# From waste to feed: the Black Soldier fly (*Hermetia illucens*) as a novel feed source for monosex tilapia (*Oreochromis niloticus*)

A thesis submitted for the degree of Doctor of Philosophy.

By

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# DECLARATION

I hereby declare that this thesis has been composed entirely by myself and has not been submitted for any other degree or qualification. All sources of information have been suitably acknowledged in the text.

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*"N'ayez pas peur de la vie, n'ayez jamais peur de l'aventure. Faites confiance au hasard, à la chance, à la destinée. Partez conquérir d'autres espaces, d'autres expériences, le reste vous sera donné de surcroît. »*
Henri De Monfreid.

*"Combattu: souvent. Battu : parfois. Abattu : jamais"*
François Athanase Charrette.

*"By endurance we conquer."*
Sir Ernest Shackelton.

# ABSTRACT

The aquafeed industry is highly dependent on fishmeal (FM) and high-protein plant substitutes. Rising costs and sustainability concerns are fueling the search for novel alternatives. Black Soldier Fly (BSF) larvae (*Hermetia* illucens) have been demonstrated to be a potential new source of sustainable protein. While they can be grown on a wide range of waste-substrates, have a short life-cycle, and a favourable nutritional profile, they can be seen as a credible candidate. In this thesis, we focused on the selection of a potential substrate in a local context, and the type of larval stage to harvest in order to optimise both production and quality of the maggot meal (MM). From these preliminary studies fruit waste were selected to grow the larvae, harvested at the "white larvae" stage to produce the MM. As the availability of MM is yet –far- from being sufficient to cover the ever-growing demand for aquafeed, a strategic use was decided in contextualised and commercially-relevant researches. In large-scale tilapia farm, all-male production is desired to optimise the production as they grow bigger and faster than females. To do so 17α-methyltestosterone is added to the feed during the first 21 days of the fry. To maximize the ingestion, low quantities but high quality feed are required. In this context, the MM was used as a feed-hormone carrier for tilapia fry (*Oreochromis niloticus*) in two experiments. Whereby the first was based on simple substitution of fish meal (FM) and commercial feed with MM (Chapter 5), the second compared 12 isoenergetic and isoproteic formulated feeds based on a prior MM digestibility analysis (Chapter 6). Results indicated that different dietary inclusions of MM did not significantly affect sex reversal rates nor fish production performance, suggesting that MM offers potential as a locally sourced feed ingredient for tilapia hatchery. This strategic application is further enhanced by the potential to co-located MM and fry-production offering producers' greater ability to manage quality assurance.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Amino acid(s) |
| ARA | Arachidonic acid |
| ADC | Apparent digestibility coefficient |
| BSF | Black Soldier Fly (*Hermetia illucens*) |
| BSFM | Black Soldier Fly Meal |
| BSF DF | Black Soldier Fly Meal Defatted |
| BSF WM | Black Soldier Fly Whole Meal |
| CEF | Controlled Environment Facility |
| CP | Crude Protein |
| DHA | Docosahexanoic Acid (22:6n-3) |
| DM | Dry Matter |
| DO | Dissolved Oxygen |
| DW | Dry Weight |
| EAA | Essential Amino Acid(s) |
| EFA | Essential Fatty Acid(s) |
| EPA | Eicosapentaenoic acid (20:5n-3) |
| FA | Fatty Acid(s) |
| FM | Fish Meal |
| FO | Fish Oil |
| Lc PUFA | Long-chain Polyunsaturated Fatty Acid(s) |
| LIDC | Low-Income Developing country(ies) |
| MM | Maggot Meal |
| N | Nitrogen |
| n-3 | Omega-3 Fatty Acid(s) |
| n-6 | Omega-6 Fatty Acid(s) |
| NFE | Nitrogen Free Extract |
| OM | Organic Matter |
| SSI | Substrate Suitability Index |
| UK | United Kingdom |
| US$ | United States Dollar(s) |
| WW | Wet Weight |

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# CHAPTER 1: GENERAL INTRODUCTION

The broader focus of the thesis is to assess the potential for insect protein as an aquafeed ingredient, being the main operating cost for intensive aquaculture. Sustainability issues are first discussed, followed by a review of conventional and novel alternative protein sources. Then the focus will be made on insect proteins, from the different species susceptible to be bred on a large scale, to their nutrient profile, production and regulatory aspects and a special analysis will be done on the Black Soldier Fly (*Hermetia illucens*) being the topic-species of this Ph.D. thesis.

A review of the outcomes from past studies on the use of this new protein source is made. Then the remaining bottlenecks, and limitations, hampering the up-scale of this potential new protein source will be assessed. From the foregoing discussion, knowledge gaps are identified and the research hypothesis and objectives of the present study framed.

## 1.1 The contribution of Aquaculture to our food system

The increasing population worldwide, expected to reach 9.77 billion by 2050 (United Nations Department of Economic and Social Affairs, 2017) is driving up the demand for animal-based food, and aquatic food in particular (Speedy, 2003; Troell *et al.*, 2014). Between 2005 and 2050 the global food demand is expected to rise by nearly 100 percent (Makkar *et al.*, 2014; Tilman *et al.*, 2011).

Fish is a rich source of vitamins, minerals essential fatty acid and protein. It plays a major role in the diets of consumers in Low Income and Developping Countries (LIDC) and middle-income countries. Many of these people are poor, malnourished and unable to afford other protein alternatives such as meat. Aquaculture plays a fundamental role in food security and economy in LIDC and middle-income countries where local small scale farms are sometimes claimed to contribute for 70-80% of the global aquaculture production (Belton *et al.*, 2018) and most of this production remains destined for domestic consumption, therefore do not divert food away from local consumers. Therefore, beside serving a role as food, the aquaculture sector is also a great source of employement, giving incentive to local economy (Belton *et al.*, 2018). In a report published by the FAO (Cai & Leung, 2017), the world fish consumption increased from 121 million tonnes in 2008 to 140 million tonnes in 2013, with a growing rate of 2.9% annually. Over 40% of this

growth is attributable to population growth, which increased during this time frame from 6.7 billion to 7.1 billion. The remaining 60% are due to the increase per capita fish consumption from 18 Kg to almost 20%, growing at a rate of 1.7% annually (Cai & Leung, 2017). Ninety percent of the growth was contributed by aquaculture (Cai & Leung, 2017). From 2008 to 2013, the per capita fish consumption increased from 16 kg to 19 kg in developing regions and declined from 26 Kg to 25 kg in developed regions. A higher growth in per capita fish consumption, together with stronger population growth, had increased the share of developing regions in world fish consumption from 74 to 78% during this time frame. Assuming that fish prices and consumer preferences remains the same toward 2020's, the per capita fish demand is expected to grow from almost 20 kg/year (in mid-2010) to 25 kg/year in early 2020's. (Cai & Leung, 2017).Therefore, the income-driven per capita fish demand increasing, combined together with a population growth, the world fish demand is expected to increase to 47 million tonnes (or 31 million tonnes under the most conservatives projections). During this time frame, this 19 million tonnes growth, would cover only 40 percent of the projected demand growth by 2020's (or 60 percent in the most conservative projections), leaving a fish demand supply gap of 28 million tonnes (or 16 million tonnes for the conservative projections). Wild fish captures has reached a peak around 95 million tonnes per year (Pauly *et al.*, 2002) since then, they remained stable for the last 20 years (OECD-FAO, 2016), and are not expected to increase considering that almost 75% of marine fish stocks are considered to be fully or over-exploited (FAO, 2007) therefore the demand for food fish is expected to be supplied by aquaculture.

While the world aquaculture production, following this growing trend is expected to grow at 4.5 percent from mid-2010's to early 2020's, it would take another 9.9 percent annual growth to cover the world fish demand supply gap required for early 2020's (Cai & Leung, 2017).

The sky-rocket development of aquaculture has allowed a fall in price for fish, making them accessible to the rural and urban poor, and created employments along its value chain, therefore contributing to both global food security and purchasing power in underdeveloped regions. (Belton *et al.*, 2018). Looking in the future, growing and wealthier populations would continue to demand more fish, and aquaculture growth is expected to be a major force to satisfy the demand growth (OECD-FAO, 2011-2016; World Bank 2013). In 2016, aquaculture contributed to almost half of the global food fish supply and models predicted a contribution by 62 % by 2030 (World Bank, 2013; FAO,

2016; Fry *et al.*, 2016). It continues to be the world's fastest growing and most diverse food production sector, with over 95.6% of total aquaculture production being realized within developing countries and the sector growing at an average rate of 6.64% per year, compared with 1.15 percent for economically developed countries (FAO, 2018). Thanks to technological progress and market improvements, culture of low – trophic species such as tilapia (*Oreochromis spp.*) are expected to expand at a faster pace than high-value species (such as salmon), It is expected that the tilapia production will be multiplied worldwide by two between 2010's and 2030 (World Bank, 2013). Being herbivorous or detrivorous, and more flexible toward feed quality, they can be good candidates for a sustainable development in tropical conditions.

## 1.2 Aquafeed sustainability challenges

### 1.2.1 Overview

With a growing trend expected to be around 4.5% from mid-2010's to early 2020's (Cai & Leung, 2017) aquaculture is considered to be a fast growing industry, contributing globally to food security. Good nutrition in animal production systems is essential to economical production of a healthy, high-quality product. In aquaculture, nutrition is critical because feedstuffs typically represent around 40-60% of the production costs in intensive and semi-intensive aquaculture. This 20% variation can be explained by the volatility of the feedstuff prices, which can sometimes jeopardise business profitability (Hasan *et al.* 2007; Rana *et al.*, 2009). Fish nutrition has improved dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture industry as it expands to satisfy increasing demand for affordable, safe, high-quality fish and seafood products.

### 1.2.2 Current challenges

In recent years, aquaculture has been presented as both a solution (Tacon & Metian, 2008) to, and a causative factor (Naylor *et al.*, 2000) of the world dwingling marine ressources.

The aquafeed industry is highly dependent on fishmeal (FM) as high-proteinaceous feedstuffs and fish oil (FO) as high-quality, and highly palatable lipid source. Energy, fatty acids and amino acid profiles offered by the FM fit perfectly with most of the fish diets requirements of most of the fishes juvenile stages (fry and fingerlings). While aquaculture can relieve pressure on wild fisheries through producing fish for human consumption, the production often requires inputs from the wild fish stocks in the form of FM and FO. The paradox stems from the diversity of farmed fish species and husbandry systems. Low trophic species (such as shellfish, some crustaceans, herbivorous fish) requires fewer inputs than higher-trophic species such as Salmonidae which require a complete, protein-rich feed that usually contains a high proportion of marine ingredients.

FM and FO are mainly derived from pelagic fisheries, fisheries by-catches, fish trimmings or offals processings from the food industry (Tacon *et al.*, 2006; IFFO, 2013). Considering that the wild fish captures have remained stable for the last 20 years, and are not expected to increase, and considering the state of the natural stocks being highly unsustainable the FM and FO prices are expected to rise (See figure 1.1). Over the past 20 years, its price more than doubled (Indexmundi, 2018), and according to recent trends, it is expected to increase between 2010 and 2030 by 90 and 70% for the FM and FO respectively (World Bank 2013). This also creates a potential adulteration incentive *i.e.* for substitution higher for lower grade FMs.

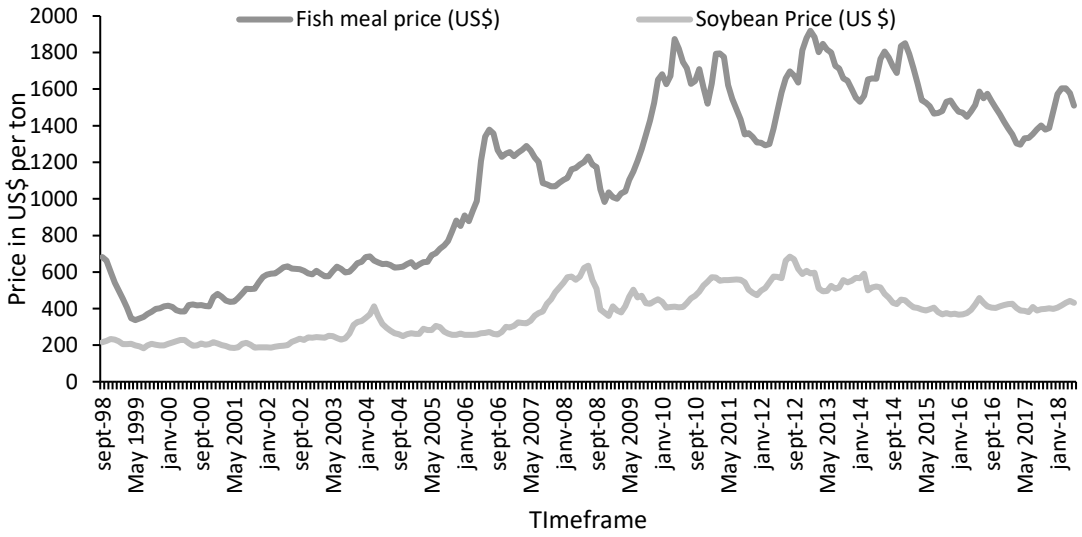



**Figure 1.21**: Price variation in USD, of the Fishmeal and Soybean over the past 10 years. After www.indexmundi.com (2018).

It was estimated in 2008 that aquaculture was the main consumer of FM, accounting for 60.8% of the global FM production (the rest being shared between pig and poultry farming) and 73.8% of the FO production (the remaining 16.2% being used for human consumption) (IFFO, 2018). In 2010 the FM used in aquaculture was 73 % (IFFO, 2018). FM and FO are produced by grinding a large proportion of the world's small pelagic fish catches (anchovy, sardines, menhaden, pilchards etc.). This production of small fish is highly variable, and influenced by regional environmental conditions (Chavez *et al.*, 2003, Hannesson *et al.*, 2006). Approximately half of the global fishmeal production comes from Peru, and is made from anchoveta (*Engraulis ringens*) (Merino *et al.*, 2010). While Peru and Chile (see figure 1.2) are the main producer, most of the FM is consumed in China.

The addition of FM and FO in aquafeed increases the feed efficiency and promotes growth through a better feed palatability. It enhances nutrient uptake, digestion, and absorption. The balanced amino acid composition of FM and fatty acid profile of FO

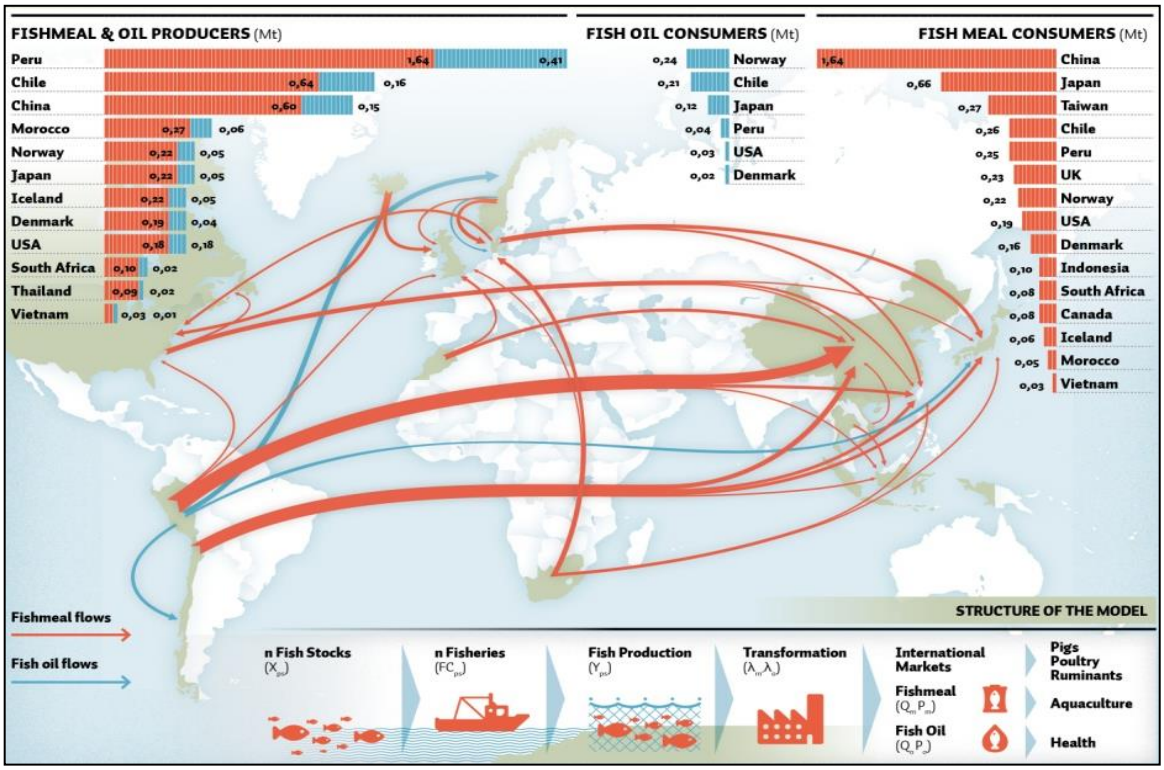



**Figure 1.2**: Fish meal and Fish oil flows worldwide, after Merino *et al.*, 2010.

complements and provides synergistic effects with other animal and vegetable proteins in the diet to promote faster growth and therefore reducing feeding costs. Fishmeal of high quality provides a balanced amount of all essential amino acids, phospholipids, and fatty acids (e.g., DHA or docosahexaenoic acid and EPA or eicosapentaenoic acid) for

optimum development, growth, and reproduction, especially of larvae and brood stock. Enhancing the digestibility of a diet, also helps to reduce pollution in the wastewater effluents by reducing the quantity of feces produced by the fish.

In the aquafeed sector, high-trophic/value carnivorus fish species, such as salmonids and some crustaceans are highly dependant on FM and FO. To a lesser extent, formulated feed for omnivorous and herbivorous fish (carps, tilapias, catfishes etc.) often contains FM and FO as secondary source of nutrient and energy or to improve the feed palatability (Tacon & Metian, 2015). However, declines in the inclusion levels of FM and FO are reported in most aquatic species formulated feeds in response to the price increase ad sustainability issues (Tacon & Metian, 2008).

Given the rapid expansion of the aquaculture industry, demand for feed and feedstuffs will certainly increase considerably. Therefore it is becoming essential to further reduce the aquaculture reliance on marine pelagic stocks by identifying more sustainable, novel feed ingredients for intensively farmed fish diets.

In addition, because the aquaculture expansion is most likely be in favour of non-carnivorous fish, the sustainable supply of animal and plant proteins, lipids and carbohydrates sources other than FM and FO will also become essential given the multiple use of these resources (livestock feeding, human consumption, biogas etc.) (Tacon & Metian, 2008). Although dietary requirements differs between each fish species and between each of their life-stages, the dispensation of feeds that meets the fish nutritional requirements is essential to ensure an optimal growth and survival.

The challenge of the aquafeed industry is therefore to identify new, sustainable, quality-consistent, and cost-efficient feedstuff which could meet the nutritional requirements for each aquatic species and their lifestages.

