 ± 0.53b	< 0.0001	< 0.0001	< 0.0001
Fat	33.2 ± 1.24d	22.5 ± 1.04g	49.0 ± 0.22a	21.1 ± 0.78g	17.1 ± 0.29h	39.3 ± 2.54c	25.5 ± 2.38f	21.1 ± 1.16g	42.3 ± 0.54b	29.7 ± 0.39e	9.5 ± 0.36i	11.4 ± 0.71i	< 0.0001	< 0.0001	< 0.0001
Ash	8.3 ± 0.08g	7.2 ± 0.08h	6.7 ± 0.17i	9.7 ± 0.44d	8.7 ± 0.01f	9.1 ± 0.17ef	10.0 ± 0.41d	8.2 ± 0.05g	9.2 ± 0.26e	10.6 ± 0.48c	12.4 ± 0.06b	15.4 ± 0.11a	< 0.0001	< 0.0001	< 0.0001
Water content	9.1 ± 0.40	10.8 ± 4.61	11.7 ± 0.46	10.3 ± 0.20	9.1 ± 0.21	10.1 ± 0.25	11.3 ± 0.24	10.4 ± 0.02	8.4 ± 0.70	9.9 ± 0.36	11.8 ± 0.20	12.1 ± 0.29	0.13	0.70	0.0279

Values presented in the table are results of triplicate analysis. Means (± standard deviation) in a row followed by different lowercase letters are significantly different ($P < 0.05$, ANOVA, LSD). B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley; W = water; Y = residual brewer's yeast; YM = residual brewer's yeast plus molasses. Supp = supplementation, Subst = Substrate.

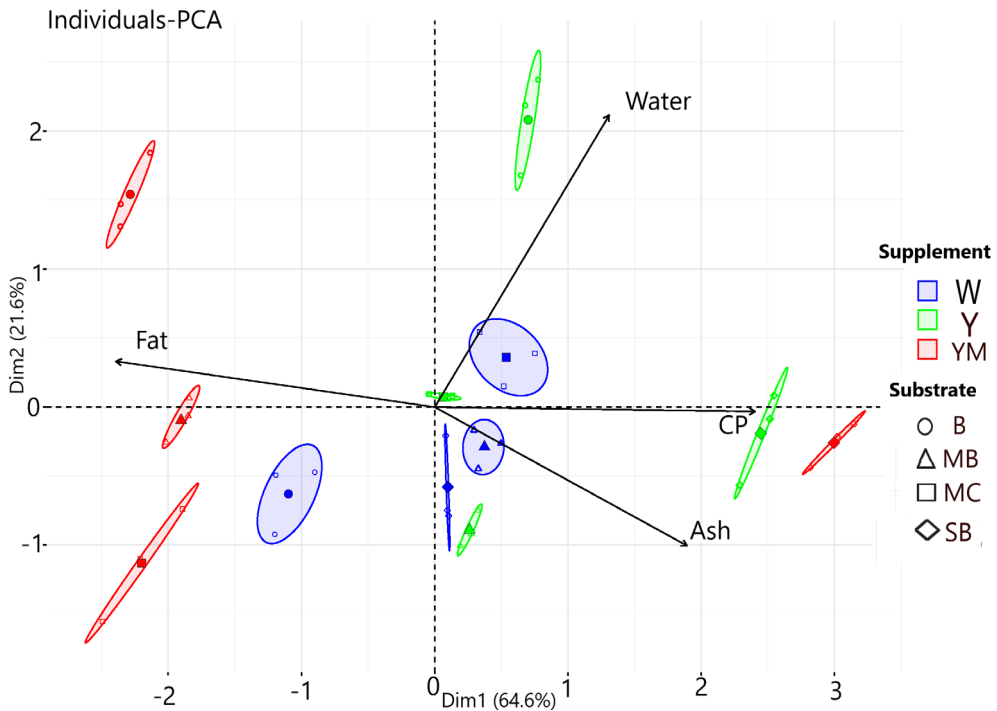


Figure 1. Principal component analysis of proximate composition of black soldier fly larvae reared on substrates composed of agro-industrial by-products. Substrates that are on the same side of a given variable have a high value for this variable while substrates that are on the opposite side of a given variable have a low value for this variable. CP = crude protein. B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley; W = water; Y = brewer's yeast; YM = brewer's yeast plus molasses.

Mineral composition

There were significant effects of substrate, supplementation and their interaction on average concentration of macrominerals and microminerals in the larvae (Table 2). Ca was the most abundant mineral in larvae across all substrates, followed by P, K, S and Mg. All other minerals such as Na, Fe, Mn and Zn were present at low concentrations. The concentration of Cu was lowest of all minerals in the larvae, independent of substrate on which they had been reared. Additionally, larvae reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses had significantly higher concentrations of all minerals compared to the rest of the substrates investigated here (Table 2). A similar result was obtained based on the principal component analysis.

Table 2. Mineral composition (g/kg dry matter) of black soldier fly larvae reared on substrates composed of agro-industrial by-products

Mineral	Treatment												P value (Two way ANOVA)		
	B			MB			MC			SB			Main effect (Substrate)	Main effect (Supp)	Interaction (Subst x Supp)
	W	Y	YM	W	Y	YM	W	Y	YM	W	Y	YM			
Macrominerals															
Ca	20.73 ± 0.208e	9.62 ± 0.121j	17.4 ± 0.20f	24.2 ± 0.20cd	14.83 ± 0.153h	16.57 ± 0.058g	23.73 ± 0.208d	13.23 ± 0.058i	20.47 ± 0.153e	28.07 ± 0.404b	24.53 ± 0.379c	31.27 ± 0.643a	< 0.0001	< 0.0001	< 0.0001
P	10.53 ± 0.153g	11.30 ± 0.173e	7.83 ± 0.135j	11.73 ± 0.058d	12.37 ± 0.058c	9.60 ± 0.053i	11.70 ± 0.100d	11.07 ± 0.058f	10.13 ± 0.058h	12.37 ± 0.058c	17.0 ± 0.10a	15.63 ± 0.322b	< 0.0001	< 0.0001	< 0.0001
K	8.20 ± 0.173h	10.53 ± 0.058d	8.7 ± 0.10g	9.77 ± 0.058e	11.63 ± 0.058c	10.40 ± 0d	9.53 ± 0.058f	11.50 ± 0c	10.4 ± 0.10d	9.80 ± 0e	16.27 ± 0.153b	17.07 ± 0.208a	< 0.0001	< 0.0001	< 0.0001
Mg	3.07 ± 0.055e	3.38 ± 0.053d	3.16 ± 0.093e	3.40 ± 0.012d	3.38 ± 0.044d	4.09 ± 0.021c	3.4 ± 0.06d	2.89 ± 0.021f	4.11 ± 0.006c	3.48 ± 0.068d	4.23 ± 0.010b	4.89 ± 0.135a	< 0.0001	< 0.0001	< 0.0001
Na	1.18 ± 0.018e	0.77 ± 0.003i	0.62 ± 0.010k	1.32 ± 0.004c	0.95 ± 0.005f	0.65 ± 0.002j	1.25 ± 0.008d	0.89 ± 0.004g	0.84 ± 0.006h	1.18 ± 0.004e	1.53 ± 0.017b	1.94 ± 0.025a	< 0.0001	< 0.0001	< 0.0001
S	4.02 ± 0.068e	3.99 ± 0.046e	2.93 ± 0.038h	4.14 ± 0.035cd	4.05 ± 0.006de	2.98 ± 0.006gh	4.16 ± 0.047c	3.87 ± 0.006f	3.06 ± 0.015g	4.18 ± 0.015c	5.55 ± 0.038a	4.99 ± 0.156b	< 0.0001	< 0.0001	< 0.0001
Microminerals															
Cu	0.016 ± 0.0002a	0.010 ± 0.0001	0.009 ± 0.0003	0.015 ± 0.0003c	0.010 ± 0.0002g	0.014 ± 0.0001d	0.014 ± 0.0001d	0.010 ± 0.0001d	0.010 ± 0.0001g	0.011 ± 0.0001g	0.015 ± 0.0001e	0.016 ± 0.0002a	< 0.0001	< 0.0001	< 0.0001
Fe	0.24 ± 0.004d	0.18 ± 0.002g	0.36 ± 0.010c	0.21 ± 0.002e	0.16 ± 0.005h	0.37 ± 0.005c	0.20 ± 0.004f	0.15 ± 0.003h	0.36 ± 0.011c	0.25 ± 0.004d	0.43 ± 0.002b	0.50 ± 0.008a	< 0.0001	< 0.0001	< 0.0001
Mn	0.25 ± 0.003c	0.09 ± 0.002k	0.19 ± 0.002g	0.22 ± 0.001d	0.11 ± 0.001j	0.195 ± 0f	0.21 ± 0.002e	0.12 ± 0.002i	0.18 ± 0.001h	0.26 ± 0.002b	0.21 ± 0.002e	0.32 ± 0.006a	< 0.0001	< 0.0001	< 0.0001
Zn	0.19 ± 0.004d	0.19 ± 0.002d	0.13 ± 0.001i	0.171 ± 0f	0.20 ± 0.002b	0.15 ± 0.001h	0.20 ± 0.002c	0.15 ± 0.001g	0.15 ± 0.001g	0.13 ± 0.002i	0.18 ± 0.001e	0.32 ± 0.007a	< 0.0001	< 0.0001	< 0.0001

Values presented in the table are mean results of triplicate analysis. Means followed by different lowercase letters are significantly different ($P < 0.05$, ANOVA; LSD). B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley; W = water; Y = brewer's yeast; YM = brewer's yeast plus molasses

The first two PCs accounted for most of the variance in the data. The first PC explained 56.9% of the variance, while the second PC accounted for 17.9% of the variation (Figure 2). There was a positive relationship between the mineral content of larvae and the substrate SB supplemented with brewer's yeast or brewer's yeast plus molasses (Figure 2). Most other substrates, particularly B, MB and MC supplemented with brewer's yeast or brewer's yeast plus molasses resulted in lower larval mineral contents. The PCA clearly separates mineral content of larvae reared on water-supplemented, brewer's yeast-supplemented and brewer's yeast plus molasses-supplemented substrates, except for SB supplemented with brewer's yeast and brewer's yeast plus molasses (Figure 2). Substrate ($P < 0.0001$), supplementation ($P < 0.0001$) and their interaction ($P < 0.0001$) significantly affected Ca/P ratio in larvae. Larvae reared on substrates supplemented with brewer's yeast only, had lower Ca/P ratio than those reared on substrates supplemented with water or brewer's yeast plus molasses (Figure 3).

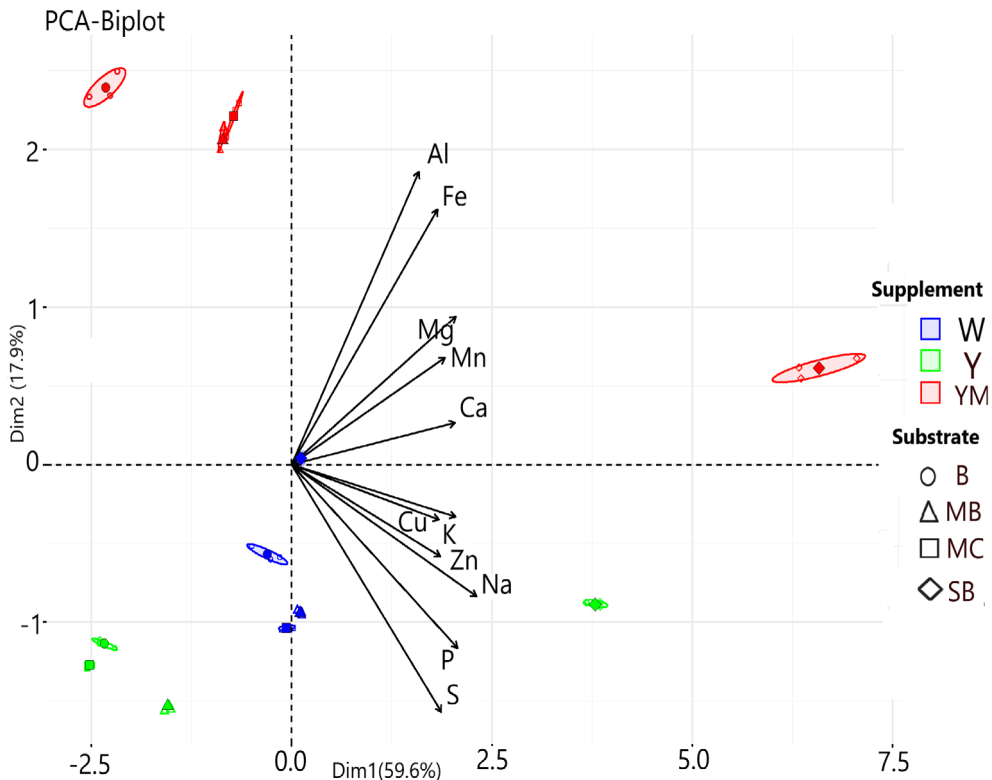


Figure 2. Principal component analysis of mineral composition of black soldier fly larvae reared on substrates composed of agro-industrial by-products. Substrates that are on the same side of a given variable have a high value for this variable while substrates that are on the opposite side of a given variable have a low value for this variable. B = spent barley; MB = spent malted barley; MC= spent malted corn; SB = spent sorghum and barley; W = water; Y = brewer's yeast; YM = brewer's yeast plus molasses.

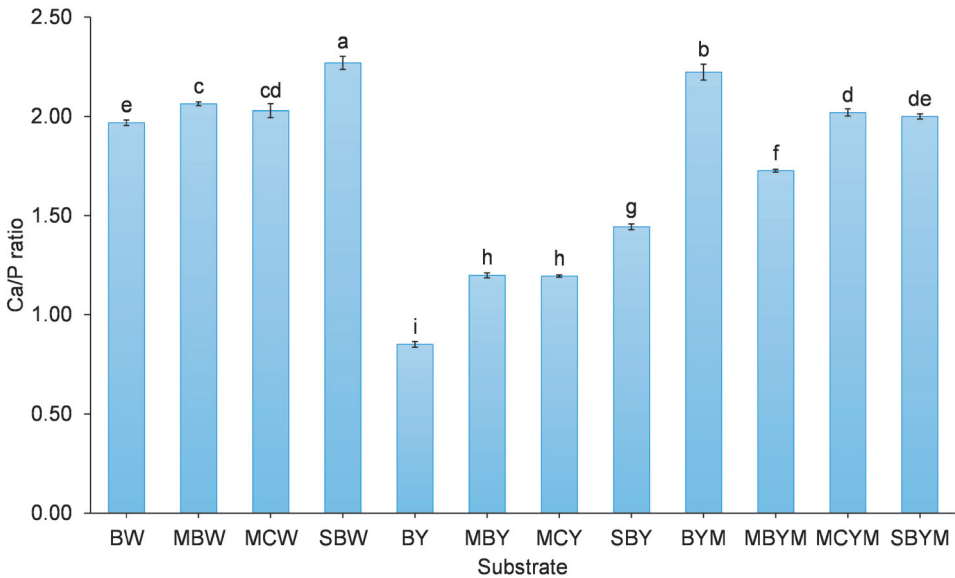


Figure 3. Calcium to phosphorus ratio (Ca/P) in black soldier fly larvae reared on substrates composed of agro-industrial by-products. Bars followed by different letters are significantly different ($P < 0.05$, LSD, mean \pm standard deviation, $n = 3$). BW = Barley/water; MBW = Malted barley/water; MCW = Malted corn/water; SBW = Sorghum/Barley/water; BY = Barley/brewer's yeast; MBY = Malted barley/brewer's yeast; MCY = Malted corn/brewer's yeast; SBY = Sorghum/Barley/brewer's yeast; BYM = Barley/brewer's yeast/Molasses; MBYM = Malted barley/brewer's yeast/Molasses; MCYM = Malted corn/brewer's yeast/Molasses and SBYM = Sorghum/Barley/brewer's yeast/Molasses.

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Amino acid composition

A total of 17 amino acids were recorded in the BSF larval samples. Of these, nine were essential amino acids while the remaining eight were non-essential amino acids (Figure 4; Table A3). The most prevalent essential amino acids were lysine, histidine and arginine, followed by: methionine, phenylalanine, isoleucine and leucine (Figure 4A). Valine was present in smaller concentration while threonine had the smallest concentration. For the non-essential amino acids, tyrosine was the most abundant amino acid, followed by proline and then glycine and alanine, whereas, aspartic acid, cystine, glutamic acid and serine were present in smaller concentrations (Figure 4B). No major differences were observed among larvae fed on the different four BSG-substrates and the three supplements.

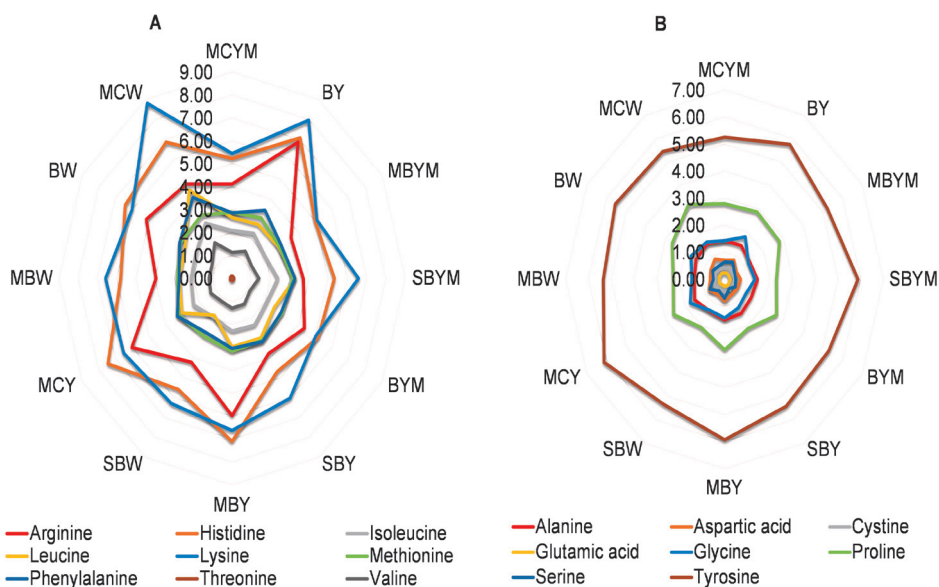


Figure 4. Concentration (mg/g dry matter) of essential and non-essential amino acids in black soldier fly larvae reared on substrates composed of agro-industrial by-products. A = essential amino acids, B = non-essential amino acids. BW = Barley/water; MBW = Malted barley/water; MCW= Malted corn/water; SBW = Sorghum/Barley/water; BY = Barley/brewer's yeast; MBY = Malted barley/brewer's yeast; MCY = Malted corn/brewer's yeast; SBY = Sorghum/Barley/brewer's yeast; BYM = Barley/brewer's yeast/Molasses; MBYM = Malted barley/brewer's yeast/Molasses; MCYM = Malted corn/brewer's yeast/Molasses and SBYM = Sorghum/Barley/brewer's yeast/Molasses.

Fatty acid composition

A total of 10 FAMEs were identified from the BSF larval samples. Of these, seven were saturated fatty acid derivatives, while the remaining three were unsaturated fatty acid derivatives (Figure 5). Lauric acid (C12:0), palmitic acid (C16:0) and stearic acid (C18:0) were the most abundant saturated fatty acids (5.9-39.4%), followed by myristic acid (C14:0) whereas, capric acid (C10:0), margaric acid (C17:0) and arachidic acid (C20:0) were detected in low proportions ($\leq 1.5\%$, $\leq 2.0\%$ and $\leq 2.5\%$, respectively) (Figure 5A). Larvae reared on SBY and SBYM had higher proportions of C18:0 than obtained for other substrates investigated (Figure 5A). The three unsaturated fatty acids detected were: linoleic acid (C18:2n-6), which is a polyunsaturated essential fatty acid and two monounsaturated fatty acids (palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9), the latter two being the most abundant unsaturated fatty acids in the present study (Figure 5B).

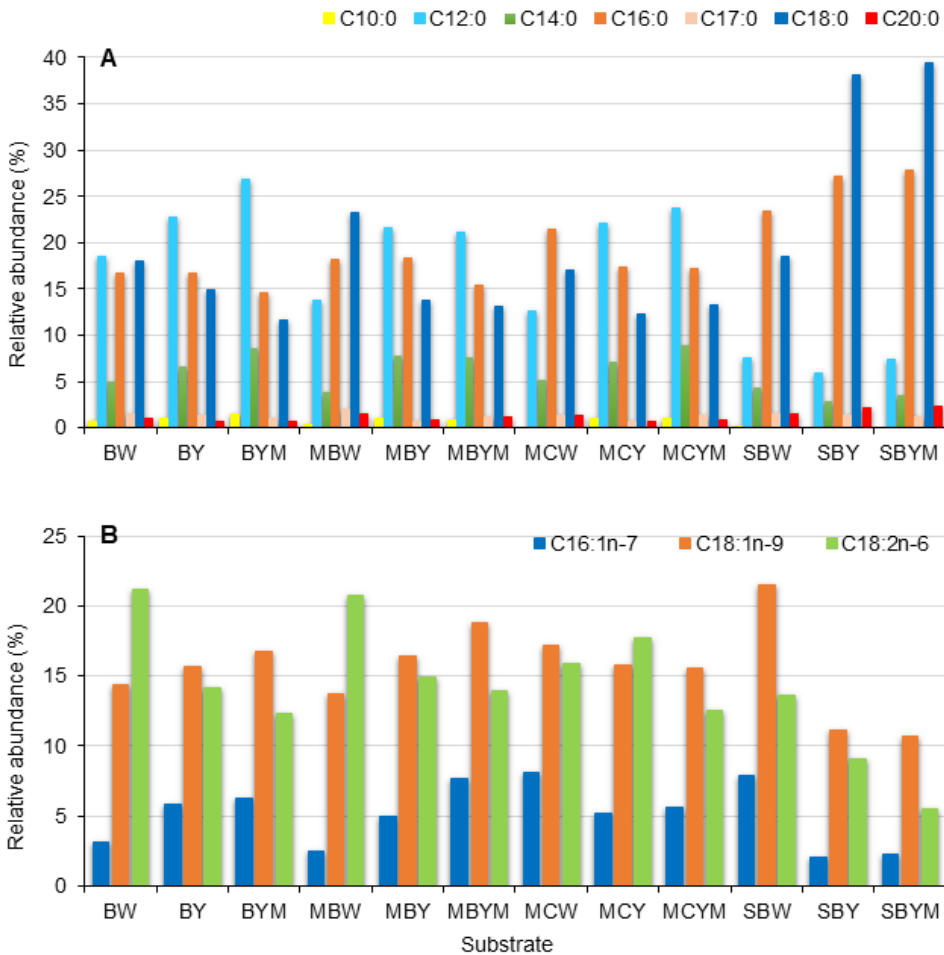


Figure 5. Relative abundance (% of total fatty acids) of fatty acids in black soldier fly larvae reared on substrates composed of agro-industrial by-products. Bars represent average ($n = 2$) proportion of total fatty acids in larvae for each substrate. A = saturated fatty acids, B = unsaturated fatty acids. BW = Barley/water; MBW = Malted barley/water; MCW = Malted corn/water; SBW = Sorghum/Barley/water; BY = Barley/brewer's yeast; MBY = Malted barley/brewer's yeast; MCY = Malted corn/brewer's yeast; SBY = Sorghum/Barley/brewer's yeast; BYM = Barley/brewer's yeast/Molasses; MBYM = Malted barley/brewer's yeast/Molasses; MCYM = Malted corn/brewer's yeast/Molasses and SBYM = Sorghum/Barley/brewer's yeast/Molasses.

Discussion

Our findings revealed that black soldier fly larvae readily fed and grew on all the organic side streams (Chia et al., 2018) and converted them into nutrient-rich biomass. However, the composition of larvae grown on each substrate combination was significantly influenced by the substrate type. The nutrient-rich larvae are suitable for several purposes, one of which is animal feeding (Liland et al., 2017; Spranghers et al., 2017; Van Huis, 2013).

Our results agree with previous reports that rearing substrate influences the nutritional composition of BSF larvae (Barragán-Fonseca et al., 2018; Spranghers et al., 2017; Tan et al., 2015; Wang & Shelomi, 2017). The supplementation of substrates with brewer's yeast, brewer's yeast plus molasses or water had significant effects on several of the recorded nutritional parameters (protein, fat, amino acid, minerals and ash contents). The significant interaction between substrate and supplementation shows that the main effect of substrate on the nutrient content of the larvae is dependent on the type of supplementation. The main effects of substrate and supplementation on water content were not significant, but their interaction was significant. This shows that there was no overall effect of either substrate or supplementation on water content of the larvae, but there was a crossover interaction by which the effect of supplementation on water content was opposite and depends on the nutritional value of the substrate (Karen, 2019).

The crude protein content of larvae in our study ranged between 30 and 46%. These values are within the range of crude protein values for BSF larvae reported in the literature (Liland et al., 2017; Meneguz et al., 2018; Spranghers et al., 2017; www.entocycle.com/process). When larvae were reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses, the resulting crude protein values were higher than obtained for the rest of the substrates investigated here. The lowest fat content values were also recorded on these substrates. Furthermore, the addition of brewer's yeast, which contains high crude protein and ash (Chollom et al., 2017) might have contributed to the high nutritional content of the larvae reared on SB. This indicates that these substrates are most suitable for rearing BSF larvae. Low fat contents have previously been reported in BSF larvae with high crude protein contents (Finke, 2013; Meneguz et al., 2018; Musundire et al., 2016).

Minerals play structural, physiological, catalytic and regulatory functions in the body (Andrieu, 2008; Suttle, 2010). The levels of Ca, Cu, Mg, Na, Mn, Fe and Zn in BSF larvae from our study are comparable to the levels reported by Spranghers et al. (2017), whereas, the levels of P, K and S were higher than the levels reported by Spranghers et al. (2017). The mineral levels in BSF larvae from our study are also similar to the levels reported by Tschirner & Simon (2015) for BSF larvae reared on different substrates. Furthermore, Makkar et al. (2014) reported higher and similar levels of Ca and P,

respectively in BSF larvae compared to our study. The difference between studies might be due to differences in life stage of the BSF analysed. For instance, Spranghers et al. (2017) reported values for BSF pre-pupae, whereas we analysed fifth instar BSF larvae. Interestingly, Ca levels in BSF larvae from the present study are comparable to the levels in fishmeal and higher than levels in soybean meal according to Makkar et al. (2014).