## 1.3 Emerging sources of proteins

### 1.3.1 Plant-based proteins

Plant proteins have been so far the main choice to replace FM and FO in fish diets. Soybean and its derived concentrates have become a common protein source in aquafeed. In 2007 it represented 25% (in weight) of the total compound aquafeed produced (Gatlin *et al.*, 2007) and in 2008, it was reported that feeds for herbivorous and omnivorous fish and some crustaceans contained in average 25% of soybean meal (Tacon *et al.*, 2011). While, virtually all fish could handle a minimum of 10% to 15% soybean meal in their diets, most of the carnivorous species, such as salmonids, being very sensitive to soybean, cannot handle more than 25% to 30%, with some species handling no more than 15% soybean (Speedy, 2003; Pratoomyot *et al.*, 2011). Therefore for such species, the inclusion levels are rapidly limited due to its intrinsic nutritional characteristic, negatively impacting fish performance (Pratoomyot *et al.*, 2011).

While the apparent protein digestibility of many plant proteins present high similarities with FM, its amino acid (AA) profile is often limiting, like a deficiency in methionine for Soybean (He *et al.*, 2013), or in lysine for cornmeal (El-Ebiary *et al.*, 2005). Tilapia fed diets with soybean meal or cornmeal often show reduced growth due to these amino acid deficiencies (He *et al.*, 2013; El-Ebiary *et al.*, 2005; El-Dahhar & El-Shazli, 1993). Although amino acid supplementation of diets has increased performance of Nile tilapia (El-Saidy & Gaber, 2002), other investigators found that fish utilize synthetic amino acids less efficiently, and excrete more nitrogenous waste, than intact protein (protein-bound) (Schumacher *et al.*, 1997; Zarate & Lovell, 1997). Also, these alternative feedstuffs can reduce the palatability of a feed or can cause health issues such as enteritis, hence, resulting in a reduction of growth and affecting fish performance (Hardy, 2010). Although the nutrient composition is important, so it is the identification of anti-nutritional factors (ANF) which can affect the fish physiology on various aspects (Francis *et al.*, 2001). Therefore, unbalanced essential amino acids, low levels of long-chain polyunsaturated fatty acids, and high levels of anti-nutritional factors limit the inclusion rates of terrestrial

plant ingredients, even in diets for omnivorous species like tilapia (El-Saidy & Gaber, 2002; Karapanagiotidis *et al.*, 2006).

The use of crops to produce feedstuff, links seafood production to terrestrial agriculture, as livestock and farmed fish rely largely on the same crops, such as soybean. Therefore, due to this sector-concurrence, the market prices vary greatly according to the rules of supply and demand. Therefore, their rising use in aquafeeds has the potential to increase price levels and volatility, and eventually worsening food insecurity among the most vulnerable populations. However it could be argued that feeding crops to livestock is much a developed country practice as 40% of cereals are fed to livestock in the U.S. whereas only 14% are fed in the African continent (FAOSTAT 2018). Globally, some 670 million tons of cereals are used as livestock feed each year, representing just over one-third of total world cereal use, and the current fraction destined for aquaculture is relatively small (around 4%) (Troell *et al.*, 2014).

In addition, the commercial livestock sector is extremely responsive to the price of cereals; whenever shortages raise cereal prices, livestock producers tend to reduce their use of cereals as feed, releasing more for food use. As a result, the food use of cereals needs to contract less than it would otherwise. Thus the use of cereals as feed may serve as a useful buffer, protecting the food supply from annual variations (Speedy, 2003).

However, one of the main criticisms for the cultivation of soybean is its environmental impact: Since, the 1960's, the production of soybean has increased over fivefold, from 18 Mt to 100 Mt per year. In some regions, its cultivation is done in deforested areas hosting great biological value (Carvalho, 1999; Osava, 1999) and contributes to biodiversity decline in South America (FAO 2006; Tritsch & Arvor, 2016; Da Costa *et al.,* 2017), use enormous quantities of water (Steinfeld *et al.*, 2006), pesticide and inorganic fertiliser utilisation (Carvalho, 1999) causing significant environmental degradation (Osava, 1999).

Therefore, tying aquaculture's rapid growth to terrestrial crop feed inputs exacerbate existing environmental problems; *i.e*. the expansion of industrial terrestrial-crop (soy, corn) associated with both biodiversity loss and climate change (OECD/FAO, 2015; El-Saidy & Gaber, 2002).

### 1.3.2 Animal-based proteins

Processed animal protein ingredients, principally terrestrial animal by-products such as offals and bone meal, blood and poultry by-product, are more comparable to FM than plants in terms of AA composition. Their nutrient composition, and digestibility is greatly variable according to the product (NRC, 2011). It is estimated that the volume of animal by-products meals would be 2 to 3 times greater than FM, being therefore the largest source of animal protein (Tacon *et al.*¸ 2006; Speedy, 2003).

For instance, poultry by-products, such as hydrolyzed feather meal can be a rich source of protein having a low ash content. Because feather meal is approximately two thirds the cost of other animal proteins on the market and is readily available from many sources, the inclusion of this protein in a commercial diet can effectively lower the price of feed production and utilize an abundant agricultural by-product, yet under-used, as an alternative source of animal protein. Hydrolysed feather, consists of processed - raw feathers. As they contain mainly keratins (containing a disulfide bounds making the proteins unavailable for fish and other animals), the steam/high pressure treatment allows to make proteins and amino acids available for animals. After processing, the apparent digestibility coefficients (ADC) for protein varied between 81 and 87%, comparable to those of standard fish meal, whereby the differences are related to processing conditions. However, a deficiency in methionine, lysine, histidine and isoleucine (Jauncey & Ross, 1982) cannot allow more than a 20 percent inclusion is fish diets without affecting the growth or the feed-conversion efficiency (Chor *et al.*, 2013; Bishop *et al.*, 1995).

Also, recently, researches have been focused on hydrolysates (concentrated proteins) that can be derived from fish by-products, improving growth and feed intake of farmed species like Atlantic salmon (Refstie *et al.*¸2004) and krill that requires more investigation in terms of processing, but has been successfully used to enhance the taste and pigmentation in fish feed (Tacon *et al.*, 2006; NRC, 2011).

However, the outbreak of bovine spongiform encephalopathy (similar to the Creutzfeldt-Jakob disease in humans) in the late 1980's, caused by the presence of prions in the animal by-products used in the animal feed which contaminated the whole food chain, the trust toward these protein sources have been seriously damaged. Therefore, the use of spinal cords, brain and other derived by-products is forbidden in animal feed. It is also important to highlight that fish fed with bone meal (or any derivate from the pork industry) are not

considered *halal*, and therefore unsuitable to consumption by Muslim people in some regions of the world (like in Malaysia or Indonesia), this greatly restricts the usage of these ingredients in the farmed-fish diets (Saidin *et al.*, 2017).

Despite the relaxation of the European Union Restrictions implemented after the outbreak to use these meal by-products in aquaculture, and evidences that the contamination risk through fish is almost inexistent, there is still a mistrust in this sector, hampering its usage in Europe (Ingrosso *et al.*, 2006; Naylor *et al.*, 2009).

### 1.3.3 Algae and unicellular-based proteins

Microalgae companies increasingly seek markets for defatted biomass that is left over after extracting omega-3 rich oil for human pharmaceutical and crude oil for fuels. Such a protein-rich co-product is a promising alternative to fishmeal in aquaculture diets (Sarker, 2018). They possess an adequate nutrient profile (Pulz & Gross, 2004; Becker 2003), are rich in proteins (47.7% for *Nannochloropsis spp*., 49% for *Phaeodactylum tricornutum* for instance, according to Daniel *et al.*, 2016) and, are rich in coloring agents such as beta carotene, used to enhance the colour of the fish (Daniel *et al.*, 2016). In addition, they present a suitable amino acid (AA) profile for fish, a correct fatty acid profile (containing for instance docosahexaenoic –DHA-, eicosapentaenoic –EPA- and arachidonic acid –ARA- and omega-3) (Nell *et al.*, 1991). Several experiments where algae were used as a replacement for conventional protein sources showed some promising results, particularly in Tilapia culture (Adarme-Vega *et al.*, 2012; Patterson & Gatlin, 2013; Moreno-Garcia *et al.*, 2017; Sarker *et al.*, 2018). Algae production offers several advantages over terrestrial crops: improved land and water-use efficiency due to higher yields per unit input, potential in-land cultivation, and lower greenhouse emissions (Beal *et al.* 2015; Gerber *et al.*, 2016; Walsh *et al.*, 2016). The productivity of such systems is very high (up to 10 000 tonnes per year for microalgae according to Richmond, 2004) and can be used as recycling system for waste waters from the agro-industry (Tacon *et al.*, 2006; Adarme-Vega *et al.*, 2012) and their integration from such industrial co-products into aquafeed could provide an additional revenue stream from algae biomass and biofuel/ pharmaceutical production. However, technical difficulties in manufacturing, drying and storing them (Borowitzka, 1997; Becker, 2003) make them difficult to upscale their production (Daniel *et al.*, 2016). However, analysts predict that

microalgae cells will quickly become a cost competitive substitute for fishmeal due to ongoing technological improvements that are expected to lower production costs for microalgae (Holland, 2016). At the moment, the cost is still higher than FM (US$1.65 per Kg against 1.30 US$ for FM according Sarker *et al.*¸2018). In other words, it may not be a viable solution for aquaculture yet (Tacon *et al.*, 2006; Olsen & Hassan, 2012).

### 1.3.4 Novel proteins with potential for local sourcing in developing countries

The growing need for sources of animal food in developing countries has unveiled the potential of underutilised, un-investigated, or endemic feedstuff used by small-holders farmers, through low-cost, farm-made feed formulations (Hasan *et al.*, 2007; PAF, 2011). These feed ingredients, which have hitherto not been used in fish feed production for various reasons; low digestibility (like the Palm Kernel Meal in some fish species) low availability, inconsistent quality (like kitchen waste), or because they can become toxic or poisonous if improperly stored or prepared (like groundnut cake which can, when mouldy, release aflatoxins). One of the main advantages reside in the lack of opportunity cost for human consumption, low cost as cheap processing by-products or otherwise unused waste products from agriculture. Studies have been made on protein sources such as leaves (like *Dialium guineense* a west-african plant from the Fabaceae family), brewer's yeast and brewer's grains (Djissou *et al.*, 2016), live or dried daphnias, rotifers, Palm Kernel Meal (PKM), Groundnut cake or Sorghum (Abowei & Ekubo, 2011). Freshwater plants like Azolla (*Azolla piñata*) can substitute upto 40% of the commercial feed in tilapia diets without compromising their growth (Djissou *et al.*, 2016), or water hyacinth (*Eichhornia crassipes*), *Ceratophyllum sp.*, or even water lilies (*Nymphea sp.*) (Abowei & Ekubo, 2011). Also, some toxic plants can be used by prior pre-processing of their leaves, like Mucuna, broad, sword and yam beans (Abowei & Ekubo, 2011). But, most of the time, due to the lack of formulation, the feedstuff used do not follow the fish requirements, potentially impairing its growth. Also, due to a high water content, the use of plants involves high processing costs required to dry them.

These non-conventional feedsources have been mainly investigated in their local context and have been considered only for low-trophic level species such as tilapia or catfish (El-Sayed, 2004; Hasan *et al.*, 2007; Sogbesan & Ugwumba, 2008). Given the growing

demand to produce larger quantities, more sustainable and economically viable alternative ingredients for animal feeds, it is important to continue investigating these potential under-utilised feed sources (El-Sayed, 2004; Rust *et al.*, 2011; Tacon *et al.*, 2011) in conjunction with development of sustainable novel technologies for cost-effective standardisation and up-scaling of production.

## 1.4 Insects as a source of feed

Entomophagy –the consumption of insects-, has been practiced by humans on almost every continent for centuries (Wang & Shelomi, 2017) with archaeological evidences demonstrating that insects were a large part of our diet in many regions (Sutton 1995; Raubenheimer & Rothman 2011). Entomophagy is now practiced in at least 113 countries, and use over 2,000 (documented) edible insect species (Jongema, 2017). The presence of insects in the cuisine plays a prominent role in some contemporary cultures, with for instance, consumption of criquets, grasshopper or coackroaches in South-East Asia, "*Mopane worms"* (*Gonimbrasia belina*, a moth) in Southern Africa, "*Akokono"* in Ghana (*Rhynchophorus ferrugineus*, a beetle) "*chicatanas"* (ants from the genus *Atta*) in Mexico, "*Zandettes"* in La Reunion Island (fried wasps from the genus *Polistes*) or fried long horned beetles grubs in Micronesia (Coleoptera from the genus *Olethrius* and *Xixuthrus*). In 1758 in his book *Systema naturae*, the Swedish naturalist Carl Von Linné even wrote about the beetle larvae "*larvae assate in deliciis habentur*" (roasted larvae are delicious). Being very proteinaceous, and containing many essential micronutrients, insects can account in some populations, for a large part of the protein income such as some regions in the Central African Republic, where at times, it can account for up to 50% of dietary protein incomes substituting less-readily available vertebrate protein sources (Raubenheimer & Rothman, 2011).

Therefore, the idea of using them as feedstuff in animal feed is not a novel concept with research into their nclusion in poultry and pig diets starting in the 1970's (Teotia & Miller, 1974; Phelps *et al.*, 1975; Newton *et al.*, 1977; Calvert *et al.*, 1969; Wang & Shelomi, 2017). A decade later, their potential as fish feed started to be assessed (Bondari & Sheppard, 1981, 1987; Tacon *et al.*, 1983; Wang & Shelomi, 2017).

To be more explicit, the number of scientific publication on the use of insects as animal feed, in the last 15 years (2000-2015) has tripled compared to the previous 30 years (1969-

1999). During the first 8 years of the 21th century (2000-2007) there were 34 peer-reviewed articles, against 56 published during the next 8 years (2008-2015). While the number of livestock feed experiments has doubled over the past 15 years, the number of feeding experiments in aquaculture has quadrupled, traducing this growing interest (Sánchez-Muros *et al.*, 2016).

Therefore, over the past 20 years, a renewed interest in insects has risen, motivated by the global need for alternative and sustainable animal source food, and as for their potential as waste management solutions.

### 1.4.1 Nutritional qualities

So far the nutritional potential of up to 24 different insect species belonging to 6 different orders (Blattodea, Coleoptera, Diptera, Isoptera, Lepidoptera, Orthoptera) has been evaluated. However, most of the work was done on Diptera and Orthoptera (representing 48 and 29% respectively of all published articles until 2015) (Sánchez-Muros *et al.*, 2016).

Most of the insect studied, have high proteins levels (Ladron De Guevara *et al.*, 1995; Ramos-Elorduy *et al.*, 1997) comprising between 30% and 65% of the total dry matter (Dobermann *et al.*, 2017). Some studies revealed that several species contains at least –if not more- the same protein levels than FM and soybean. The highest protein values (based on a dry matter basis) recorded were found in the Orthoptera order, where *Boopedon flaviventris, Melanoplus mexicanus*, and *Sphenarium histrio* contain 76, 77.1, 74.8% respectively. In Coleoptera, the larvae of the the Dysticidae, *Ranthus atricolor* host 71.1% proteins and the cactus weevil (*Metamasius spinolae*) up to 69.1%. Regarding the Diptera, *Drosophila melanogaster* larvae can contain up to 70.1% proteins (Sanchez-Muros *et al.*, 2014).

Levels of protein, fat and energy vary across insect species and also within species depending on what the insects have fed on, stage of development, sex and environmental factors (Bukkens, 1997; Ramos-Elorduy *et al.*, 2002; Finke & Oonincx, 2014). However, general ranges have been estimated in Table 1.1.

**Table 1.1**: Protein, fat and energy content of some insects. The values are expressed based on dry matter percentages, while the energy is expressed as kcal/100g. Data from Rumpold & Schlüter (2013a); Barroso *et al*., (2014); Devic (2016). Specific species were selected as examples if they deviated significantly from the average, or are one of the most popularly consumed species. If there was more than one entry for a specific species, the average was calculated.

| | Proteins (%) | Lipid (%) | Energy (Kcal/100g) |
|---|---|---|---|
| **Coleoptera (mean)** | 40.69 | 33.4 | 490.3 |
| *Rhyncophorus phoenicis* (palm tree weevil – larvae) | 32.86 | 36.86 | 478.87 |
| *Tenebrio molitor* (mealworm- larvae) | 48.35 | 38.51 | 557.12 |
| *Zophobas morio* | 53.5 | 6 | 122.76 |
| **Diptera (mean)** | 49.48 | 22.75 | 409.78 |
| *Musca domestica* -larvae (common housefly) | 46.9 | 15.3 | 209.5 |
| *Hermetia illucens* (Black Soldier Fly) | 36.2 | 36.5 | 501,3 |
| *Lucilia sericata* (Common bottlefly) | 53.5 | 13.2 | -- |
| **Hemiptera (mean)** | 48.33 | 30.26 | 478.99 |
| **Hymenoptera (ants,bees, wasps) (mean)** | 46.46 | 25.09 | 484.45 |
| *Oecophylla smaragdina* (Weaver ant) | 53.46 | 13.46 | -- |
| **Isoptera (termites) (mean)** | 35.34 | 32.74 | -- |
| **Lepidoptera (butterflies, moths) (mean)** | 45.38 | 27.66 | 508.89 |
| *Bombyx mori* (silkworm caterpillar) | 61.8 | 8.81 | 389.6 |
| *Cirina forda* (Shea caterpillar) | 47.48 | 11.5 | 359 |
| *Galleria mellonella* (waxworm caterpillar) | 38.01 | 56.65 | 650.13 |
| *Samia Cynthia ricinii* (ailanthus silkworm pupae) | 54.7 | 25.6 | 463.63 |
| **Odonata (dragonflies, damselflies) (mean)** | 55.23 | 19.83 | 432.33 |
| ***Orthoptera* (crikets, grasshoppers, locusts) (mean)** | 61.23 | 13.41 | 426.25 |
| *Acheta domesticus* (house criket, adult) | 65.04 | 22.96 | 455.19 |
| *Schistocera sp.* | 61.05 | 17 | 427 |
| *Sphenarium purpuracens* | 61.33 | 11.7 | 404.22 |
| *Ruspolia differens* | 44.3 | 46.2 | -- |

Between 46% to 96% of all AA are present in evaluated insect proteins, although there are usually limited amounts of tryptophan and lysine (Bukkens, 1997; Ramos-Elorduy *et al*., 1997; Dobermanh *et al.*, 2017). Relative to fish meal, insect meals are usually deficient in histidine, lysine and threonine, but contains usually more lysine methionine and tyrosine than soy meal.

Two major AA profiles can be identifed between the different insects orders: Diptera are, very similar to FM, while Orthoptera and Coleoptera orders are closer to soybean meal (Barroso *et al.*, 2014). Diptera also have levels of histidine, methionine, lysine and threonine comparable to FM with a higher proportion of phenylalanine than fish meal or soybean. However, Diptera have a relative deficit in leucine that does not occur in Orthoptera or Coleoptera. The percentages of tyrosine and valine were higher in all analysed insects than in fish meal. (Barroso *et al.*, 2014)

The lipid content of insects is very variable, and generally ranges between 7 to 77 g/100g (on a dry weight basis), the maximum recorded being the Mexican caterpillar of the moth *Phassus triangularis* where the lipid content exceed 77% of the body composition (on a dry matter basis). However, with proteins, the lipid content varies greatly during the development stage, and for holometabolous insects, which have a larval and pupal stages, it is higher in larvae and pupae than in adults (Sánchez-Muros *et al.*, 2014; Ramos-Elorduy *et al.*, 1997). Soft-bodied insects such as isopteran, also display a high lipid content (around 30%), while adult insects with a hard exoskeleton, such as Coleoptera, or Orthoptera are at the lower end f the lipid spectrum (Bukkens, 1997).

Lipid content averages about 20% (DM basis), and therefore tends to be higher than fishmeal (usually around 8%) or soy meal (around 3%). This has some advantages: it could be useful when formulating high energy diets for animal livestock, like broiler chickens (Sánchez-Muros *et al.*, 2016), or –if properly extracted could serve other purposes: some authors have even proposed use of insect oil to produce biodiesels, and the resulting enriched-protein paste could be used for animal feed (Manzano-Agugliaro *et al.*, 2012).

The substrate used to feed insects greatly influences their fatty acid profile. For instance, it has been demonstrated, that feeding fish offals to Black Soldier Fly larvae enrich them with n-3 LcPUFA, EPA, DHA acids. When fed to trouts, this enriched larvae successfully improved the Omega-3 and 6 FA levels in fish muscle (Sealey *et al.*, 2011).

Based on the substrate, species, and lifestage of the studied insects change the FA profile, some broad trends can be identified. Insects meal show reduced levels of linoleic acid (18:2n-6) compared to soybean, however, compared to FM it exhibits higher levels of PUFAS n-6 (Omega-6 fatty acids) and low levels of n-3 PUFAS, especially 20:5 n-3 (EPA) and 22:6 n-3 (DHA) (Rumpold & Schlüter, 2013a; Calder, 2017). However, while being low, the levels of Omega-3 and 6 FA are still higher for many insects than any other animal livestock such as beef, pork and chicken. The inclusion of PUFA is essential

because they affect many important biological functions in vertebrates (Sánchez-Muros *et al.*, 2016).

The low levels of n- 3 impose a limit on the inclusion of insect meals in diet formulations, and particularly fish diets when fishmeal is replaced by insect meal. In fact, when a 25% substitution of FM by BSF meal is realised, it starts affecting the n-3 FA levels in the fish (St-Hilaire *et al.*, 2007).

The possibility to manipulate insects FA composition through choice of feed substrate is a great advantage to overcome the lack of LcPUFA, essential for carnivorous and marine fish species. Alternatively, defatting can also be used to to improve the quality of insects meals to remove unnecessary lipids and FA not matching with the fish dietary requirements and to concentrate proteins (Fasakin *et al.*, 2003; Kroeckel *et al.*, 2012; Henry *et al.*, 2015).

Rumpold & Schlüter (2013) published an exhaustive review on the mineral and vitamin contents found in different insect species. As stated earlier, it varies with the order, species and lifestage, however, insects generally contain low levels of calcium, potassium and sodium (Hwangbo *et al.*, 2009), and contain high levels of phosphorous. The magnesium levels are very high in Hemiptera and Orthoptera (Rumpold & Schlüter, 2013). The iron, magnesium, manganese, selenium, zinc, and copper content allows insects to be used as a good source of micronutrients for livestock (Rumpold & Schlüter, 2013; 2015).

Insects are generally rich in Vitamin B2 (between 0.11-8.9 %), pantothenic acid and biotin (FAO, 2004; Bukkens, 1997). It can be highlighted that Orthoptera and Coleoptera shows high levels of Vitamin B12, B1, and folic acid (Finke, 2002; Bukkens, 1997). On the other hand, insects are generally deficient in Vitamin A, C, niacin, and vitamin E (FAO, 2004).

Recently, it has been demonstrated that house crickets, BSF and mealworms can synthesise *de novo* vitamin D2, D3 when exposed to ultraviolet light, similarly to vertebrates (Oonincx *et al.*, 2018).

Several Anti-Nutritional Factors (ANF) in insects were identified, such as oxalate, phytate, tannin and hydrocyanide, but were far below toxic levels, therefore can be considered negligible (Omotoso, 2006; Ekop *et al.*, 2010).

The presence of chitin may also impair feed performance (Shiau & Yu, 1999; Olsen *et al.*, 2006; Rust, 2002). Chitin is an n-acetylated polysaccharide playing a major role in the structure of the insect's cuticle. It is always covalently bound to catechol compounds and sclerotin-like proteins (Majtan *et al.*, 2007). Its proportion varies greatly amongst

insects' orders and different lifestages (typically, it is usually higher in adults than larvae, and higher in highly sclerotized insects like Coleoptera) (Barroso, 2014).

With a caloric content of 17.1 $KJ.g^{-1}$, chitin could constitute a substantial percentage of the total energy intake. However, the β1,4 bond in chitin is indigestible for several fish species (Rust 2002 *in* Sanchez-Murros *et al.* 2015) and, in some studies, its presence might have affected the growth performance by influencing the feed intake, availability and digestibility of nutrients, including proteins (Longvah *et al.* 2011) or lipids (Kroeckel *et al.* 2012), leading to a reduction in growth suggesting that it could be an ANF (Sanchez-Muros et al 2015). The hydrolysis of chitin requires the involvement of chitinase and chiobiase enzymes.

However, this nitrogen-containing polysaccharide, can account for 5-8% of the total nitrogen in insects (Kumar, 2000). Therefore its presence may lead to an over-estimation of the crude protein content (Diener *et al.*, 2009) when the protein levels are only tested using the Kjedahl method, and when no correction measures are applied (more details in Chapter 4).

The removal of chitin during meal manufacture is being investigated. Chitosan can be extracted (Guangdong Entomological Institute, in a patent developed in 2015) or alternatively chitin could be degraded by enzymatic methods before being added to diets as a product of hydrolysis (*i.e.* Chito-oligosaccharides, acetylglucosamine or chitosan) or via an alkaline extraction (DeFoliart *et al.* 1982; Belluco *et al.* 2013; Shiau & Yu, 1999; Se-Kwon & Niranjan, 2005; Lin *et al.* 2012a,b). Its removal could improve the protein quality of the feed, making it more digestible (Newton *et al.*, 2005; Sheppard *et al.*, 2007; Rumpold & Schlüter, 2013).

Also, if properly removed and purified, chitin could potentially become a high value by-product. Chitin and chitosan can be used as antioxidant, anti-inflammatory, drug delivery-media and plastic (Park & Kim, 2010). Several recent studies, highlight the fact that at low levels, incorporated in a feed, chitin may have a positive effect on the functions of the immune system. Having antimicrobial properties (Rinaudo, 2006), it can act as an immunostimulator effective on a short term basis (Mastan, 2015). However, at the moment, the technical and economic feasibility of chitin extraction have yet to be determined (Diener *et al.*, 2011).

Several antibacterial and antifungal peptides have been detected in numerous insect species, potentially improving the shelf life of insect-based feed (Ravi *et al.*, 2011; Zhao *et al.*, 2010). Methanol extracts of BSF larvae showed antibacterial activity that strongly

inhibited proliferation of *Klebsiella pneumonia*, *Neisseria gonorrhoeae* and *Shigella sonnei*. It also had the unique property of virtually blocking the viability of the bacteria (Choi *et al*., 2012). This methanol extract was later narrowed down to hexanedioic acid, which also showed antibacterial properties that effectively inhibit the growth of bacteria like *Staphilococus. aureus* meticilline resistant (MRSA), *K. pneumonia* and *Shigella dysenteriae* (Choi & Jiang, 2014; Park *et al.*, 2014). The antimicrobial substances in BSF larvae could be a potential source of novel antimicrobial-like compounds for infection control (Park *et al.*, 2014).

While the nutritional value of insects varies with the species, stage and substrate they were fed on, insect remain a potentially valuable and proteinaceous feedstuff, ranging in the acceptable levels of proteins, lipids and micronutrients of any other feed source. The fact that the fatty acid profile can be easily (and widely) modulated is a great advantage for a future specific feed formulation, targeting special requirements.

### 1.4.2 Waste remediation

One-third of the food produced for human consumption, around 1.3 billion tonnes annually, is currently lost or wasted (HLPE, 2014; FAO, 2018), with an estimated value of 680 billion US$ in industrialized countries and 310 billion US$ in developing countries (Charles *et al.*, 2010). Along with the intensification of the livestock industry expected to generate twice more manure by 2050 (FAO, 2011) sustainable solutions to deal with this waste streams are required. The use of low-value, or even better, waste not used in any other value chain, could be a favourable framework to develop the insect industry for cost-efficiency and sustainability. Thanks to the ability of several farmed-insect species to develop upon a wide range of organic substrates, their production could follow an integrating bio-system approach where organic waste that could impair the global environment if not appropriately treated or disposed, could be used to generate animal feed, reducing the greenhouse gases emission, and improve environmental safety, contributing to close the loop for nutrients flows, resulting in a zero-waste industry.

Although challenges remain, related to the water content, transportation or risk of contamination, there is an opportunity for an insect-based waste remediation. Besides generating insect meal, the production of frass at the end of the bioconversion process could be used as a biofertiliser for crop production. The value chain that is developing

around this industry is circular and based on the relationship between environment (ecological impact and benefits) and the market demand for this protein source and biofertiliser, the efficient use of insects can close the loop the nutrient loop applying the principles of circular economy (Vedlkamp *et al.*, 2012; Van Huis *et al.*, 2013).

However, care should be used if the insects function as waste converter: as said previously, the nutritional profile of arthropods is the reflection of what they eat, therefore, they can potentially bio accumulate chemical contaminants. While, the larvae analysed in most studies, generally possessed levels of chemical contaminants below the recommended maximum concentrations (according to the European Commission, the World Health Organisation and Codex Alimentarius Commission, 2010), exception can happen, like high levels of Cadmium found in in *Musca domestica* larvae (Diener *et al.*, 2009, Charlton *et al.*, 2015). While the black soldier fly has a high tolerance for heavy metals (Cai *et al.*, 2017), it makes it even more important to prevent contamination because it might go unnoticed when present.

Antibiotic contamination with veterinary medicine like Nicarbazin is also a source of concern, Charlton *et al.* (2015) showed that larvae of *Musca domestica* could accumulate this chemical, when fed with contaminated feces.

Recently a study conducted by Cai *et al.* (2018a) demonstrated that Black Soldier Fly larvae could successfully breakdown tetracycline –a commonly used antibiotic for livestock- through its digestive tract. A follow-up study (Cai *et al.*, 2018b) even demonstrated that the frequency of antibiotic resistant genes in the microbial community remaining in the waste after digestion is reduced. The same species is also known to successfully degrade three commonly used pharmaceuticals (carbamazepine, roxithromycin and trimethoprim) along with two pesticides (azoxystrobin and propiconazole) with no bioaccumulation detected in the larvae (Lalander *et al.*, 2016).

Therefore, potentially acting as bioaccumulator, the insect meal mass-production should be carefully monitored to ensure that no heavy metals, pesticides nor drugs are present in the final feedstuff, and this prevention start first with the feeding substrate given to the larvae (Van Der Spiegel *et al.*, 2013).

### 1.4.3 Insect farming: insect as mini-biorefining factories

Insect mass production is essential to supply the growing demand for proteins in both human and animal nutrition. Their production could be a sustainable way to avoid the over-exploitation of wild resources (Sanchez-Muros *et al.*, 2016). Insect farming is not a new practice as commercial mass-production systems have exited for centuries for apiculture, and sericulture (silkworms), or to support the biological control of agricultural pests (Rumpold & Schlüter, 2013). Also, more recently, the mass breeding of mosquitoes, for the Sterile Insect Technique, is proven successful. In a nutshell, the idea is to breed massively insect vectors, discard the females (which draw blood to oviposit), sterilise males (by irradiation or the use of the bacteria Wolbachia to affect their fecundity) and release them in the wild so they could mate with wild females, and transfer a sterile sperm, hampering the fecundity and therefore the vector population in the wild (Oliva *et al.*, 2012).

This knowledge was the basis for the development of mass-rearing of edible insects. Insect-farming ensures a traceability, constant quality, and consistency of the insect-based products (Rumpold & Schlüter, 2013) and is not different than any other conventional livestock systems: The insects need access to water and feed (substrate) to supply energy and nutrients for growth and excrete intestinal content (frass). The production is impacted by the physical conditions (small scale/large scale, low or high level of technological management solutions etc.) and the level of biosecurity in place to prevent introduction of e.g. microorganisms from the surrounding environment (wildlife, neighbouring animal farms, waste management units etc.). In principle, there are no differences in the farming system regarding rearing of insects for feed or for food although they have to comply with different legislative frameworks (discussed in Chapter 1.3.4).

In European insect farms, insects are kept in a closed environment, in boxes/cages, where the atmosphere, substrate, water etc. can be controlled. Currently no hormones, antibiotics or chemicals are used for the existing insect farming systems except for biocides to disinfect the production environment in between batches of insects (European commission, 2015).

At the moment, 5 main species are mass-reared for a food and feed target, mostly in Asian countries (Figure 1.3). While Orthoptera (Mainly *Acheta domesticus*, the house criquet),

due to their high commercial value (around 23 US$ per kilo on a dry weight basis, Jesse Willems Pers. Comm., 2018) are more targeting the insect-as-food market, Mealworms (mostly *Tenebrio molitor*), the Black Soldier Fly –BSF- (*Hermetia illucens*) and *Musca domestica* (the common housefly) are expected to be used mostly (but not only for the case of mealworms) as an animal feed source.

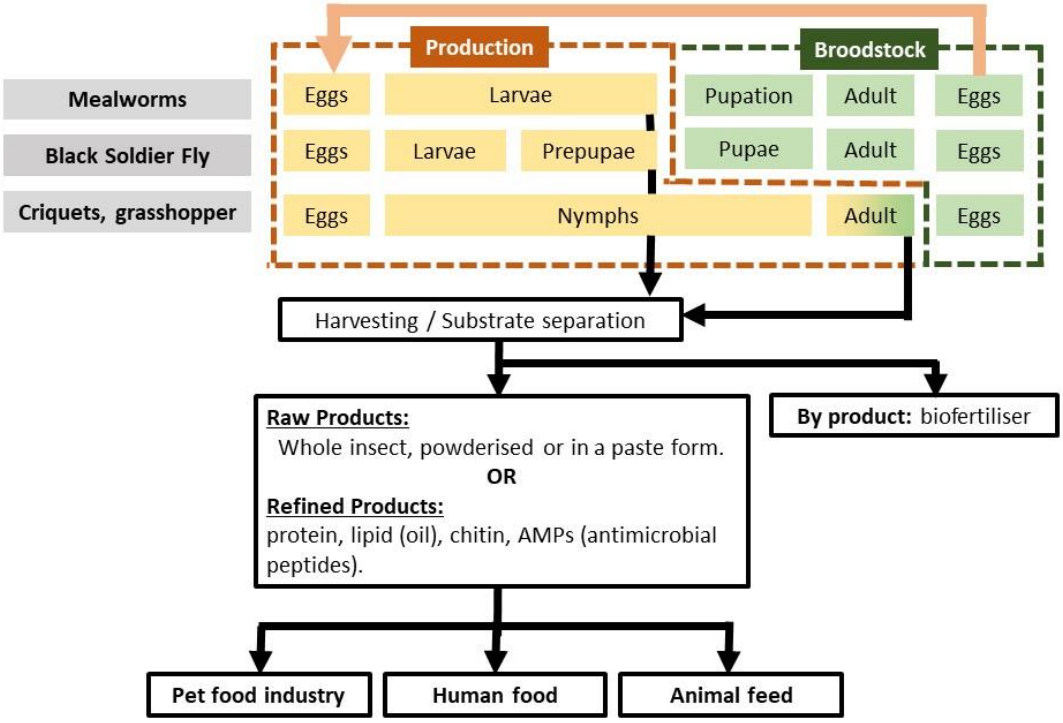



**Figure 1.43**: Overview of the chain from production to consumer. The main farmed species, used as food or feed, are grouped by the lifestages of harvesting. The lifecycle is divided in two parts: the first one being used for the production of the insect meal and the biofertiliser, while the second one is kept for broodstock and reseed the substrate for the production.

Regarding mealworms, very few companies have yet upscaled their production. We can cite as an example Ynsect in France, which pilot-production factory is aiming to produce 17 tonnes of fresh larvae per year (Thévenot *et al.*, 2018). Despite having a fairly long lifecycle (it takes up to 10 weeks at 28°C to obtain the larvae), the main advantage is that, due to the dryness of the substrate (around 18 percent according to Wu, 2009), harvesting the larvae is very easy, and can be done by a simple sieving (Morales-Ramos *et al.*, 2011b).

However the biggest issue, is the fact that they require the use of a dry and formulated substrate. Often, agricultural by-products like wheat bran are used with raw vegetables as supplements to balance the nutrient ratio of the feed, as some important nutrients are missing in wheat bran such as vitamins, essential fatty acids, and sterols (Van Broekhoven *et al.*, 2015; Morales-Ramos *et al.*, 2011a). Therefore, a substrate already use for animal feed, is turned to grow insects…to be themselves used later as animal feed. Efforts should focus on identifying alternative sources of feed for insects that have lower environmental impacts, as shown by Smetana *et al.* (2016) and Halloran *et al.* (2016). For example, mealworms could be fed low-value agricultural by-products such as palm tree pulp flour (Alves *et al.*, 2016), dried distillers grain or spent grain from makgeolli (Kim *et al.*, 2016). For the moment this contradiction, yet to be addressed, forces to research yet un-used agricultural by-products to increase the economic viability, and decrease the environmental footprint of this production systems.

Regarding the housefly, the breeding is very easy, as it can be bred on a large range of organic substrate, and has a rapid lifecyle (it takes 3-6 days to obtain market-size larvae at 28°C). However, one of the main constrains comes with biosecurity: the common housefly is an anthropophilous species, and colonise sewage, rotten material, food waste etc. Therefore, in case of escape, it can be a major source of nuisance for nearby habitations. While the adults feed (contrary to the BSF), they can transmit disease through their saliva. Transmission takes place when the fly makes contact with people or their food and can be a vector to a great number of diseases including enteric infections (like dysentery, diarrhoea, typhoid, cholera and certain helminth infections), eye infections (such as trachoma and epidemic conjunctivitis), poliomyelitis and certain skin infections (such as yaws, cutaneous diphtheria, some mycoses and leprosy) (WHO, 1986). In addition, adults can transmit fungi (Phoku *et al.*, 2014) *Escherichia coli* (De Jesùs *et al.*, 2004), and *Salmonella enterica* (Holt *et al.*, 2007) through their droppings. Their larvae, not only do not reduce the bacterial load of the substrate, can host -and seed- new substrates with these bacteria. Therefore, if not sterilised, the meal produced from these larvae can be potentially pathogenic for the animals, like the example presented by Davies & Wray (1993) where chicken fed with contaminated larvae of *Lucilia sericata* (a close relative to *Musca domestica*) caused a salmonella infection in the birds.
The particular case of the Black Soldier Fly is detailed in the next dedicated Chapter (1.5).