Micronutrients, though required in trace amounts are important for the growth, health and immunity, and reproduction of animals. For instance, Fe is an essential component of the respiratory pigments and enzymes involved in tissue oxidation and therefore plays an important role in oxygen and electron transport within the body. Zn is a component of enzyme systems and plays essential roles in lipid, protein, and carbohydrate metabolism. It plays an active role in the synthesis and metabolism of proteins (Tacon, 1987; Vallee & Falchuk, 1993). A deficiency in Zn decreases leukocyte function and increases susceptibility to bacterial infections (Sordillo et al., 1997). Copper is a component of the enzyme ferroxidase and plays a vital role in Fe metabolism, and therefore haemoglobin synthesis and red blood cell production and maintenance (Tacon, 1987). Deficiency of Cu decreases neutrophil killing capability of mammary gland immunity and increases susceptibility to bacterial infection (Sordillo et al., 1997). Manganese is an essential micro-mineral required for bone formation, regeneration of red blood cells, metabolism of carbohydrates and the reproductive cycle in animals (Tacon, 1987).

Overall, the mineral levels in BSF larvae in the present study are in compliance with the requirements of poultry and pigs (NRC, 1994, 1998). Therefore, BSF larvae reared on agro-industrial substrates used in the present study are promising alternatives to fishmeal and soybean meal in terms of mineral content. While all macrominerals are important, Ca and P are the most abundant and functionally related macrominerals in the body. These minerals are particularly implicated in bone formation and eggshell formation (Li et al., 2017; Pelicia et al., 2009; Yang, 2019; Zotte et al., 2019). A deficiency or an excess of either of these minerals affects the utilization of the other. Animal feed is often deficient of these two minerals (Mcdowell, 2003; Suttle, 2010) and requires supplementation to meet dietary requirements of the animals. In the present study, Ca and P were the predominant macrominerals recorded in BSF larvae. An ideal Ca/P ratio is important in reducing nutritional secondary hyperparathyroidism in insectivorous animals such as cattle egrets, reptiles, amphibians and cats. This is a metabolic bone disease that results from insufficient dietary intake of Ca or excessive intake of P in the diet (Boykin, 2019; Krook et al., 1963; Lock, 2017; Phalen et al., 2005). The Ca/P ratio in the present study is lower than the 8.4 value for BSF larvae reported by Makkar et al. (2014). However, the values found in our study largely fall within the recommended range (1-2) for animal diets (IPNI-International Plant Nutrition Institute, 1999; Li et al., 2017; Makkar, 2014; Olson & Hale, 2001; Pelicia et al., 2009; Stewart, 2017; Zotte et al., 2019). In addition, our values are similar to the Ca/P ratio in fishmeal and higher than

values for soybean meal, housefly maggot meal, mealworm, locust meal, house cricket, Mormon cricket, silkworm and pupae meal (Makkar et al., 2014). Our data agree with previous reports that BSF larvae contain a higher concentration of Ca than P (Boykin, 2019; Dierenfeld & King, 2008; Finke, 2013; Klaphake, 2010). Larvae reared on BSGs supplemented with brewer's yeast showed significantly lower Ca/P values compared to those reared on substrates supplemented with water or brewer's yeast plus molasses. Brewer's yeast has been shown to contain lower levels of Ca than of P (Onofre et al., 2017). This might have contributed to lower concentrations of Ca and high P concentrations in the larvae and may explain the low Ca/P ratio. However, Ca/P values are within the recommended range, indicating that the addition of brewer's yeast to the substrates can still result in the production of high-quality BSF larvae with well-balanced Ca/P ratio, which will contribute to the total dietary Ca/P requirement of farmed animals. Furthermore, our results show that calcium is the most abundant essential mineral in the larvae. This is consistent with previous reports (Liu et al., 2017; Makkar et al., 2014; Schmitt et al., 2019; Spranghers et al., 2017; Wang & Shelomi, 2017). Overall, our data show that larvae reared on these substrates represent a promising alternative source of minerals for animal feeds.

The amino acid profiles of BSF larvae in the present study are similar to those previously reported in the literature (Makkar, 2014; Sealey et al., 2011). However, information on the amino acid profiles of BSF larvae reared on BSGs is limited. Although statistical analysis was not possible due to limited number of replicates, the results of the present study show that substrates on which the larvae were reared did not influence the amino acid concentrations of the BSF larvae. Overall, lysine, which is one of the most limiting essential amino acids in pigs and poultry was most prevalent in the BSF larvae. Histidine and arginine were also abundant in the BSF larvae in this study. In line with the literature (Makkar, 2014), cystine had the lowest concentration in larvae independent of the substrates we studied. However, the variation in the concentration of amino acids across different substrates was largely within narrow limits. Therefore, we conclude that all substrates used in the present study are suitable for producing BSF larvae that contain balanced amino acids for animal feed.

Analyses of fatty acids in the present study indicate that fatty acid composition of the larvae was influenced by the rearing substrate. For instance, larvae reared on SBY and SBYM had high proportions of C16:0 and C18:0 and low proportions of unsaturated fatty acids, which is consistent with the low crude fat contents of the larvae recorded for these substrates in the proximate analysis. This further indicates that these substrates could be used to produce protein-rich BSF larvae, with reduced fat contents for animal feeds. Consistent with previous studies, the fatty acid profiles of larvae in the present study revealed a composition of mostly saturated fatty acids (seven out of ten fatty acids detected) (Meneguz et al., 2018; Ramos-Bueno et al., 2016; Surendra et al.,

2016; Makkar, 2014). Of the saturated fatty acids, the high proportion of C12:0 in BSF larvae corresponds to earlier reports for larvae reared on other side streams. For instance, when reared on fruit waste, palm decanter cake and treated sewage sludge, BSF larvae were richest in C12:0 compared to the rest of the saturated fatty acids (Leong et al., 2015).

Studies have demonstrated antimicrobial properties of C12:0 on gastrointestinal bacteria and the ability to supply energy for piglets. The use of BSF larval meal in animal feed therefore represents a nutraceutical advantage.

This means that the BSF larval meal in animal feed not only plays a nutritional role but also a therapeutic role in the health of the animal and, thus offers an excellent opportunity for reduced use of antibiotics in animal production, which is currently a major concern (Devi & Kim, 2014; Lee & Chiang, 1994; Skřivanová et al., 2006; Spranghers et al., 2018). It has also been reported that increased inclusion of BSF larval meal in broiler diets led to increased saturated fatty acid content of resulting edible meat products (Schiavone et al., 2017). Meat with a higher ratio of polyunsaturated to saturated fatty acids is more desirable for human consumption (Wood et al., 2003). Therefore, while the saturated fatty acid content of the BSF larvae could benefit the animal in terms of energy and antimicrobial activity (Çetingül & Yardımcı, 2008; Devi & Kim, 2014; Lee & Chiang, 1994; Skřivanová et al., 2006), these fats could be more useful in applications other than animal feed, for example in the production of cosmetic products (www.protenga.com/products/; www.bangkokpost.com/business/1168653/lord-of-the-flies; <http://sflyproteins.com/>).

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Furthermore, unsaturated fatty acids are the most important fatty acids in animal diets, two of which are essential and required in animal diets (Çetingül & Yardımcı, 2008; Makkar, 2014; Syadati et al., 2012). In particular, C16:1n-7 and C18:1n-9 detected in BSF larvae in the present study are important for animal health. For example; studies have shown that C16:1n-7 plays a vital role in preventing apoptosis as well as promote growth and proliferation of pancreatic β -cells (Diakogiannaki et al., 2007). In addition, monounsaturated fatty acids and polyunsaturated fatty acids play a role in reducing serum low density lipoprotein cholesterol levels (Grundy, 1989).

While fatty acids are generally important for human and animal health, the composition of omega 3 (n-3) and omega 6 (n-6) fatty acids, as well as the n-6/n-3 ratio are crucial. A high n-6/n-3 ratio is associated with cardiovascular diseases, inflammatory and autoimmune diseases. A ratio of n-6/n-3 fatty acids smaller than five is more desirable and considered optimal for human health (Simopoulos, 2002). In the present study, only n-6 (C18:2n-6) was detected in the larval samples. As reported in the literature, C18:1n-9 was the only polyunsaturated fatty acid detected in the present study (Meneguz et al., 2018). The limited unsaturated fatty acids, particularly the polyunsaturated fatty acids detected in the present study agrees with previous reports, which showed that BSF

larvae contain far lower levels of polyunsaturated fatty acids compared to housefly larvae, mealworms, and adult crickets (Leong et al., 2015; Makkar et al., 2014). Furthermore, BSF larvae reared on household waste were deficient in essential fatty acids in favour of saturated fatty acids (Kawasaki et al., 2019). To compensate for this deficiency in essential fatty acids, varying the fatty acid contents in the substrates used to rear BSF larvae could help improve the polyunsaturated fatty acids (Barroso et al., 2017; Ooninx et al., 2019). The inclusion of fish offal in BSF larval diets can enhance the unsaturated fatty acid content of the larvae. Fat deposition in BSF larvae is largely dependent on the substrate they feed on (Barragan-Fonseca et al., 2019; Sheppard et al., 1994; Spranghers et al., 2017; St-Hilaire et al., 2007).

In livestock feed formulation, ingredients are selected based on their nutrient content, availability, palatability and cost (www.poultryhub.org/nutrition/feed-formulation/). Key nutrients in feed ingredients are amino acids (contained in proteins), vitamins and minerals. Fats and protein provide energy needed to support metabolic processes and growth, whereas minerals are needed for bone formation, enzyme activation and egg shell development in laying hens. Against this background and from an overall evaluation of our data, BSF larvae reared on BSGs can successfully provide protein-rich meal for feed. Dietary essential and non-essential amino acids are necessary for the survival, development, growth, health and reproduction of animals. In poultry diet, methionine and lysine are two most limiting amino acids. In pigs, lysine, threonine, methionine, tryptophan and valine are the limiting amino acids in feed (Dalibard et al., 2014; Toride, 2004; Wu, 2014). Our data demonstrate a high nutritional value of BSF larvae containing most of the limiting amino acids in livestock diets.

Furthermore, the high content of Ca, P and other essential microminerals such as Fe and Zn recorded in the present study represents a high potential of the larvae as a feed component in livestock feed. The inclusion of BSF larval meal can therefore minimise the additional use of external Ca supplementation in diets (Dierenfeld & King, 2008).

Conclusions

This study provides insight into the effects of different agro-industrial side streams, especially BSGs on the nutritional value of BSF larvae and demonstrates that BSF larvae reared on these substrates are nutrient-rich and suitable for animal feed. While the different substrate combinations would generally produce high quality larvae in terms of nutrient composition, SB supplemented with brewer's yeast or brewer's yeast plus molasses appear to be more suitable as a rearing substrates and result in larvae with higher levels of crude protein and minerals. Overall, these findings confirm that BSGs are a valuable resource for rearing BSF larvae. Supplementing these substrates with residual brewers' yeast and cane molasses can enhance the larval quality for animal feed.

However, the unsaturated fatty acid profile of the larvae needs to be enhanced by ensuring that substrate nutrient content meets the nutritional requirements of the larvae. Our findings show that BSF larvae can be mass-produced on these substrates, while providing an alternative strategy for managing brewery waste streams, when the conventional uses of these substrates such as direct feeding to livestock are not sufficient.

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Chapter 7

Effect of dietary replacement of fishmeal by insect meal on growth performance, blood profiles and economics of growing pigs in Kenya

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Abstract

Pig production is one of the fastest growing livestock sectors. Development of this sector is hampered by rapidly increasing costs of fishmeal (FM), which is a common protein source in animal feeds. Here, we explored the potential of substituting FM with black soldier fly larval meal (BSFLM) on growth and blood parameters of pigs as well as economic aspects. At weaning, 40 hybrid pigs, i.e., crossbreeds of purebred Large White and Landrace were randomly assigned to five iso-nitrogenous and iso-energetic dietary treatments: Control (0% BSFLM and 100% FM (T0)), and FM replaced at 25% (T25), 50% (T50), 75% (T75) and 100% (T100) with BSFLM. Average daily feed intake (ADFI), average daily gain (ADG), body weight gain (BWG) and feed conversion ratio (FCR) were calculated for the whole trial. Hematological and serum biochemical parameters, the cost–benefit ratio (CBR) and return on investment (RoI) were evaluated. No significant effect of diet type was observed on feed intake and daily weight gain. Red or white blood cell indices did not differ among diets. Pigs fed T25, T75 and T100, had lower platelet counts compared to T0 and T50. Dietary inclusion of BSFLM did not affect blood total cholesterol, triglycerides, low-density lipoprotein and high-density lipoprotein. CBR and RoI were similar for the various diets. In conclusion, BSFLM is a suitable and cost-effective alternative to FM in feed for growing pigs.

Keywords: growing pigs; blood parameters; insect larval meal; alternative protein; animal feeds; cost benefit analysis; return on investment

Introduction

Pig production is one of the fastest growing livestock sectors globally, with most of the growth occurring in the developing countries (Githigia et al., 2012). Pigs are of socio-economic value to smallholder farmers and provide a safety net in times of financial crisis (Githigia et al., 2012). A short breeding cycle, high fecundity, high feed conversion efficiency and increasing demand are major drivers of growth in this sector. In communities currently experiencing a shift from ruminant to non-ruminant livestock production, pig farming is becoming relevant (Githigia et al., 2012; Serem et al., 2017). However, expansion and profitability are constrained by increasing feed costs, especially the protein ingredients (Githigia et al., 2012). Feed costs represent 60%–70% of total costs in intensive pig production, which is especially due to costs of protein. In East Africa, major protein ingredients such as fishmeal (FM) and soybean meal are increasingly unavailable and expensive for smallholder farmers (Onsongo et al., 2018). Consequently, farmers resort to alternative feed sources considered to be cheaper without knowledge of their influence on the physiological response and animal growth (Etim et al., 2014a; Etim et al., 2014b; Etim et al., 2014c; Serem et al., 2017). Considering the importance of pig production in livelihoods of smallholder farmers and the growing scarcity of protein ingredients as well as the environmental implications of producing these resources (Masuda & Goldsmith, 2009; Tacon & Metian, 2008), dependence on FM and soybean meal is not sustainable (Van Huis et al., 2013).

Insects have high protein and fat content and have been considered promising high-quality feed components (Bosch et al., 2014; Rumpold & Schluter, 2013; Veldkamp et al., 2012). Insects could replace 25% to 100% of FM or soybean meal in feeds for livestock and aquaculture, depending on the insect, livestock and fish species (Makkar et al., 2014). The black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) is distributed worldwide in the tropics and warm temperate regions (Sheppard et al., 1994). BSF larvae feed on organic resources such as fruit remains, animal manure, vegetables and brewers' spent grains (Chia et al., 2018; Li et al., 2011; Makkar et al., 2014; Meneguz et al., 2018; Myers et al., 2008; Nguyen et al., 2013; Pinotti et al., 2019; Rehman et al., 2017; Salomone et al., 2017) and convert these resources into high-quality insect protein and fat. In contrast to other dipteran species such as the house fly *Musca domestica*, BSF is not considered a pest and its larvae can reduce populations of harmful bacteria (Erickson et al., 2004; Liu et al., 2008). On a dry matter basis, BSF larvae contain 37% to 63% crude protein, 7% to 39% fat (Barragan-Fonseca et al., 2017; Bosch et al., 2014), 8% calcium, 1% to 2% phosphorus, 0.1% to 0.3% sodium and 0.4% to 1% magnesium (Barragan-Fonseca et al., 2017; Dierenfeld & King, 2008; Finke, 2013; Newton et al., 2005a). BSF larval meal has been used as an ingredient in feed for fish (Cummins Jr et al., 2017; Devic et al., 2018; Dumas et al., 2018; Kroeckel et al., 2012; Li et al., 2016;

Magalhaes et al., 2017; Sealey et al., 2011; Zarantoniello et al., 2018), poultry (Maurer et al., 2016; Onsongo et al., 2018; Schiavone et al., 2017) and pigs (Biasato et al., 2019; Driemeyer, 2016; Newton et al., 1977; Spranghers et al., 2018), with promising results. However, there is no exhaustive information on the influence of BSF larval meal (BSFLM) on performance and health response of growing pigs. The few studies on the inclusion of BSFLM in pig feeds have focused on piglets, replacing either FM or soybean meal with low (less than 10%) levels of BSFLM over short (10–40 days) periods of feeding (Biasato et al., 2019; Driemeyer, 2016; Newton et al., 1977; Spranghers et al., 2018). The short experimental periods do not allow for a complete growth phase under the dietary supplementation and, therefore, do not reflect the common practice of pig feeding. In addition, higher levels of BSFLM inclusion in pig feed have not been assessed. In the present study, we subjected growing pigs to feeds with higher (25%–100%) dietary replacement levels of FM with BSFLM over an extended period (>60 days) of feeding, covering the complete pig grower phase and measured the effect of diet on performance and economics of pig feeding.

Hematological and biochemical parameters are affected by several factors, including diet (Etim et al., 2014a). Nutritional deficiencies and diseases influence clinical health status of animals (Wilson et al., 1972). In the case of nutritional deficiencies, blood profiling can provide an indication of the clinical health status as well as the extent to which dietary deficiencies impact physiological status of the animal, which allows farmers to adjust the diets of the animals to ensure that they receive adequate feed ingredients for optimal production (Ameen et al., 2007; Etim et al., 2014a; Shanmugam et al., 2017). BSF larvae represent a novel protein source in animal feed. Studies have reported unaffected growth performance, blood parameters, nutrient digestibility, gut morphology and histological features of piglets as well as gut antimicrobial potential of the inclusion of full-fat or partially defatted BSFLM in piglet feed at the rate of 4%–10% to replace soybean meal or FM (Biasato et al., 2019; Driemeyer, 2016; Spranghers et al., 2018), but there is little information on the effect of complete replacement of FM by full-fat BSFLM in pig feed. Utilization of insect meal as an animal feed ingredient is attracting interest from researchers because conventional feed ingredients are increasingly becoming unaffordable to resource-poor farmers due to rapidly rising costs. This requires us to search for alternative protein ingredients that can economically supplement conventional feed ingredients used in feed formulation without adverse effects on the health and performance of the animals. The inclusion of ingredients in feed formulation does not only aim at a balanced nutrient content for optimal growth performance, but also considers profitability of the production process (Spring, 2013). Therefore, the present study aimed at evaluating the effect of substituting FM with BSFLM on (a) growth performance, (b) hematological and serum biochemical indices and (c) economic implications of BSFLM inclusion in growing pig diets.

Materials and Methods

The present study was conducted at the pig rearing facility of the Non-ruminant Research Institute (NRI) of the Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha, Kenya. The general care and management of the animals followed accepted guidelines as described by the Federation of Animal Science Societies (FASS, 2010).

BSF larval meal (BSFLM) and experimental diets

Diets were formulated to meet growing pig requirements (NRC, 1979). Isonitrogenous and isoenergetic diets were prepared by replacing the FM content of a control diet (T0) at 25%, 50%, 75% and 100% (T25, T50, T75 and T100, respectively) with BSFLM, as experimental diets (Table 1). Maize meal, wheat pollard and rice polishing were included as energy sources. BSFLM and FM served as major protein sources. Vitamin and mineral premix, salt, limestone and bone meal served as vitamin and mineral sources (Table 1). FM, maize meal, rice polishing, salt, limestone, vitamin and mineral premix, lysine, methionine and bone meal were purchased from commercial animal feed retailers. BSF larvae were obtained from the International Centre of Insect Physiology and Ecology (*icipe*), reared following the BSF rearing procedure of the Animal Rearing and Containment Unit (ARCU), *icipe*. BSF larvae were reared on a mixture of brewers' spent grains (BSGs) obtained from Kenya Breweries Limited (KBL). After harvest, larvae were sterilized by washing in warm water (84 °C) for 10 minutes and then oven-dried using a commercial stainless-steel fruit/vegetable/meat/fish drying machine model (CT-C-III Series hot air circulating drying oven, Henan Forchen Machinery Co., Ltd., Henan, China). The machine can dry 360 kg/batch of fresh insects for 2.5 h at 120 °C. Dried larvae were ground into larval meal using a hammer mill (Newton et al., 1977).

Proximate, amino acids and mineral composition of experimental diets

The dry matter content of formulated feed samples was gravimetrically determined after the loss of water. The samples were heated to 103 ± 2 °C for 3 h to constant weight. Ash content was determined by ignition of samples at 550 °C in a muffle furnace. Dried and ground samples were exposed to an electromagnetic scan in the absorbance mode using near infrared (NIR) spectroscopy (CROPNUTS, Nairobi, Kenya). The crude protein, fat, starch, oil, acid detergent fiber, neutral detergent fiber, sugar and digestibility (NCGD) values were determined following standard laboratory procedures and energy values were calculated (Núñez-Sánchez et al., 2012; Rosales et al., 2011). Essential and non-essential amino acid contents of BSF larvae and experimental diets (Table 1) were analyzed by AMINOLab® (Evonik Industries, Hanau, Germany) using an amino acid

analyzer (Biochrom 30 plus, Biochrom Ltd. Cambridge, UK) (Al Sagan et al., 2018; Llamas & Fontaine, 1994; Powell et al., 2017; Windham, 1995; Zampiga et al., 2018). Feed samples were homogenously ground with an Ultra Centrifugal Mill RETSCH-ZM 200 to pass through a 0.5 mm sieve. Finely ground samples were weighed using an analytical balance, display accuracy ± 0.01 mg into 50 mL laboratory bottles, with thread of DURAN glass (Schott, Mainz, Germany), red polybutylene terephthalate (PBTP) caps with silicone/Teflon seal. Then, 25 mL of hydrochloric-phenol reagent was added to the sample in the bottles and the mixture was introduced into a thermostatically controlled heating oven (UT 6060 AR, Thermo Electron LED, Langenselbold, Germany) at 110 °C, with loose screw tops for one hour and then with tightened screw tops for 23 h to complete the sample hydrolysis. Methionine and cystine samples were prepared through performic acid oxidation procedures followed by the acid hydrolysis–sodium metabisulfite method (Slump & Bos, 1985; Windham, 1995). The resulting hydrolysate solutions were then introduced into the amino acid analyzer and the sample amino-grams were detected at 570 nm and 440 nm. Amino acid concentrations in the samples were determined in duplicate.

The mineral content of feed samples (Table 2) was analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-OES; CROPNUTS, Nairobi, Kenya). Sample preparation involved microwave-assisted acid digestion. Aliquots of ground feed samples were transferred to a glass tube of the microwave system. Then, a mixture of nitric acid and hydrochloric acid was added to the sample and allowed to digest. The resulting solution was filtered into a volumetric flask and used for ICP-OES analysis to determine the following minerals: Boron, molybdenum, iron, copper, zinc, cobalt, manganese, sodium, sulphur, magnesium, potassium, phosphorus and calcium (Baralkiewicz et al., 2007; Plotka-Wasyłka et al., 2018; Santos et al., 2012; Sreenivasulu et al., 2017).

Table 1. Ingredients, composition of black soldier fly larval meal and experimental diets

Parameter	BSFLM	Diets				
		T0	T25	T50	T75	T100
		Ingredient (kg)				
Maize meal	-	12.0	14.0	14.0	14.0	15.0
Wheat pollard	-	52.0	35.0	33.0	35.2	34.2
Rice polishing	-	22.0	30.5	32.0	29.8	28.3
Fishmeal	-	10.0	7.5	5.0	2.5	-
BSFLM	-	-	9.0	12	14.5	18.5
Lysine	-	1.0	1.0	1.0	1.0	1.0
Methionine	-	1.0	1.0	1.0	1.0	1.0
Salt	-	0.2	0.2	0.2	0.2	0.2
Bone meal	-	0.8	0.8	0.8	0.8	0.8
Limestone	-	0.8	0.8	0.8	0.8	0.8
Vitamin and mineral premix ^a	-	0.2	0.2	0.2	0.2	0.2
Dry matter (DM) (%)	94.9	92.6	94.0	92.4	93.2	94.0
Crude protein (% DM)	46.6	15.4	15.3	15.0	15.7	14.8
		Essential amino acids (% DM)				
Lysine	2.9	1.0	0.8	0.9	1.0	0.9
Methionine	0.8	0.5	0.4	0.5	0.5	0.5
Methionine + Cystine	1.1	0.7	0.6	0.8	0.7	0.7
Isoleucine	1.8	0.5	0.5	0.5	0.5	0.5
Leucine	2.9	1.0	1.1	0.9	1.0	0.9
Threonine	1.7	0.5	0.5	0.5	0.5	0.5
Phenylalanine	2.2	0.6	0.6	0.5	0.6	0.6
Valine	2.6	0.7	0.7	0.7	0.7	0.7
Arginine	2.2	0.9	0.9	0.8	0.8	0.8
Histidine	1.3	0.3	0.4	0.3	0.3	0.3
		Nonessential amino acids (% DM)				
Alanine	3.0	0.8	0.9	0.8	0.9	0.8
Aspartic acid	3.9	1.1	1.2	1.1	1.1	1.0
Cystine	0.4	0.3	0.3	0.2	0.2	0.2
Glutamic acid	4.9	2.3	2.3	2.0	2.1	2.0
Glycine	2.5	0.8	0.8	0.7	0.8	0.7
Proline	2.4	0.8	0.9	0.8	0.8	0.8
Serine	1.8	0.6	0.6	0.6	0.6	0.6

BSFLM = black soldier fly larval meal. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. DM = dry matter. Amino acid values are means of duplicate analyses. ^a Premix provided per kg diet: Vitamin A 6,000,000 IU; Vitamin D₃ 1,000,000 IU; Vitamin E 5,000 IU; Vitamin K₃ – KASTAB 3,000 mg; Vitamin B₂ – riboflavin 4,500 mg; Vitamin B₃ – nicotinic acid 22,000 mg; Vitamin B₅ – pantothenic acid 16,000 mg; Vitamin B₆ – pyridoxine 2,250 mg; Vitamin B₉ – folic acid 350 mg; Vitamin H – biotin 50 mg, Vitamin B₁₂ – cobalamin 22 mg; choline chloride 150,000 mg; antioxidant 125,000 mg; iron (Fe) 40,000 mg, manganese (Mn) 40,000 mg; zinc (Zn) 100,000 mg, copper (Cu) 25,000 mg; iodine (I) 1,000 mg, cobalt (Co) 250 mg, selenium (Se) 100 mg.