The breeding process of all these species, beside producing the insect meal, ends up with an feces ("frass") enriched substrate, called biofertiliser. As most of the studies were realised on the BSF frass, this topic will be discussed in the next chaper (1.5).
The production of insect meal results in less greenhouse gas emission, requires less land compared to conventional livestock production (the production trays can be stacked vertically to maximise the production surface), therefore resulting in a lower ecological footprint (Oonincx *et al.*, 2010; Oonincx & De Boer, 2012). Insect susceptible to this mass-rearing are highly prolific species, and if their growth is done on organic waste, can reduce the environmental impact on the livestock sector (Oonincx *et al.*, 2015; Van Zanten *et al.*, 2015). In addition, insect meals will soon not only offer the raw product but could be purified, to produce derivated products, such as protein concentrates, chitins, oils, antimicrobial peptides.

### 1.4.4 Regulatory framework

The General Food Law (Regulation (EC) 178/2002) sets the framework for EU legislation on food and feed, and applies to all stages of food and feed production. Since, 2013, under strict conditions, the EC regulation 56/2013 lifted the ban on the use of non-ruminant Processed Animal Proteins (PAP) in farmed non ruminant, including aquaculture, feed.
When farmed insects or products derived from farmed insects are produced to be used as food for humans, or as feed for food-producing animals, the industry has to comply with rather complex legal requirements. These requirements are related to the insects themselves, the feed or substrate fed to the insects, the firm producing the insects, and the ultimate marketing for use as food or feed (Van Der Spiegel *et al.*, 2013).
As farmed insects are non-pathogenic invertebrates species for humans of animals, they are considered as Category 3 material (EC regulation 1069/2009). While the re-authorised Processed Animal Proteins (EU regulation 142/2011) are derived category 3 materials and must answer to constraining requirements during collection, processing and slaughter in certified abbatoirs, the regulation (EC) 1099/2009, does not mention insects (Halloran & Münke, 2014).
As no requirements for insects are laid down in Annex III of Regulation (EC) 853/2004, establishments handling insects do not need approval prior to the start of operation (but should be registered to the proper authorities). This prescription is posed by regulation

(EC) 183/2005, which defines safety and hygiene standards for animal feed products. The breeding of insects is part of "primary production," the processing of insects into food is not. Thus, companies breeding and converting insects into food should comply with Annex I and II of Regulation (EC) 852/2004. It only allows to breed seven species of insects which are: *Hermetia illucens* (BSF), *Musca domestica* (Common housefly), *Tenebrio molitor* (yellow mealworm), *Alphitobius diperinus* (Lesser mealworm), *Acheta domesticus* (House criket), *Gyllodes sigillatus* (banded criket), *Gryllus assimilis* (field criket), only if they have been processed by according methods set in the chapter III, annex IV in the regulation (EU) N° 142/2011.

Insects may only be fed with materials of vegetal origin. Some exceptions are however admitted for the following products having an animal origin (category 3 materials): Fishmeal, blood products (from non-ruminants) di and tricalcium phosphate of animal origin, hydrolysed proteins from non-ruminants, hydrolysed proteins from hides and skins of ruminants, gelatine and collagen from ruminants, eggs and eggs derivated-products, milk, milk-based products, milk-derived products, colostrum, honey and rendered fat. The feeding substrate of the insects shall not have been in contact with any other material of animal origin, beside those mention above, and the substrate shall not contain manure, catering waste or other waste (EU, L138/111, 2017).

In a nutshell, at this moment, the feeding of farmed insect shall use only agricultural waste, or animal-based waste as listed before, but cannot be fed with slaughterhouse or rendering derived products, manure, or catering waste. The same ban applies to the use of unsold products from supermarkets or food industries (e.g. unsold products in reason of manufacturing or packaging defects) that contain meat or fish.

Today, farmed animals derived proteins are banned for use in feed for ruminant (e.g. cows) and monogastric animals (e.g. pigs and poultry animals). More commonly known as the EU 'feed ban', such prohibition was introduced by EU public authorities in reaction to the Bovine Spongiform Encephalopathy (BSE) outbreak in the early 2000'. The feed ban rules are contained in Regulation No 999/2001 (see article 7 and Annex IV), which is more commonly known as the 'TSE Regulation'. Insect based-feed is allowed for pet food and fur animals (e.g. mink). This ban is not applicable to insect oil.

The regulation N° 2017/893 (effects starting on the 1st July, 2017) adopted by the European Commission on 24 May 2017, authorises the use of insect proteins coming from seven species –listed above- in feed for aquaculture animals. The European Commission

services are currently exploring the possibilities for proposing a new revision of the feed ban rules in order to authorise pig and insect proteins in poultry feed.
To sum up, it is possible to breed in the EU seven insect species, if they were bred on agricultural by-product (except manure) and some specific products having an animal origin. The insect meal can be used as feed, for pet food, fur animal diet, aquaculture, but not yet in poultry or pig industry. Insect oil can be used for all livestock.

The EFSA Scientific Committee (2015) highlighted that risks of contamination (like heavy metals, as detailed above) are still high through the value chain, and needs to be assessed. Regarding the occurrence of prions, while their expression or replication is not considered possible in insects, a contamination is still possible, as the insects can be a passive vector. Therefore the total prion infectivity carried by the insects would depend on the amount of infectivity present in the substrate the insects are fed on and can only be equal to or small than this (Thackray *et al.*, 2012, 2014a, b).
This report concluded that for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species and developmental stage, as well as the methods for further processing will all have an impact on the occurrence and levels of biological and chemical contaminants in food and feed products derived from insects. While hazards related to the environment are expected to be comparable to other animal production systems. Given the lack of knowledge identified around these risks, it was suggested that more studies should evaluate these hazards risks when insect are used as food or feed. In other countries insects are listed as acceptable and safe ingredients (Halloran & Münke, 2014). In Ghana, Mali, Kenya and Uganda, there is no legislation preventing their use as feed, while in China or Thailand, Insect meal is listed in the Feed Material Catalogue as suitable animal feed ingredients (Halloran & Münke, 2014).

## 1.5 Black Soldier Fly

With the emergence of insect as feed industry, the Black Soldier Fly have attracted attention thanks to its short lifecycle, capacity to develop on a wide range of substrates including low –or no value organic waste, and the fact that it is virtually distributed worldwide without causing ecological troubles. This sub-chapter will highlight the

distribution, ecological significance, lifecycle of the fly, followed by a review of its farming and environmental benefits, nutritional value and experiments performed on several livestock, and will conclude on the current bottlenecks hampering its up-scale.

### 1.5.1 Biology

The Black Soldier Fly *Hermetia illucens* is a Diptera. (from the Greek *di*: two and *Ptera*: wings). While this species seems to be almost cosmopolitan (See figure 1.4), the black soldier fly has been proposed an American origin (Rozkošný, 1983) and can be found in the tropical regions of South and Central America (Copello, 1926; Furman *et al.* 1959; Marshall *et al.*, 2015), and the North America continent (for the detailed list of states in the US and Canada see Marshall *et al.*, 2015). This extremely-temperature and habitat-tolerant animal has a great potential to inhabit new territories at more Northern situations than it has formerly been presumed, therefore it spreads rapidly around the globe. The species has been accidentally introduced to Southern Europe where it was first recorded from Malta in 1926 (Lindner, 1936) and after World War II, became widespread in the Western part of the Mediterranean sub region. Within the Palearctic region, it can be found between 49°N and 40°S (Martínez-Sánchez *et al.* 2011; Roháček & Hora, 2013; For the full European records, see Leclercq, 1997; Martìnez-Sànchez *et al.* 2011; Fauna Europea, 2017; Beschovski & Manassieva, 1996; Ssymank & Doczkal, 2010; Üstüner *et al.* 2003; Tsagkarakis *et al.*, 2017; Richoux, 2009; Dauphin, 2003; Chevin, 1986; Franco, 2013; Sauter, 1989; Tòth, 1994). However, it remains unclear when *Hermetia illucens* firstly arrived in Europe. In 1984, Professor Gino Fornaciari exhumed the remains of Isabella d'Aragona (1470-1524) buried in the Abbey of San Domenico Maggiore, in Italy, for paleo-pathological studies (D'Errico *et al.*, 1988). Fornaciari took this occasion to sample the entomofauna associated to the remains in the sarcophagus. Near the queen's skull, two body parts belonging to the Black Soldier Fly larva were found. Considering that the fly larvae requires abundant quantities of decaying organic matter (Fornaciari, 2006), and the sarcophagus having been sealed, it is unlikely that the body was contaminated later by the maggots. This finding acknowledged the presence of this fly almost 400 years before the first European record in Malta, suggesting that it was probably present in Italy four centuries before. To this finding, Benelli *et al.* (2014) offer three hypothesis: a) it was introduced from America earlier than we thought: Isabella

d'Aragona died the 12th February 1524, almost three decades after the America's discovery by Columbus. At that time, many Spanish gallions were trading in the port of Naples, allowing an accidental introduction; b) the apparent American origin is wrong and it was native from the Palearctic region, even if it remained unknown until 1926, or c) the fly larvae does not belong to *H. illucens* but to a new close related species or a cryptic one. More similar cases have to be found to confirm any one of the 3 possibilities. The BSF is recorded from many Asian countries, (for the full records see Kim *et al.* 2008; Kim, 1997; Morimoto & Kiritani, 1995; Marshall *et al.* 2015; Caruso *et al.* 2013; Roháček & Hora, 2013; Oliveira *et al.* 2015; May, 1961; McCallan, 1974; INPN, 2017). Very few articles attest its presence in Africa (for the full records see INPN, 2017; Oliveira *et al.* 2015; Marshall *et al.* 2015; Lardé, 1990).

**Figure 1.54**: Distribution of the Black Soldier Fly (*Hermetia illucens*).

As for most of its distribution pattern the BSF seems to be more frequent along the costal line. To this pattern, Marshall *et al.* (2015) and Martìnez-Sànchez *et al.* (2011) suggests that maritime transport might have played a major role in repeated accidental introductions.

The fly seems to cohabit peacefully with local entomofauna where it is introduced. Its proportion is always very low *in natura*. In Brazil, in a study using household waste as an attractant, it was demonstrated that BSF larvae represented 1% of the total fly

population colonising the trap (Ferrari *et al.* 2009), while in Ghana this ratio was less than 0.2% (Maquart, unpublished). A competition still occurs however between BSF and local saprophageous flies, which explains the low representation of BSF amongst fly populations. The BSF is very susceptible to competition on its early stages (the first 3-4 days), and having a longer development time than most of the other flies, they cannot colonize quickly enough a substrate to –later- inhibit the oviposition of other species. The presence of BSF larvae is known to inhibit the oviposition of housefly in a substrate (Furman *et al.*, 1959; Bradley & Sheppard, 1984) but only if the substrate is already well colonised: this inhibition is only observed when large density of BSF larvae is present in the substrate, and when the larvae are old enough. Interestinly, this inhibition also occurs on the frass left by the bioconversion.

There is –at the moment- no record of ecological problems due to the introduction of BSF in introduced area, comforting its status of "non-pest" species. Recently one article published by Hasim *et al.* (2017) allegedly attributed the collapse of wild bee hive colonies to BSF larvae in peninsular Malaysia. However, this very unusual colonisation might have happened because of a drastic change in climate (with an increase of rainfall and humidity due to the entrance to the raining season), and the fact that honey started to ferment, hence potentially attracting female flies to oviposit. The abundance of bee material (rotten propolis, fermented honey and bee bread) becoming a viable substrate for the larvae. This is to this day, the only record case of BSF infestation.

Like all holometabolous insect, the BSF cycle is characterised by 4 distinct stages: the egg, the larvae, the pupae and the adult (see Figure 1.5). The eggs, measuring about 1 mm, are usually laid in clusters of 200- 650. Having an ovoid shape they are laid in crevices or rugose textures near a favourable substrate (Tomberlin & Sheppard, 2001; Tomberlin *et al.*, 2002). Their coloration changes from beige to yellowish/beige during the incubation period, which lasts from a little bit more than 4 days, at 27–29°C (Booth & Sheppard, 1984) to about 2 days at 30°C. Once the eggs hatch, the neonates larvae crawl in the feeding substrate. They pass through 5 instars, lasting about 15 days (at 30°C). They can be recognized by their stout shape and large size (up to two centimeters long for the last larval stage). The body is dorso-ventrally flattened contrary to housefly larvae which body is very plump. The larvae are highly photophobic and remains inside the substrate. The $6^{th}$ instar is called prepupae. This stage is characterized by a marked colour change from beige to dark brown, a cessation of feeding and the migration outside

the feeding substrate (referred as “self-harvesting”) (Schremmer, 1986; Diener *et al.*, 2011). The prepupal stage usually takes less than 10 days (at 30°C). At this stage they are at their maximum size, with a large store of fat to sustain them through metamorphosis. Once a suitable place for pupation is located, the prepupae ceases its activity, and enters in pupation stage. The outer skin (pupae) is used as a shield to protect the nymph through pupation. This stage last between 14 to 17 days.

Once the adults emerge, they do not need feed as they largely rely on the energy reserves built during their larval development (Newton *et al.*, 2005a, b), but these supplies can be supplemented by sugary water to increase their lifespan. The environmental factor that intimately governs their life expectancy is the access to water: adults having access to water may live up to 14 days, whereas those deprived of water hardly survive more than 8 days (Tomberlin *et al.*, 2002; Olivier, 2004; Tomberlin *et al.*, 2009). Males often emerge earlier than females (Tomberlin *et al.*, 2002; Kim *et al.*, 2008) and after 2 days, mating takes place (Tomberlin, 2001; Tomberlin *et al.*, 2009; Tomberlin and Sheppard, 2002). Another 2 days are needed before egg laying. Imagos live only 5 to 14 days, its life expectancy being unquestionably dependent on body size (and associated energy reserves) and on access to water (Tomberlin *et al.*, 2002; Olivier, 2004; Tomberlin *et al.*, 2009).

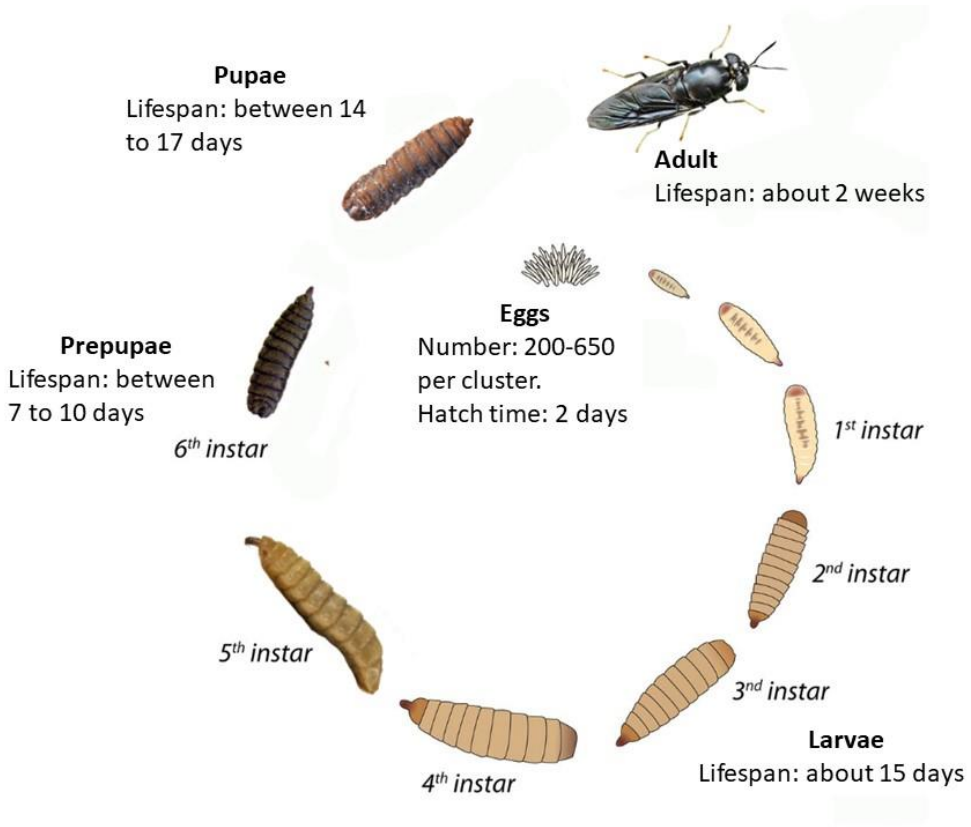



**Figure 1.55**: Life cycle of the Black Soldier Fly (*Hermetia illucens*). The time frame given is expressed for 30°C.

## 1.5.2 Environmental benefits

The larvae are saporphageous, and can be fed on wide range of substrates, and more interestingly on low or no-value organic waste, like animal manures (Newton *et al.*, 2005), fruits (Nguyen *et al.*, 2013), and vegetables (Malloch, 1917), or even some indigestible food, such as coffee (Diener *et al.*, 2009) and beeswax (Malloch, 1917). Therefore the BSF larvae are considered as great recycler (Lardé, 1990).
While feeding on the substrate Larvae reduce significantly pathogenous bacteria like *Escherischia coli* or *Salmonella enterica* (Erickson *et al*., 2004; Liu *et al*., 2008), but also, showed reductions of Adenoviruses, Reoviruses and Enteroviruses (Lalander *et al.*, 2014). Therefore the substrate after the bioconversion is considered pathogen-free (Erickson *et al*., 2004; Liu *et al*., 2008).

The adults rely only on water to survive (Skidmore, 1985; Tomberlin *et al.*, 2002) making the farming process easier as they don't have to be fed. BSF is harmless to humans and

livestock, therefore the risks of disease transmission are null (Bradley & Sheppard, 1984; Leclerq, 1997; Rozendaal, 1997; Sanchez-Arroyo, 2011). In addition, due to the fact that this species is very prolific (a female lay between 1.5 to 2.3 mg of eggs –so around 600-920 eggs- during its lifetime according to Tomberlin *et al.*, 2002; Heussler *et al.*, 2018) and has a short lifecycle (the larvae can be harvested within 15-20 days) it indicates a great potential for mass rearing.

The Black Soldier Fly Larvae (BSFL) may be grown on a wide range of organic substrates including manures and other waste substrates (Oonicx 2015, Diener *et al.*, 2011, Lalander *et al.*, 2014) whose management has public health, environmental, biosecurity and logistical challenges in both the developed and developing world (Halloran *et al*., 2016). Therefore, they can efficiently be used as a waste remediation system by efficiently converting nutrient-poor, low or no value waste that are costly to treat or dispose into valuable biomass, reducing effectively the biomass, effluents, and odours thereby reducing sanitation issues and risks of pollution (Sheppard *et al.*, 1994; Newton *et al.*, 2005a; Myers *et al.*, 2008; Diener *et al.*, 2011b; Lomas, 2012; Lalander *et al.*, 2013). In contrast to other solutions for waste management, such as composting or anaerobic digestion, BSFL can reduce waste loads by around 70% in comparatively short time scales to either composting or anaerobic digestion (Diener *et al*., 2011) and can reduce pathogenic loads (Lalander *et al.*, 2014; Erickson, *et al.*, 2004; Liu *et al.*, 2008) which anaerobic digestion does not. It may therefore contribute to solving waste management problems at the same time as providing valuable feed ingredients to livestock industries and fertilisers and therefore of particular interest to regions that have limited access to quality feed ingredients, organic fertilisers and also have waste management issues, such as in Africa.