Table 2. Mineral and proximate composition of experimental diets

Parameter	Diets				
	T0	T25	T50	T75	T100
Boron (ppm)	4.2	2.8	2.3	2.7	3.1
Molybdenum (ppm)	1.3	1.1	0.5	1.0	1.2
Iron (ppm)	750.5	731.0	632.6	518.4	529.2
Copper (ppm)	22.6	501.1	80.5	16.7	22.8
Zinc (ppm)	121.4	110.6	114.1	102.3	112.6
Cobalt (ppm)	3.4	0.5	0.4	0.5	0.4
Manganese (ppm)	295.4	257.0	264.1	243.0	222.9
Sodium (ppm)	2439.8	1648.6	1097.8	1138.8	998.9
Sulphur (%)	0.4	0.6	0.3	0.3	0.3
Magnesium (%)	0.8	0.7	0.7	0.6	0.6
Potassium (%)	0.9	0.9	0.9	0.9	0.9
Phosphorus (%)	0.9	0.7	0.8	0.8	0.7
Calcium (%)	2.5	2.8	2.1	1.7	1.8
Sugar (%)	4.5	5.1	6.2	6.5	8.7
Starch (%)	29.2	25.3	27.6	20.7	26.9
Ash (%)	10.5	10.6	10.1	10.1	8.9
Acid detergent fiber (%)	15.3	19.0	17.0	20.7	15.4
Neutral detergent fiber (%)	37.7	42.8	41.6	47.0	39.7
Digestibility (NCGD) (%)	79.3	73.5	75.0	70.0	76.9
Oil (%)	5.0	8.1	8.9	9.8	12.2
Net energy (MJ/kg)	9.5	9.8	10.5	9.9	12.0

T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fish-meal with BSFLM. NCGD = neutral cellulase gammadase digestibility, ppm = parts per million.

Experimental animals and housing

Before the commencement of the experiment, forty (20 male and 20 female) hybrid pig weaners, which consisted of a cross between purebred Large White and the Landrace with mean body weight of 18.25 ± 0.34 kg were sourced from Farmer's Choice Limited, Nairobi, Kenya. The pigs were randomly assigned to five dietary treatments, each replicated four times per sex (four males and four females). Pigs were housed in concrete floor pens (3.65 m \times 1.85 m) each containing two pigs (one male and one female). Each pen was provided with a one-sided self-feeder (1.80 m \times 0.20 m \times 0.18 m). Pigs were adapted to the pens for 14 days before the start of the experiment, during which they were fed a commercial starter feed. At the start of the experiment, each pen was labeled with a number and diet type while each animal was identified with a unique number by ear tattooing. A layer (~0.25 m thick) of dry wood shavings was carefully placed at one corner of the floor of each pen, which served as bedding for the pig and provided warmth. Pig pens were cleaned every day by scrubbing the floor using Teepee straight brooms (c27, Chandarana Foodplus, Nairobi, Kenya) and water. Each pen was provided with a nipple drinker fitted to the wall and the distance between the nipple and floor was adjusted as the pigs increased in height. Experimental animals were allowed ad libitum access to feed and water throughout the experiment.

Growth performance

Individual pig body weight was recorded on a weekly basis using a 150 kg × 500 g suspended weighing scale (Salter, model 235, Bilston, England), with the sides covered with a wire mesh to prevent uncontrolled exit of the animal. The entry gate of the weighing cage was opened sideward and the animal was led into a stable, non-moving floor after which the gate was closed. Once inside the cage, the animal was allowed to settle, and the weight value read on an analogue display screen above the weighing cage. After every reading, the pig was released from the weighing cage into its pen and the scale was moved to the next pen on two wheels fitted on the front end of the cage. On the day of weighing, pigs were only provided with feed after recording their body weight. The weekly body weights were used to calculate average daily weight gain (ADG). Feed offered to the pigs and unconsumed portions were weighed daily using a digital platform weighing scale (XK3190-A12, >300 kg, Gromy Scale Co., Ltd., Hangzhou, China) to calculate average daily feed intake (ADFI). The trial lasted nine weeks. Total body weight gain and feed consumed were used to calculate feed conversion ratio (FCR) for each dietary treatment.

Blood characteristics

At the end of the growing pig phase, three randomly selected pigs from each dietary treatment were starved for 12 h, with access to drinking water only. After this period, two blood samples (5 mL each) were drawn from the peripheral ear vein using flashback blood collection needles and 9 mL vacutainer blood collection tubes (VP4082, Sunphoria Co., Ltd., Taipei, Taiwan). One of the samples was treated with the anticoagulant ethylene diamine tetra acetic acid (K2EDTA) and the other with serum clot activator. These samples were transported immediately to the laboratory for further analysis.

Hematological and serum lipid parameters

A 5-part white blood cell (WBC) differential and complete blood cell count was performed using the automated IDEXX ProCyte Dx™ Hematology analyzer (IDEXX, Westbrook, ME, USA) by laser flow cytometry, optical fluorescence and laminar flow impedance. Each ethylene diamine tetra acetic acid (EDTA)-anticoagulated whole blood sample was mixed thoroughly for seven minutes on a sample rocker. Once the processing was over, the sample details were entered and the type of analysis to be carried out was indicated. Thereafter, the blood samples were loaded into the analyzer and automatically run to generate the following parameters: Red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count, total white blood cell count

(WBC), neutrophil percentage, lymphocyte percentage, monocyte percentage, eosinophil percentage and basophil percentage. The WBC differential counts were qualitatively verified through Romanowsky-stained thin blood smear examination using a light microscope at the oil immersion objective (100×). Clotted blood samples were centrifuged at 4000 revolutions per minute (rpm) for 10 minutes. The serum lipoprotein (HDL), triglyceride and low-density lipoprotein (LDL) levels in the samples were measured on an automatic Cobas Integra 400 plus Chemistry Analyzer (Roche, Rotkreuz, Switzerland) using enzymatic colorimetry.

Economic analysis

Two key parameters, the cost–benefit analysis (CBA) and return on investment (RoI) (Onsongo et al., 2018) were used to evaluate the economic implication of replacing FM in pig diets with BSFLM. The cost–benefit ratio (CBR), as an indicator in CBA, was used to summarize the economic value of replacing FM with BSFLM in pig diets. Here, it was assumed that all other costs of production were constant for all dietary treatments, except the cost of the feed, which was considered in the CBR and RoI calculations. Feed costs were calculated from the ingredient prices based on quantities of each item incorporated in the dietary treatments. The total revenue from the pigs was estimated by considering 3.0 US \$/kg of pig's live body weight, assumed to represent all the benefits that would be received from the production. The ratio between the production revenue and the production cost represents the CBR. A CBR value greater than one suggests that the benefits of the production exceeded the production costs and vice versa. RoI is a measure of gain/loss generated from an investment relative to the money invested. The higher the RoI value the better the returns of the project under consideration (Aok, 2012; Onsongo et al., 2018).

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Statistical Analysis

General linear modeling was used to assess the effect of diet on growth performance, blood parameters and economic parameters of pigs fed BSFLM-based diets and a control diet over a nine-week period. Collinearity of variables was checked to obtain independent covariates. The model for each analysis included all independent variables, which were removed one by one until the Akaike information criterion (AIC) was at a minimal level. For growth performance, diet, sex and their interaction effect were included for the analysis of ADG, body weight gain (BWG) and final body weight (FBW). For weekly body weight (BW), diet, sex, time (week) and their interaction effects were included in the model. Two pigs (female and male) per replicate were provided with feed in the same trough. Hence, ADFI analysis by sex was not possible. Three randomly selected pigs per dietary treatment were used for the blood parameter assessment, with diet as the explanatory variable. Similarly, the economic analysis was based on ADFI,

hence, diet was included as the explanatory variable in the model. Mean effects were considered statistically significant at $P < 0.05$, with a least significant difference test (LSD) as the post-hoc test. All statistical analyses of the data were implemented using R software (version 3.5.1).

Ethical approval

Ethical approval for the study was provided by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO)–Veterinary Science Research Institute (VSRI); approval Code No.: KALRO-VSRI/IACUC019/30082019.

Results

Growth performance and feed conversion

Pigs readily accepted experimental diets and no mortality was recorded. Neither diet nor sex affected initial weight, final body weight (FBW) or average daily weight gain (ADG); the interaction between diet and sex was also not significant (Table 3). Weekly body weight (BW) differed significantly among diets ($P < 0.001$) and sexes ($P = 0.005$). BW increased significantly ($P < 0.001$) from week 1 to week 9 for male and female pigs (Figure 1). The interaction between diet and sex on BW was significant ($P < 0.001$). There was no significant interaction between diet and week ($P = 0.110$) or between sex and week on BW ($P = 0.388$). Furthermore, the interaction between diet, sex and week on BW was also not significant ($P = 0.345$). Body weight gain for the entire experimental period (BWG) was neither affected by diet ($P = 0.351$; Figure 2) nor by sex ($P = 0.486$) and neither was the interaction between diet and sex ($P = 0.340$). ADFI did not differ among diets (Figure 3). FCR differed significantly among diets ($P = 0.011$). When fed T75 or T100, FCR of the pigs was significantly higher compared to T25 (Figure 4).

Table 3. Effects of dietary BSFLM inclusion on growth performance of growing pigs.

Parameter	Sex	Diets					P value (GLM)								
		T0	T25	T50	T75	T100	Diet	Sex	Diet x Sex						
Initial weight, kg	F	19.5 ± 1.73	16.8 ± 0.63	17.1 ± 0.66	17.0 ± 0.87	18.5 ± 0.71	0.080	0.152	0.628						
	M	18.8 ± 1.56	17.1 ± 0.85	18.5 ± 0.89	18.5 ± 0.79	20.8 ± 0.83									
	Overall	19.1 ± 1.09	17.0 ± 0.49	17.8 ± 0.57	17.8 ± 0.61	19.6 ± 0.66									
Final weight, kg	F	56.3 ± 4.3	50.8 ± 2.65	56.3 ± 1.45	47.8 ± 1.76	50.0 ± 1.43				0.474	0.295	0.395			
	M	53.8 ± 3.56	53.5 ± 3.68	53.9 ± 4.90	53.0 ± 1.97	56.9 ± 0.99									
	Overall	55.0 ± 2.63	52.1 ± 2.16	55.1 ± 2.41	50.4 ± 1.58	53.4 ± 1.53									
Average daily gain, kg	F	0.61 ± 0.04	0.56 ± 0.04	0.62 ± 0.04	0.50 ± 0.03	0.54 ± 0.03							0.126	0.408	0.641
	M	0.59 ± 0.04	0.57 ± 0.04	0.62 ± 0.04	0.57 ± 0.04	0.59 ± 0.03									
	Overall	0.60 ± 0.03	0.57 ± 0.02	0.62 ± 0.02	0.53 ± 0.02	0.57 ± 0.02									

BSFLM = black soldier fly larval meal. General linear model (GLM) $P < 0.05$. For each diet, $N = 4$; F = female; M = male. Overall = data for female and male pigs pooled together. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM.

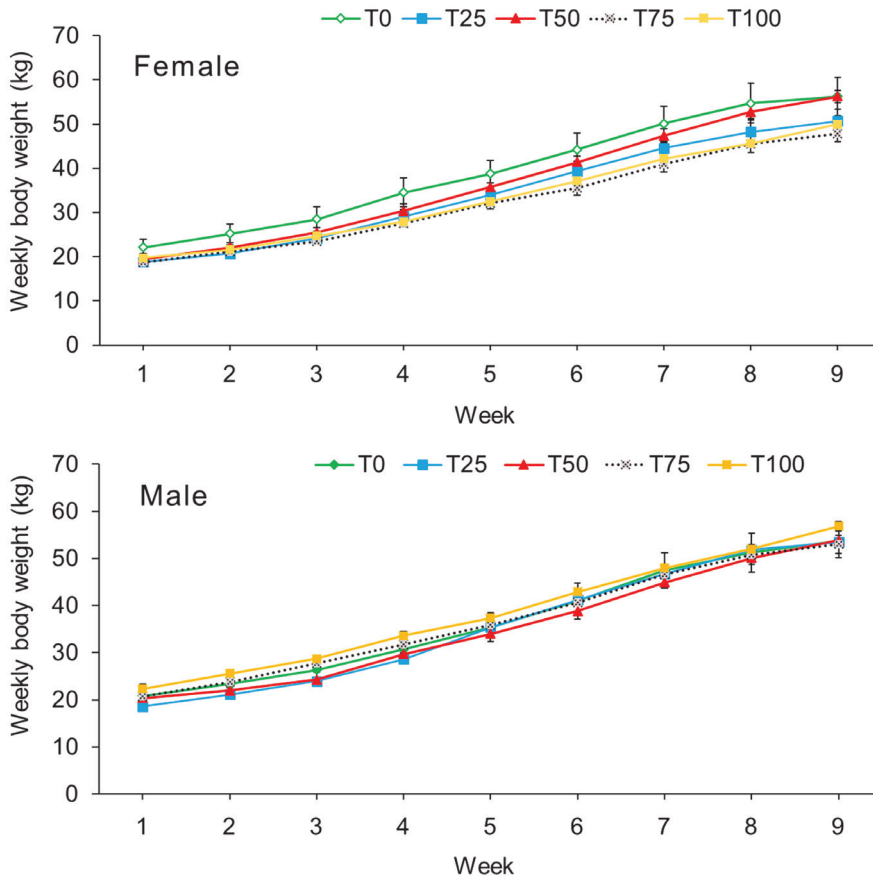


Figure 1. Mean (\pm SE) weekly body weight of pigs fed BSFLM-based diets and a control diet. BSFLM = black soldier fly larval meal. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, 4 males and 4 females were investigated.

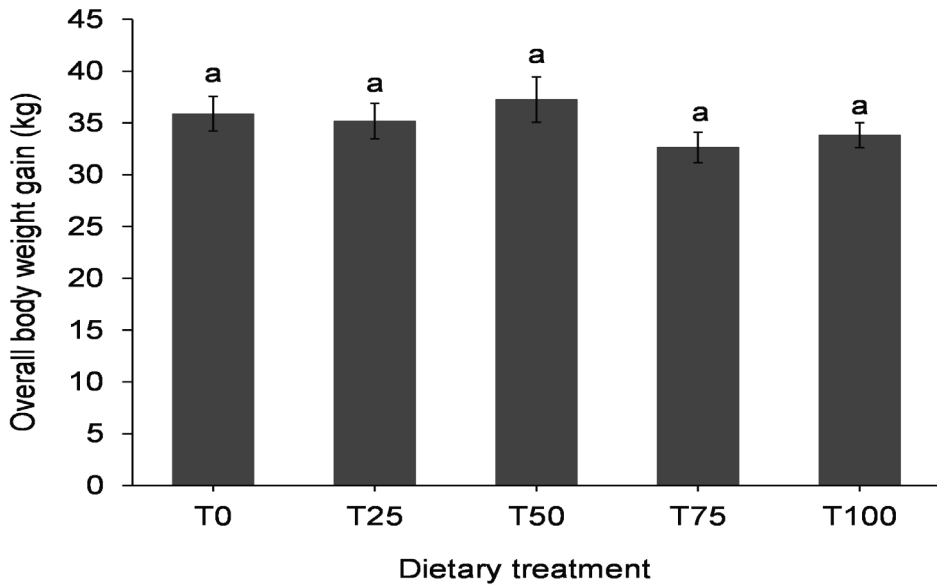


Figure 2. Body weight gain (mean \pm SE) of pigs fed BSFLM-based diets and a control diet for the whole trial. Bars followed by the same letter are not significantly different: $\alpha = 0.05$, general linear model (GLM, LSD). BSFLM = black soldier fly larval meal. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, 4 males and 4 females were investigated. Data for female and male pigs pooled together because there is no significant effect of sex. N = 8 per bar.

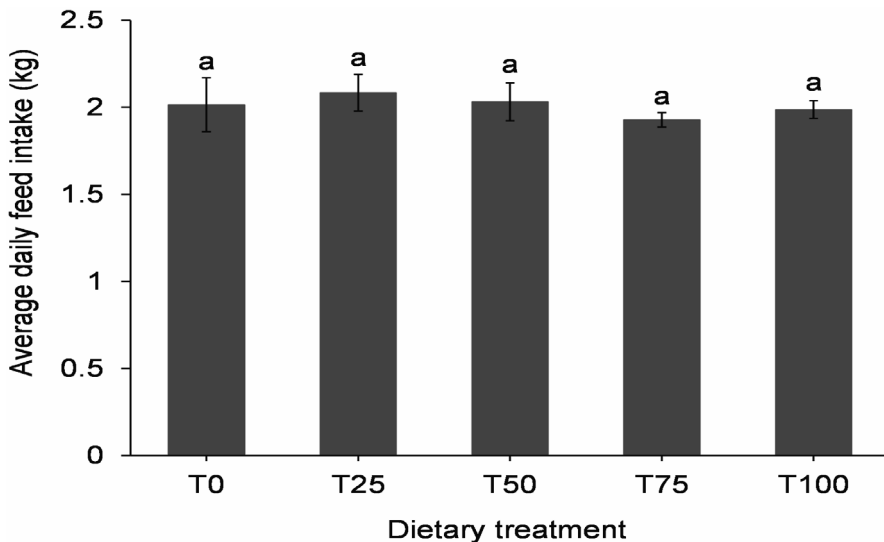


Figure 3. Average daily feed intake (\pm SE) in pigs fed BSFLM-based diets and a control diet. Bars with same letters are not significantly different: $\alpha = 0.05$, general linear model (GLM), BSFLM = black soldier fly larval meal. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. N = 8 per bar.

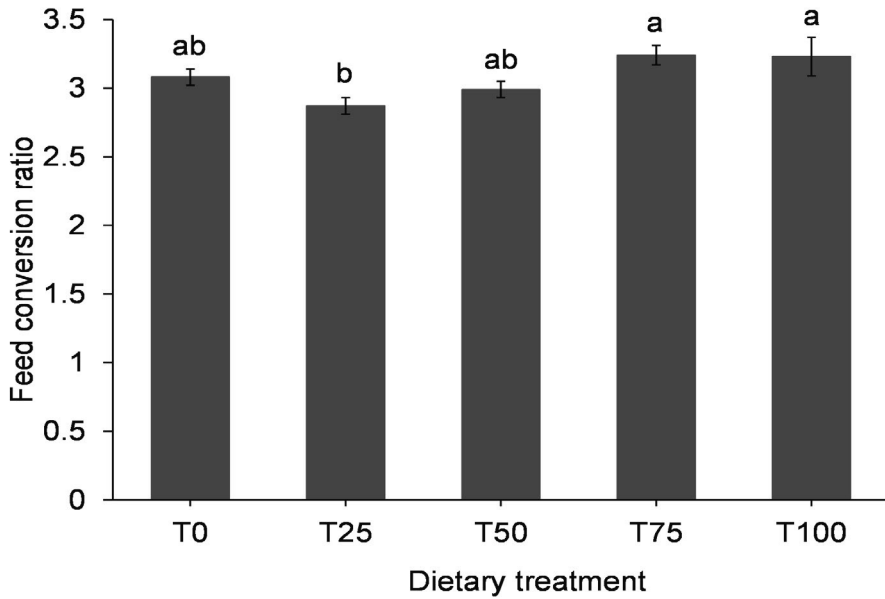


Figure 4. Feed conversion ratio (mean \pm SE) for pigs fed BSFLM-based diets and a control diet. Bars with different letters are significantly different: $P < 0.05$; general linear model (GLM), LSD. BSFLM = black soldier fly larval meal. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. N = 8 per bar.

Hematological and serum lipid parameters

Red blood cell indices did not differ among dietary treatments (Table 4). Hb at T25-T100 was within the normal range, whereas at T0, Hb was slightly below the normal range. MCHC values at T25-T100 were below the normal range compared to T0. Platelet count differed significantly among diets (Table 4). At T0, platelet count was and within the normal range compared to treatments T25, T75 and T100 (Table 4).

WBC count did not differ among pigs fed with different diets ($P = 0.463$). At T0 and T50, WBC counts were within the normal range, whereas the WBC counts of pigs from the other dietary groups were slightly above the normal range (Table 5). Diet significantly affected neutrophil counts (Table 5). At T75 and T100, neutrophil counts were significantly higher and within the normal range compared to T0 and T25 (Table 5). Lymphocyte counts did not differ among diets (Table 5). At T100, lymphocyte count was within the normal range, whereas at T0 – T75 the values were above the normal range. Monocyte, eosinophil and basophil counts did not differ among diets and were all within the normal range (Table 5). Serum lipid parameters did not differ among diets. All serum lipid parameters investigated were within the normal range (Table 5).

Table 4. Effects of dietary BSFLM inclusion on red blood cell indices and platelet count of growing pigs

Parameter	Diets					P value (GLM)	Normal range
	T0	T25	T50	T75	T100		
RBC ($\times 10^{12}$ /L)	5.4 ± 2.3	7.0 ± 0.2	7.4 ± 0.1	6.8 ± 0.6	6.9 ± 0.1	0.725	5.0 - 8.00
Hb (g/dL)	10.2 ± 4.3	13.3 ± 0.2	13.8 ± 0.4	12.8 ± 1.0	13.5 ± 0.3	0.719	10.7 - 16.7
Hct (%)	35.5 ± 15	48.0 ± 1.0	47.6 ± 1.7	45.2 ± 3.2	47.0 ± 2.1	0.704	32.0 - 50.0
MCV (fl)	64.4 ± 1.7	69.1 ± 3.0	64.4 ± 3.2	66.5 ± 1.8	68.1 ± 2.4	0.593	50.0 - 68.0
MCH (pg)	19.3 ± 0.5	19.1 ± 0.8	18.6 ± 0.8	18.8 ± 0.4	19.6 ± 0.3	0.803	17.0 - 21.0
MCHC (g/dL)	30.0 ± 1.5	27.8 ± 0.1	28.9 ± 0.2	28.3 ± 0.8	28.8 ± 0.7	0.449	30.0 - 34.0
RDW (%)	20.5 ± 1.7	21.8 ± 0.2	22.0 ± 0.5	21.6 ± 1.0	22.2 ± 0.4	0.706	15.0 - 27.0
Platelet (K/uL)	382 ± 7.0 ¹ a	209 ± 49c	328 ± 33ab	229 ± 27bc	251 ± 28bc	0.042	300 - 700

BSFLM = black soldier fly larval meal. RBC = red blood cell, Hb = haemoglobin, Hct = haematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, RDW = red cell distribution width. (¹) n = 2. Means (± SE) within a row followed by different letters are significantly different: P < 0.05, general linear model (GLM), LSD. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, 3 pigs were investigated.

Table 5. Effects of dietary BSFLM inclusion on white blood cell and serum biochemical indices of growing pigs

Parameter	Diets					P value (GLM)	Normal range
	T0	T25	T50	T75	T100		
WBC (k/L)	17.3 ± 1.6	24.5 ± 2.7	20.6 ± 2.5	24.0 ± 3.6	24.8 ± 5.0	0.463	11.0 - 22.0
Differential count (%)							
Neutrophils	24.2 ± 1.0c	24.7 ± 0.4c	27.1 ± 1.1bc	29.9 ± 2.9ab	31.8 ± 0.4a	0.019	28.0 - 51.0
Lymphocytes	69.4 ± 1.4	66.8 ± 0.8	64.8 ± 1.2	62.4 ± 4.0	60.9 ± 1.2	0.092	39.0 - 62.0
Monocytes	3.5 ± 0.7	5.6 ± 0.5	4.8 ± 0.9	4.1 ± 0.8	4.3 ± 1.2	0.496	2.00 - 10.0
Eosinophils	2.8 ± 0.5	3.0 ± 0.1	3.3 ± 0.3	3.5 ± 0.3	2.9 ± 0.5	0.684	0.50 - 11.0
Basophils	0.13 ± 0.03	0.08 ± 0.01	0.07 ± 0.03	0.15 ± 0.03	0.10 ± 0.0	0.585	0.00 - 2.00
Blood serum indices (mmol/L)							
Total Chol	1.90 ± 0.16	2.11 ± 0.13	2.11 ± 0.20	2.50 ± 0.12	2.19 ± 0.12	0.185	1.68 - 5.81
Total Trig	0.69 ± 0.08	1.03 ± 0.23	0.99 ± 0.17	0.89 ± 0.03	0.89 ± 0.07	0.479	0.11 - 1.13
LDL	0.67 ± 0.11	0.88 ± 0.08	0.75 ± 0.12	0.71 ± 0.11	0.65 ± 0.04	0.50	< 3.00
HDL	1.23 ± 0.09	1.23 ± 0.07	1.37 ± 0.09	1.77 ± 0.23	1.53 ± 0.09	0.066	> 1.00

BSFLM = black soldier fly larval meal. WBC = White Blood Cell. Means within a row followed by different letters are significantly different: P < 0.05, general linear model (GLM), LSD. BSFLM = Black soldier fly larval meal. Chol = cholesterol, Trig = triglycerides, LDL = Low density lipoproteins, HDL = High density lipoproteins. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, 3 pigs were investigated.

Economic analyses of the inclusion of black soldier fly larval meal in pig diets

Replacing fish meal by BSFLM in pig diet did not affect the profit accrued from the sale of pigs (Table 6). Cost benefit ratio and return on investment did not differ among diets (Table 6).

Table 6. Economic analyses of replacing fishmeal by BSFLM in growing pig diets

Parameter	Diets				P value (GLM)
	T0	T25	T50	T100	
Cost feed (US\$/kg)	0.50	0.52	0.52	0.51	-
Cost of protein ingredient in feed (%)	0.24	0.31	0.31	0.30	-
Total feed consumed (kg/pig)	126.6	128.5	127.9	126.0	-
Cost of feed consumed per pig (US\$), C_p	62.89	67.29	66.14	64.43	-
Final body weight of pig (kg)	55.0 ± 2.63	52.1 ± 2.16	55.1 ± 2.41	50.4 ± 1.58	0.474
Amount at final weight (US\$/live weight), S_p	165.0 ± 7.89	156.4 ± 6.49	165.2 ± 7.22	151.7 ± 4.42	0.477
Profit, P_r	102.1 ± 7.89	89.1 ± 6.49	99.0 ± 7.22	89.7 ± 4.42	0.499
Cost benefit ratio, CBR	2.6 ± 0.13	2.3 ± 0.10	2.5 ± 0.11	2.5 ± 0.07	0.318
Return on investment, RoI	162.4 ± 13	132.4 ± 9.64	149.7 ± 11	144.8 ± 7.13	0.318

BSFLM = black soldier fly larval meal. (-) value were not calculated. Cost (US\$/kg) of protein ingredients used in the dietary treatments: Fishmeal (*Rastrineobola argentea*) = 1.20; BSFLM = 0.85. Live weight of pig = 3.00 US\$/kg; $P_r = S_p - C_p$; CBR = S_p / C_p ; RoI = $P_r / C_p * 100$. Final body weight, S_p , CBR and RoI are expressed as mean ± standard error of the mean. General linear model (GLM), $P < 0.05$. T0 = 0% (control), T25 = 25%, T50 = 50%, T75 = 75% and T100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, 8 pigs were investigated.

Discussion

In Africa, to the best of our knowledge, the current study is the first to report the positive impact of non-defatted BSF larvae as a protein-rich ingredient in pig feeds. Protein is an important component of animal feeds required for growth and development. The source of protein is crucial because it affects availability and utilization of the essential amino acids (Wallace et al., 2017). The crude protein (CP) content of BSF larvae largely depends on the substrate used to rear the larvae and varies from 39% to 44% (Lalander et al., 2019), which is comparable or superior to that of the commonly used soybean and FM (Onsongo et al., 2018). BSFLM is a suitable ingredient in pig feed (Makkar et al., 2014) and feeds containing BSFLM are as palatable as those containing soybean meal (Newton et al., 1977). Our results agree with earlier studies in which partially defatted or full-fat BSF inclusion levels of only 4%–10% in partial replacement of either FM or soybean meal did not result in a significant difference in growth performance of the piglets (Biasato et al., 2019; Driemeyer, 2016; Spranghers et al., 2018), when compared to the conventional FM /soybean diets. The present study shows that much higher replacement levels, up to 100% were acceptable and well tolerated by the pigs. No significant decline in growth parameters or mortality was recorded. Acceptability and suitability of BSFLM has also been successfully recorded for fish and chicken (Pieterse et al., 2018; Sealey et al., 2011). Thus, our study shows that BSFLM can successfully replace FM as a sustainable protein-rich ingredient in growing pig diet as reflected in the growth performance and feed conversion rate, which were similar for all the treatment and control diet groups of pigs. Rejection of feed due to texture, palatability or inclusion of BSFLM was not observed, which is in line with the observations by Ramos-Elorduy et al. (2002) for broiler chickens.

In animal production, ADG is a critical index of growth performance (Wu et al., 2018). We did not observe a significant effect of dietary treatment on ADG of the pigs, which is a clear indication of adequate nutrient supply by the different formulated diets. Tolerance of insect-based protein-rich diets for pigs has been documented in other studies (Coll et al., 1992; Medhi, 2011; Medhi et al., 2009a; Medhi et al., 2009b; Newton et al., 2005b). In India, silkworm meal was used to completely replace FM in the diet of growing and finishing pigs without altering carcass and meat quality and blood parameters (Medhi, 2011; Medhi et al., 2009a; Medhi et al., 2009b). Similarly, in Nigeria, feeding early weaned pigs with a 3:1 mixture of dried rumen content and maggot meal in the diet replacing 10% wheat offal did not have adverse effects on performance (Adeniji, 2008). The inclusion of 10% and 15% of defatted BSFLM in the diet of growing quails (from 10 to 28 d of age) led to comparable production performances and carcass traits with those of quails fed conventional soybean meal and oil-based diets (Cullere et al., 2016). The suitability of BSFLM for the growing pigs in our experiments can be attributed to the high level of digestibility, which is consistent with other studies on pigs fed with

BSF larvae (Newton et al., 1977), and broilers fed with housefly pupal meal (Pretorius, 2011).

In contrast to the current study, Newton et al. (2005b) reported that a complete replacement of dried plasma with BSF pre-pupal meal in the diet of early weaned pigs reduced performance of the pigs by 3%–13%. Poor performance of weaner pigs fed on pre-pupal meal might be attributed to the higher chitin levels in the pre-pupae than in larvae as used in our study, which has been reported to contribute to decreased digestibility resulting in reduced nutrient utilization and growth performance in animals when higher substitution rates are used (Alegbeleye et al., 2012; Newton et al., 2005b). Low digestibility of BSF pre-pupal protein in animal feeds is also supported by Bosch et al. (2014), who attributed this to higher cuticular protein-sclerotization in the pupae. The similarity in feed intake and average daily weight gain recorded for all the treatment groups in the current study can be attributed to utilization of the 5th instar larval meal instead of the pre-pupal meal, which contributes sufficient nutrients in the diets with high level of digestibility. BSFLM has also been shown to be of good nutrient composition for reptiles (Klaphake, 2010).

In the present study, the values of the hematological parameters RBC, Hb, Hct, MCH, MCV, MCHC and RDW for pigs fed BSFLM were not significantly influenced by the replacement levels. The values for RBC, Hb, Hct, MCH and RDW fell within the physiological range for pigs, which is a clear indication of a good health status of the animals, implying that the quality of the test diets was adequate to maintain good health of the pigs. Dietary replacement of FM with BSFLM at the rates of 25%, 50%, 75% and 100% in pig diet improved RBC, Hb, Hct and RDW, which had higher values compared to the control FM diet group of pigs. The RBC counts and Hb concentration in blood increased to a level of 37% and 35.3%, respectively, at a FM replacement level of 50% with BSFLM. These results are consistent with the reports by Marono et al. (2017) and Loponte et al. (2017), who reported that dietary BSFLM inclusion positively affected the blood profile of laying hens and Barbary partridges, in terms of higher globulin levels. Our results may be attributed to high digestibility of insect-based protein and high levels of minerals such as iron, which is required for the formation of haemoglobin in the pigs. The higher the haemoglobin concentration, the better the oxygen circulation in the body, hence, better performance of the animal (Olugbemi et al., 2010).

The results of the present study show that the composition of the various treatments significantly affected platelet count in pigs. The replacement of FM with BSFLM at rates of 25%, 75% and 100% in the feed was associated with significantly lower blood platelet counts out of the normal range than observed with 50% replacement of FM with BSFLM or with the control diet without BSFLM. This implies that diet composition in the present study significantly influenced the developing hematological system with some unknown factors suppressing the normal developmental increase in platelet

counts in growing pigs. Low platelet concentration implies that blood clotting might be impaired, resulting in blood loss in case of injury (Etim et al., 2014a). According to Martin et al. (1983), bleeding time largely depends on both platelet counts and mean platelet volume.

The largely similar WBC count obtained in this study implies that the ability of the pigs to respond to and eliminate infection was not compromised with the inclusion of BSFLM to replace FM in the diets. The normal monocyte levels may indicate that the pigs did not react to any infections during the experimental period. Furthermore, the similarity in basophil levels indicates that the pigs showed no hypersensitivity reaction to the inclusion of BSFLM in diets while the normal levels of eosinophils might indicate that the pigs did not suffer from parasitic infections during the experimental period (AACC, 2019; Konlan et al., 2012). However, higher (75% and 100%) levels of replacement of FM with BSFLM significantly improved the neutrophil count to the normal physiological range compared to the control FM diet. Neutrophils play an important role in immune responses, especially in wound healing through microbial sterilization and macrophage attraction (Nathan, 2006). BSF larval fat contains medium-chain saturated fatty acids with antimicrobial properties (Skřivanová et al., 2006; Spranghers et al., 2018). For instance, lauric acid has been identified as the most predominant medium chain saturated fatty acid found in BSF larvae. It has been shown that inclusion of coconut oil, which contains medium-chain saturated fatty acids in rabbit feeds significantly increases leucocytes and neutrophil counts (Ahlante et al., 2010). The increase in neutrophil count in the present study could be an indication of the antimicrobial response in pigs fed high BSFLM-based diets. According to (Ahlante et al., 2010), increased mobilization of leucocytes and neutrophils in animals fed with coconut oil-based feed resulted from the stimulation of the pluripotent haemopoietic stem cells from which leucocytes are produced in the presence of a growth inducer and differentiation inducer, which are proteins. Neutropenia is a consequence of reduced neutrophil and leucocyte levels, which leads to reduced body immunity. Therefore, the inclusion of BSFLM in pig feed is highly recommended due to the valuable nutrients available to the growing pigs. The lymphocyte counts of pigs fed 100% FM diet, 25%, 50% and 75% BSFLM diets were higher and out of the normal range. This implies that these diets might have stimulated both cellular and humoral immune response systems of the pigs to protect against intracellular and extracellular pathogens such as *Mycobacterium*, *Listeria*, *Bruceella*, *Pasteurella* or *Salmonella*, viruses and fungi (Dudek et al., 2006).

The replacement of FM by BSFLM up to 100% did not affect the serum biochemical indices that are indicators of pig health. It is worth noting that although the use of hematological and biochemical indices have been considered as a fast means of assessing nutritional and health status of farmed animals, this has rarely been used in pig veterinary practices (Biasato et al., 2019). The observation that replacing FM by BSFLM does

not affect serum biochemical indices in pigs supports the conclusion that BSFLM could form the basis of a valuable component in grower pig diets.

The cost–benefit analysis, which assesses whether an investment is sound and if-and by how much—profits outweigh costs, allows for comparing costs and benefits of alternative investments (David et al., 2013) as in the present study. The similarity in results obtained for the ‘control’ (100% FM diet) and BSFLM-based diets indicate that BSFLM is not only a valuable component of pig feed from a performance perspective but also from an economic perspective. This supports the need to further investigate the economic prospects of using BSFLM in large-scale pig feed formulation and feeding programs. A key advantage of insects as a feed ingredient, especially the BSF larvae over other conventional protein sources is their ability to convert waste into high-value biomass and closing nutrient cycles as they reduce pollution and costs of managing organic waste (Wang & Shelomi, 2017).

Conclusion

Dietary replacement of FM up to 100% with full-fat BSFLM did not adversely affect growth, blood characteristics or economic parameters. Although some changes in blood cell counts were observed, values were largely similar among diets. Pigs did not show visual signs of illness or abnormal behavior. Moreover, serum biochemical parameters were all within normal range for pigs. The present study indicates that a complete replacement of FM with full-fat BSFLM as an ingredient in growing pig feed is feasible, with reduced predisposition to heart diseases associated with high total cholesterol and LDL. Cost–benefit analysis results of the present study indicate that the inclusion of BSFLM in pig feed is a worthwhile investment for pig farmers. Finally, there is little evidence to suggest that adverse health effects should be expected in pigs following BSFLM consumption. Further studies are required to assess the effect of feeding BSFLM to pigs on meat sensory attributes and consumer perceptions.

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Chapter 8

Black soldier fly larval meal in feed enhances growth performance, carcass yield and meat quality of finishing pigs

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Abstract

The price of protein ingredients (soybean and fishmeal - FM) commonly used in feed for meat production has increased drastically due to reduced availability. Black soldier fly (BSF) larvae are a novel source of animal protein whose use in animal feeds rapidly increases. Yet, little is known about the effect of dietary inclusion of BSF larval meal (BSFLM) on finishing pig growth, carcass yield and meat quality. The present study evaluated the effect of replacing FM with BSFLM on growth performance, carcass traits and meat quality of pigs. A control diet (including FM: 0% BSFLM) was compared with four dietary levels of replacement of FM with BSFLM at 25, 50, 75 or 100%. Forty hybrid pigs (crossbreeds of purebred Large White and Landrace) were randomly allocated to the five different dietary treatments. Feed intake, body weight gain and feed conversion ratio were measured. After 98 days of feeding, all eight pigs per treatment were slaughtered for the evaluation of carcass and nutritional content of the organ and muscle tissues. The results show that diet significantly affected average daily weight gain, final body weight, average daily feed intake, and feed conversion ratio. Fasted weight and carcass weight of pigs fed diets with 50 – 100% of FM replaced with BSFLM were significantly greater than in the control group. Back fat depth was significantly greater in the 75 and 100% replacement groups than in the 0-50% replacement groups. Belly, ham and loin cuts weighed significantly more in the 50-100% replacement groups than in the control group. The crude protein content of pork tissues on dry matter basis (DM) was high, ranging between 65 - 93% across all dietary groups. BSFLM proves to be a suitable feed ingredient in finishing pig feed and can completely replace FM with beneficial consequences on growth, carcass and nutritional quality of edible pork by-products. These findings are relevant for commercial pig feed production and provide for the first time a nutritional perspective on pork derived from pigs raised on insect-based feeds.

Introduction

Approximately one third of global meat production is represented by pig meat (Bruinsma, 2003). In Sub-Saharan Africa, pig production has a great potential for raising household income of millions of resource-poor smallholder farmers especially women in the rural and peri-urban communities (Ouma et al., 2014; Pezo et al., 2014). The production and consumption of pork has recently increased in many countries in Sub-Saharan Africa. Further studies revealed that with the growing *per capita* income, population and urbanization, this trend is predicted to continue, surpassing the increase in demand for cereals and root tubers (Pica-Ciamarra & Otte, 2009).

In Kenya, pig production is an important source of household income for pig farmers (Kagira et al., 2010a; Kagira et al., 2010b; Mutua et al., 2010). Although 70% of the producers are small-holder farmers (Githigia et al., 2012; Mbuthia et al., 2015), pig meat ranked the second highest livestock export earner for Kenya at USD \$1,122,000 in 2002 (Kagira et al., 2010a). However, a major barrier for producers is the lack of quality feed ingredients, especially the major protein sources such as fishmeal (FM) and soybean meal. The reduced availability of these common protein sources in the market throughout the year has led to an increase in feed costs, representing 60 - 70% of the total costs of pig production. Thus, cheaper and sustainable sources of protein for inclusion in animal feed to substitute FM are urgently needed (Ardjosoediro & Neven, 2008; Fiaboe & Nakimbugwe, 2017; Githigia et al., 2012). Although FM has a good amino acid profile and minerals that can positively impact nutrition and meat quality of farmed animals (Cho & Kim, 2011), it is mainly obtained from silver cyprinid fish *Rastrineobola argentea*, which constitutes the largest catch by weight of fishery in Lake Victoria (Kolding et al., 2014). The high demand for this fish has led to excessive fishing activities in Lake Victoria, prompting the lake regulatory authorities to institute periodic fishing bans. As a result, FM is not available in sufficient quantities throughout the year, leading to increased prices (Ardjosoediro & Neven, 2008). Therefore, novel protein sources, including edible insects are needed to supplement the current sources of protein (Van Krimpen & Hendriks, 2019).

Insects contain high-quality protein, fats and minerals, and are suitable alternatives to FM or soybean meal in animal feed (Makkar et al., 2014). The black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) is widespread in the tropics and warm temperate regions of the world (Sheppard et al., 1994). BSF larvae (BSFL) feed on a variety of substrates including organic side streams (Chia et al., 2018a; Chia et al., 2018b; Meneguz et al., 2018; Rehman et al., 2017), converting residual nutrients into high-quality insect biomass (Barragan-Fonseca et al., 2017; Barragan-Fonseca et al., 2018; Diener et al., 2009; Lalander et al., 2015). BSF larval meal (BSFLM) has been used extensively in fish (Belghit et al., 2019; Xiao et al., 2018; Zarantoniello et al., 2018) and poultry feeds

(Moula et al., 2018; Onsongo et al., 2018), but its use in pig feed formulation has only been assessed occasionally (Biasato et al., 2019; Newton et al., 1977; Yu et al., 2019).

A pig production cycle produces mature pigs either for slaughter or reproduction and feed is the most critical input in every growth phase (piglet-grower-finisher) in commercial pig production. The finishing phase of pigs, a growth phase which typically precedes slaughter or market stage of pigs, is marked by considerable lean growth (Kim et al., 2005). The inclusion of BSFLM in pig feed has not yet been extensively assessed and has thus far been focused on the piglet stage. Yu et al. (2019) replaced soybean meal with BSFLM in finishing pig feeds at the rate of 4 and 8% and investigated the colonic microbiota and bacterial metabolite production in the animals. They report that the inclusion of BSFL in pig feed may enhance mucosal immune homeostasis of pigs through altering bacterial composition in the intestinal (colon) mucosa and the bacterial metabolite profile. In the present study, we replaced FM with BSFLM in feed that was provided to finishing pigs until market weight, and assessed their growth performance, as well as carcass traits and meat quality. Market weight is an important economic factor in pig production, impacting pork quality and profit. Furthermore, the values of different cuts of pig carcasses are different; hence, information on the proportion of primal cuts can be important in assessing carcass yield to optimize profit (Kim et al., 2005).

Body weight gain in pigs and feed composition are important factors in pork production (Muns et al., 2018). About 80% of feed used in a farrow-finish operation is consumed in the grower-finisher phase (Njoku et al., 2015). Inadequate feed intake affects growth performance, thus impacting the cost of production. Reduced carcass fat content, especially in animal carcasses set for the consumer market is preferred (Zak & Pieszka, 2009).

Edible meat by-products including internal organs such as liver, lungs, heart, spleen and kidneys are generated through slaughter of pigs. These by-products make up an important portion of the animal's live weight and can provide essential nutrients to consumers who are limited in meat and meat products (Alao et al., 2017; Fayemi et al., 2018; Irshad & Sharma, 2015). However, their consumption is limited and varies among cultures and economic status (Seong et al., 2014). The commercial value of these by-products is also low and limited information on their nutritional quality may be responsible for their low consumption rates (Seong et al., 2014). Information on the nutritional composition of edible pork by-products when fed insect-based feeds, can influence the consumer's decision to accept these by-products. This information can also promote the use of edible pork by-products and can contribute to ensuring the widespread use of insect meal in pig feed formulation.

Farmed animals are often assessed for growth on farm or at the market gate based on live body weight (Birteeb et al., 2015). However, weighing heavier animals is challenging

to resource-poor farmers because they cannot afford the expensive weighing scales. Simple linear body measurements can therefore provide a basic assessment of the animal in smallholder farms. The present study aimed at evaluating the effect of replacing FM with BSFLM on growth performance and carcass yield of finishing pigs as well as the nutritional composition of some major pork by-products. The study also established the correlation between live body weight and linear body measurements using principal component analysis (PCA).

Materials and Methods

Study site

The experiment was conducted at the Non-ruminant Research Institute (NRI) of the Kenya Agricultural and Livestock Research Organization (KALRO) located in Naivasha, Kenya.

Insect meal and experimental diets

Black soldier fly larvae used in the study were obtained from the Animal Rearing and Containment Unit (ARCU), icipe, Nairobi, Kenya. The larvae were reared on a mixture of brewers' spent grains (BSGs) from Kenya Breweries Limited. BSF larvae were sterilized by washing in warm water (84 °C) for 10 minutes and then oven-dried using a stainless-steel fruit/vegetable/meat/fish drying machine model (CT-C-III Series hot air circulating drying oven, Henan Forchen Machinery Co., Ltd, China). Dried larvae were ground into larval powder meal using a hammer mill (Newton et al., 1977). Diets were prepared to partially or completely replace FM with BSFLM and were formulated to be isonitrogenous and isoenergetic to meet requirements for finishing pigs (National Research Council, 1979). FM content of a control (D0) diet was replaced at 25, 50, 75 and 100% with BSFLM to obtain: D25, D50, D75 and D100, respectively, as experimental diets (Table 1).

Proximate, amino acid and mineral composition of experimental diets

The dry matter content of formulated feed samples was gravimetrically determined after loss of water. The samples were heated to 103 ± 2 °C for 3 h until constant weight, when the weight of samples was constant for two consecutive readings. Ash content was determined by ignition of samples at 550 °C in a muffle furnace. Dried, ground samples were exposed to an electromagnetic scan in the absorbance mode using near infrared (NIR) spectroscopy (Cropnuts, Nairobi, Kenya). The crude protein, fat, starch, oil, acid detergent fibre, neutral detergent fibre, sugar and digestibility (NCGD) values

were determined following standard laboratory procedures (Núñez-Sánchez et al., 2012; Rosales et al., 2011). The amino acid composition of experimental diets (Table 1) was analyzed by AMINOLab® (Evonik Industries, Hanau, Germany) using an amino acid analyzer (Biochrom 30 plus, Biochrom Ltd. Cambridge, UK) (Al-Sagan et al., 2018; Llamas & Fontaine, 1994; Powell et al., 2017; Windham, 1995; Zampiga et al., 2018). Feed samples were homogeneously ground with an Ultra Centrifugal Mill RETSCH-ZM 200 to pass through a 0.5 mm sieve. Finely ground samples were weighed using an analytical balance, display accuracy ± 0.01 mg into 50 ml laboratory bottles, with thread of DURAN glass (Schott), red PBTP caps with silicone/Teflon seal. Then, 25 ml of hydrochloric-phenol reagent was added to the sample in the bottles and the mixture was introduced into a thermostatically controlled heating oven (UT 6060 AR, Thermo Electron LED, Langensfeld, Germany) at 110 °C, with loose screw tops for one hour and then with tightened screw tops for 23 hours to complete sample hydrolysis. Methionine and cysteine samples were prepared through performic acid oxidation procedures followed by acid hydrolysis–sodium metabisulfite method (Powell et al., 2017; Slump & Bos, 1985). The resulting hydrolysate solutions were then introduced into the amino acid analyzer and the sample aminograms were analyzed at 570 nm and 440 nm. Amino acid concentrations in the samples were determined in duplicate.

Mineral composition of feed samples (Table 2) was analyzed by Inductively Coupled Plasma-Optical Emission spectrometry (ICP-OES) (Cropnuts, Nairobi, Kenya). Ground feed samples were transferred to a microwave digestion system. Then, a mixture of nitric acid and hydrochloric acid was added to the sample and allowed to digest the sample. The resulting solution was filtered into a volumetric flask and used for ICP-OES analysis to determine the following minerals: boron, molybdenum, iron, copper, zinc, cobalt, manganese, sodium, sulphur, magnesium, potassium, phosphorus and calcium (Baralkiewicz et al., 2007; Plotka-Wasyłka et al., 2018; Santos et al., 2012; Sreenivasulu et al., 2017).

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Animals and Housing

Hybrid grower pigs (crossbreeds of purebred Large White and Landrace) comprising of 20 boars and 20 gilts, with mean body weight (BW) of 54.3 ± 0.93 kg were randomly assigned to the five dietary treatments, with eight replicate pigs (four boars and four gilts) per treatment. Pigs were placed individually in pens (3.7 m x 1.9 m) with concrete floors. Each pen was provided with a one-sided feeding trough (1.8 x 0.2 x 0.2 m) and a nipple drinker. Each pen was labelled with a number and diet type while each animal was identified with a unique number by ear tattooing. Pig pens were cleaned every day by scrubbing the floor using Teepee straight brooms with handle (c27, Chandarana Foodplus, Nairobi, Kenya) and water. Pigs were allowed *ad libitum* access to feed and water throughout the experiment.

Growth performance

Initial body weight of the pigs was recorded at the start of the experiment and subsequently the pigs were weighed on a weekly basis using a 150 kg x 500 g suspended weighing scale (model 235, England, Salter). The sides of the scale were covered with a wire mesh to prevent uncontrolled exit of the animal. The entry gate of the weigh cage was opened sideward and the animal was led into a stable, unmoving floor after which the gate was closed. Once inside the cage, the animal was allowed to settle, and the weight value read on an analogue display screen above the weigh cage. Pigs were only provided with feed after recording their body weight if this coincided with the weekly body weight measurement. The experiment lasted 14 weeks and the weekly body weight values were used to calculate average daily weight gain (ADG). Feed offered to the pigs and the unconsumed portions were weighed daily using a digital platform weighing scale (XK3190-A12, Gromy Scale Co., Ltd, Hangzhou, China) to calculate average daily feed intake (ADFI). Feed conversion ratio (FCR) was calculated as total feed consumed divided by the body weight gain at the end of the experiment.

Table 1. Composition of experimental diets

Parameter	Diets				
	D0	D25	D50	D75	D100
	Ingredient (kg)				
Maize meal	13.0	14.0	14.5	14.0	14.0
Wheat pollard	46.5	35.0	34.0	32.3	36.0
Rice polishing	27.5	33.8	33.0	34.0	30.0
Fishmeal	7.0	5.3	3.5	1.8	0.0
BSFLM	0.0	6.0	9.0	12.0	14.0
L-Lysine	1.0	1.0	1.0	1.0	1.0
DL-Methionine	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral premix*	1.0	1.0	1.0	1.0	1.0
Bone meal	1.3	1.3	1.3	1.3	1.3
Salt	0.5	0.5	0.5	0.5	0.5
Limestone	1.3	1.3	1.3	1.3	1.3
Dry matter (DM) (%)	92.6	93.1	93.6	92.2	94.1
Crude protein (% DM)	12.9	14.9	15.3	14.8	16.3
	Essential amino acids (% DM)				
Lysine	1.1	1.2	1.3	1.2	1.3
Methionine	0.6	0.7	0.7	0.5	0.5
Methionine + Cystine	0.9	0.9	0.9	0.7	0.8
Isoleucine	0.4	0.4	0.5	0.4	0.5
Leucine	0.8	0.9	0.9	0.9	1.0
Threonine	0.4	0.5	0.5	0.4	0.5
Phenylalanine	0.5	0.6	0.6	0.5	0.6
Valine	0.6	0.6	0.7	0.6	0.8
Arginine	0.8	0.8	0.8	0.7	0.9
Histidine	0.3	0.3	0.3	0.3	0.4
	Nonessential amino acids (% DM)				
Alanine	0.7	0.8	0.8	0.8	0.9
Aspartic acid	0.9	1.0	1.1	1.0	1.2
Cystine	0.2	0.2	0.2	0.2	0.2
Glutamic acid	1.9	2.0	2.0	1.8	2.1
Glycine	0.7	0.7	0.8	0.7	0.8
Proline	0.7	0.8	0.8	0.8	0.9
Serine	0.5	0.6	0.6	0.5	0.6

BSFLM = black soldier fly larval meal. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. DM, crude protein, essential and non-essential amino acids are results of chemical analysis of feed samples. Amino acid values presented are mean values of two subsamples analyzed. * Premix contained per 2 kg: Vitamin A 3,000,000 IU; Vitamin D3 500,000 IU; Vitamin E 2,500 IU; Vitamin K3 – KASTAB 2,000 mg; Vitamin B2 – riboflavin 2,500 mg; Vitamin B3 – nicotinic acid 20,000 mg; Vitamin B5 – pantothenic acid 10,000 mg; Vitamin B6 – pyridoxine 500 mg; Vitamin B12 – cobalamin 12 mg; choline chloride 100,000 mg; zinc bacitracin 10,000 mg; antioxidant 125,000 mg; iron (Fe) 40,000 mg, manganese (Mn) 25,000 mg; zinc (Zn) 80,000 mg, copper (Cu) 25,000 mg; iodine (I) 500 mg, cobalt (Co) 250 mg, selenium (Se) 100 mg.