The residues from the bioconversion process consist of undigested substrate residues and insect feces (Alvarez, 2012; Čičková *et al.*, 2012a). Frass represent between 80 to 95 percents of the total "fresh" outputs (*i.e.* larvae + frass) of the bioconversion process (Calvert, 1979; Čičková *et al.*, 2012b; Wang *et al.*, 2013; Caruso *et al.*, 2014) and several authors suggested to valorizate the frass by further hydrolysing into fermentable sugars suitable for the food industry (like ethanol fermentation); processing to facilitate storage and handling, vermicomposting (Newton *et al.*, 2005b; Li *et al.*, 2011c; Čičková *et al.*, 2015; He *et al.*, 2016).

The frass chemical composition is comparible to other conventional biofertilisers, with optimal levels of N, P, K to supplement soils (Choi *et al.*, 2009; Wang *et al.*, 2013; Lalander *et al.*, 2014).

Very few researches were done on the possibility of using BSF residues as biofertiliser, however, it was proven successful for slow-growing crops (such as pepper, ockro) but not suitable for quick-growing crops such as maize, as an organic fertilizer releases slowly its nutrient into the soil. The results of the agronomy trial conducted by Devic (2016) showed that there is some positive effects on the use of frass as a biofertiliser, for plant growth and soil fertility. The structure of the soil was improved, it was more aerated compared to the unamended soil and the soil improved with NPK fertilisers. This improvement is a characteristic of organic soil conditioners that usually encourage the development of a root system and therefore the growth of the plants.

In addition to the soil structure improvement, according to Vickerson *et al.* (in a patent developed by Enterra in 2015) BSF frass have been shown to have properties that confer protective effects against plant pathogens by reducing or inhibiting pest damage to a susceptible crop. Field plots demonstrated this activity against several *Agriotes* species and *Limonius canus* (Coleoptera: Elateridae). The results showed that BSF frass treatment exerted a protective effect against wireworm feeding damage, but also showed entomocid activity. For example, mixing BSF frass and soil (8% in soil, dry weight frass/dry weight frass plus soil) killed 90-100% *A. lineatus* within 1 -6 days. Similarly, at a rate of 7.5% (dwt/dwt) it killed from 28% to 88% *A. lineatus* under 5 days. Similarly, using 10% frass (dwt/dwt) mixed with soil, killed 100% and 80% respectively of *A. obscurus* and *L. canus* after 24 hours. The frass also exhibited insecticidal activity against European chafer (Coleoptera: Scarabidae), with 20% more chafer larvae killed after 20 days of exposure to 8% frass (dwt/dwt), compared to controls. The same activity was observed against cabbage root maggots, killing larvae and pupae as well and reducing fly emergence. Further experiments needs to be done to properly assess these faculties.

Therefore, BSF-based frass could be a competitive product on the overall composts/biofertilisers markets due to its availability and thanks to a composition comparable to classical compost or vermicompost, which are high-value products but available in limited quantities.

### 1.5.3 Commercialisation trends

As demonstrated before, the BSF industry can be threefold: production of sustainable feedstuffs, waste management and biofertiliser ("frass") production.
Under the paradigm of insects as an alternative protein feedstuff to mitigate the effects of climate change or rising population, the advantages of the BSF might reasonably put it as the insect of choice, as not only it does not compete with humans nor livestock for food or feed, does not have specialized diets, nor require significant environmental manipulation (Van Itterbeeck & Van Huis, 2012) but provides also an economic and ecological service in processing the waste substrates that it feeds on. No other insect comes close to closing so many material flow loops and creating nearly self-sustaining food production cycles. Rearing BSFL on wastes, could be a self-financing form of pollution reduction (Gabler & Vinnerås, 2014), although it is not yet known what are the best ratios of insects to substrate and what conditions will provide the optimal mix of larval production and waste recycling. BSFL thus show strong promise as part of a sustainable system (Katayama *et al.*, 2005)

At the moment, several international commercial pilot productions have started to sprout around the world, like in Canada (Enterra), South-Africa (AgriProtein) Spain, the Netherlands (Protix), France (Innovafeed), Malaysia (Entofood) or China (Van Huis *et al.*, 2013; Drew & Pieterse, 2015; Henry *et al.*, 2015; Pastor *et al.*, 2015). Starting with a capacity of substrate input of 200 to 1,000 tons per day, their main focus is protein production rather than waste management. Therefore, homogenous and pure input materials such as brewery waste, food waste or chicken manure, are required to allow a controllable and stable production of high value animal protein. However, competition with biogas or composting plants can have a negative influence on the availability and thus the price of these resources (Diener *et al.*, 2015).
Due to Intellectual Properties restrictions, very little information are available on their technology nor advancements in the field. With legislation changes, the industry will be able to use a broad range of substrates, therefore paving the way into large-scale waste management industry along their protein-based production industry.
On the other end of the spectrum are decentralised small-scale household composting systems (like households, private chicken farmers and hobbyists) operating BSF

composting for personal use (Olivier & Hyman, 2011). But they are more focusing on very-local waste treatment, and are motivated by the idea of self-sufficiency, by feeding raw dried or live maggots to their livestock or pets. Their insect production is considered negligible in the wider context.

But, between these two extremes, there is a market gap for medium-scale BSF treatment facilities, potentially treating 10 tonnes of waste per day, serving a local and contextualised market. This is of great interest given the high share of organic material in the waste streams – especially in low- and middle-income countries (LMIC) – and the growing demand for locally produced animal feed. As such, BSF technology could provide an opportunity for local entrepreneurs, serving not only the aforementioned demands but also creating employment (Diener *et al.*, 2011).

Yet, very few pilot scale system disclose their current production levels and technology. Burtle *et al.* (2012) designed a system in the USA which can theorically produce 3,750 tonnes of BSF meal per year, using 360 tonnes of food waste and swine manure weekly. In Indonesia, the EWAG project, claimed to produce between 750kg – 1 750 Kg of larvae (wet weight) out of 9-21 tons of food waste weekly (Dortmans *et al.*, 2017).

As for now, given the regulatory restrictions (see 1.3.4 above), maggot meal (MM) are mainly used in the petfood industry and has just started to be used in aquaculture. The pet food industry has a high purchasing power, which has rapidly identified the potential of these products as high-quality feed industry. Meanwhile, research keeps investigating the applicability and safeness of MM-based products as feed ingredients for various livestock and fish species.

### 1.5.4 Nutritional profile

The fact that Dipteran meal share similarities with FM suggest a high potential as a feed ingredient including aquatic species (see Table 1.2). The whole body composition of the BSF varies greatly with the substrate the larvae are fed on (Barranga-Fonseca *et al.*, 2017; Sanchez-Muros *et al.*, 2016; Wang & Shelomi, 2017), and allows a manipulation of its nutritional profiles, especially FA profile (St-Hilaire *et al.*, 2007; Biancarosa, 2016; Barranga-Fonseca *et al.*, 2017). But the effects are not linear: it was demonstrated in an experiment were food waste mixes were used as a substrate, and where the protein and

lipid composition were known, that it did not correlate with the larval fat content (Oonincx *et al.*, 2015). The body composition of the BSFL according to the substrate, for protein, lipids and micronutrients is resumed in the Chapter 3 dedicated to identify potential substrate to produce the BSFL in Ghana, the following table resumes (Table 1.2), as an example, the nutritional profile of BSFL fed on brewery waste (Barroso *et al.*, 2014).

But despite these variations, some generalities can be made:

The dry matter ranges between 20 to 44% according to the lifestage, being higher for the later larval stage called prepupae (Diener *et al.*, 2009; Finke, 2013; Nguyen *et al.*, 2015; Oonicx *et al.*, 2015, Sheppard *et al.*, 2008; Barranga-Fonseca *et al.*, 2017).

The protein content can vary between 37-63% (on a dry matter basis) (Barranga-Fonseca *et al.*, 2017). The amino acid is relatively constant amongst the studies, and therefore is not considered to change too much according the substrate the larvae are fed on. BSFL are particularly rich in lysine (6-8% of the protein content) (Sheppard *et al.*, 2008) and is similar to the levels observed in other animal feedstuff (Newton *et al.*, 1977). When produced on pig manure, the levels of lysine, leucine, phenylalanine and threonine are similar to soybean (Newton *et al.*, 2005b), and the levels of alanine, methionine, histidine and tryptophan are higher. When defatted BSFM can have crude proteins levels over 60% (Spranghers *et al.*, 2017). The defatting of the larvae requires processing which can be achieved either chemically by solvent extraction, using hexane (Kim *et al.*, 2016) or mechanically by cold-pressing the larvae prior to grinding (Russin *et al.*, 2011). But these solutions have financial, environmental and logistical costs out of reach for developing world countries.

**Table 1.2:** Proximate composition and amino acid profile of Black Soldier Fly larvae, fishmeal and soybean according Barroso *et al.* (2014).

| | **BSFL** | **FM** | **Soybean** |
|---|---|---|---|
| **Proximate composition (% dry matter)** | | | |
| Crude protein | 36.2±0.3 | 73.0±0.8 | 50.4±0.2 |
| Crude fat | 18.0±1.6 | 8.2±0.0 | 3.0±0.0 |
| Ash | 9.3±0.3 | 18.0±0.2 | 7.8±0.0 |
| Nitrogen-free exctract | 36.5±1.0 | 0.8±0.7 | 38.8±0.3 |
| **Amino acid (% total AA)** | | | |
| Arginine | 8.24 | 7.42 | 8.03 |
| Histidine | 5.29 | 7.86 | 3.28 |
| Isoleucine | 5.76 | 5.04 | 5.47 |
| Leucine | 6.87 | 7.81 | 8.01 |
| Lysine | 7.60 | 8.78 | 6.34 |
| Methionine | 1.50 | 2.93 | 1.01 |
| Phenylalanine | 6.88 | 5.38 | 5.79 |
| Proline | 6.16 | 4.76 | 4.99 |
| Threonine | 5.39 | 6.26 | 4.17 |
| Tyrosine | 6.35 | 3.91 | 2.93 |
| Valine | 6.31 | 5.56 | 5.45 |

Regarding the lipid profile, it ranges between 6.6-39.2% (Newton *et al.* 1977; Barragan-Fonseca *et al.*, 2017; Wang & Shelomi, 2017), between 58-72% of it are saturated fatty acid, and the rest, shared between mono- and polinsaturated fatty acids (Kroeckel *et al.*, 2012; Li *et al.*, 2011c; Makkar *et al.*, 2014; Surendra *et al.*, 2016). The fatty acid profile is closely related to the composition of the diet. As said previously, when fish offals are used as a substrate the larvae can incorporate n-3 FA like α-linolenic acid or eicosapentaenoic acid (Sealey *et al.*, 2011; St-Hilaire *et al.*, 2007a). The fatty acid potentially present in the BSF larvae are resumed in the Table 1.3:

**Table 1.3**: Fatty acid profiles, (expressed as % of total fatty acids) on the black soldier fly larvae fed from different substrates. (according Barranga-Fonseca *et al.*, 2016; Kroeckel *et al.*, 2012; Li *et al.*, 2011a; Oonincx *et al.*, 2015b; Sealey *et al.*, 2011; St-Hilaire *et al.*, 2007a; Zheng *et al*., 2012). Values expressed either as mean±SD when the sampling number was sufficient, or otherwise as single values.

| Fatty acids | Cattle manure (n=3) | Chicken feed (n=2) | Cattle manure + fish offals (n=2) | By products (high fat) (n=2) | By-products (low fat) (n=2) | Swine manure (n=1) | Restaurant waste (n=1) |
|---|---|---|---|---|---|---|---|
| Capric acid | 3.1 | 0.9 | - | 0.7; 0.8 | 0.3; 1.2 | - | 1.8 |
| Lauric acid | 26.7± 7.8 | 47; 46.6 | 34.1; 37.1 | 28.9; 38.4 | 48.4; 50.7 | 49.3 | 23.4 |
| Myristic acid | 3.9± 1.6 | 6.5; 9.2 | 6.3; 6.5 | 7.4; 7.8 | 9.9; 9.5 | 6.8 | - |
| Palmitic acid | 16.9± 2.6 | 15; 12.7 | 14.3; 17.3 | 14.4; 17 | 11.6; 11.8 | 10.5 | 18.2 |
| Palmitoleic acid | 5.1± 1.8 | 3.4 | 7.6 | 2.9; 3.4 | 4.7; 6.6 | 3.5 | 9.4 |
| Stearic acid | 5.3± 1.5 | 2.2; 2.1 | 2; 2.4 | 2.4; 2.8 | 1.8; 2 | 2.8 | 5.1 |
| Oleic acid | 26.1± 5.2 | 10.2- 14 | 16.5; 18.8 | 15.9; 18.1 | 10.3; 10.8 | 11.8 | 27.1 |
| Linoleic acid | 4.5± 2.4 | 9.4 | 3.9; 5.9 | 8.3; 17.1 | 3.6; 6 | 3.7 | 7.5 |
| α-linoleic acid | 0.2 | 0.6; 0.8 | 0.5; 0.7 | 0.8; 1.5 | 0.6; 1 | 0.1 | - |
| Stearidonic acid | - | - | 0.5 | - | - | - | - |
| Arachidonic acid | 0.04 | 0.1 | 0.2 | 0.1; 0.2 | 0.1; 0.6 | - | - |
| Eicosapenta-enoic acid | 0.07± 0.1 | - | 1.8; 3.5 | - | - | - | - |
| Docosapenta-enoic acid | 0.01 | 0.1 | 0.1; 0.4 | - | - | - | - |
| Docosahexa-enoic acid | 0.06 | 0.1 | 0.4; 1.7 | - | - | - | - |

The micronutrient profile, while depending on the substrate the larvae are fed on, shows some relatively constant profiles: BSFL accumulate calcium and manganese, but display low levels of sodium or sulphur (Spranghers *et al.*, 2017). The calcium levels are higher than in any other insects accounting between 6.6-9.3% dry weight, compared to less than 1% for other insect species- (Makkar *et al.*, 2014; Spranghers *et al.*, 2017; Finke, 2013). This level is higher than in FM (Makkar *et al.*, 2014) giving a considerable advantage over other insects nutritionally. They also provide adequate levels of other essential minerals and vitamins with levels at least as good as, if not superior to the other insect species (Finke, 2013).

BSFL shows high levels of C12:0 medium-chain fatty acid (Spranghers *et al.*, 2017), and especially Lauric acid, known to have a profound antiviral and antibacterial effect (Gasco *et al.*, 2018), and can act as probiotic effects on the microbiota of livestock (Devi & Kim, 2014) and antibiotic effects on gastrointestinal disease-causing bacteria (Skrivanova *et*

*al.*, 2006). The fat of prepupae reared on organic waste streams with high amounts of starch can host up to 60% lauric acid (Spranghers et al., 2017). Recently, an *in vitro* trial demonstrated that BSF prepupal fat (0.58 g C12:0/100 ml) inhibited the growth of *lactobacilli*, and had strong antibacterial effects against *D-streptococci* infections in pigs (Spranghers et al., 2018). The possible antimicrobial effects of fat (C12:0) of the BSF could therefore provide an important added value when they are used as a protein source in the feed of monogastrics (Spranghers et al., 2017; Gasco *et al.*, 2018). So, in addition to the characteristic of chitin, proven to stimulate the immune system (see chapter 1.3.1) BSF could be considered as a good alternative to in-feed antibiotic.

### 1.5.5 Review of nutritional studies

Motivated by the global need for alternative and sustainable protein sources, the BSF have been investigated in many aquaculture article. As stated previously, while the number of livestock feed experiments has doubled over the past 15 years, the number of feeding experiments in aquaculture has quadrupled, traducing this growing interest (Sánchez-Muros *et al.*, 2016).

Flies are not particularly part of the natural fish feed intake in the wild, but other arthropods are, suggesting that farmed fish might beneficiate from BSFM as a feed ingredient. Several comprehensive reviews were performed on its use as a livestock ingredient (Sànchez-Muros *et al.*, 2016; Barragan-Fonseca *et al.*, 2017; Wang & Shelomi, 2017) or aquatic species (Rumpold & Schlüter, 2013; Barranga-Fonseca *et al.*, 2017, Makkar *et al.*, 2014; Riddick, 2014; Henry *et al.*, 2015; Wang & Shelomi, 2017).

#### 1.5.5.1 Aquaculture nutrition studies

The early studies on the incorporation of BSF in fish feeds mainly investigated herbivorous/omnivorous fish species such as catfish, tilapia or carp. Over the last decade, interest has risen, and many feeding experiments are now looking at a wider spectrum of fish species, including carnivorous species.

The first studies, targeted semi-intensive aquaculture which is well developed in LIDC. As low-quality FM or low protein feeds are commonly used (Tacon, 1996; Tacon & Da Silva, 1997; Heuzé & Tran, 2013), the potential of BSFM should not be neglected. In

addition, due to the difficulties involved in the separation of larvae from the substrates, both larvae and frass (assuming that the substrate presents no risks, and is nutritionally favourable) could be dispensed to fish with other feedstuffs after drying and blending (Spinelli, 1980).

Several studies, showed that BSF used as a supplementary feed to Blue or Nile tilapia, Channel catfish or African catfish with low quality or cheap feed allowed a significantly better growth performance than the fish fed the supplementary feed only (Bondari & Sheppard, 1981; Ebenso & Udo, 2003; Madu & Ufodike, 2003; Oyelese, 2007; Kareem & Ogunremi, 2012). However, when used solely compared to a commercial diet it would depressed severely the growth, mostly because the BSFM does not cover entirely the dietary requirements of the fish (Bondari & Sheppard, 1987).

For instance, in Channel catfish (*Ictalurus. punctatus*) the substitution of commercial diet with 50 and 75% of fresh Black Soldier Fly larvae was tested during 10 weeks in polyculture with channel catfish and blue tilapia. The results indicated that fish body weight and total length were not affected, and the aroma and texture of the fish fed with Black Soldier Fly were acceptable to the consumer (Bondari & Sheppard, 1981). However, a later study, by the same authors (1987) was less favourable: replacement of 10% fishmeal with 10% dried BSLM resulted in slower growth over a 15-week period for sub-adult channel catfish grown in cages. However, the replacement did not reduce the growth rate significantly when channel catfish were grown in culture tanks at a slower growth rate. Feeding 100% larvae did not provide sufficient dry matter or protein intake to enable a good growth for channel catfish in culture tanks and therefore was linked to a problem with meeting the fish dietary requirements. (Bondari & Sheppard, 1987).