Table 2. Mineral and proximate composition (dry matter basis) of experimental diets

Parameter	Diets				
	D0	D25	D50	D75	D100
Boron (ppm)	2.6	3.7	2.6	2.6	1.68
Molybdenum (ppm)	1.1	1.0	0.8	0.8	10.5
Iron (ppm)	374.2	522.9	404.4	433.1	499
Copper (ppm)	13.2	11.6	13.7	10.7	24.4
Zinc (ppm)	66.3	92.6	82.4	74.8	123
Cobalt (ppm)	0.1	0.2	0.2	0.1	0.44
Manganese (ppm)	159.7	232.5	203.3	200.2	209
Sodium (ppm)	2833.7	3744.5	3049.2	3384.1	942
Sulphur (%)	0.4	0.4	0.3	0.3	0.3
Magnesium (%)	0.5	0.6	0.5	0.5	0.58
Potassium (%)	0.8	1.1	0.9	0.9	0.93
Phosphorus (%)	0.8	1.0	1.0	0.9	0.64
Calcium (%)	2.5	3.0	2.6	2.1	1.58
Sugar (%)	2.5	3.6	6.2	5.6	8.66
Starch (%)	32.3	17.1	25.5	25.4	29.8
Ash (%)	8.5	12.3	8.3	9.3	9.53
Acid detergent fiber (%)	16.8	25.7	19.8	20.7	18.1
Neutral detergent fiber (%)	39.1	50.1	41.1	42.3	40.2
Digestibility (%)	77.8	67.9	75.4	75.0	72.9
Oil (%)	4.7	6.5	8.6	8.6	11.3
Net energy (MJ/Kg)	9.3	7.6	10.2	10.0	13.4

D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fish-meal with black soldier fly larval meal; ppm = parts per million. Values presented in the table are results of single analyses

Linear and live body measurements

Body measurements studied included: rump height (RH), distance from the highest point of rump to the ground; heart girth (HG), length of body circumference just behind the forelegs; body length (BL), distance between the middle point of the shoulder blade and the base of the tail, measured on the midline along the back; ear width (EW), measured at the widest part of the ear; head width (HdW), measured at the widest point of the head; body height (BH), distance from the top of the mid-point of the trunk to the ground; Fore limb (Flb), length from the point of attachment of the foreleg to the tip of the foreleg; hind limb (Hlb), length from the point of attachment of the hind leg to the tip of the hind leg; Trunk depth (TD), measured from the mid-point of the trunk (at the top of the back) to the lowest point of the stomach; Shoulder width (SW), distance from left to right upper arm at the widest point of the shoulder; Hip width (HW), distance from left to right at the widest point of the hip; flank circumference (FC),

measured immediately in front of the hind legs; back height (BaH), measured from the middle point of the upper hips to the floor; neck circumference (NC), and the live body weight (BW) of each pig. Each pig was restrained while taking the body measurements. The linear body traits were recorded using a measuring tape in centimeters (Adeola et al., 2013; Zaragoza, 2009).

Slaughter and Carcass Characteristics

Forty pigs, consisting of eight (4 boars and 4 gilts) pigs per dietary treatment were slaughtered and analyzed for carcass yield, cut-up parts, organ weight, and composition of muscle, bone, fat and skin in primal cuts at the end of the experiment. All pigs were slaughtered at the slaughter facility of the Non-Ruminant Institute, Kenya Agricultural and Livestock Research Organization, Naivasha, Kenya where the feeding trial on pigs was conducted. Pigs were weighed and fasted for about 18 hours after which the fasted weight of each pig was recorded before it was stunned. Pigs were carefully slaughtered following prescriptions according to the Kenya Society for the Protection and Care of Animals (KSPCA), Naivasha, Kenya. Pigs were immobilized by head stunning, using a stunning gun and bled by incision, cutting through the jugular vein between the skull and the atlas after which complete dehairing was done. Following dehairing, the stomach of each pig was opened along the greater curvature and eviscerated. After evisceration, the remaining part was weighed within 45 min postmortem to determine the hot carcass weight (HCW) and later expressed as percentage of the ending live weight to obtain the dressing percentage (DP). The head was removed by cutting through the occipito-atlas joint. The trotters were removed by sawing through the hock joint at a right angle to the long axis of the leg.

The carcass was split longitudinally by sawing along the dorsal midline and the right half of each carcass was dissected into main cuts (ham, shoulder, loin and belly) and into other parts (collar butt, tenderloin and rump). The ham was separated by locating the division between the second and third sacral vertebrae and then sawing perpendicularly to the long axis of the carcass. The shoulder was separated from the loin and belly by a straight cut between the second and third ribs. The shoulder cut was further separated into the shoulder picnic (portion closest to the knee) and collar butt (portion closest to the spine of the pig). The middle portion of the carcass side was divided into the loin and belly by a straight cut from the edge of the tenderloin muscle on the ham end through the front rib tight against the protruding edge of the split backbone. The rump is the portion posterior to the loin and anterior to the tail and was separated by cutting just below the exposed pelvic or aitchbone (Njoku et al., 2015; Sheridan et al., 1991). For each cut, the weight was recorded using a digital weighing scale. The four main cuts (ham, shoulder picnic, loin and belly) were deboned and the skin plus subcutaneous fat

removed. The weight of the dissected lean (muscle), bone, skin plus fat of each of the main cuts was recorded and expressed as a percentage of the weight of the cut.

The unskinned loin portion of the half carcass was sectioned between the 10th and 11th rib and after exposing the loin eye of the *Longissimus dorsi* muscle (LELD), the surface of the LELD was covered with a transparent plastic grid (20 x 15 cm) marked out into squares of one centimeter (cm²) and the number of squares covered by the grid counted to obtain the loin eye area (LEA).

The 10th rib back fat depth (TRFD) was measured at three quarters of the distance of the lumber muscle from the dorsal process of the vertebral column (Lowell et al., 2018), using a digital venier caliper (VonHaus 6" 150 mm Digital Caliper Micrometer Vernier Gauge Tool, UK).

The fat-free index (FFI) was estimated using the following equation, described in procedure 1 for ribbed carcasses (Burson, 2010) and used by Lowell et al. (2018).

$$\text{FFI} = (8.588 + (0.465 \times \text{HCW, lb}) - (21.896 \times \text{TRFD, in}) + (3.005 \times \text{LEA, in}^2)) / \text{HCW, lb} \times 100$$

Where FFI = fat free index, HCW = hot carcass weight, TRFD = 10th rib fat depth, LEA = loin eye area.

Proximate and mineral composition of pork tissues

The heart, kidney, liver, *Longissimus dorsi* (LM), lung and spleen tissues from slaughtered pigs reared on five different diets (D0-D100) were freeze-dried using a Benchtop Freeze Dryer (VirTis AdVantage 2.0, SP Scientific, New York, USA). Dried tissue samples were crushed into powder using a blender (Preethi Trio Mixer Grinder 500W, India). Samples were analyzed following the procedures described by AOAC (1990). The nitrogen content (%) was determined using the Kjeldahl method. The crude protein (CP) was then determined by multiplying the nitrogen content by the factor 6.25. Fat content was determined using the Velp solvent extractor (SER 148/6) with ethyl ether as extractant. Ash content was determined by heating at 550 °C overnight. The organic matter (OM) was then determined by subtracting ash content from 100.

Pork tissues samples were microwave-digested using a mixture of 65% nitric acid, 37% hydrochloric acid and 30% hydrogen peroxide-ACS grade, (Sigma-Aldrich, USA) (Mohammed et al., 2017). Minerals including calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulphur (S), and zinc (Zn) in the digested samples were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES).

Statistical Analysis

Statistical analyses of the data were performed with R software (version 3.5.1) by using a general linear model, which included: diet, sex and their interaction as independent variables for the analysis of ADG, ADFI, FCR, body weight gain (BWG) and final body weight (FBW), fasted body weight, carcass weight, DP, LEA and FFI. For the cut up parts, visceral organs and composition of primal cuts, diet was included in the model as the independent variable. The model for each analysis included all independent variables which were removed one by one until the Akaike Information Criterion (AIC) was at a minimal level. Mean effects were considered statistically significant at $P < 0.05$, with least significant difference test (LSD) as post-hoc test.

Ethical approval

Ethical approval for the study was provided by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO) - Veterinary Science Research Institute (VSRI); approval Code No.: KALRO-VSRI/ IACUC019/30082019.

Results

Growth performance

All pigs showed healthy growth throughout the experimental period. Diet significantly affected ADG (Figure 1). At higher levels of replacement of FM with BSFLM, ADG was higher than obtained for lower levels of replacement of FM with BSFLM. Sex did not affect ADG ($P = 0.30$) and the interaction between diet and sex was not significant ($P = 0.90$). Diet significantly affected BWG (Figure B1). At higher levels of replacement of FM with BSFLM, BWG was higher than obtained at lower levels of BSFLM. Sex significantly affected BWG ($P = 0.027$), but the interaction between diet and sex was not significant ($P = 0.37$).

Neither diet nor sex affected ADFI and also the interaction between diet and sex was not significant (Table 3). FBW differed significantly among diets (Figure 2). At higher levels of replacement of FM with BSFLM, FBW was higher than at a lower levels. FBW differed significantly between boars and gilts ($P = 0.014$), but the interaction of diet and sex on FBW was not significant ($P = 0.13$). Diet significantly affected FCR (Figure 3). At higher levels of replacement of FM with BSFLM, FCR was lower compared to the lower levels of replacement of FM. Sex did not affect FCR ($P = 0.07$) and there was no interaction effect of diet and sex on FCR ($P = 0.72$).

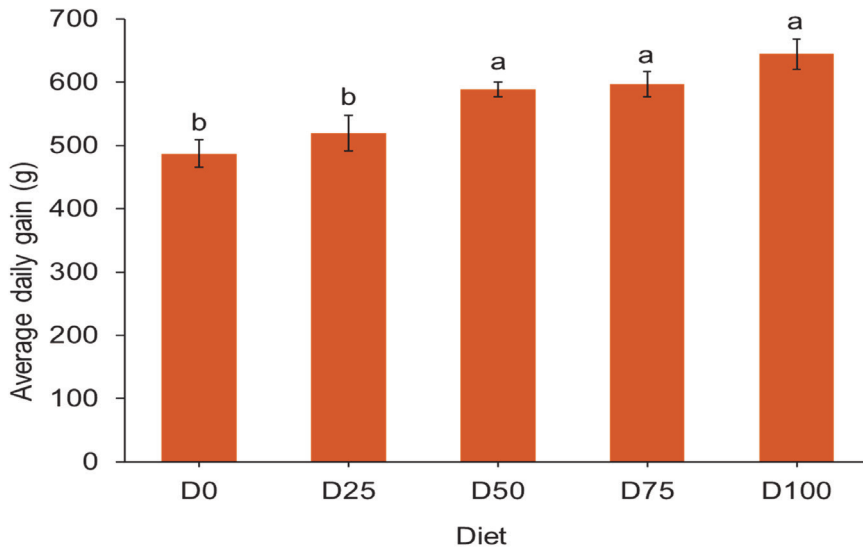


Figure 1. Average daily body weight gain (ADG) in finishing pigs fed BSFLM-based diets and a control diet. Means followed by different letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. LSD = Least Significant Difference test. For each diet, $N = 8$.

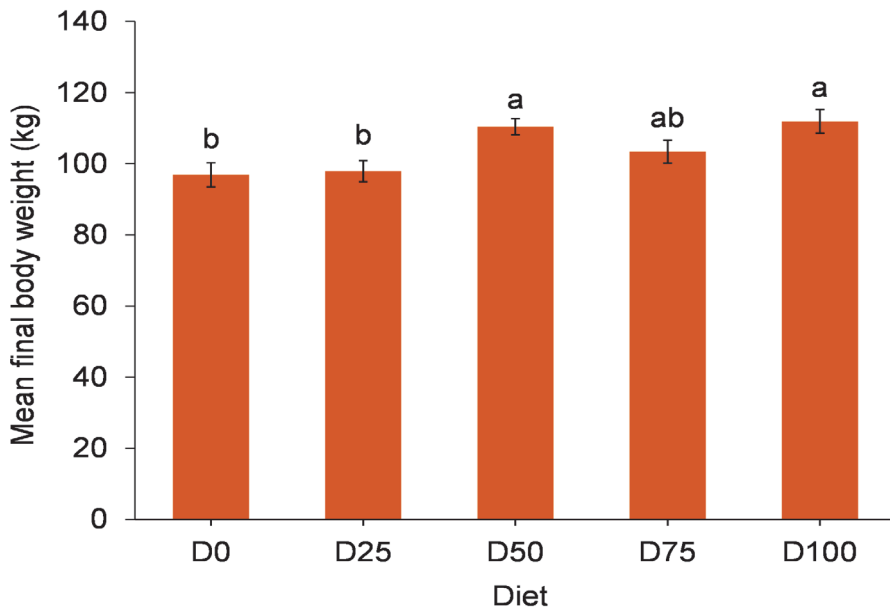


Figure 2. Mean (\pm SE) final body weight of finishing pigs fed BSFLM-based diets and a control diet. Means followed by different letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal, D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, $N = 8$.

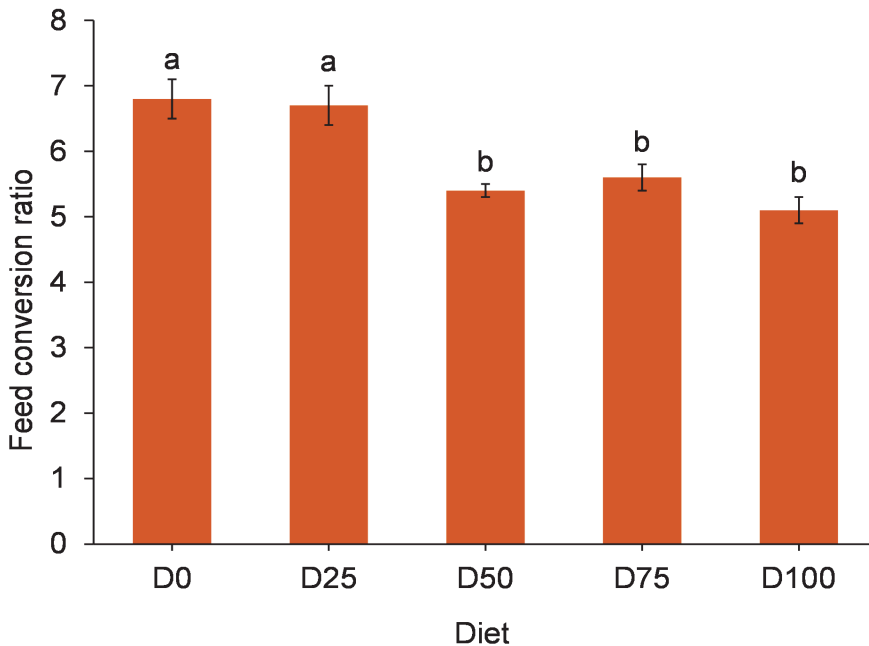


Figure 3. Mean (\pm SE) feed conversion ratio (FCR) for finishing pigs fed BSFLM-based diets and a control diet. Means followed by different letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal, D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, $N = 8$.

Carcass yield

Fasted weight and carcass weight differed significantly among diets (Table 3). Fasted weight and carcass weight were higher at higher levels of replacement of FM with BSFLM than at lower levels of replacement of FM with BSFLM. Sex significantly affected fasted weight and carcass weight (Table 3). There were significant interactions between the effect of diet and sex on fasted weight and carcass weight (Table 3). Dressed percentage (DP) did not differ among dietary treatments (Table 3). Sex did not affect DP, but there was an interaction effect of diet and sex on DP (Table 3). LEA did not differ among diets. Sex did not affect LEA, and there was no interaction effect of diet and sex on LEA (Table 3). Fat free index (FFI) was significantly lower for boars and gilts at higher levels of replacement of FM with BSFLM (Table 3). Back fat depth measured at the 10th rib increased with increased replacement of FM with BSFLM (Figure 4). Overall, back fat depth differed significantly ($P = 0.004$) among diets with higher values for higher replacement rates of FM by BSFLM (Figure 4).

Table 3. Effect of dietary replacement of fishmeal by black soldier fly larval meal on feed intake and carcass yield in finishing pigs

Parameter	Sex	Diets						P value (GLM)	
		D0	D25	D50	D75	D100	Diet	Sex	Diet x Sex
Initial body weight (kg)	Boar	54.1 ± 3.85	56.4 ± 3.61	55.5 ± 4.53	54.8 ± 1.94	57.1 ± 1.14			
	Gilt	56.1 ± 4.43	51.3 ± 2.63	57.1 ± 1.43	49.6 ± 4.61	50.9 ± 1.14			
	Overall	55.1 ± 2.74	53.8 ± 2.28	56.3 ± 2.22	52.2 ± 1.70	54.0 ± 1.40	0.71	0.18	0.47
Average daily feed intake (ADFI) (kg)	Boar	2.75 ± 0.11	2.89 ± 0.05	2.82 ± 0.05	2.78 ± 0.02	2.93 ± 0.01			
	Gilt	2.79 ± 0.11	2.89 ± 0.05	2.87 ± 0.02	2.76 ± 0.09	2.76 ± 0.07			
	Overall	2.77 ± 0.02	2.89 ± 0.02	2.85 ± 0.02	2.77 ± 0.03	2.84 ± 0.03	0.37	0.67	0.48
Fasted weight, kg	Boar	96.1 ± 4.03	104.6 ± 2.77	117.0 ± 3.18	119.4 ± 3.81	129.9 ± 1.56			
	Gilt	99.9 ± 1.60	95.4 ± 2.19	114.1 ± 1.96	105.1 ± 3.78	111.9 ± 2.16			
	Overall	98.0 ± 2.13 ^c	100 ± 2.39 ^c	115.6 ± 1.81 ^{ab}	112.3 ± 3.66 ^b	120.9 ± 3.62 ^a	< 0.001	< 0.001	0.005
Carcass weight, kg	Boar	74.0 ± 3.38	80.9 ± 2.20	90.9 ± 2.51	93.2 ± 3.54	104.7 ± 1.72			
	Gilt	77.6 ± 1.69	75.3 ± 1.22	90.2 ± 1.11	83.4 ± 2.24	84.0 ± 1.61			
	Overall	75.8 ± 1.87 ^b	78.1 ± 1.56 ^b	90.6 ± 1.28 ^a	88.3 ± 2.68 ^a	94.4 ± 4.06 ^a	< 0.001	< 0.001	< 0.001
Dressed percentage, (DP) %	Boar	77.0 ± 1.31	77.3 ± 1.01	77.7 ± 0.34	78.1 ± 1.28	80.6 ± 1.16			
	Gilt	77.7 ± 0.63	79.1 ± 1.40	79.1 ± 0.64	79.5 ± 0.85	75.1 ± 0.87			
	Overall	77.3 ± 0.69	78.2 ± 0.87	78.4 ± 0.42	78.8 ± 0.76	77.9 ± 1.24	0.68	0.92	0.005
Loin eye area (LEA), cm ²	Boar	36.8 ± 2.29	34.5 ± 2.40	32.8 ± 1.75	34.8 ± 0.75	37.5 ± 3.07			
	Gilt	34.5 ± 2.10	32.8 ± 1.11	36.8 ± 1.44	33.5 ± 0.65	34.0 ± 2.12			
	Overall	35.6 ± 1.50	33.6 ± 1.27	34.8 ± 1.29	34.1 ± 0.52	35.8 ± 1.85	0.76	0.44	0.36
Fat free index (FFI), %	Boar	57.8 ± 1.08	55.9 ± 0.92	54.6 ± 0.65	53.4 ± 0.76	53.3 ± 0.63			
	Gilt	56.6 ± 0.78	56.5 ± 0.59	55.1 ± 0.52	54.7 ± 0.25	53.4 ± 1.45			
	Overall	57.2 ± 0.66 ^a	56.2 ± 0.52 ^{ab}	54.8 ± 0.39 ^{bc}	53.3 ± 0.73 ^c	53.3 ± 0.73 ^c	< 0.001	0.62	0.62

Means (± standard error) within a row (overall) followed by different superscript are significantly different, General Linear Model (GLM), P < 0.05, LSD. Overall = boar and gilt data pulled together. D0 = 0%, D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. LSD = Least Significant Difference test. Dressed percentage = Carcass weight / fasted live body weight) x 100. FFI = ((8.588 + (0.465 × Carcass weight, lb) - (21.896 × 10th rib fat depth, in) + (3.005 × LEA, in²))/(Carcass weight) × 100, (Burson, 2010). For each diet 4 boars and 4 gilts were investigated.



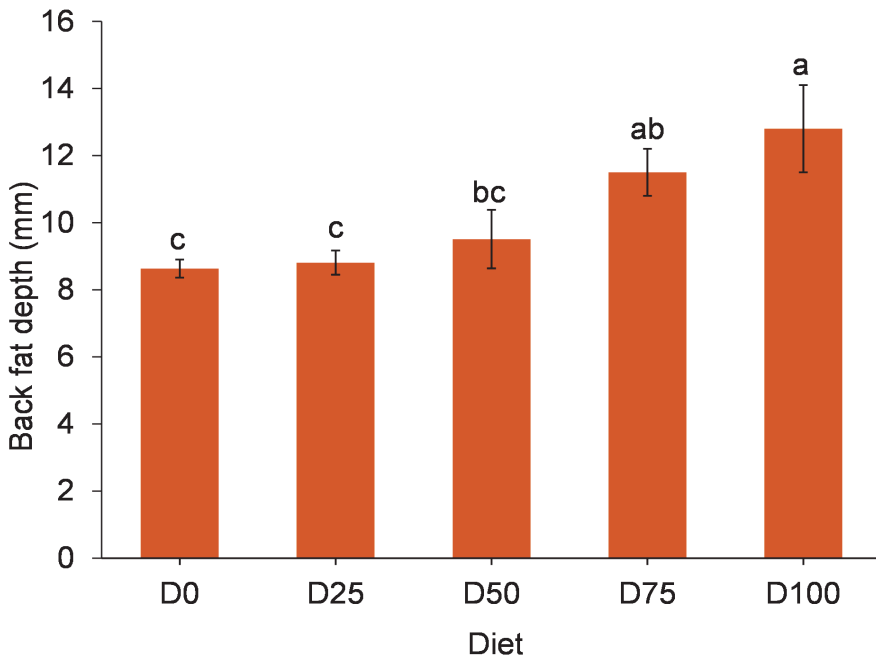


Figure 4. Mean (\pm SE) 10th rib back fat depth in carcasses of finishing pigs fed BSFLM-based diets and a control diet. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. Means followed by different letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal. For each diet, $N = 8$.

Cut-up parts and organ weight

The weight of primal cuts (belly, ham and loin) differed significantly among diets (Figure 5). At higher rates of replacement of FM by BSFLM, primal cuts weighed more than for pigs fed lower levels of BSFLM. The head weight, tenderloin weight, rump weight and tail weight differed significantly among dietary treatments (Table 4). Spleen and testes weighed significantly more when pigs were fed higher levels of BSFLM than those fed lower levels of BSFLM, whereas, the heart, liver, kidneys, lungs and ovaries had similar weights across dietary treatments (Table 4). Diet significantly affected the amount of fat-skin in primal cuts (Figure B2). At D100, fat-skin was higher compared to D0, except in the shoulder cut for which similar values were recorded (Figure B2). The ham had the lowest FS (Figure B2). In ham, shoulder, loin and belly, the weight of muscle and bone tissues was similar among diets, except at D100 where bones from the loin weighed less compared to the rest of the dietary treatments (Table B1).

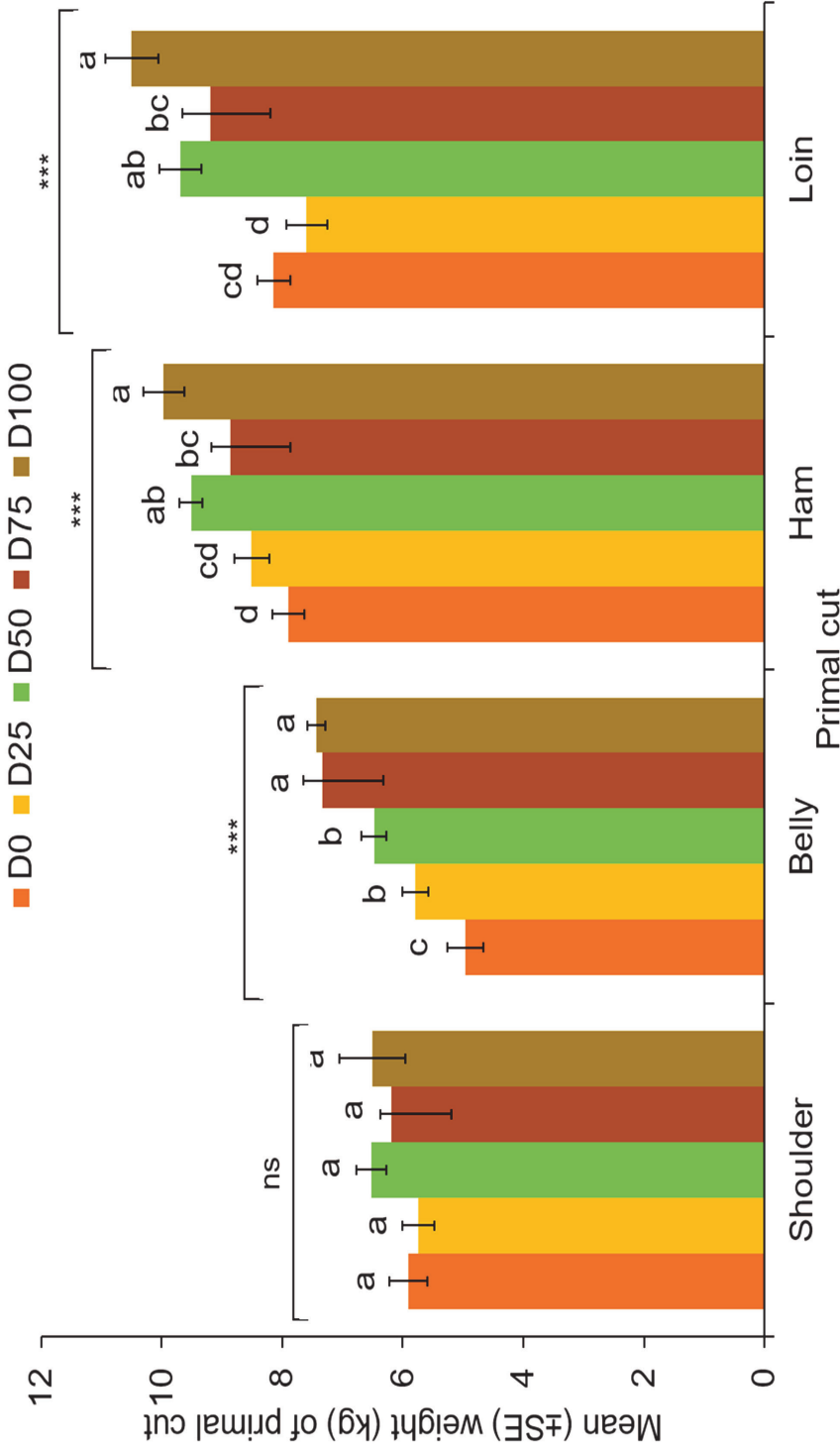


Figure 5. Mean weight (\pm SE) of primal cuts of half carcass in finishing pigs fed BSFLM-based diets and a control diet. Means within each primal cut followed by different letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. Significance level: ***: $P < 0.001$, ns: not significant. For each diet, $N = 8$.

Table 4. Effect of dietary inclusion of black soldier fly larval meal (BSFLM) on cut-up parts of carcasses and visceral organs in slaughtered pigs

Parameter	Diets				P value (GLM)	
	D0	D25	D50	D100		
	Cut-up parts (kg)					
Head weight,	6.27 ± 0.13 ^b	6.25 ± 0.25 ^b	7.26 ± 0.22 ^a	7.07 ± 0.22 ^a	6.89 ± 0.35 ^{ab}	0.014
Collar butt weight	3.86 ± 0.13	3.88 ± 0.14	4.15 ± 0.19	3.99 ± 0.24	3.70 ± 0.31	0.67
Tenderloin weight	0.61 ± 0.05 ^c	0.69 ± 0.05 ^{bc}	0.78 ± 0.04 ^{ab}	0.82 ± 0.04 ^a	0.81 ± 0.02 ^a	0.003
Rump weight	2.34 ± 0.16 ^d	2.69 ± 0.09 ^{cd}	3.34 ± 0.15 ^{ab}	2.94 ± 0.15 ^{bc}	3.60 ± 0.29 ^a	< 0.001
Trotters weight	1.67 ± 0.05	1.70 ± 0.07	1.90 ± 0.07	1.85 ± 0.07	1.90 ± 0.10	0.08
Tail weight	0.18 ± 0.01 ^c	0.18 ± 0.02 ^c	0.25 ± 0.01 ^b	0.28 ± 0.02 ^{ab}	0.30 ± 0.02 ^a	< 0.001
	Organ weight (kg)					
Heart weight	0.37 ± 0.02	0.40 ± 0.03	0.46 ± 0.04	0.44 ± 0.02	0.43 ± 0.03	0.09
Liver weight	1.62 ± 0.07	1.47 ± 0.07	1.63 ± 0.06	1.50 ± 0.04	1.67 ± 0.06	0.12
Kidneys weight,	0.33 ± 0.02	0.29 ± 0.02	0.34 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.17
Lungs weight	0.74 ± 0.06	0.64 ± 0.04	0.67 ± 0.04	0.67 ± 0.04	0.72 ± 0.07	0.69
Spleen weight	0.13 ± 0.01 ^c	0.13 ± 0.01 ^c	0.17 ± 0.01 ^a	0.16 ± 0.01 ^{ab}	0.17 ± 0.01 ^a	0.017
Testes weight	0.82 ± 0.08 ^c	1.03 ± 0.05 ^b	1.12 ± 0.03 ^{ab}	1.24 ± 0.08 ^a	1.31 ± 0.06 ^a	<0.001
Ovaries weight	0.02 ± 0.004	0.02 ± 0.007	0.02 ± 0.005	0.02 ± 0.002	0.02 ± 0.001	0.59

Means (±standard error) within a row followed by different superscripts are significantly different, General Linear Model (GLM), $P < 0.05$, LSD. D0 = 0%, D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, $N = 8$.

Linear and live body measurements

The PCA revealed two main groups of highly correlated sets of variables, which explained 53.98% of the total variance (PC1 = 41.54%, PC2 = 12.44%) (Figures B3 and B4). First, the variables HdW, BH, Hlb, RH, BaH, BW, BL, FC, HG, Flb, TD and NC were positively correlated. Secondly, HW and SW were positively correlated, but negative correlated with EW. Of all these variables, RH, BaH, BW, FC and TD showed the highest contribution to the PC, with BW closely related with BaH, FC, RH and HG (Figure B3). A cluster plot of PC1 and PC2 shows an overlap between pigs fed D0 and those fed D25, D50, D75 and D100, although the degree of overlap between D0 and D100 is smallest (Figure B4).

Proximate and mineral composition of pork tissues

CP content differed significantly among diets (Figure 6). At D50, overall CP content was higher compared to other dietary treatments investigated. Tissue type significantly affected CP ($P < 0.001$). The interaction effect of diet and tissue type on CP content was significant ($P < 0.0001$). The average CP content ranged between 65% (heart tissue) and 93% (lung tissue) (Figure 6). The heart and LM tissues from pigs fed insect-based diets (D25, D50, D75 and D100) had higher crude fat contents than those fed the control diet (D0) (Figure 6). OM content differed significantly among diets (Figure 6). Overall, tissue samples at D100 had higher OM content than the other diets investigated. Tissue type significantly affected OM ($P < 0.001$). Water content was only slightly affected by diet type. Overall, diet type had no significant effect ($P = 0.554$) on water content of tissues, whereas there was a significant effect ($P < 0.0001$) of tissue type on water content. Liver and LM tissues from pigs fed D25, D50, D75 and D100 had significantly higher ($P < 0.0001$ and $P = 0.012$, respectively) water contents than samples from pigs fed D0, whereas the water content of heart, kidney, lung and spleen tissue samples were not influenced by diet type ($P = 0.141$, $P = 0.592$, $P = 0.462$ and $P = 0.289$, respectively) (Figure 6). There was no significant interaction between diet and tissue type on water content ($P = 0.167$).

K, P, S and Na were the major macro-minerals detected in all tissues across all diets. In heart, lung, LM and spleen tissues, K was the most abundant macro-mineral followed by P and S. Kidney tissues exhibited similar levels of K and P, while in liver tissues, P was the most abundant mineral (Figure 7). Spleen, liver and LM tissues from pigs fed BSFLM-based diets had higher concentrations of K than those from pigs fed the control diet (D0), except in the heart where D25 and D100 had lower K contents (Figure 7). In all tissues, Ca had the lowest concentrations among the macro-minerals detected. Among micro-minerals, Fe was most abundant in all tissues, except LM in which Zn had the highest concentration.

Overall, Fe and Zn were the most abundant micro-minerals followed by Cu (Figure 8). The LM tissue from pigs fed D100 contained higher concentrations of Fe and Zn than those fed D0 (Figure 8).

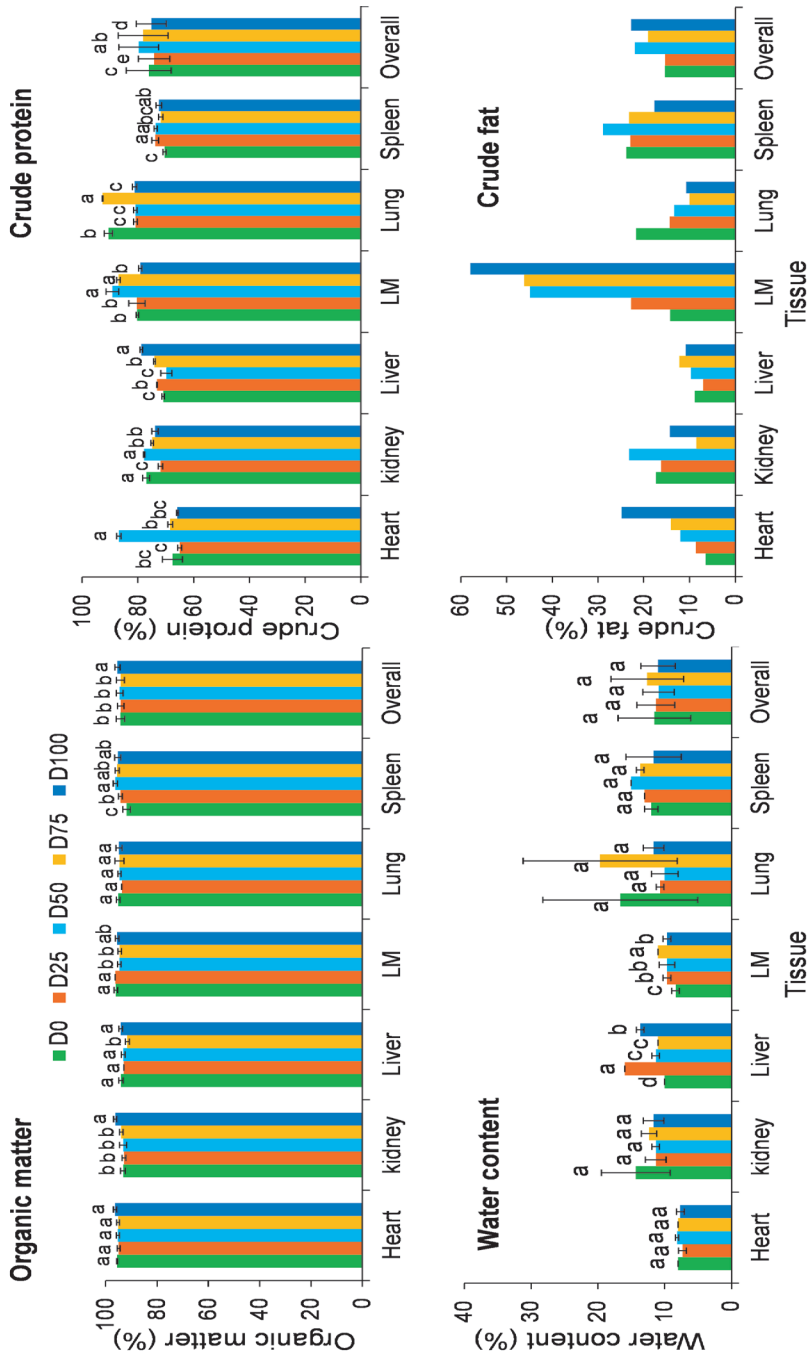


Figure 6. Proximate composition (% dry matter basis) (mean \pm SD) of organ and muscle tissues of finishing pigs. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. LM = *Longissimus dorsi* (loin muscle). Bars followed by different letters are significantly different (GLM, $P < 0.05$; LSD). Bars for crude fat represent mean value of two subsamples analysed. No statistical analysis was performed on the crude fat data.



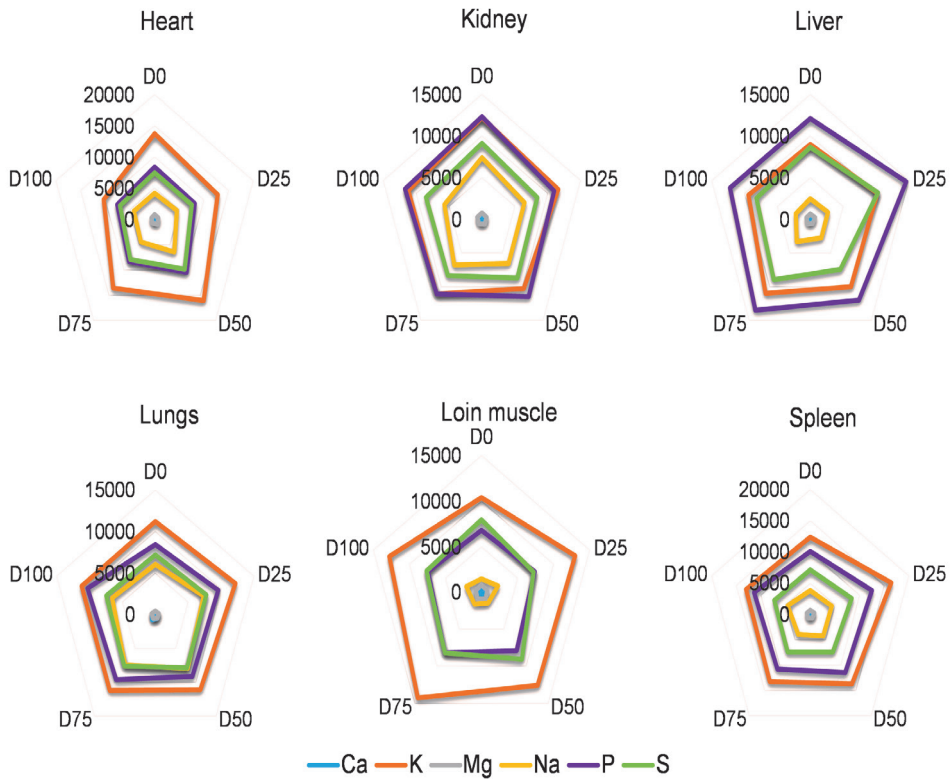


Figure 7. Composition (mg/kg) of macro-minerals in muscle and organ tissues of slaughtered pigs fed BSFLM-based diets and a control diet. BSFLM = black soldier fly larval meal. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. Ca = calcium, K = potassium, Mg = magnesium, Na = sodium, P = phosphorus, S = sulphur. Values presented are results of single analyses. Loin muscle = *Longissimus dorsi*.

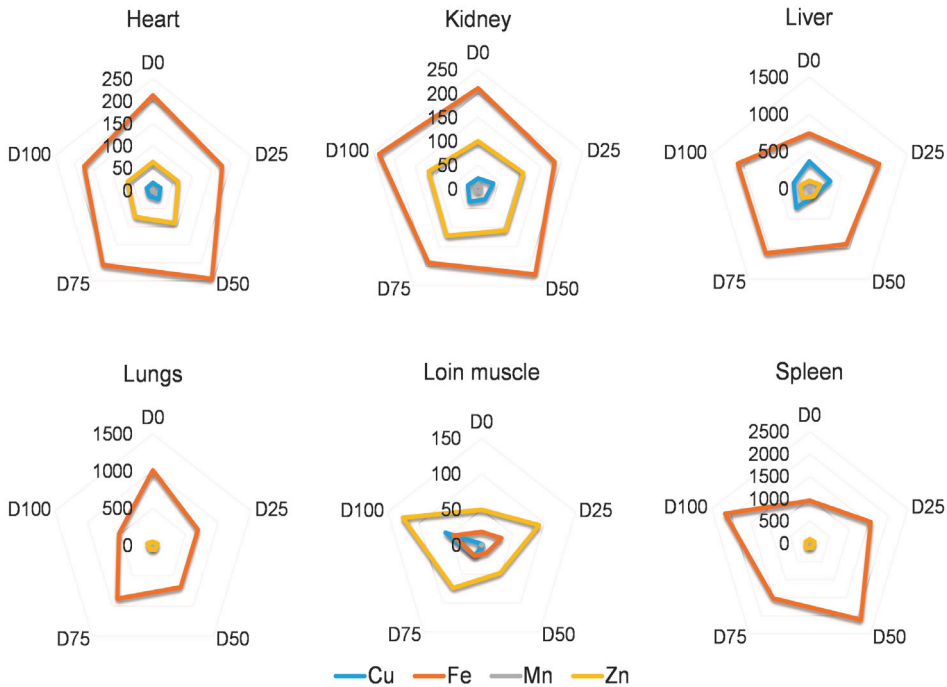


Figure 8. Composition (mg/kg) of micro-minerals in the muscle and organ tissues of pigs fed BSFLM-based diets and a control diet. BSFLM = black soldier fly larval meal. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. Values presented are results of single analyses. Cu = copper, Fe = iron, Mn = manganese, zinc (Zn). Values presented are results of single analyses. Loin muscle = *Longissimus dorsi*.

Discussion

Black soldier fly larvae are an alternative source of animal protein and have been used in aquaculture, poultry and piglet feeds to replace either FM or soybean meal (Makkar et al., 2014). The present study used partial or complete dietary replacement of FM by BSFLM to demonstrate for the first time the value of BSFLM on growth performance, carcass traits and nutritional value of finishing pigs. Identifying BSFLM as a feed ingredient that guarantees high-quality pork products will not only help to limit dependence on the traditional feed protein sources, but will also provide an alternative protein source for farmers in times of scarcity.

Most importantly, our results have revealed important positive effects of the insect-based diets on growth performance, feed conversion and carcass traits of finishing pigs. Our study shows that higher levels (50-100%) of replacement of FM with BSFLM, resulted in higher body weight, higher feed conversion and higher carcass yield than the FM-based control diet (D0). Pork by-products had high (65-93%) CP. The proportion of

fat in the primal cuts was affected by diet while the muscle and bone tissues were not (except in the loin).

Previous studies had shown that inclusion of dried BSFLM in replacement of soybean meal, FM or dried plasma, either did not affect or positively influenced piglet growth performance parameters (Biasato et al., 2019; Newton et al., 1977; Newton et al., 2005; Spranghers et al., 2018). Moreover, replacing soybean meal by BSFLM in finishing pig feed results in beneficial changes in the microbiota and metabolites of the colonic digesta and mucosal immune gene expression in finisher pigs (Yu et al., 2019). For instance, replacing soybean meal by BSFLM in diet increases the abundance of health-promoting *Lactobacillus spp.*, decreases the abundance of pathogenic *Streptococcus spp.* and upregulated the expression of anti-inflammatory cytokines in finishing pigs (Yu et al., 2019). Our data provide the first insight into the potential of BSFLM as a nutrient-rich feed ingredient to support maximal lean gain towards attending market weight in finishing pigs. Our data show that BSFLM results in better performance of finishing pigs than when fed with conventional feed based on FM.

The improved performance in the present study is a clear demonstration of feed tolerance in the pigs, a balanced nutrient content and high digestibility of the insect-based diets, which led to optimal feed efficiency and body weight gain. In the literature, studies under other feed regimes showed that pig growth highly depends on the nutrient content of their diet. A protein-rich diet with a balanced amino acid profile leads to high performance (Kim et al., 2005; Nam et al., 1995). Feed intake and quality play essential roles in animal growth performance (Patience et al., 2015). The improved growth performance of pigs with increasing levels of dietary replacement of FM with BSFLM in the present study can be attributed to increased palatability of the diets which resulted in sufficient consumption of digestible nutrients, particularly protein, which support rapid growth (Esonu et al., 2002). This conclusion is supported by the increased ADG, BWG, FBW and low FCR for BSFLM-based diets that we recorded.

Pig market weights vary considerably depending on the region, consumer preference or the processing method of the pig when slaughtered. For instance, in Kenya, the recommended PMW ranges between 90 and 100 kg live body weights according to Farmer's Choice Ltd., the main pig market provider in the country (<http://farmerschoice.co.ke/>). In the present study, the average fasted live body weight of pigs at slaughter ranged between 98 and 121 kg. The weight range in our study falls within the recommended ranges for most other countries (Kim et al., 2005). It is worth noting that pigs fed diets with FM fully replaced by BSFLM had the highest fasted live body weight of 121 kg while pigs fed the control diet without BSFLM had the lowest live fasted body weight of 98 kg at slaughter. This corresponds to the higher ADG recorded for pigs at 100% BSFLM and demonstrates that the replacement of FM with BSFLM in pig feed contributes to faster weight gain, which implies that pigs attain the market weight faster

compared to pigs raised on control diets. This is also supported by the low feed conversion ratio values recorded for BSFLM-based diets in this study.

In Kenya, Farmer's Choice recommends and accepts pigs with market sizes ranging from 40 to 120 kg cold carcass weight. Carcasses within this range are either consumed locally, processed into other pork products or exported whole to other countries. In the present study, the overall carcass weight of pigs ranged between 76 and 94 kg, which is well within the recommended range for pork in Kenya. In our study, pigs fed BSFLM-based diets produced heavier carcasses than those fed the control FM-based diet. Carcasses from all dietary groups had similarly well-formed loin eye, which is one of the quality control features for commercial pork. Therefore, BSFLM is a suitable alternative to FM in commercial pig feed formulation for the Kenyan and international markets.

Daily fat intake is essential for humans because fat provides energy, enhances food palatability and absorption of vitamins (Seong et al., 2014). The fat content of pork tissues in our study was generally higher than values reported in the literature, which could be due to differences in the feeding regime, breed, body weight at slaughter, or processing method of the meat samples (De Lange et al., 2003; Okrouhla et al., 2009; Park et al., 2012; Seong et al., 2014; Skobrák & Bodnár, 2012; Valaitiene et al., 2017; Williams, 2007). The full-fat BSFLM used in our study might have contributed to the crude fat content of the pork tissues. For example, the overall result of fat content shows that pork tissues from pigs fed diets with 50-100% replacement of FM with BSFLM had higher crude fat levels than those from pigs fed diets with 0-25% replacement of FM with BSFLM. Moreover, the high fat content in the LM is consistent with the significantly higher value of the subcutaneous fat of the loin cuts recorded for pigs fed D100.

Although the pigs in the control group had a significantly higher fat free index than in the D100 group, the fat free index values were generally above 50% across all dietary groups, which is an indication of the suitability of BSFLM for finishing pig growth and carcass quality. However, it would be necessary to evaluate this parameter under restricted feeding regimes, because unlimited access to feed and high body weight as in this study might have contributed to an increased fat deposition, consequently impacting the fat free index in pigs in the D100 group (Kim et al., 2005).

As mentioned above, a good fat-free index value above 50% was recorded in the present study, indicating that BSFLM is suitable for both growth and lean quality of pigs, although the 100% BSFLM diet showed a lower value compared to the FM-control diet. Fat content is one of the desirable attributes of meat quality used to assess suitability of meat for retail or further processing and is mainly affected by feeding strategy (Rosenvold & Andersen, 2003). In pigs, many dietary components are absorbed from the feed in the intestines into the muscle and fat tissues, which subsequently affect pork quality (Rosenvold & Andersen, 2003). In the present study, the belly had the highest fat

content among the primal cuts, which is similar to that reported by Dunshea & Souza, (2003), who indicated that the belly of pigs is one of the major parts of the body where fat deposition takes place. Furthermore, the back fat depth of pigs fed FM-based diets in the present study was significantly lower than obtained for BSFLM-based diets especially at 75 and 100% levels of replacement of FM with BSFLM. This may have resulted from the high fat content of the BSFLM-based diets used.

The content and distribution of fat in various primal cuts are important characteristics of meat for various market segments (Dunshea & D'souza, 2003). In the present study, ham, loin and belly of pigs fed D100 had significantly more fat than in pigs fed the control diet (D0), which can be due to the high fat level of BSF (Wang & Shelomi, 2017). Furthermore, the subcutaneous adipose tissue is the major site of fat deposition in pigs and fat deposition could result from consuming high-carbohydrate diets or pre-formed fats in diets (Dunshea & D'souza, 2003). Live body weight is another factor that affects fat deposition in farmed animals (Dunshea & D'souza, 2003). In the present study, pigs fed D100 had higher fat content in primal cuts and were heavier than pigs fed D0, which is in line with that of the studies described by Dunshea & D'souza (2003).

In the present study, K and P were the most predominant macro-minerals in pork tissues, whose role in human nutrition has been well documented (Gupta & Gupta, 2014; He & MacGregor, 2008). Micro-minerals, which are essential for proper cell functioning were also abundant in pork tissues. The high concentration of minerals in pork tissues in the present study indicates that BSFLM can be considered as a valuable component in pig feed formulation with no adverse effects on nutritional quality of the meat.

In pig production, feed represents an important input. The efficiency with which pigs utilize dietary nutrients for growth, maintenance, lean gain and lipid accretion is crucial and has economic implications for individual and industrial production (Patience et al., 2015). Feed conversion ratio is an important measure of feed efficiency in animal production. A low FCR implies that less feed is required to produce a unit of pork weight, thereby leading to less feed required in a production system. This impacts profitability, feed demand and the competitive position of a feed ingredient against other feed sources (Patience et al., 2015). Our data show a significant reduction in FCR at higher levels of replacement of FM with BSFLM, implying that BSFLM-based feed can reduce feed cost in terms of quantity demanded in a finishing pig production. This reduction in FCR positively correlated with body weight gain in the pigs, thus further increasing the value of BSFLM in feed.

Live body weight of farmed animals is the most important growth and economic trait used by livestock producers and processors of animal products in sub-Saharan Africa to inform farm management decisions. Smallholder farmers, however, can barely afford the expensive weight scales, especially for heavier animals (Birteeb et al., 2015). Simple

linear body measurements are therefore, necessary. In the present study, PCA identified the most important variables which were affected by diet. The correlation between live body weight and the linear body measurements indicates that these body traits can be used to predict body weight of pigs in situations where this parameter cannot be measured directly as has been reported previously for pigs and other livestock (Adeola et al., 2013; Machebe & Ezekwe, 2010).

Conclusion

Our study clearly demonstrates that BSFLM is a suitable alternative to FM in feed of finishing pigs. This study is unique in its approach as it presents a comprehensive dataset that combines growth performance, carcass traits and nutritional quality of pigs fed BSFLM-based feeds for the first time. Replacement of FM with BSFLM at 50 - 100% in pig feeds clearly demonstrated a significant increase in feed conversion, growth performance (body weight gain), carcass traits and meat quality of finishing pigs. Our results, therefore, do not only provide important information for novel insect-based feeds for pig production industries but also provides information on meat nutritional quality for human consumption. Thus, these research advances hold important opportunities for addressing the increasing scarcity of protein-rich feeds, which is an impediment in the pig value chain. This will facilitate the creation of employment and open new economic opportunities for income generation among the resource-constrained groups such as women and youths.

Acknowledgments

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Chapter 9

General Discussion

Introduction

While the global human population is expected to approach 10 billion people by 2050, more than 50% of the increase will take place in the developing world, particularly Africa (United Nations, 2017). This growth in the global human population together with rising incomes, urbanization and shifts in diets drive an increasing demand for protein-rich animal products such as meat, eggs, fish, and milk (Van Huis, 2013). Although a growth in the demand for these products is expected to support human health, the aquaculture and livestock industries face challenges related to the availability of feeds and feed ingredients (Ranganathan et al., 2016). As a result, researchers, policy makers and commercial producers are looking for alternative ways to meet this demand, while minimizing adverse impacts on the environment. In this regard, insects which have long been part of the natural diet of humans and animals are increasingly considered as a potential novel protein source for animal feed production (Van Krimpen & Hendriks, 2019).

The growing interest in insects as feed ingredients can be explained by their high nutritional quality, high reproductive rate, high feed conversion efficiencies and ability to exploit a wide variety of food substrates, including organic side streams (Ooninx et al., 2015; Ortiz et al., 2016; Van Huis, 2013). While several insect species possess these features, the black soldier fly (BSF) *Hermetia illucens* L. (Diptera: Stratiomyidae) is considered to be the most suitable species for animal feed (Makkar et al., 2014; Van Huis et al., 2013; Veldkamp et al., 2012). As mentioned in Chapters 1 and 2 of this thesis, the BSF is not a pest, it is not considered to transmit diseases and the larvae can upcycle various organic side streams by converting them into high-quality biomass (Cickova et al., 2015). To exploit the potential of this insect and produce much-demanded sustainable alternative proteins, the capacity to produce the required quantities is crucial. Despite the growing interest in insects as feed, large-scale insect farming is still novel. Insect rearing at industrial scale is the most important means to produce the required quantities (Van Huis et al., 2015). Little is known about availability and suitability of substrates on which insects are reared. An understanding of these aspects is essential for the production of high-quality larvae for feed as well as scalability of the production system (Gold et al., 2018; Ortiz et al., 2016). Insects convert their feed to high-quality biomass more efficiently than livestock animals (Van Huis et al., 2013), but without the right environmental conditions, their feeding, growth and development rates are adversely affected. For example, temperatures below or above the optimum threshold will reduce insect performance and productivity (Ortiz et al., 2016). In captivity, insects are generally unable to locate the right food and maintain optimum ambient temperatures. Therefore, these conditions must be provided to ensure optimal growth and overall farm productivity. Insects are naturally eaten by fish and some livestock, and their high

nutrient content makes them suitable for animal feed. So far, poultry, fish and to a lesser extent, pigs have been targeted in feeding trials involving insect meal, especially BSF larval meal (BSFLM) (Makkar et al., 2014). Little is known regarding the use of BSFLM on pig performance.