But now, studies focus more on using BSFM as a protein replacement source to FM or Soybean in fully formulated feeds destined to intensive aquaculture.

Recently, promising results were obtained during a 65 day trial in yellow catfish (*Pelteobagrus fulvidraco*) were, among a formulated feed, the FM was replaced by BSFM at incremential levels (0, -control- 13, 25, 37, 48, 68, 85 and 100%) (Xiao *et al.*, 2018). Compared with the control group, the growth performances (e.g. weight gain rate increased by 21.7%) and immune indexes (e.g. serum lysozyme activity increased by 6.8%) of yellow catfish fed, with diets in which a maximum of 48% FM protein was replaced by BSFL meal protein, were significantly better. Overall, the diet in which 25% FM was replaced by BSFLM resulted in the greatest growth performance (e.g. weight gain rate increased by 29.1%) and immune indexes (e.g. serum lysozyme activity

increased by 31.9%) as well as the lowest feed conversion ratio (FCR) (0.9) among all diets tested (Xiao *et al.*, 2018). Correspondingly, a study from Zhang *et al.*, (2014a) demonstrated the same beneficial effects with 25% FM replacement by BSFM in the same fish species.

For carnivorous species, especially Salmon (*Salmo salar*), Turbot (*Psetta* maxima), Trout (*Oncorhynchus mykiss*) and seabass (*Dicentrarchus labrax*), the results shows a limit in the use of BSFM in their diet, probably because of a low palatality, digestibility and poor FA profile of this new protein source. In adult Atlantic salmon diet, Lock *et al.* (2014) tested the replacement of FM with BSFM (0, 25, 50, 100%). The feed intake and the FCR decreased with the increase of BSFM inclusion, however, the lipid digestibility, histology and organoleptic properties of the fillets, were similar across the treatments. The authors concluded that a favourable AA and FA profiles are needed to make BSFM a promising ingredient in Atlantic salmon diet. In adults Rainbow trout (*Oncorhynchus mykiss*), BSF prepupae could be used to replace 25% of the FM and 38% of the FO components of a commercial diet with no negative impacts on production (St-Hilaire 2007; Sheppard, *et al.*, 2007). However, levels above were systematically associated with a decrease in growth and affected fish performance (St-Hilaire *et al.*, 2007; Sealey *et al.*, 2011). In addition, lowering the FO levels in the feed by substitying it with BSF full fat feed would significantly decrease the n-3 LcPUFA contents (St-Hilaire *et al.*, 2007). However, Sealey *et al.* (2011) indicated that BSF prepupae reared on dairy cattle manure and trout offal, could replace up to 50% of FM portion of a practical trout diet for 8 week with no impact on the FA profile of the fish, nor the sensory quality of resulting rainbow trout fillets. In gilthead seabream juveniles, BSFM from prepupae may replace up to 30% FM without affecting fish performance (Karapanagiotidis *et al.*, 2014). In juveniles turbot (*Psetta* maxima) the inclusion of BSFM in juvenile turbot was possible up to 33%, without significantly affecting the feed intake, FCR, and protein retention (Kroeckel *et al.*, 2012). However, feed intake and specific growth rate decreased with increasing BSFM incorporation, probably due to low palatability and digestibility of the feed (Kroeckel *et al.*, 2012). Similarly, slightly better results were obtained in an experiment on European seabass, where the replacement of FM by BSF prepupae meal up to 45% did not affect growth performance (Magalhães *et al.*, 2017).

For Tilapia species results are promising. Some of the earliest work by Bondari & Sheppard (1981) demonstrated that the growth rate of Blue tilapia (*Oreochromis aureus*) in polyculture with catfish, when fed diets containing 50-75% and 100% fresh soldier fly

larvae over a 10 week period was comparable with control fish fed with commercial diets. However the complex design of the experiment made interpretation of the results problematic however as it was impossible to control for different and possibly competitive feeding behaviours between the two species. A second trial by the same author (1987), found that in a monoculture of blue tilapia, fed with chopped or whole larvae *ad libitum* led to severely depressed fish growth compared to standard diet. But the use of fresh (rather than dry) larvae by the authors also raises issues around potential commercialisation. First, fresh larvae reduce the dry matter and protein intake compared to a 'dry' diet. Secondly prepupae were used, as it is the easiest larval stage to collect because of their wandering and 'self-harvesting' behaviour prior to pupation; but their elevated chitin content, makes it hardly digestible. Comparisons between these and other studies are complicated by a range of experimental design factors. Ogunji *et al.* (2008) used a dry, low-protein BSFM (28.6% DM basis) and reported that the fish growth was significantly lower than the fishmeal fed fish for the treatments containing 150 and 300g/kg maggot meal. However, the dietary formulation method employed resulted in neither a non-isonitrogenous nor iso-caloric diets, making them hard, if not impossible, to compare. A more recent study on Nile tilapia (Devic *et al.* 2017) used the white larvae dry meal to formulate isonitrogenous and isoenergetic diets with maggot meal inclusions at 0, 30, 50 and 80 g/kg substituting gradually three conventional expensive feedstuffs: fish meal, fish oil and soybean meal. Results showed no significant difference in growth parameters (final weight; weight gain and SGR), feed utilization efficiency (FCR and feed intake) between treatments. Similarly fish whole body composition (dry matter, crude protein, lipid, ash and fibre) was unaffected by the treatments except the fatty acid compositions which mirrored that of the diets. Thus the study confirmed the substitution potential of BSF white maggot meal as a potential replacement for other commonly used dietary protein sources with respect to biological performance. Similar promising results were obtained recently, (Ushakova *et al.*, 2018) where the use of BSF prepupae (at a rate of 0.5g/Kg of feed) in the diet of juveniles Mozambique Tilapia increased significantly the mass of the fish, and the digestibility of the diet. The biological activity of the prepupae as a feed additive was largely manifested in the increase in the number of spermatozoa in the lumina of the seminiferous tubules. The authors attributed the stimulation of spermatogenesis to the presence of $Mn2^{+}$ (Ushakova et al., 2017), in a biologically active form in the insect feed additive.

Typically, for most species, inclusions up to 30-40% are possible, in a formulated diet meeting the fish dietary requirements, without affecting nor the growth, FA profile or the flavour of the fish. Maximum replacement level of FM by BSFM is, however, dependent on its quality. Sealey *et al.* (2011) showed that 50% FM could be successfully replaced by fish offal-enriched BSFM in diets for adults rainbow trout, while replacement by normal BSFM would negatively affect fish growth. Also, of two types of BSFM tested for Atlantic salmon, only one allowed a total FM replacement of the diets without affecting fish performance (Lock *et al*., 2016).

#### 1.5.5.2 Poultry nutrition studies

Black Soldier Fly have been used in poultry feed as a total or partial replacement for maize or soybean, mainly because chickens are naturally insectivorous and BSF naturally colonise and breaks down poultry manure (Bradley *et al.*, 1984; Bradley & Sheppard, 1984). In broiler chicken feed, Oluokun (2000) examined the effects of treatments with either FM or BSF larvae meal against a full fat soybean as control. On average live weight gains of broiler fed with FM or BSFM were higher than those of the control diet. Oluokun also reported that the diet upgraded with the BSF did not affect the weight gain nor the feed consumption. Moreover there were improvements in the carcass yield, internal organ measurments (kidney, gizzard, liver), and abdominal fat in animal fed with larvae diet regarding FM or control diet. Borrelli *et al.* (2017) demonstrated that BSF-fed laying hens had an increased body weight gain and increased frequency of CD4+ T lymphocyte, serum lysozyme activity, and spleen lymphocyte proliferation. BSFL reinforced bacterial clearance and increased survivability of broiler chicks against *Salmonella gallinarum.* These data suggested that BSFL has prophylactic properties with stimulating non-specific immune responses, as well as reduced bacterial burden against *S. gallinarum*. The same probiotic effect was demonstated in broiler chicken by Schiavone *et al.* (2016 & 2017).

The same beneficial results were observed by Moula *et al.* (2018), where the commercial feed was substituted by fresh BSF larvae. The broiler chicken were significantly heavier than the normal-fed control birds.

In Guinea Fowls, the same positive results in gradually substituting FM by BSFLM (0-20-40-60-80 and 100%) in a formulated feed (where the initial FM level was 3%) were

observed, where not only the final weight, daily weight gain and feed intake was improved by the presence of BSF in the meal, but the meat tasted also better for the BSF fed birds (Wallace *et al.*, 2017; 2018).

In summary, BSLF inclusion guaranteed satisfactory productive performances, carcass traits and overall meat quality, thus suggesting that BSLF could be a promising new feed ingredient for chickens and could improve the organoleptic properties of the final meat.

#### 1.5.5.3 Other nutritional studies

Several experiments have been conducted on the use of BSFL as pet food. For instance, in young alligator (*Alligator mississipiensis*) – typically insectivorous- a complete replacement of commercial feed by BSF prepupae resulted in a lower consumption and growth compared to the commercial feeds (Bodri & Cole, 2007). This could be explained by the fact that BSF prepupae did not meet the nutrients requirements of the alligators and the fact that the pellet size was smaller than those of commercial diets, lowering its acceptability toward the reptiles. A similar experiment was conducted on Mountain Chicken frogs (*Leptodactylus fallax*), where whole dried, BSF larvae substituted a commercial feed (Dienefeld & King, 2008). The growth of the frogs was lower, probably due to a poor digestibility: the calcium digestibility of the whole BSF was only 44% compared to 88% when the BSF had been previously grinded. They even observed that whole larvae may pass intact and poorly digested through the gastrointestinal tract unless the cuticle is pierced. However, when grinded, the BSF larvae could potentially be used as feed for insectivorous reptiles and amphibians. In fact they have been proven successful on numbers of captive lizards and amphibian species (Dierenfeld & King, 2009). It is also interesting to note that BSF frass was successfully tested on giant river prawns (*Macrobrachium rosenbergii*) where growth, palatability and survival were similar to the control-commercial diet (Tiu, 2012).

### 1.5.6 Current bottlenecks and constraints

Like any emerging industry, the BSF business is subjected to limitations (see legal limitations in Chapter 1.3.4) and technological bottlenecks. The breeding of the BSF is still challenging and a strong knowledge of its biology is still required to enhance, and

optimise, its production for an adaptation to site-specific conditions (which can be overcome by using Environmental-controlled chambers).

At the moment, the production of large and consistent amounts of BSF eggs is one of the main bottlenecks for a sustainable and successful mass production system, and biotic and abiotic factors affecting broodstock husbandry are yet to be fully understood (Gobbi, 2012; Wang & Shelomi, 2017; Sheppard *et al.*, 2002).

Also, being a worldwide-spread insect, strains can be identified and are known to affect the global production system. For instance, Zhou *et al.* (2013), showed that three strains of the BSF (one from Texas, one from Guangzhou, China and the last one from Wuhan, China) displayed very different profiles: The Wuhan strain appeared to be fitter, having a reduced time to reach the prepupal stage than those from Guangzhou (17.7% quicker) or Texas (29.9%) and were heavier (14.4–37.0% more than those from Guanghzhou or Texas respectively). In terms of waste reduction, that same strain reduced the dry matter of the substrate by 46.0% (swine manure), 40.1% (dairy), and 48.4% (chicken manure) more than the Guangzhou strain and 6.9, 7.2, and 7.9% more than the Texas strain. They demonstrated that the phenotypic plasticity (development and waste conversion) could vary greatly across populations of BSF and has to be taken into account when establishing a production facility. Furthermore, like any other livestock production, inbreeding has to be taken into account when mass-producing the larvae, as it could really hampered their production (Badenhorst, 2017).

Having no known disease yet affecting the production, and while it is known that the presence of BSF reduce bacterial activity in the substrate (Liu *et al.*, 2008), inhibits and controls the oviposition and development of *Musca domestica* (Sheppard 1983; Bradley and Sheppard 1984) pupation is a sensitive development stage. So far 2 parasitoidic wasps are known to feed on the BSF pupae. The first one belonging to the genus *Trichopria sp.* (Hymenoptera: Diapriidae) was recorded from the USA (state of Georgia) (Bradley *et al.*, 1984). It has also been observed in Indonesia (Caruso *et al.*, 2014). Unfortunately, in both cases, specimens were not identified to a species level. The second species, *Dirhinus giffardii* Silvestri, 1914 (Hymenoptera: Chalcididae) was identified in 2015 affecting the pupal development of the larvae in BSF production systems in Ghana and Mali (West-Africa) (Devic & Maquart, 2015). *D. giffardii* is a solitary parasitoid attacking young host pupae (2-3 day old) when they enter to the pharate stage (stage specified in Barros-Cordeiro *et al.* 2014). However, they never parasite pupae older than 8 days (Dresner, 1954 on Tephritid flies, but also confirmed in Ghana for *H. illucens* from our

observations). *D. giffardii* can strongly impact the emergence of BSF pupae, causing a reduction of up to 70.7% of the production, and decreasing hatching rates to only 8.5% (Devic & Maquart, 2015). *D. giffardii* was used as a biological control agent against tephritid flies. Described in 1914 from Nigeria, it became widespread and can now be found in more than 20 countries across the globe. Its presence in a BSF production system can lead to a drastic reduction in pupae hatching rates and therefore can represent a real constrain. However, protecting the early stages of the pupation of *Hermetia illucens*, by keeping them into a box covered by a fine mesh (<1mm wide), remains the best prevention system to ensure a successful BSF production in countries where these parasitoid occurs.

While these aspects refer to a lack of knowledge yet to be filled, or zootechnic issues yet to be overcomed, the main challenge remains on the industrialization of the farming, process, and its economic viability and cost-competitiveness which should at least meet or exceed systems producing conventional protein sources for aquaculture. This could be achieved through optimizing the production and processing process, using economically competitive and sustainable resources (Rumpold & Schlüter, 2013; Van Huis *et al.*, 2013; Pastor *et al.*,2015).

Therefore the choice of the substrate remains a key element to up-scale insect production, as it will affect the nutritional composition (especially the FA profile), potentially improve the quality of the larvae as a feedstuff. But this should also account several key points such as sustainability, being quality-constant, availability, co-location and cost (Rumpold & Schülter, 2013; Van Huis *et al.*, 2013). Furthermore safety aspects concerning the substrate should be taken into consideration as the presence of heavy metals could lead to bioaccumulation in the larvae (Borowska *et al.*, 2004; Tylko *et al.*, 2005, Diener, 2010; Borowska & Pyza, 2011; Diener *et al.*, 2011a) which would become a risk for the fish after ingestion.

For the moment, while the regulatory framework is loosening, the volume of MM that can be produced is still one of the main constrain for its use in aquaculure. But this is related to a better understanding of the fly's biology and zootechnical improvement which are expected to improve, allowing a gradual upscale of the insect production.

The economic aspect of the MM production is an important point to consider as it is one of the main driver of using an alternative feedstuff (Rust *et al.*, 2003). The market price is defined by the profit margin and the production costs, and to answer the vast market demand for aquafeed, insect-based products have to be supplied at a competitive price

compared to other conventional sources of protein. Coming from an animal origin, this price is expected to be higher than plant proteins because of its better digestibility.

Production costs in industrialized countries under a temperate climate, is expected to be high because of the need for environment controlled facilities (to maintain a constant humidity, light, and temperature) to keep the colony and the production facility running. Also, as the labor is expensive, such industry should require a fully-automatized production to decrease the labor cost.

In LIDC, small or medium production systems are less demanding economically, and might be profitable, providing that some improvements of the current methods to improve cost and productivity are made (Caruso *et al.*, 2014; Dortmans *et al.*, 2017). Several studies have concluded that such system could be economically viable in LIDC, as replacement to expensive, quality-inconsistent (if not poor) FM (Ajani *et al.*, 2004; Sogbesan *et al.*, 2008; Ezewudo *et al.*, 2015).

According to Drew & Pieterse (2015), owning the large-scale company AgriProtein in South-Africa, once the legislation framework will open in favour of insect-based products in animal feed, and the possibility to use low value organic waste, the remaining challenges will be aligned with the price of the other conventional feed, therefore producing a feedstuff for less than 1000 US$ per tonne, and would become a profitable business and an immediate incentive to use MM in aquafeeds.

## 1.6 Research hypothesis and objectives

From the foregoing discussion, it is clear that MM currently has a very limited capacity to meet the ever growing demand for aquafeed ingredients. This points to a need for a more targeted, strategic use. Secondly, the high variability in the nutritional profile of MM precludes more prescriptive advice regard its dietary inclusion for various fish species and lifestages.

Therefore, contextualised and commercially relevant research should investigate, where and how insect-based products could be integrated in aquaculture. The environmental and socio-economic context should be assessed prior investigating their use in aquafeed.

It is expected that a strategic use of consistent and high-quality MM can meet the specific requirements of various fish species cultures in different aquaculture systems, thereby

contributing to food security. Critical part of intensive aquaculture process, such as the fry production, could benefit from insect meal as a suitable FM substitute.

The main objectives of this study is to assess which suitable substrate and which larval stage of the BSF should be used to produce the MM, and if this MM could be suitable as a nursery feed during the sex-reversal in Nile tilapia (*Oreochromis niloticus*). Specifically the objectives are:

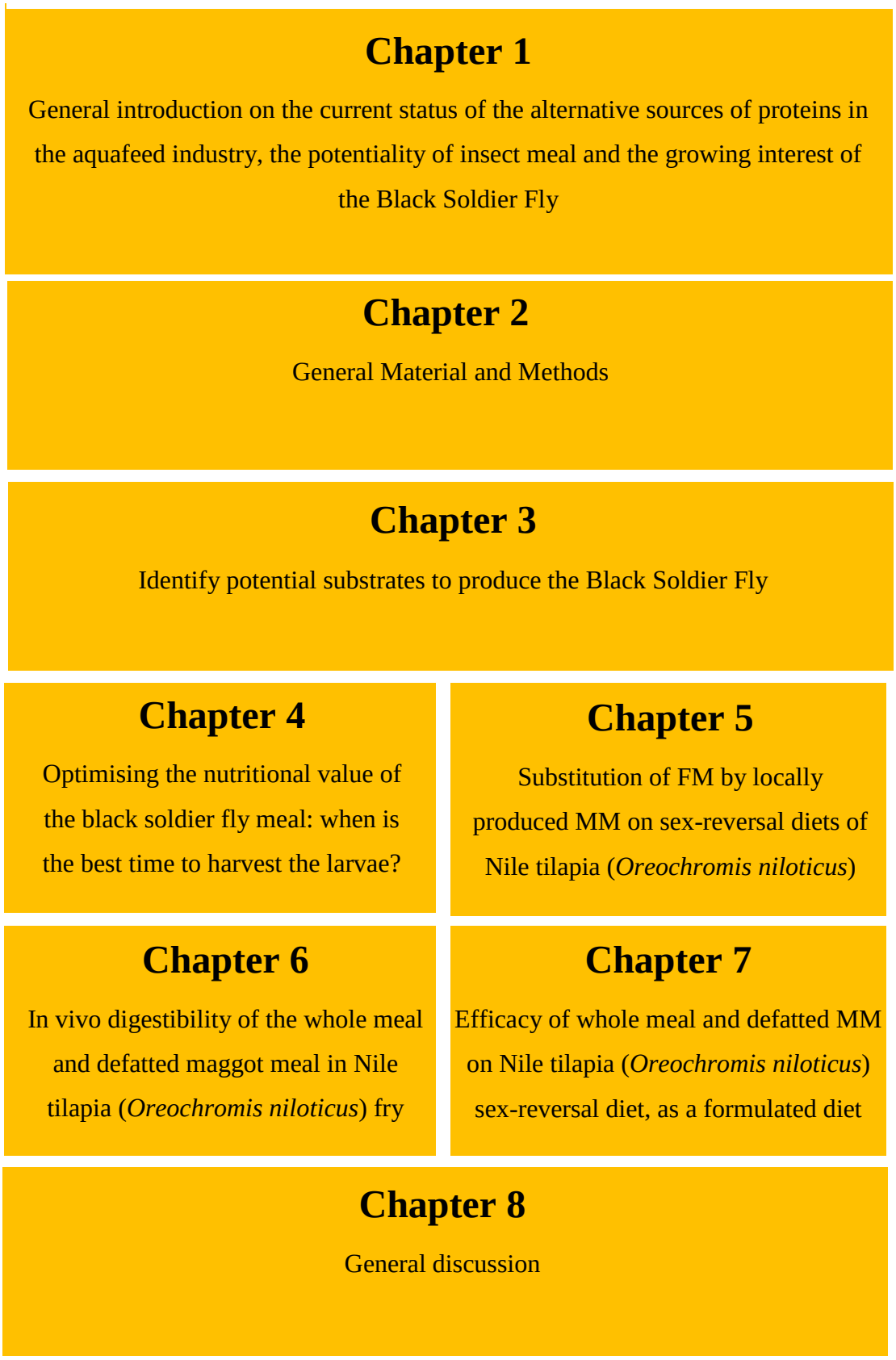
- (1) To develop a 'substrate sustainability index' (SSI) for commercialisation of BSF production in Ghana, W. Africa as an example of an emerging economy with a favourable tropical climate.