The main objective of this thesis was to assess the potential of BSF larvae as animal feed component and to investigate farmers' willingness to accept insect-based feed for animal production in Kenya. In the following sections, I discuss the possible implications of the results presented in the different chapters of this thesis. As indicated in Chapter 1, the establishment and sustainability of an insect for feed sector will depend on: 1) market acceptance of and demand for insect-based feed, 2) availability of suitable substrates for mass rearing insects as feed, 3) suitable environmental conditions for insect development and reproduction, 4) nutritional quality of the insects to meet nutritional requirements of the consuming animal species and, 5) the effect of insect meal on animal growth performance and resulting animal products.

Willingness to pay for insect-based feeds in Kenya

Recently, the use of insects as a sustainable alternative to the resource-intensive and increasingly unaffordable fishmeal (FM), and soybean meal in animal feed has received considerable interest due to their high nutritional value, low environmental footprint in terms of land and water requirements and greenhouse gas emission as well as their high conversion efficiencies (Ortiz et al., 2016; Van Huis, 2013; Van Huis et al., 2013). It is worth noting that the development of a new market commodity is conditional upon the acceptance and use by the target consumer (Alemu et al., 2015). Therefore, assessing the willingness to pay (WTP) is important because it provides an indication of the potential demand for a new market product prior to production.

In Chapter 3, we assessed knowledge, attitude and WTP for insect-based feeds among poultry, pig and fish farmers in Kenya. This research demonstrated that in the study areas, farmers are aware of insects as feed and consider insects as a good source of protein. Furthermore, farmers accept and are willing to pay more for insect-based feed. Therefore, the results not only indicate that there is a demand for insect-based feed, but also serve as a baseline for future investigations. The high WTP for insect-based feeds recorded among farmers in this study is in line with a report by Van Huis (2013), who stated that the inclusion of insects in feed for aquaculture and aviculture production will not be considered a problem by consumers. This willingness may be considered as a reflection of the need for suitable alternatives to the conventional protein sources for feed. This finding corresponds to the positive attitudes of the farmers with respect to insects as a good feed ingredient as well as their willingness to pay more for insect-based feed in this study.

The acceptance of the different insect species in this study shows a similar pattern for poultry, pig and fish farmers and appears to be based upon the common knowledge on insects. For example, the BSF, which is relatively new and considered as a novel insect species for feed had the lowest percentage acceptance compared to termites, grasshoppers and crickets, which are widely known and eaten by humans. To support the implementation of insect-based feed in Kenya and elsewhere in Africa, farmer education on the different insect species for feed and their potential in terms of production and nutritional value is therefore crucial. Furthermore, the high WTP for insect-based feed raises the need for the development of training programs to build farmers' capacity to rear and market insects as a feed ingredient in Kenya. This is highly recommended because farmer participation, including both men and women, will increase local production and availability of insect ingredients and facilitate the establishment of intensive insect-based agribusiness enterprises in Kenya and beyond. Small scale insect farming through inclusive business models can strengthen local economies through income generation from increased production and sell of the insects to local livestock farmers as well as feed millers (Chapter 2).

In future intervention strategies, the incorporation of insect-based feed into aquaculture and livestock nutrition is relevant and will have a greater impact if the government, non-governmental organizations, research organizations and farmer organizations are involved in the awareness technology transfer campaign in Kenya and Africa at large. Most importantly, future intervention strategies must ensure that the demand for insect-based feed can be met, which means that consumers need to know where to obtain insect-based products. This therefore calls for raising general awareness of edible insects as feed to widen the market reach of the new insect-based feed sector.

Agro-industrial by-products as substrates for rearing BSF larvae

Brewers' spent grains (BSG) are a highly valuable by-product commonly used as feed for ruminant livestock, but have a poor feeding value for growing pigs and poultry (Waldron, 2009). Moreover, the high moisture content of BSG makes it an excellent medium for the growth of bacteria, yeast and fungi. This limits its maximum storage duration to less than a week in warm climates (Heuzé et al., 2017). Alternative uses of these substrates are therefore necessary. On the other hand, insect farming can provide an alternative protein-rich feed resource for aquaculture and non-ruminant livestock production. Artificial diets or feed meant for other livestock have successfully been used to produce BSF larvae (Nguyen et al., 2015; Sheppard et al., 2002). However, sustainability in insect-based feed production can only be achieved if insects are reared on

organic side streams that are no longer fit for human food or livestock feed (Bosch et al., 2019; Veldkamp et al., 2012). Agro-industrial processes generate large quantities of by-products which can support insect production (Meneguz et al., 2018). Therefore, Chapter 4 of this thesis assessed the effect of combining BSG with brewer's yeast, and cane molasses as larval diets on the life-history parameters of the BSF.

The BSF larvae were found to successfully grow and reach the pre-pupal stage in all substrate combinations investigated (Chia et al., 2018b). Most importantly, larval survival was high across substrates and the development time of the larvae was comparable to values reported by Gligorescu et al. (2018) and shorter than values reported by Myers et al. (2008). This shows that these by-products are suitable substrates for rearing BSF larvae.

Larvae reared on substrates supplemented with brewer's yeast at 20-30 °C (Chia et al., 2018a) had significantly shorter development time and higher larval weight than those reared on un-supplemented substrates at the same temperature. This indicates that brewer's yeast had improved substrate quality such as palatability that led to increased food intake, resulting in faster growth of larvae. Larval food intake was, however, not measured in the present study and is regarded as an important topic for future investigation (Bosch et al., 2019). Furthermore, previous studies showed that nutrient imbalance and low temperatures resulted in slower development of BSF (Barragan-Fonseca et al., 2019; Gligorescu et al., 2018; Harnden & Tomberlin, 2016; Tomberlin et al., 2009).

These findings have implications in the production of high-quality BSF larvae as an alternative to FM and soybean meal in aquaculture and livestock production. The suitability of BSG for rearing BSF larvae has been reported (Meneguz et al., 2018), but no study has so far assessed the effect of combining BSG with brewers' yeast or molasses. These results therefore demonstrate for the first time that these by-products, which are abundantly generated by the Kenyan brewery and sugar companies are valuable substrates for rearing BSF larvae. These data reveal an innovative strategy in solving important environmental and economic problems. Firstly, BSF larvae can be used in degrading brewery waste streams to reduce unpleasant smells emanating from abandoned solid wastes and composts, thereby reducing environmental pollution. Secondly, the BSF larvae convert these low-grade by-products, which are available throughout the year, accessible, and less competitive for food or feed to nutrient-rich insect larvae as an alternative protein ingredient for animal feed formulation.

Temperature and insect development and reproduction

Temperature is one of the most important abiotic factors that affect cold-blooded organisms, which cannot regulate their body temperature that controls the rate of biological processes. Lower and upper temperature thresholds, and the optimum temperatures have implications for developmental processes, where within a specific range, a temperature change results in an increase or decrease in the rate of development (Mirhosseini et al., 2017; Wagner et al., 1984). An understanding of temperature thresholds has important implications for establishing insect populations, whose activities occur in the context of daily and seasonal fluctuations in temperature (Best et al., 2012).

Insects are cold-blooded animals and their internal body temperature varies depending on the ambient temperature. The normal activity of an insect species occurs within a narrow zone, below or above which the temperatures may become lethal and this varies depending on the species (Dreyer & Baumgartner, 1996; Infante, 2000; Ju et al., 2011; Ratte, 1984; Taylor, 1981; Yang et al., 1994). Although temperatures below or above the zone of normal activity may not be sufficiently extreme to kill the insect, the surviving insect fails to achieve optimum reproduction to maintain its population (Ju et al., 2011). In the case of farming insects for feed, this will greatly impact productivity. Therefore, Chapter 5 of this thesis studied BSF larvae at nine constant temperatures (10 – 42 °C) and determined their growth rate and reproductive potential.

The results show that when BSF larvae were reared at 30 °C, the development time was significantly shorter than obtained at lower or higher temperatures. Furthermore, the population growth rate of the BSF was higher at 30 °C with higher intrinsic rate of natural increase and shorter doubling time compared to the other temperatures investigated (Chia et al., 2018a). The low intrinsic rate of natural increase, higher doubling time and longer mean generation time of BSF at 20 and 25 °C might have been caused by reduced metabolic rates which led to a decrease in food intake and, hence, slowing growth and the reproductive potential of the insect (Clarke & Fraser, 2004; Gligorescu et al., 2018). Temperature affects the rate of metabolism which controls resource uptake from the environment and energy allocation for growth, development, reproduction and excretion (Brown et al., 2004). Threshold and thermal requirements have been determined for crop insect pests to predict their activities in the field and implement control measures that match with target insect life stages (Dahi et al., 2017; Padmavathi et al., 2013; Rao et al., 1989), but little is known about threshold temperatures of BSF. Information on optimal temperature is vital for large scale, year-round production. Therefore, our findings are valuable for colony maintenance and optimization of commercial rearing procedures of BSF under variable temperature conditions. The estimated thermal thresholds could be used to predict BSF growth activity in rearing facilities

for their effective management.

Our study provides evidence to support the silver-spoon hypothesis, which states that development under favourable conditions will lead to better performing adults under all adult conditions (Minias et al., 2015; Scharf et al., 2015; Song et al., 2018).

Nutritional value of BSF larvae

One of the reasons for the growing interest on insects as an alternative feed ingredient in animal feed is their favourable nutritional composition (Van Huis et al., 2013; Yin et al., 2017). The nutrient composition of insects varies considerably across species and is influenced by the substrates used in rearing (Meneguz et al., 2018; Spranghers et al., 2017). In Chapter 6, BSG supplemented with brewer's yeast, cane molasses or water were fed to BSF larvae. The nutritional value of edible insects can be assessed by their chemical composition, alongside their digestibility within the consuming species, and their nutrient requirements Makkar et al. (2014). The digestibility of BSF larvae and the nutritional requirements of livestock fulfilled by the larvae were not determined in this thesis, which might be interesting in future investigations. However, larvae were rich in protein and fat, with excellent amino acid and fatty acid profiles respectively, and minerals, all vital nutrients for animal growth performance. The results revealed significant effects of both the BSG and the supplements on the nutrient composition of the larvae. Larvae reared on substrates supplemented with brewer's yeast generally had higher crude protein contents than those reared on substrates supplemented with brewer's yeast plus molasses or supplemented with water. Also, larvae reared on substrates supplemented with brewer's yeast plus molasses were higher in crude fat compared to larvae reared on other substrates investigated in this study. Therefore, the nutritional composition of the BSF larvae can be enhanced through supplementation of BSG to produce protein- and fat-rich BSF larvae. For use as a feed ingredient, the protein content and the amino acid profile of the larvae are important. The crude protein content of BSF larvae ranged between 30 and 46%, which is comparable to values reported in the literature (Lalander et al., 2019; Liland et al., 2017). The BSF larvae in this study contained the essential amino acids lysine and methionine, which are the major limiting amino acids in livestock diets (Farkhoy et al., 2012). This favours the inclusion of BSF larvae as nutritious and suitable ingredients in animal feed.

Calcium (Ca) and phosphorus (P) constitute the major part of the mineral content of bones such that a deficiency or an excess of one affects the proper utilization of the other. These two often appear in limited quantities in common livestock feedstuffs (Mc-dowell, 2003). In this thesis, the Ca/P ratio ranged from 0.8 to 2.3, which largely meets the requirements of poultry, fish and pigs (Andrews et al., 1973; Reinhart & Mahan, 1986; Sakamoto & Yone, 1973; Tschirner & Simon, 2015; Van Krimpen et al., 2013).

BSF larvae were also rich in micro-minerals including iron and zinc, which complied with the required values for non-ruminants. Therefore, BSF larvae reared on the agro-industrial by-products are a suitable ingredient for farmed animals and can potentially replace minerals contained in the conventional FM in animal feeds.

The larvae of BSF contained both saturated, monounsaturated and polyunsaturated fatty acids. In animal nutrition, fats are as important as proteins and carbohydrates and play important roles in feed conversion and faster growth rate of animals (Çetingül & Yardımcı, 2008). In Chapter 6 of this thesis, BSF larvae were predominated by saturated fatty acids including lauric acid. This is well in line with reports from other studies in the literature (Belghit et al., 2019; Ushakova et al., 2016). Lauric acid is easily absorbed and oxidized, and therefore yields energy when ingested. However, taken together with other medium chain fatty acids such myristic acid and palmitic acid, lauric acid can be hypercholesterolaemic (Dalle Zotte et al., 2018; Ulbricht & Southgate, 1991). In a wider context, lauric acid has membrane-disruptive properties and has an antimicrobial effect in the consuming species (Dalle Zotte et al., 2018; Ulbricht & Southgate, 1991), which can be exploited for novel molecules in the chemical and pharmaceutical industries other than the animal feed industry.

The potential of the agro-industrial by-products to improve the nutritional value of BSF larvae in this thesis confirms their suitability as substrates for rearing BSF larvae (Chapter 4), while valorizing the low-grade by-products into high-quality biomass (Chapter 6). This can particularly be valuable when the supply of these by-products exceeds demand for direct consumption by livestock since their use as substrates for rearing BSF larvae would mean saving the environment from pollution. For instance, molasses, a thick, sticky brown syrupy liquid can cause environmental pollution through aesthetic degradation if it spills. It can also cause water pollution if major spills or factory effluents drain into water bodies (M'Ndegwa, 2016).

Black soldier fly larvae as feed ingredient for pigs

FM is conventionally used as a major source of high-quality animal protein, with well-balanced amino acid profiles, digestible energy, mineral and vitamin contents in animal feeds. However, ocean fish stocks are being depleted by overfishing and increasing restrictions on unregulated fishing often result in reduced availability of FM (Dobermann et al., 2017). Overall, the scarcity and associated high prices of FM create perverse incentives to increase animal production (Delgado et al., 2003). Therefore, the use of alternative protein sources in animal feed is indispensable. Insects present such an alternative (Merino et al., 2012; Van Huis, 2013; Van Huis, 2016). While several species are being considered for animal feed, BSF is widely considered as the most promising

fly species because its larvae can convert a wide range of organic side streams, including manure, into high-quality insect protein (Diener et al., 2009; Sheppard et al., 1994). In Chapters 7 and 8, pigs were provided with feeds, in which FM content was replaced at 25, 50, 75 or 100% level of inclusion of BSFLM. The reported feed intake and weight gain in Chapter 7 showed that BSFLM was as suitable in supporting growth of weaning piglets as FM. Furthermore, red or white blood cell indices, as well as the cholesterol levels of pigs were not affected by the replacement of FM by BSFLM in pig feed, which indicates that the health of the animals was not compromised by the inclusion of BSFLM in their feeds.

In Chapter 8, the inclusion of BSFLM resulted in significant weight gain, improved feed conversion, and higher carcass yield of finishing pigs. Nonetheless, higher levels of replacement of FM with BSFLM also resulted in increased backfat depth of pigs, which consequently reduced the fat free lean index of the carcass. However, this is not specific to the BSFLM. Backfat thickness in pigs is reported to show a linear increase with increase in live body weight between 110 and 140 kg, and a decreased percentage fat-free lean (Kim et al., 2005; Wu et al., 2017). The weight of pigs at slaughter in this study ranged from 112-121 kg for pigs reared on diets with 50-100% levels of replacement of FM with BSFLM, which is within the reported range. Limiting back fat deposition is possible through feed restriction as opposed to unlimited access to feed by finishing pigs (Kim et al., 2005; Nieto et al., 2012; Wu et al., 2017).

While Biasato et al., (2019) reported that a dietary inclusion of up to 10% of partially defatted BSFLM could be used for weaning pigs without any adverse effects on the growth performance, blood profile, nutrient digestibility, gut morphology or histological features of the piglets, my results show that a complete replacement of FM with BSFLM is possible. Furthermore, pork by-products from pigs fed insect-based feed were rich in protein and essential minerals. This shows that BSFLM is nutritionally a valuable component in pig feed. Moreover, the Kenyan Bureau of Standards has accepted a standard for insects as animal feed, which provides legal approval for the inclusion of insect meal in animal feed in Kenya (Kenya Standard, DKS 2711:2016).

In summary, my results of the inclusion of BSFLM in pig feed are relevant at many levels. To individual farms, this presents a suitable alternative to consider when the supply of FM is limited, without adverse effects on growth or health of animals. The lower FCR, the comparable CBR and RoI estimates in this thesis have economic implications. For instance, low FCR means that less feed is required per unit kilogram of pork produced when BSFLM is included in feed. To the pork sector, higher carcass yields can be obtained when pigs are reared on BSFLM-based feed compared to FM-based feeds. The pork consumer is also assured of meat that is rich in essential nutrients.

Future perspectives

The ability of BSF larvae to thrive on organic waste material has inspired commercial applications. Nutritionally, the BSF larvae are a valuable protein source for animal feed. Such quality, combined with the need for affordable and sustainable alternatives to the current protein sources for a global population projected to increase by two billion people in the next three decades (United Nations, 2019), present an opportunity for small and large scale BSF production (Chapter 2). However, to achieve its full potential and effectively compete with the current protein-rich FM and soybean meal, a key challenge to be addressed is the scalability of insect production (IPIFF, 2018). To meet the demand for large quantities of insect biomass, a steady source of safe food substrates will be required. While BSF larvae can exploit a wide range of food sources, accessing substrates with consistent nutritional composition will constitute a major challenge. As shown in this thesis, manipulation of rearing substrates through supplementation with nutrient-rich by-products will be a potential way to stabilize the nutritional composition of insect meal. Furthermore, the current reliance of insect rearing on organic side streams for environmental sustainability may be challenging in the future. For instance, the available organic waste streams today may soon become highly demanded and expensive as insect food as the insect sector grows. An integrated insect-livestock farming approach should be encouraged to ensure regular supply of substrates for insect rearing and competitiveness of the sector.

Consumers demand nutritious, safe and healthy animal products from animal producers. Insect producers are therefore required to produce high-quality insect products that meet nutritional needs of animals (IPIFF, 2018). Moreover, this thesis has shown that farmers in Kenya are willing to use insect-based protein as a feed component in animal production. However, little is known about consumer acceptance of animal products derived from insect-based feed. In the future, therefore, research should investigate the effect of BSF-based feed on sensory attributes of animal products in Kenya. In BSF colonies, studies have shown that feeding adult BSF increases the oviposition period (Bertinetti et al., 2019; Chia et al., 2018b). However, little is known about the number of egg batches an adult BSF can lay in its life time. It is worth investigating if multiple egg laying is possible and whether feeding adult BSF can impact its reproductive capacity. Globally, over 70% of the poor live in rural areas. An improvement in their livelihoods would substantially contribute towards global food security.

Therefore, policies and interventions seeking to improve rural livelihoods should involve rural farmer groups through inclusive business models, in the production of BSF larvae for their own use and to supply local markets (Chapter 2). Strengthening insect farming initiatives through cooperatives and farmer associations can help farmers better organize themselves, and allow more access to opportunities to become less dependent

on imported feed ingredients, while at same time gaining income from production and sell of insects as a feed component. Insect farming can substantially contribute to meeting the increasing demand for animal sourced food through bioconversion of organic waste streams and closing of nutrient cycles (Van Krimpen & Hendriks, 2019).

Conclusion

The results presented in this thesis reveal that livestock farmers in the areas in Kenya studied are aware of insects as feed and are willing to pay for insect-based feed for poultry, pig and fish production. The survival and development of BSF larvae reared on agro-industrial by-products, composed of BSG, brewer's yeast and cane molasses, demonstrated the suitability of these substrates in the mass production of BSF larvae as an alternative protein component in animal feed. The optimum temperature for population growth and reproduction of BSF is 30 °C. The high nutrient content of BSF larvae reared on the different agro-industrial by-products further confirmed the suitability of these substrates for their mass production. Improved growth performance and carcass yield of pigs as recorded in this thesis support the use of low-cost, insect-based feed as an alternative to the conventional protein sources to achieve better livestock feeding. Generally, the findings from this study favour the production of BSF larvae as an alternative protein source in livestock feed and in the sustainable management of organic waste streams.

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Summary

Current trends predict a global population increase to about 10 billion people by 2050, forcing an increased food or feed production, which exerts pressure on the environment for resources. With the increasing human population, increased urbanization and income rise, there is an increased demand for animal-based foods such as beef, dairy, pork, chicken, eggs and fish. Efficient animal production requires adequate feeding to produce high-quality animal products. Aquaculture and livestock industries face challenges related to the availability of sustainable and affordable feed ingredients. Fishmeal (FM) and soybean meal are the major protein sources in animal feed, which are increasingly becoming scarce and unaffordable, thereby increasing the total cost of production. High costs of production adversely affect growth of production and livelihood of smallholder producers, particularly in developing countries. Currently, interest in using insects as an alternative nutrient source is growing and the need to establish an insect sector has become important, but for insect-based feed to make a substantial contribution in substituting the conventional protein-rich FM and soybean meal, large quantities of insect biomass are required, which makes insect mass rearing an inevitable step. Knowledge on sources of substrates for rearing insects, suitability of insect meal as feed ingredient, as well as acceptance and demand by end users is therefore important for a sustainable insect-based feed sector. The study described in this thesis focused on the potential of black soldier fly (BSF) larvae as a feed ingredient and farmers' willingness to pay (WTP) for insect-based feed in Kenya.

Chapter 2 presents an overview of the current literature on the potential of insects as an alternative animal feed ingredient and the role insect farming can play in inclusive business models for smallholder farmers, with a focus on the BSF. Insects are highly nutritious, with protein contents comparable to FM and soybean meal. The BSF is the most suitable insect species reared for animal feed because it is not considered as a pest or vector of diseases and can its larvae exploit a wide range of food substrates, including organic side streams such as manure. The larvae can upgrade low value organic side streams to a high-quality feed ingredient. Moreover, insect farming fits well into the concept of a circular economy and has great prospects for animal feed and waste management, with valuable consequences for many societal aspects, which align with various Sustainable Development Goals, including reduction of poverty, hunger and food waste.

In **Chapter 3**, farmers' knowledge of and attitude towards insects as feed, and their WTP for insect-based feed were assessed using a contingent valuation method. We found that poultry and fish farmers are aware that insects can be used as a feed ingredient. Farmers have a positive attitude that insects are a nutritious feed ingredient for animals. Furthermore, farmers are willing to pay for insect-based feed. In addition, factors such as age of farmer, gender, education level, marital status, distance to feed trader, awareness of insects as feed, attitude towards insects as feed, acceptance of insect species,

availability of agricultural inputs, training, and market information affect farmers' WTP for insect-based feed. These findings imply that there is a potential demand for insect-based feed in Kenya and suggest that the implementation of extension programmes to educate farmers on the nutritional value of insects as well as provide market information may improve farmers' attitude towards insects, create more awareness and enhance WTP for insect-based feed. Our findings also highlight the need for increased training programmes towards capacity building of local insect producers in order to increase local production and availability of insects for the animal feed value chain, which is currently recognized by the Kenyan government.

Availability and accessibility of suitable substrates is key to a successful insect mass rearing operation. In **Chapter 4**, we explored the potential of rearing BSF larvae on a combination of agro-industrial by-products composed of brewers' spent grains (BSG) and brewer's yeast from beer brewing, and molasses from cane sugar production. Immature and adult life history parameters including: larval development, survival, adult emergence, adult longevity and fecundity of the BSF were assessed. Our results show that BSF larvae successfully developed to pre-pupae in all substrate combinations, with 86-99% larval survival. When provided with water or a sugar solution upon emergence, adult BSF lived 2-3 times longer than adults BSF that were not fed. Unfed female BSF died without ovipositing, whereas the water- or sugar- female BSF successfully oviposited. These findings imply that the BSG are a suitable substrate for rearing BSF larvae. Supplementing BSG with brewer's yeast or a combination of brewer's yeast and molasses supports larval growth and, therefore, is a valuable strategy to manage brewer's yeast and cane molasses. These findings are valuable in the production of BSF larvae as feed as well as in the management of organic waste streams through bioconversion of substrates. The results also provide insight into the management of adult BSF colonies to achieve optimal performance.

The time taken for most insects to complete a specified growth stage is largely dependent on temperature and food. In **Chapter 5**, BSF larvae were reared on BSG supplemented with brewer's yeast or un-supplemented at nine constant temperatures. Our results show that supplementing BSG with brewer's yeast resulted in higher weight of BSF larvae compared to the un-supplemented substrates. Furthermore, the population growth rate of BSF was most favourable at 30 °C with a higher intrinsic rate of natural increase and shorter doubling time compared to the other temperatures evaluated. These findings are valuable for the optimization of rearing conditions of the BSF under various environmental conditions and the prediction of population dynamics.

The BSF larvae convert organic material into high-quality insect biomass that can be used as a feed ingredient. The nutrient composition of the larvae may vary depending on the substrates on which the larvae are reared. In **Chapter 6**, we therefore assessed the nutritional quality of BSF larvae reared on agro-industrial by-products composed

of BSG, brewer's yeast and molasses (Chapter 4) for animal feed. Our results show that larvae reared on all the substrates investigated were rich in protein, with a balanced amino acid profile, fats, predominated by saturated fatty acids, as well as macro- and micro-minerals. Furthermore, supplementing BSG with brewer's yeast resulted in higher crude protein content of the BSF larvae than obtained on substrates without brewer's yeast. The BSF larvae contained 30-46% crude protein, which is comparable to values recorded for BSF larvae reared on other substrates such as fruit and vegetable wastes in other studies. These findings imply that BSF larvae reared on these agro-industrial by-products are nutritionally suitable for animal feed.

FM is the major source of protein in animal feed, but its rapidly reducing availability leads to increased prices. Alternative sources of protein are therefore needed for animal feed production. The BSF larvae are a novel protein source widely used in aquaculture and poultry feeds, but little is known about the effect of substituting FM with black soldier fly larval meal (BSFLM) on growing and finishing pigs. In **Chapters 7 and 8**, the potential of a partial or complete replacement of FM by BSFLM on growth and economic performance, carcass yield and nutritional quality of pigs was investigated. Pigs were fed different diet types: control (no BSFLM: 0%), 25, 50, 75 and 100% replacement of FM by BSFLM for the grower and finisher phases of the pigs. Our results show that the replacement of FM by BSFLM did not affect feed intake, daily weight gain, red or white blood cell counts as well as serum lipid indices of pigs in the grower phase (**Chapter 7**). Moreover, the inclusion of BSFLM in feed for growing pigs did not affect the value of return on investment (RoI) estimated, but the cost benefit ratio showed a value greater than 1.0, which means that BSFLM can be considered as an ingredient in commercial production of pig feed.

In the finishing phase (**Chapter 8**), the inclusion of BSFLM greatly improved feed conversion and daily weight gain of the pigs. Replacing FM with 50-100% levels of inclusion of BSFLM in feed led to higher carcass weights than at lower levels of BSFLM. Furthermore, pork tissues were rich in crude protein and minerals across all diet types tested. BSFLM proves to be a suitable ingredient in finishing pig feed and can completely replace FM with beneficial consequences for growth, carcass and nutritional quality of edible pork by-products. These findings are valuable for the production of insect-based feed for pigs. In **Chapter 9**, the key findings of this research and the implications for BSF production in Kenya are addressed and recommendations for future investigations are provided.

In conclusion, the results of the research described in this thesis show that farmers are aware that insects can be used as a feed component and are willing to pay for insect-based feed in Kenya. The combination of agro-industrial by-products, composed of BSG, brewer's yeast and molasses is suitable for rearing BSF larvae. Moreover, the addition of brewer's yeast to BSG leads to higher protein content of the BSF larvae.

Furthermore, the inclusion of BSFLM in pig feed improves pig growth and carcass quality. Farmer sensitization on the nutritional value of the different insect species, especially the BSF is necessary to increase awareness and enhance WTP for insect-based feed among farmers. Organoleptic studies are recommended on pork derived from pigs raised on insect-based feed to investigate consumers' perceptions with regards to sensory characteristics of the meat. The findings presented in this thesis are relevant to animal feed producers seeking to include insect meal in their feed formulae, to smallholder BSF farmers and to livestock producers. Additionally, these findings are valuable to non-governmental organizations, policy makers and intergovernmental bodies seeking to implement policies to improve livelihoods of smallholder farmers, and enhance access to alternative sources of high-quality protein ingredients for animal feeds.

Appendix A

Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products

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Submitted

Analysis of amino acids

Column conditions: The chromatographic separation was achieved on the Agilent 1260 Infinity Binary Liquid Chromatograph system (Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a ZORBAX SB-C18, 4.6 × 250 mm, 3.5 μm column with a heater turned at 40 °C and an auto sampler tray maintained at room temperature. As the mobile phases, LC grade water (A) and LC grade methanol (B) each containing 0.1% formic acid were used. The flow rate is maintained at 0.5 ml/min and the gradient is programmed as shown in the following Table A1. Free amino acids were identified using a Mass Selective Detector Agilent 6120 quadrupole LC/MS operating on ESI-positive mode at a mass range of m/z 70-600 and 70eV cone voltage.

Table A1. Flow rate of liquid chromatography

Time (minutes)	% A	% B
0	90	10
8	90	10
14	0	100
19	0	100
21	90	10
25	90	10

Table A2. Calibration equations for external quantification of amino acids in black soldier fly larvae reared on substrates composed of agro-industrial by-products

Amino Acid	Equation (y)	R ²	Calibration curve range (microgram)
Alanine	$3E+07\ln(x) + 1E+08$	0.9946	0.0223 to 0.4455
Arginine	$7E+07\ln(x) + 2E+08$	0.9507	0.0436 to 0.8710
Aspartic Acid	$7E+06\ln(x) + 3E+07$	0.9915	0.0333 to 0.6655
Cystine	$9E+06\ln(x) + 4E+07$	0.9965	0.0300 to 0.6008
Glutamic Acid	$1E+07\ln(x) + 5E+07$	0.994	0.0368 to 1.1033
Glycine	$2E+07\ln(x) + 1E+08$	0.9894	0.0188 to 0.5630
Histidine	$4E+07\ln(x) + 1E+08$	0.9898	0.0388 to 1.9400
Isoleucine	$3E+08\ln(x) + 9E+08$	0.9129	0.0328 to 1.6400
Leucine	$6E+07\ln(x) + 2E+08$	0.9472	0.0328 to 1.6400
Lysine	$8E+07\ln(x) + 2E+08$	0.9894	0.0366 to 1.8275
Methionine	$2E+08\ln(x) + 5E+08$	0.9405	0.0376 to 1.8650
Phenylalanine	$3E+08\ln(x) + 8E+08$	0.9025	0.0413 to 1.6520
Proline	$6E+07\ln(x) + 2E+08$	0.9917	0.0863 to 1.4388
Serine	$1E+07\ln(x) + 5E+07$	0.994	0.0263 to 0.5255
Threonine	$1E+07\ln(x) + 6E+07$	0.9956	0.0298 to 1.4888
Tyrosine	$2E+08\ln(x) + 4E+08$	0.9675	0.1359 to 2.2650
Valine	$1E+08\ln(x) + 4E+08$	0.9676	0.0293 to 1.4650

Table A3. Amino acid concentration (mg/g dry matter) of black soldier fly larvae reared on substrates composed of agro-industrial by-products

Amino acid	Treatment											
	B			MB			MC			SB		
	W	Y	YM	W	Y	YM	W	Y	YM	W	Y	YM
Essential amino acids												
subsample 1												
Lysine	6.6	7.2	5.15	7.6	5.9	4.88	9.5	6.09	5.491	6.67	5.86	7.4
Histidine	6.6	6.75	4.84	5.96	6.97	4.60	7.03	7.05	4.88	5.52	3.4	5.75
Arginine	5.9	6.43	4.333	4.23	5.3	3.11	4.99	5.77	3.9	4.28	3.48	3.97
Methionine	3.140	3.025	3.052	2.867	3.194	2.75	3.31	3.241	2.846	2.947	3.155	3.32
Phenylalanine	3.24	3.15	2.985	2.774	3.11	2.66	4.4	3.11	2.74	2.681	3.150	3.62
Isoleucine	2.221	2.17	2.12	2.28	2.22	2.02	3.00	2.278	2.048	1.925	2.376	2.605
Leucine	2.95	2.86	2.570	3.04	2.70	2.62	4.86	2.969	2.56	1.94	3.13	3.8
Threonine	0.097	0.096	0.098	0.092	0.103	0.087	0.113	0.107	0.092	0.092	0.100	0.102
Valine	1.240	1.21	1.12	1.32	1.21	1.06	2.01	1.236	1.144	1.128	1.244	1.6
subsample 2												
Lysine	5.4	8.75	4.90	5.6	7.4	5.30	8.1	6.91	5.400	5.93	6.19	5.72
Histidine	6.24	7.41	5.49	5.61	7.24	5.38	6.7	7.84	5.57	5.66	6.0	4.89
Arginine	4.4	7.3	4.356	3.67	6.68	3.98	4.51	6.27	4.33	4.16	4.10	3.43
Methionine	3.064	3.060	2.956	2.817	3.163	2.90	3.07	3.158	2.915	2.890	3.071	3
Phenylalanine	3.04	3.71	2.914	2.735	2.99	3.22	3.74	3.46	2.96	2.763	3.200	2.91
Isoleucine	2.132	2.37	2.02	1.98	2.37	1.92	2.56	2.357	2.134	1.957	2.319	2.167
Leucine	2.66	2.58	2.493	2.42	3.26	2.93	4.04	3.051	2.75	1.77	2.86	2.7
Threonine	0.099	0.100	0.094	0.090	0.102	0.088	0.098	0.109	0.102	0.088	0.100	0.092
Valine	1.158	1.5	1.0	1.09	1.38	1.2	1.59	1.311	1.131	1.137	1.326	1.2
Non-essential amino acids												
subsample 1												
Tyrosine	5.520	5.61	5.255	5.60	5.81	5.01	4.9	6.03	5.213	5.41	5.12	6.29
Alanine	1.478	1.39	1.392	1.352	1.476	1.253	1.539	1.517	1.33	1.341	1.435	1.57
Aspartic acid	0.725	0.72	0.682	0.636	0.73	0.603	0.94	0.79	0.63	0.595	0.627	0.8
Cystine	0.472	0.470	0.421	0.418	0.473	0.397	0.42	0.498	0.433	0.421	0.450	0.453
Glutamic acid	0.254	0.239	0.246	0.240	0.258	0.226	0.255	0.265	0.228	0.237	0.264	0.265
Glycine	1.748	1.70	1.16	1.59	1.14	1.247	1.575	1.87	1.22	1.314	1.11	1.51
Proline	2.679	2.65	2.631	2.49	2.49	2.55	3.47	2.564	2.67	2.084	2.099	2.56
Serine	0.664	0.67	0.542	0.519	0.65	0.479	0.581	0.783	0.58	0.495	0.371	0.535
subsample 2												
Tyrosine	5.608	5.90	5.347	5.10	6.06	5.45	6.0	6.24	5.293	5.29	5.73	5.47
Alanine	1.437	1.50	1.316	1.309	1.528	1.307	1.497	1.526	1.53	1.348	1.478	1.33
Aspartic acid	0.668	0.92	0.639	0.622	0.90	0.573	0.77	0.782	0.79	0.675	0.669	0.61
Cystine	0.447	0.410	0.438	0.410	0.464	0.406	0.430	0.505	0.431	0.431	0.457	0.437
Glutamic acid	0.243	0.251	0.237	0.229	0.256	0.230	0.245	0.263	0.241	0.232	0.264	0.237
Glycine	1.673	1.93	0.99	1.30	1.72	1.168	1.596	1.62	1.63	1.322	1.32	1.17
Proline	2.648	3.09	2.666	2.04	2.73	3.04	2.91	2.642	2.91	2.036	2.091	2.01
Serine	0.568	0.78	0.561	0.472	0.78	0.541	0.517	0.714	0.70	0.489	0.438	0.449

B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley; W = water; Y = residual brewer's yeast; YM = brewer's yeast plus molasses. Values presented in the table are duplicate concentrations (subsamples 1 and 2) of amino acids for black soldier fly larvae for each substrate.

Appendix B

Black soldier fly larval meal in feed enhances growth performance, carcass yield and meat quality of finishing pigs

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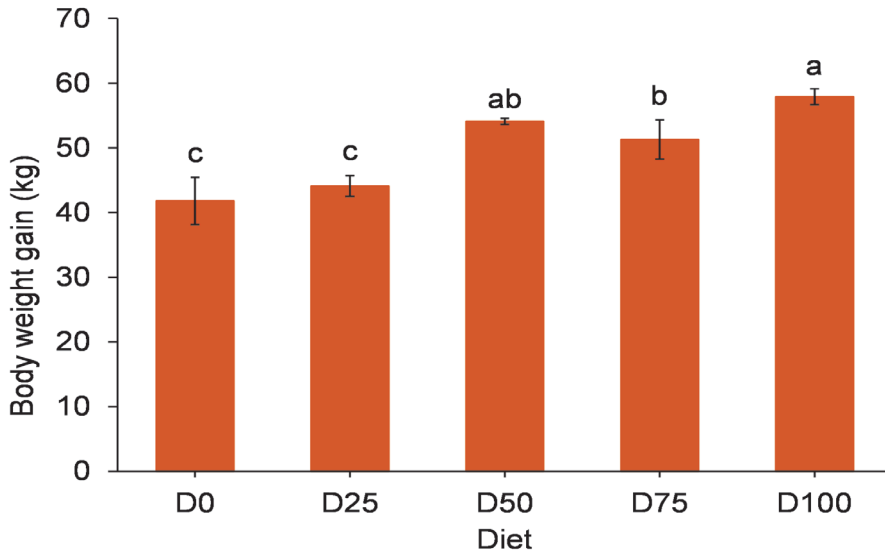


Figure B1. Overall mean body weight gain in finishing pigs fed BSFLM-based diets and a control diet. Means (\pm SE) followed by different lowercase letters are significantly different (GLM, $P < 0.05$; LSD). BSFLM = black soldier fly larval meal, D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, $N = 8$.

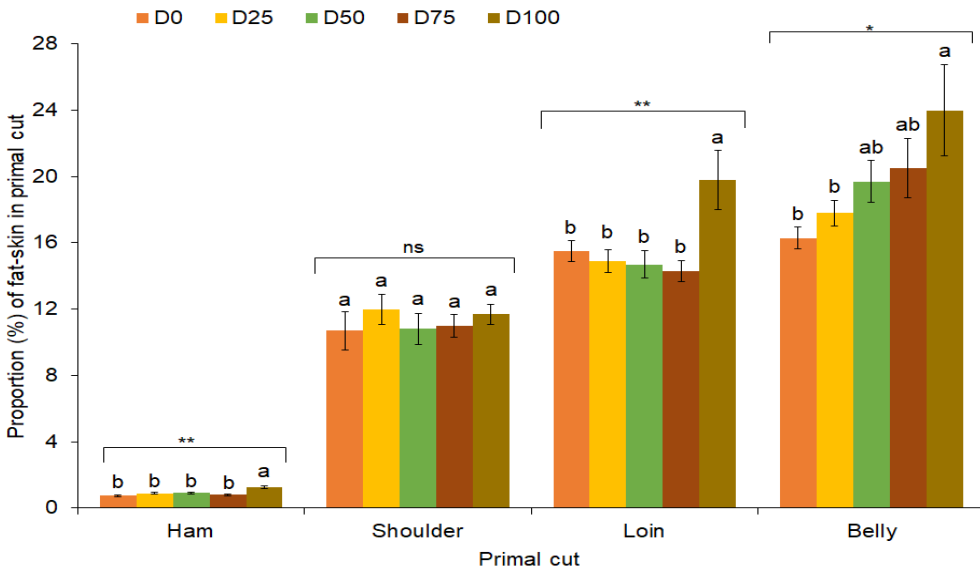


Figure B2. Composition of fat and skin in primal cuts of half carcasses of finishing pigs fed BSFLM-based diets and a control diet. Means (\pm SE) within each primal cut followed by different letters are significantly different (GLM, $P < 0.05$; LSD). D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. Significance level: **: $P < 0.01$, *: $P < 0.05$, ns: not significant.

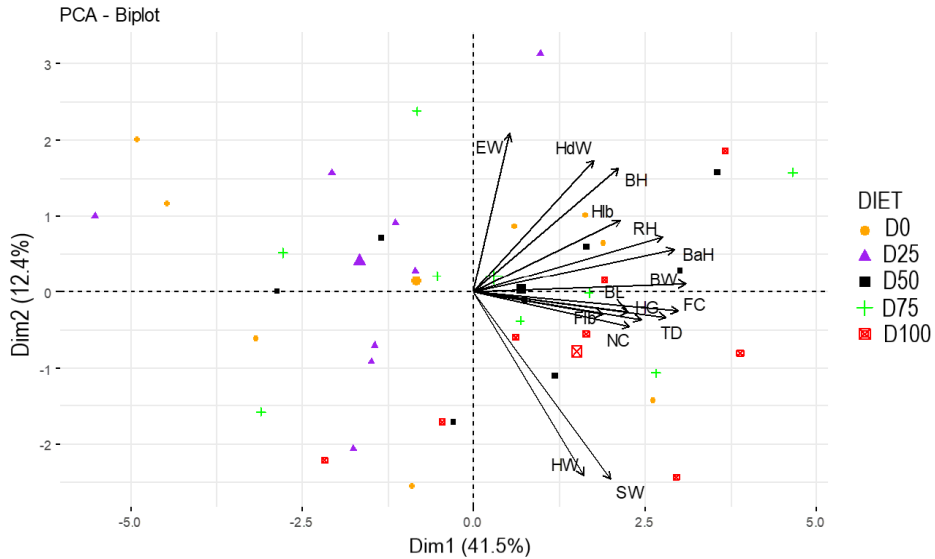


Figure B3. Principal component analysis (PCA) for pig traits assessed for the five diets. The factor map identifies clusters of correlated variables. Principal components 1 and 2 (Dim1 and Dim2) show the space where variables are expressed (> 50% of variance). Variables (vectors) close to the center are less represented in the first two PCs. Variables analyzed: BW = live body weight of pig, BL = body length, BH = body height, RH = rump height, HG = heart girth, Flb = fore limb, Hlb = hind limb, EW = ear width, TD = trunk depth, SW = shoulder width, HW = hip width, FC = flank circumference, BaH = back height, HdW = head width, NC = neck circumference. Diets: D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM.

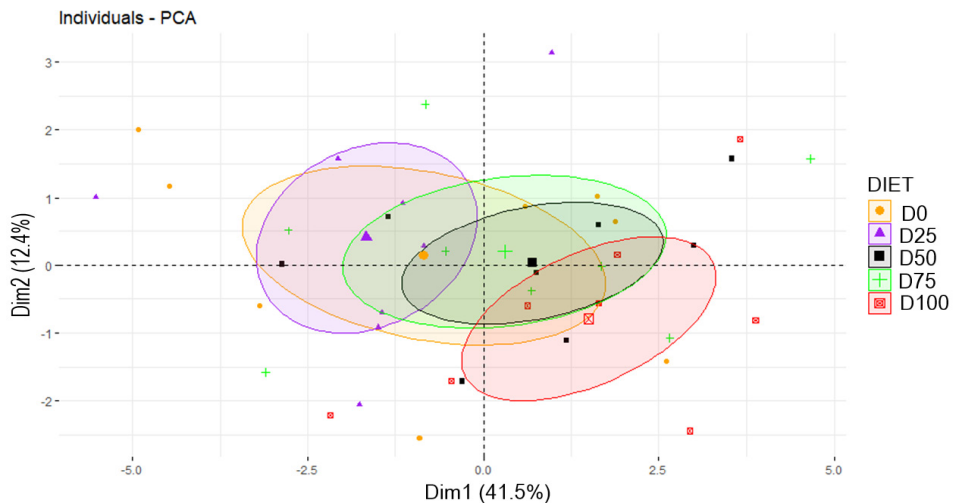


Figure B4. Principal component analysis (PCA). The ellipses represent the treatment groups and show the overlap between the control diet and the BSFLM-based diets. Principal components 1 and 2 (Dim1 and 2) show the space where variables are expressed (> 50% of variance). Diet: D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM.

Table B1. Proportion of muscle and bone in primal cuts of half carcasses in pigs fed diets with different levels of black soldier fly larval meal (BSFLM)

Parameter (%)	Diet					P value, GLM
	D0	D25	D50	D75	D100	
Ham						
Muscle	75.1 ± 0.51	73.4 ± 0.52	74.6 ± 0.90	73.3 ± 1.07	71.9 ± 1.05	0.09
Bone	15.0 ± 0.50	15.2 ± 0.52	14.3 ± 0.57	15.5 ± 0.83	13.5 ± 0.52	0.13
Shoulder						
Muscle	72.7 ± 1.29	68.8 ± 1.04	71.7 ± 0.92	71.1 ± 0.83	71.9 ± 0.79	0.09
Bone	16.5 ± 0.67	18.0 ± 0.52	17.1 ± 0.69	17.3 ± 0.48	15.8 ± 0.51	0.11
Loin						
Muscle	58.6 ± 1.47	57.9 ± 1.46	58.5 ± 1.16	57.7 ± 0.67	56.9 ± 1.57	0.91
Bone	25.3 ± 1.23 ^{ab}	26.4 ± 0.63 ^a	25.9 ± 0.92 ^a	27.6 ± 1.05 ^a	23.0 ± 0.84 ^b	0.022
Belly						
Muscle	62.9 ± 2.96	64.6 ± 1.08	63.0 ± 1.13	60.5 ± 1.84	60.4 ± 2.26	0.52
Spare ribs	20.5 ± 2.97	16.98 ± 0.79	16.75 ± 0.95	17.5 ± 0.78	15.1 ± 0.72	0.16

Means (± SE) within a row followed by different superscripts are significantly different, General Linear Model (GLM), $P < 0.05$, LSD. D0 = 0% (control), D25 = 25%, D50 = 50%, D75 = 75%, D100 = 100% levels of replacement of fishmeal with BSFLM. For each diet, N = 8.

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**Curriculum vitae
&
Publications**



Shaphan Yong Chia was born on 10 June 1987 in Baingo, Cameroon. After completing his primary and secondary education, he enrolled in Government High School, in Wum and obtained his Advanced Level (AL) certificate in 2007. Shaphan then joined the University of Buea, Cameroon from 2008 to 2011, where he pursued a Bachelor of Science (BSc) degree in Zoology (major) and medical laboratory technology (minor). Later on, from 2013 to 2015, Shaphan did a Master of Philosophy (MPhil.) degree in Agricultural Entomology at the University of Ghana, Accra, Ghana, under the African Postgraduate

Programme in Insect Science (ARPPIS), sponsored by the German Academic Exchange Service (DAAD). During his MPhil, he assessed the species composition and host association of thrips in the Eastern and Greater Accra Regions, Ghana. After completing his MPhil, he joined the Institute of Research and Agricultural Development (IRAD), Bambui-Bamenda, Cameroon as a research volunteer. In 2016, Shaphan enrolled for a Sandwich PhD program between Wageningen University & Research, and the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. His research focused on black soldier fly larvae as a sustainable animal feed ingredient. The results of this research are presented in this thesis. He was actively involved in and led the *icipe* scholars association (IScA) (2016-2017), which seeks to advance student interest in science through participation in academic exchange programmes as well as promote social interaction among scholars at *icipe*. Shaphan's ambition is to continue conducting research on the sustainable production of high-quality insects as a novel feed component for animal production and improvement of livelihood.

List of publications

In peer-reviewed journals

- Chia S.Y.**, Tanga C.M., van Loon J.J.A. and Dicke M. (2019). Insects for sustainable animal feed: inclusive business models involving smallholder farmers. *Current Opinion in Environmental Sustainability* 41:23-30 (chapter 2 in this thesis)
- Chia S.Y.**, Tanga C.M., Osuga I.M., Mohamed S.A., Khamis F.M., Salifu D., Sevgan S., Fiaboe K.K.M., Niassy S., van Loon J.J.A., Dicke M. and Ekesi S. (2018). Effects of waste stream combinations from brewing industry on performance of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae). *PeerJ* 6:e5885 (Chapter 4 in this thesis)
- Chia S.Y.**, Tanga C.M., Khamis F.M., Mohamed S.A., Salifu D., Sevgan S., Fiaboe K.K., Niassy S., van Loon J.J.A., Dicke M. and Ekesi S. (2018). Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. *PLoS ONE* 13:e0206097 (Chapter 5 in this thesis)
- Chia, S. Y.**, Tanga, C. M., Osuga, I. M., Alaru, A. O., Mwangi, D. M., Githinji, M., Subramanian S., Fiaboe K.K.M., Ekesi S., van Loon J.J.A. and Dicke, M. (2019). Effect of dietary replacement of fishmeal by insect meal on growth performance, blood profiles and economics of growing pigs in Kenya. *Animals*, 9, 705. (Chapter 7 in this thesis)

Submitted

- Chia S.Y.**, Tanga C.M., Osuga I.M., Cheseto X., Ekesi S., van Loon J.J.A. and Dicke M. Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products (chapter 6 in this thesis)
- Chia S.Y.**, Tanga C.M., Macharia J., Diiro G.M., Ekesi S., van Loon J.J.A. and Dicke M. Knowledge and willingness of smallholder farmers in Kenya to pay for insect-based feeds (Chapter 3 in this thesis)

In preparation

- Chia S.Y.**, Tanga C.M., Isaac M. Osuga, Alphonse O. Alaru, David M. Mwangi, Macdonald Githinji, Ekesi S., van Loon J.J.A. and Dicke M. Black soldier fly larval meal in feed enhances growth performance, carcass yield and meat quality of finishing pigs (Chapter 8 in this thesis)

Education statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (4.5 ECTS)

- Insects as high quality protein ingredient for poultry, fish and pig feed industries

Writing of project proposal (4.5 ECTS)

- Assessing the potential of black soldier fly as protein subsidy in poultry, fish and pig feed in Kenya

Post-graduate courses (12.6 ECTS)

- Introduction to insect taxonomy, identification and insect photography; International centre of insect physiology and ecology (ICIPE), Nairobi, Kenya (2016)
- Introduction to GIS and remote sensing; ICIPE (2016)
- Introduction to behavioural and chemical ecology; ICIPE (2016)
- Introduction to molecular biology and bioinformatics; ICIPE (2016)
- Principles of biostatistical reasoning: an introduction to statistical methods in biological research; ICIPE (2016)
- Ecological modelling; ICIPE (2016)
- Biostatistics: introduction to R software; ICIPE (2016)

Laboratory training and working visits (4.5 ECTS)

- Effect of insect meal on pig performance; Non ruminant Research Institute (NRI)- Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha, Kenya (2018)

Competence strengthening / skills courses (6.8 ECTS)

- Introduction to research ethics; ICIPE (2016)
- Health and safety; ICIPE (2016)
- Introduction to GIS and remote sensing; ICIPE (2017)

- Scientific writing and publishing; ICIPE (2017)
- Information literacy training, reference management; ICIPE/WUR (2017-2018)
- Communication with the media and the general public; WUR (2018)
- Interpersonal communication for PhDs; WUR (2018)
- Critical thinking and argumentation; WUR (2018)
- Reviewing a scientific paper; WUR (2018)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.5 ECTS)

- PE&RC Day (2018)
- PE&RC Midterm weekend (2018)
- PE&RC Last years weekend (2019)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- Plant health theme monthly meetings; ICIPE (2016-2017)
- ICIPE Scholars association-science club meetings; ICIPE (2016-2017)
- Student dry run programme; ICIPE (2016-2017)
- PhD Lunch meetings; WUR (2018-2019)

International symposia, workshops and conferences (9.3 ECTS)

- International conference on legislation and policy on the use of insect as food and feed in East Africa; Kisumu, Kenya (2016)
- 22nd Meeting and conference of the African association of insect scientists: towards securing human welfare through management of insect diversity in a changing world; Wad Medani, Sudan (2017)
- Annual general meeting of ICIPE governing council; poster presentation; Nairobi, Kenya (2017)
- The 2nd International conference: Insects to Feed the World (IFW); oral and poster presentation; Wuhan, China (2018)
- 30^e Nederlandse entomologendag; poster presentation; Ede, the Netherlands (2018)
- Symposium earth futures; WUR, the Netherlands (2018)
- Annual general meeting of ICIPE governing council; poster presentation; Nairobi, Kenya (2018)

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Propositions

1. Dietary replacement of fishmeal by black soldier fly larval meal enhances growth and carcass yield in finishing pigs.
(this thesis)
2. Predominance of saturated fatty acids in black soldier fly larvae reduces their nutritional value.
(this thesis)
3. Pest outbreaks in crops can be prevented by increasing biodiversity in and around agricultural land.
4. Market access and trade interactions involving livestock enhance the spread of HIV/AIDS.
5. Multidisciplinary approaches are vital in assessing gut health of animals in feeding trials.
6. Mosquito vector control is more justified by instrumental value than by intrinsic value of the organism.
7. The key to sound leadership is service.

Propositions belonging to the thesis, entitled:

“Black soldier fly larvae as a sustainable animal feed ingredient in Kenya”

Shaphan Yong Chia

Wageningen, 20 December 2019