- (2) To evaluate proximate nutrient and yield characteristics of BSFL larvae at different life stages (prepupae and earlier white larval stages) and morphological indicators of development status.

- (3) To compare the sex-reversal ratio and production performance of tilapia fry fed with MM and FM in two trials (i) directly substituted and as (ii) part of a nutritionally complete formulated diet.

This thesis is structured into eight chapters as shown in the Figure 1.6.

**Figure 1.6.** Structure of the Ph.D. thesis



# CHAPTER 2: GENERAL MATERIALS AND METHODS

# 2.1 Experimental diets and feed ingredients

## 2.1.1 Maggot meal production

The Black Soldier Fly Meal (BSFM) and the feedstuffs were produced and/or sourced locally.

For the Chapters 3 and 4, the BSFM was produced in Ghana, at the EntoPrise production pilot site, located in the Greater Accra Region, in Adenta at the Animal Research Institute - Frafraha road (5°43'42.5"N 0°08'18.2"W -using WGS84 system-). BSFM was obtained from 450 degree-days old (hence 15 days-larvae produced at 30°C) BSF larvae fed on a substrate constituted of fresh fruit waste. The mix used consisted of hand-chopped fruits in a mix composed of 60% watermelon, 20% papaya, 20% avocado.

Briefly, the larvae were harvested and placed into a bowl with saw dust to make them empty their gut overnight, then after a sieving through a 0.5mm sieve to remove the saw dust, they were killed and dried in an electrical oven at 55°C during 2 days. The dried larvae were then grinded using a grinder (Binatone Blender BLG-699) until it was flour-size particles. All feed was sieved using a fine mesh (<1mm) to homogenize the size of the feed by discarding large particles. The BSFM used for the experiment in Chapter 5 was produced over a 3 months period and was kept frozen during this time to prevent its adulteration. The feedstuffs –other than BSFM- used in Chapter 5 were sourced from Ranaan Fish Feed West Africa LTD. (Greater Accra Region, Prampram, Ghana). For Chapter 5, the BSFM used was not defatted, for technical and logistic issues inherent to the local context.

For Chapters 6 and 7, the BSFM was sourced in Malaysia (Entofood Sdn Bhd; Kuala Lumpur). The larvae used were also 450 degree-days old, and were produced on food waste, due to IP reasons, the exact composition of the substrate remains unknown. The BSFM used was spray-dried, turning it into a very fine powder. As a matter of consistency, all the BSFM was sieved to ensure an homogenisation in the size of the feed particles. Two types of meal were used: the full fat BSFM, and the defatted BSFM. For the later, the powder obtained from the grinding was cold-pressed to extract the oil. All the other ingredients used in the production of the tested diets were sourced in Thailand. Their specific origin is specified in the Chapters 6 and 7.

### 2.1.2 Experimental diets

For Chapter 5, the whole-meal BSFM was used as a substitute for the 'Super Start Feed SS0' produced by Ranaan Fish Feed Company West-Africa, commonly used for the sex-reversal process in Ghanean hatcheries, and as a substitute to pure locally-sourced fish meal. Sex-reversal tilapia fry (*Oreochromis niloticus*) were fed simple diets prepared on-farm in Ghana (Troppo Farm, Asetsuare, Volta Region) by replacing totally or partially the feed (which commercial name is 'SS0' or pure Fish meal) by whole BSF larvae meal. Being a basic-substitution experiment, while the treatments were not isoenergetic nor isonitrogenous, they all meet the requirements of the species according to their lifestage (NRC, 2011).

For Chapters 6 and 7, conducted in Nam Sai Farms, (Prachinburi, Thailand) both whole and defatted BSFM were used as ingredients in the formulation of complete and balanced diets. Chapter 6 consisting of a digestibility analysis of the meal, different protein sources (including defatted MM and Whole-meal BSFM) were tested against each other to analyse their digestibility values in swim-up tilapia fry. In Chapter 7, 12 isoenergetic and isocalorific diets (including 2 controls: a positive and a negative) were examined as sex-reversal tilapia fry-feed. 10 different diets consisted in incremental use of BSFM were tested, in which 5 formulations were used for Whole-meal BSFM, and 5 others for defatted BSFM.

Diet formulation, manufacture and storage are detailed in each relevant chapter. Complete diets were formulated to satisfy the nutritional requirements of the species according to their lifestage (Jauncey, 1998; Hayashi et al., 2002; NRC, 2011).

## 2.2 Experimental designs and set-up

### 2.2.1 On-farm Experiments

All experiments were conducted on-farm using the facilities and benefited from the technical assistance of commercial running systems in Ghana and Thailand. Local, and widely accepted commercial husbandry practices were reproduced at an experimental scale to ensure the commercial relevance, and applicability of the results.

Working in a commercial hatchery brings numerous constraints that have to be taken in consideration. Firstly, precautions were taken to ensure that the experiments did not interfered in anyway with the good commercial practices taking place in the ponds nearby. Hence, all the experiments took place in geographically separated ponds, isolated from the commercial units. Because the staff from the farms was involved in the experiments, it was important that such research-related activities did not impair with the well-functionning activities of the farm itself, and its productivity. Thus, while ensuring a scientific approach and validity of the results, experiment and their related activities such as feeding, harvesting, measuring D.O., temperature etc., which were time consuming given the size of the trials, had to be conducted simply and efficiently. In addition, each experiment was adapted to the space, material, fish and pond availability.

### 2.2.2 Experimental design

In each chapter the design, number of treatments, replication, fish number, feed quantity and sampling methods are described. Although all the trials were conducted in similar condition to commercial practices, the availability of BSFM was always the limiting factor for the size of the experiments. Through all experiments, four replicates were used; fish were maintained in experimental units: tanks (Chapter 6) and hapas (Chapters 5 and 7). The hapas were smaller compared to commercial units, but all the fish were stocked at commercial density (*i.e.* 12 fish/l for the day-old swim-up fry). To ensure a consistency of the water quality, all the hapas were placed inside the same pond, filled 3 weeks prior every experiment to allow the water to go green, and randomly placed to avoid any location effect on the different treatments.

Nile tilapia fry (*Oreochromis niloticus*) was considered for these trials due to its economic significance in Ghana and Thailand. The duration for the trials in the Chapters 5 and 7 was 21 days, post-hatching. The time frame used by commercial hatchery to give an hormone-impregnated feed in order to perform an all-male production. After these 21 days, all the fish, regardless of their treatments were fed with a classical commercial feed used in the local hatcheries for another 6 weeks in order to be able to be sexed. During this time the fish were stocked in bigger hapas at a density of 1 fish/l, following classic commercial procedures. For the digestibility experiment conducted in Chapter 6 the fish

were kept in tanks for 21 days only, to be able to collect enough feces to perform the digestibility analysis.

While all investigations were initiated with juveniles, the experimental periods allowed a significant increase in body weight, of at least 300% as recommended in the NRC guidelines (2011).

In all experiments, water quality was monitored daily using appropriate and available equipment (thermometer, DO meter and spectrophotometer). For every experiment in Chapter 5 and 7, the hapas were changed every 10 days to be maintained clean at all time by preventing excessive fouling of the nets.

The experiments realised in Chapter 6-7, in Thailand, were approved by the ethical committee (AWERB/1617/202/New Non ASPA and AWERB/1718/060 New Non ASPA respectively).

### 2.2.3 Experimental sampling

Fish were systematically weighted at the beginning of each experiment, and at the termination of the experiment. The frequency of the samplings between the start and the end of each experiments is detailed in every chapter. Due to the number of fish involved in all trials, fish were carfully bulk weighted, and 3 sub-samples representative of the total population, were taken randomly, bulk weighted and individually counted. At the end of each experiment –at the end of the 21 days period- fish were graded into 3 sizes using a hand grader with mesh of 7.5 and 9mm to assess the uniformity of the fish.

Fish were euthanised by administration of anaesthetic overdose of metacaine sulfonate MS-222, in order to be able to be sexed at the end of each experiment.

## 2.3 Biochemical analysis

Proximate composition of the feedstuffs, diets and feces were analysed at the University of Stirling in accordance with the Association of Official Analytical Chemists (AOAC, 1990) or standard methods detailed below. Dry matter was tested on whole and fresh material, while proteins, ash, lipids, energy and chitin analyses were performed on finely grinded, and homogenised dried samples as the diets and feedstuff. Two methods for the lipid analyses were used and described below. Each biochemical analysis was replicated twice.

### 2.3.1 Dry matter

To calculate the DM, 1.0g of feedstuff, or 20g of previously blended and homogenised fish body samples (wet weight) were placed in a drying oven (Gallenkamp Oven 300) at 110°C overnight until constant weight was achieved (AOAC, 1990). Then the sample was placed in a dessicator to be weighted on a precision scale.

Faeces samples were freeze-dried using a Christ Alpha 1-4 LSC Freeze dryer (Osterode am Hartz, Germany) at -50°C under vacuum during 48h until constant weight was achieved.

### 2.3.2 Crude protein

The protein content of the samples was estimated using the Kjedahl method, (*i.e.* inferred from the nitrogen content of the sample).

Briefly, 250 mg of each sample was placed in a Kjedahl digestion tube with 2 mercury Kjedahl tablets and 5 ml of concentrated sulfuric acid, and then heated to 420°C for an hour. After cooling at room temperature, distillation was carried out using the Tecator Kjeltec TM 2300 analyser (Foss, Warrington, U.K.) according to the standard method (Persson, 2008) and the manufacturer's instructions.

Once the nitrogen content is known, as proteins consists of amino acids contain nitrogen (N) in the amino group, it is inferred that proteins contains 16% nitrogen, therefore using this ratio, the protein level can be calculated.

### 2.3.3 Crude lipid

Whereas the fatty acid composition has to be determined or not, two methods were used to estimate the lipid method. The first one, the Folch method, is non-destructive and allows to proceed with the fatty acid determination in the samples, but is time consuming. The Soxhlet extraction is a destructive method, but less time consuming than the first one. According to the future use of the sample one method could be preferred to another. In every chapter the method used is specified.

#### 2.3.3.1 Folch method

Folch is a non-destructive method applied to samples to extract the crude lipid used for subsequent fatty acid analyses. Briefly, the total lipid were extracted from 0.5g of sample by homogenising in 20 volumes of ice-cold chlorophorm/methanol (2:1 v/v) using a Ultra-turax tissue disruptor (Fisher scientific, Loughborough, U.K.) according to Folch *et al.* (1957) and determined gravimetrically afteran overnight dessication under vacuum. The samples were then kept in 2ml glass vials and the ambient atmosphere was replaced by nitrogen, before being kept at -20°C for further fatty acid analysis.

#### 2.3.3.2 Soxhlet method

When the samples were not subjected to a fatty acid analysis, the lipid were treated according to the Soxhlet extraction with petroleum ether (Christie, 2003) following an acid hydrolysis with HCL. Acid hydrolysis was performed with 1.0g of grinded and homogenised samples using a fully automated hydrolysis apparatus (Tecator Hydrotech™ 8000, Foss analytical, Hillerød, Denmark), according to the manufacturer's instructions, and hydrolysed samples were then dried at 60°C for 16-18 hours and transferred to the Soxhlet apparatus (Tecator Soxetc system 2050 auto extraction apparatus, Foss analytical Hillerød, Denmark)..

### 2.3.4 Fatty acid profile

To analyse the Fatty Acid Methyl Esters (FAME) we used the lipid extracts obtained from the Folch method. FAME were prepared from total lipid re-dissolved in a chlorophorm/methanol solution (2:1v/v) at a concentration of 10 mg/ml by acid-catalysed transesterification at 50°C for 16 hours (Christie, 1993). The extraction and purification of FAME were conducted using the protocol described in Tocher & Harvie (1988) and then quantified by gas-liquid chromatography using a Fison GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30X0.32mm i.d. x 0.25µm ZB-wax column (Phenomenex, Chershire, UK), *on column* injection and flame ionisation detection. Hydrogen was used as a carrier gas with initial oven thermal gradient of 50°C at 150°C at 40°C.min$^{-1}$ to a

final temperature of 230°C at 2°C.min$^{-1}$. Individual FAME were identified by comparison with known standards (Supelco™ 37-FAME mix; Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher & Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 1.19, Thermoquest Italia S.p.A., Milan, Italy). Fatty acid content (g/100g of sample) was calculated using heptadecanoic acid (17:0) as internal standard.

### 2.3.5 Ash

The total ash content was determined by the incineration of 1.0g of sample placed in a pre-weighted porcelain crucible in a muffle furnace (Gallenkamp Muffle Furnace) at 600°C for 16 hours (AOAC, 1990).

### 2.3.6 Gross energy

Gross energy of feedstuffs and diets was measured by bomb calorimetry (Parr 6200 bomb calorimeter, calibrated with benzoic acid) according to the manufacturer instructions. Briefly, 1.0g of sample was placed in a container filled with pure oxygen, and then combusted with a fuse. The heat released and the temperature variation was used by the instrument to calculate automatically the energy content of the sample in KJ.g$^{-1}$.

### 2.3.7 Amino acid composition

The Amino acid composion of feedstuff and diets was proceeded by ALS Food & Pharmaceutical (Cambridgeshire, U.K.) and Eurofins Food and Feed Testing (Moss, Norway) using High-Performance Liquid Chromatography (HPLC) method according to their standard commercial techniques.

### 2.3.8 Chitin estimation

The chitin analysis was performed using the protocol from Black & Swartz (1950). Firstly, 2g of BSFM previously dried and homogenised were placed into a 250mL beaker. 50 mL of 1M HCl were added into the mix, and then placed into a boiling water bath for an hour. The content was filtered through a N°40 ashless whatman filter paper (pre-dried and weighted). The precipitate was washed 3 times with about 75 mL of near boiling distilled water. Then the precipitate and the filter paper were placed into a new 250mL beaker. 100mL of 5% NaOH solution were added and then placed in a near-boiling water bath for an hour. The content was then filtered again using another N°40 ashless whatman filter paper (pre-dried and weighted). The overall precipitate was washed twiced with pure acetone. It is then placed in pre-ashed crucible (weighed) and dried overnight at 110°C. The overall is weighted the next morning and incinerated in a muffle furnace for two hours at 550°C. The overall is weighted for the final time, the loss in weight between the dry weight and the ash weight is the chitin content.

## 2.4 Digestibility analysis

To analyse the digestibility of different feedstuff (see Chapter 6), we placed 0.1% (at inclusion level) of Yttrium oxide ($Y_2O_3$) included as an inert marker in the diets of 21 days-old fry. Then the Yttrium oxide levels in the initial diets and feces of fish (collected by deposition in the bottom of the tank and collected before each feeding session) was measured using an acid digestion technique by Inductively-Coupled Plasma Mass-Spectrophotometry (ICP-MS). Briefly, 0.1g of sample (feedstuff, diets and feces) were digested in 5 ml of concentrated nitric acid in a CEM Mars Xpress microwave digester for 20 minutes at 190°C. Each tube was then filled up to 25 ml with distilled water and 400 µl of the solution was then further diluted to 10 ml with distilled water. Samples were then analysed in a Thermo Scientific Series 2 ICP-MS (Cheshier, UK).

Differences in the ratios of the parameters for dry matter, protein, lipid, gross energy to Yttrium oxide in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficient ($ADC_{diet}$) for each of the nutritional parameters examined in each diet based on the following formula (Maynard & Loosli, 1979)

$$ADCdiet = 1 - (\frac{Yt\ diet\ \times Parameter\ Faeces}{Yt\ faeces\ \times Parameter\ diet})$$

Where $Yt_{diet}$ and $Yt_{faeces}$ represent the Yttrium oxide content of the diet and faeces, respectively, and $Parameters_{diet}$ and $Parameters_{faeces}$ represent the nutritional parameters of concern (dry matter, protein or energy) content of the diet and faeces, respectively. Digestibility values for each diet are presented in table 4. The digestibility value for each of the test ingredients in the tested diets examined in this study were calculated according to the formula:

$$\text{Nutr. ADingredient} = \frac{(ADtest\ \times Nutrtest\ - (ADbasal\ \times Nutrbasal\ \times 0.7))}{(O.3\ \times Nutringredient)}$$

Where $Nutr.AD_{ingredient}$ is the digestibility of a given nutrient from the test ingredient included in the test diet at 30%. $AD_{test}$ is the apparent digestibility of the test diet. $AD_{basal}$ is the apparent digestibility of the basal diet, which makes up to 70% of the test diet. $Nutr_{ingredient}$, $Nutr_{test}$ and $Nutr_{basal}$ are the level of the nutrient of interest in the ingredient, test diet and basal diet respectively (Sugiura *et al.,* 1998).

## 2.5 Statistical analysis

All statistical analysis were carried out using IBM SPSS Statistics (Version 21). A significance level of 5% ($p<0.05$) was chosen for all analysis. Data were presented as the arithmetic mean along with the standard deviation of the mean (mean±SD) unless stated otherwise.

Normal distribution of the data sets was verified using Shapiro-Wilk test and homogeneity of the variance was tested with Levene's test. Significant differences between treatments ($p<0.05$) were assessed using one-way analysis of variance (ANOVA) parametric test or Kruskal-Wallis non-parametric test when preliminary assumptions were violated. In the case of significant differences, Tukey's HSD post-hoc test was then applied to rank the groups.

# CHAPTER 3: IDENTIFYING POTENTIAL SUBSTRATES FOR UP-SCALING BLACK SOLDIER FLY PRODUCTION IN GHANA

# 3.1 Introduction

Protein are amongst the most expensive ingredients in animal feeds. The limitations of fish meal, its growing cost and sustainability concerns underpin the need to look for alternative protein sources. Vegetables sources such as soybean, or other terrestrial crops, are widely used substitutes but compete with primary crops for land, can contribute to deforestation (Carvalho, 1999; Osava, 1999) and use large amounts of pesticides and water (Osava, 1999; Carvalho, 1999). Their limited amino acid profiles and the presence of anti-nutritional factors can also limit their disgestibility and nutritional value (El-Ebiary *et al.*, 2005; He *et al.*, 2013; Pratoomyot *et al.*, 2011, Hardy, 2010). Novel alternative protein sources include algae, fungi, and insects. Insect farming has potential to optimise land use as they can be stacked vertically in trays (Van Huis *et al.*, 2013), have low water requirements, and insects can be fed a wide range of substrate (Nguyen *et al.*, 2013; Leong *et al.*, 2016).

One of the most studied species is the Black Soldier Fly (*Hermetia illucens* –BSF). BSF can tolerate a wide range of environmental conditions. While it can develop in any thermal condition above 10°C, it is now widespread across the globe. It spread widely during and after World War II, and within the Palearctic region it can be found between 49°N and 40°S (Martìnez-Sànchez *et al.* 2011). The BSF is recorded from many Asian and African countries (INPN, 2017; Oliveira *et al.*, 2015; Marshall *et al.*, 2015; Lardé, 1990). Where it has been introduced the fly seems to cohabit with no apparent negative impacts on local entomofauna, its proportion being always very low *in natura*. Therefore the BSF can be qualified as a "non-pest" species and its breeding requires no specific precautionary measures. Larval development time is short, reaching harvest size after only 15 days at 30°C. The BSF reduces the presence of harmful bacteria (Liu *et al.*, 2008) and inhibits the oviposition of *Musca domestica*, the common housefly (Furman *et al.*, 1959; Bradley & Sheppard, 1984).

A key advantage of the BSF compared to other mass-reared insect species, is their ability to convert a wide range of substrates (Table 3.1). BSF larvae are saprophageous, and use their powerful mandibles to degrade the substrate. The enzymatic activity of their digestive system and their symbiotic flora contribute to efficient break down and conversion of substrates into usable energy. High enzyme activity levels of leucine arylamidase, α-galactosidase, β-galactosidase, and α-mannosidase, lipases and proteases

have been reported by Kim *et al.* (2011). This is augmented by symbiotic flora such as *Bacillus subtilis*, *B. amyloliquefasciens*, *B. stratosphericus* and *Proteus mirabilis* (Jeon *et al.*, 2011; Yu *et al.*, 2011; Zheng *et al.*, 2013). This enables BSF larvae to feed on a wide range of substrates and precludes dependence on specific, or niche food sources.

Consequently BSF have been employed to recycle animal waste (Sheppard *et al.*, 1994; Nguyen *et al.*, 2013), faeces (Diener *et al.*, 2009; Lalander *et al.*, 2016), or vegetable and agricultural wastes (Rachmawati *et al.*, 2010; Diener *et al.*, 2009; Green & Popa, 2012; Gujarathi & Pejaver, 2013; Kalová & Borkovcová, 2013; Nguyen *et al.*, 2013). Upcycling them into a high quality ingredient for animal feed (Hale, 1973; Newton *et al.*, 1977; Veldkamp *et al.*, 2012; Tschirner & Simon, 2015) and compost (Table 3.1).

This ability is particularly interesting in the realm of waste remediation services, where the management of organic substrate can affect public health, and have environmental, biosecurity, and logistical challenges in both developed and developing worlds (Halloran *et al.*, 2016). Therefore, the BSF larvae can efficiently convert nutrient-poor, with low or no value, waste which are otherwise costly to dispose, into valuable biomass. By doing so, the waste volume will be reduced, biomass, effluents and odours resulting from it will be minimised, improving sanitation and diminishing the risks of pollution, while creating a valuable and sustainable protein source and biofertiliser (Sheppard *et al.*, 1994; Newton *et al.*, 2005a; Myers *et al.*, 2008; Diener *et al.*, 2011b; Lomas, 2012; Lalander *et al.*, 2013).

Consequently, the BSF larvae could be used to sustain a circular economy, where raw materials would be derivated from low or no value waste streams – acting as a waste remediation service- where all the outputs of the bioconversion would be used, generating near-zero waste.

This capacity to act as a waste management agent is particularly interesting in urban and peri-urban areas as solid waste disposal is often considered to be the second most pressing problem that urban city dwellers are facing after unemployment (Da Zhu *et al.*, 2008). While a poor management can negatively affect human health, it can be a major set-back for the economic growth (Diener *et al.*, 2009; Kalová & Borkovcová, 2013). On the contrary, when properly managed, such system usually results in job creation and income generation both for the formal and informal sector (Diener *et al.*, 2009).

**Table 3.1**: Breeding substrates for *Hermetia illucen*s larvae in the literature.

| **Manures** | |
|---|---|
| **Chicken manure – broilers** | Sheppard *et al.* 1994 ; Guitterez *et al.* 2004 ; Diener *et al.* 2009 |
| **Chicken manure – layers** | Bondari & Sheppard, 1987; Sheppard *et al.* 1994 |
| **Dairy manure** | Myers *et al.* 2008 |
| **Cow manure** | Newton *et al.* 1977; Newton *et al.* 2008 |
| **Pig manure** | Newton *et al.* 2005 |
| **Horse manure** | Mutafela *et al.*, 2015 ; Julita *et al.*, 2018 |
| **Sheep manure** | Julita *et al.*, 2018 |
| **Human faeces** | Bradley, 1930; Diener *et al.*, 2009 ; Lalander *et al.*, 2013 ; Banks *et al.*, 2014. Makkar *et al.* 2014; Choudhury *et al.*, 2018 |
| **Agricultural by-products** | |
| **Fish offals** | St-Hilaire *et al.* 2007 |
| **Coffee pulp** | Lardé, 1989; Lardé, 1990 |
| **Brewery waste** | Tschirner & Simon 2015 |
| **Dried distillers' grains (with solubles from barley, corn wheat and sugar syrups)** | Tschirner & Simon 2015 |
| **Palm Kernel Meal (PKM)** | Hem *et al.* 2008 ; Rachmawati *et al.*, 2010; Caruso *et al.* 2013 |
| **Sugar beet pulp** | Tschirner & Simon 2015 |
| **Mixture of middlings from a feed mill** | Tschirner & Simon 2015 |
| **Rice straws** | Manurung *et al.*, 2016 |
| **Fruits** | Nguyen *et al.*, 2013 |
| **Rotting fruits** | Green & Popa 2012; Makkar *et al.* 2014 |
| **Bee wax** | Malloch, 1917 |
| **"Vegetable waste"** | Spranghers *et al.*, 2017 |
| **Biogas digestate** | Spranghers *et al.*, 2017 |
| **Organic "waste"** | |
| **"Organic waste"** | Sawangkeaw & Ngaprasertisth, 2013; Kalová & Borkovocová 2010: |
| **Household waste** | Barry 2004; Diener *et al.* 2011 ; Newby, 1997 |
| **Restaurant and Kitchen waste** | Nguyen *et al.* 2015; Spranghers *et al.*, 2017b |
| **Municipal waste** | Diener *et al.* 2011 ; Kalová & Borkovocová, 2010 ; Mutafela *et al.*, 2015 |

Accra, being the largest city in Ghana with a population of 3.9 million, is one of the fastest growing cities in Africa in terms of population and economic performance (ISSER, 2016) and is good example of this problem. The lack of adequate capacity of most municipal and state governments to deal with municipal solid waste has resulted in refuse being dumped in both water bodies and urban landscape. Obviously, the indiscriminate disposal of waste, results in its accumulation in public places regardless of being hazardous or non-hazardous (Mwesigye *et al.*, 2009).

In this context, the BSF system could be of interest as an alternative waste disposal management agent. However, to implement such system, a major constraint is the identification of a proper feeding substrate to breed the insect. Therefore, the holistic assessment of available substrates, and creation of a Substrate Suitability Index (SSI) are the first critical steps toward the establishment of a viable commercial BSF production. In the present chapter, we use a field-based approach from the EntoPrise Project in Ghana, to assess and identify potential rearing substrates for BSF larvae in the context of a small-scale BSF production system.

## 3.2 Material and Methods

This case study was carried out in Ghana, at the EntoPrise site (5°43'42.5"N 0°08'18.2"W, using WGS84 system), located at the Animal Research Institute, in Adenta, Greater Accra region in Ghana. This site was chosen for the project due to its proximity with Accra (being in its peri-urban area), and for its all-year round favourable environmental conditions.

To identify a potential breeding substrate, a literature review was performed, to list all the known substrates for BSF larvae. This was then cross-referenced with substrates available in the Greater Accra Region *i.e.*: abattoir rumen waste, vegetable and fruit market waste, plantation agriculture and food processing waste. The substrate list was established in partnership with the Animal Research Institute staff, which provided location and contact with the waste generator industries or people in charge.

Due to a lack of legislation in the context of insect production, all substrates were considered regardless being allowed or not in other countries (like in E.U.). Each waste source was systematically visited and mapped using a GPS to assess its distance from the production site. Characteristics of waste streams were identified and various values weighted, through dialogue with local stakeholders and secondary data gathered in the literature.

To identify a potential substrate for a medium scale bioconversion system using the Black Soldier Fly, several key factors had to be considered, forming the basis of the Substrate Suitability Index ("SSI" - see Figure 3.1):

- The first criteria taken in consideration was the **cost and availability**. The purchasing cost per ton in US dollars, along with the transportation costs was assessed using a key informant interview. The transportation cost was based on the rental of a medium-size pickup truck (available at the Animal Research Institute). The price was fixed: for a collection range up to 30, 60 and up to a 100 Km the price was respectively 20.7; 41.4; 62.1 US$. The seasonality and quantity, were assessed using both key informant interviews and literature review (when it was possible).

- The second criteria was regarding **health and safety**, to assess if the substrate could potentially contain heavy metals, pesticides or antibiotics, which could have been bioaccumulated in the larvae, or contain harmful bacteria/viruses rending its handling by workers risky. These data were assessed using both key informant interview (when possible) and literature review.

- The third and last criteria was about **nutrient composition and larval performance**. Nutrient composition of the most promising-potential substrates was assessed according to AOAC (1990) procedures, and a larvae growth performance test was tested by placing 12kg of substrate into a 90x40x15cm tray, and seeded with 0.50g 5 day-old (post-egg haching) larvae. Then, 100 larvae were randomly collected daily and weighted to assess the growth rate, until they reached the prepupal stage. A single-factor repeated measures (ANOVA) followed by a Tukey's HSD for post-hoc was used to test significance ($p<0.05$) between the means of the weight of 11days-old larvae (when they are supposed to be harvested). Nutrient composition was only tested when the substrate was not prone to a prohibitive cost, high risk of heavy metal contamination or very low quantities involved.

Once all the potential substrates were listed, and when all relevant data associated to each studied variables were gathered, a table was done using Excel. To help the decision process, a rating system was defined as follows to give a score to the final index: "1" for acceptable values, "2" for a criteria subjected to caution, and "3" for a prohibitive criteria (for instance: high risk of heavy metal contamination). This rating system was only

associated to the categories “cost and availability” and “Health impact”, and not with the proximate composition of the substrate as it was not always possible to analyse them. Substrate with a score greater than 15, or having a score of “3” in one of its categories, were considered as non-viable. Then, based on the growth data, a model was designed and tested at the EntoPrise site in Ghana. The system ran for 18 months, the total length of the EntoPrise Project.

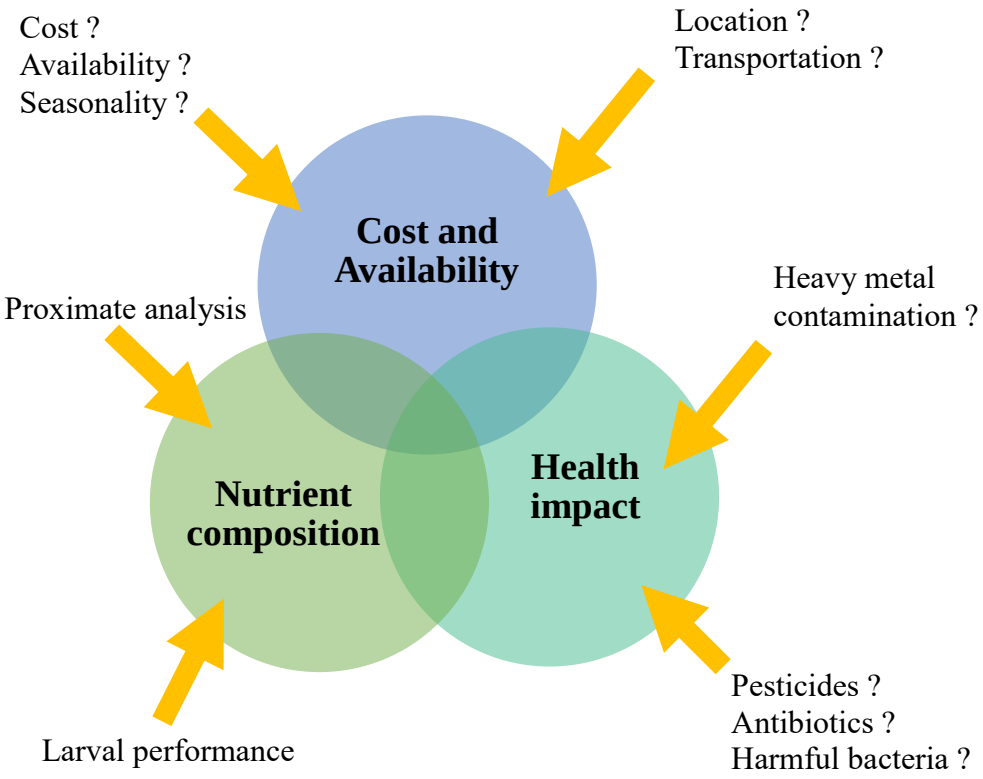



**Figure 3.2**: Substrate Suitability Index (SSI) showing criteria used for the substrate selection.

## 3.3 Results

The substrate were listed in Table 3.2. Substrates displaying a score of 3 in one of the different categories –considered as containing a prohibitive criteria- or an index score above 15 were not implemented in the system. The cow and pig manure were discarded because their collectability was extremely difficult (and was scored “3”) as they are not

pooled at the same place, but on the contrary, scattered on meadows, or –for the case of pig manure- rarely on an industrial 'feed-lot' scale. All animal offals (abattoir waste, cow blood, rumen waste) were discarded as the presence of bovine tuberculosis in Ghana is high, therefore occupational health risks involved in their processing was scored as very high. The brewery waste along with the wheat bran were not useable as there is a high opportunity cost for alternative uses, thus their price is too high (62.1 and 142.2 US$ respectively). The Golden Exotic and Blue Skies fruit waste, were located too far away from our production site (nearly 100 kilometers away) and were being utilised by their owners to produce compost for local use. The municipal waste were not useable either as there is no primary sorting of the waste, which would have required a high man power, and the risks of heavy metal and other contamination were too high. This left 3 substrates from the original panel: chicken manure from broilers, from layers and vegetable market waste (fruits only).

The growth rate of the BSF larvae (Figure 3.2) fed with the 2 remaining substrates (along with cow and pig manure to assess the growth on different kinds of manures) showed that the larvae performed poorly on manures, but had a significantly better growth when fed with market fruit waste ($p<0.01$). The growth rate of the larvae grown on *layer* chicken manure (containing saw dust) is not presented as it was null.

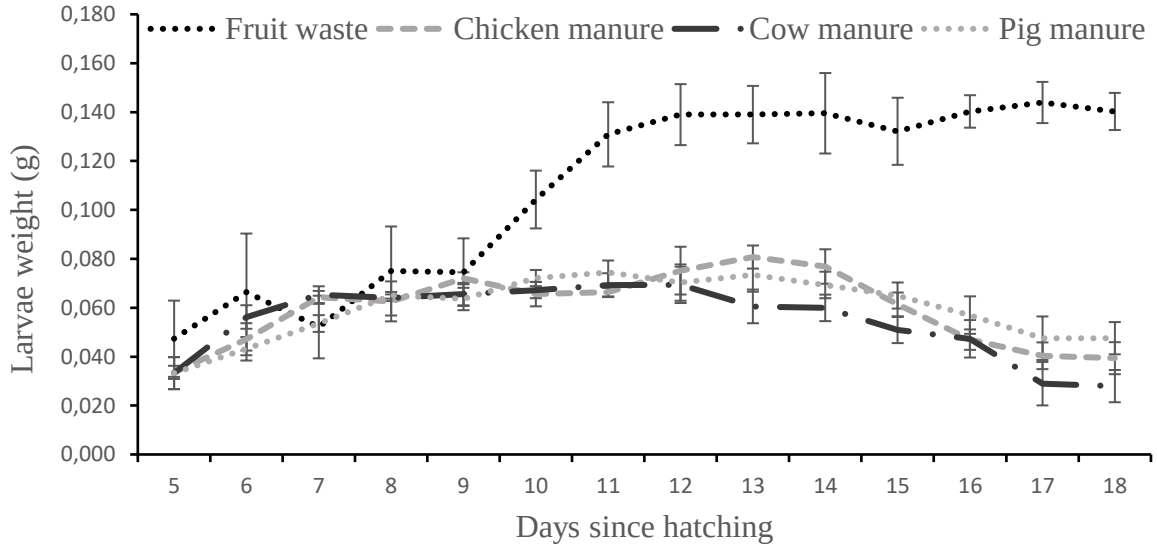



**Figure 3.3**: Growth rate of BSF larvae reared on market waste and different types of manures.

**Table 3.2**: Substrate Suitability Index (SSI) applied in the Greater Accra Region (Ghana) context.

| Substrate | Cost & Availability | | | | | | | Contamination risk | | | Nutrient composition (% on dry matter value, presented 'as is') | | | | | Index score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from site (Km) | Transport cost (US$[1]) | Collectability | Are waste sorted ? | Seasonality | Tons/day | US$/ton[1] | Heavy metal risk | Pesticide risk | Bacteriological risk | Moisture | Protein | Lipid | Ash | Fiber | |
| **Manures** | | | | | | | | | | | | | | | | |
| Chicken manure (Broiler)[2] | 1 | 20 .7 | H (depends) | Yes | C | 270 | 20.7 | L | L | M | 9 | 4.5 | 9.8 | 89.6 | 4.5 | 13 |
| Chicken manure (layer)[2] | 1 | 20 .7 | H (depends) | No (with saw dust) | C | 686.08 | 20.7 | L | L | M | 20 | 9.8 | 0.05 | 37.1 | 14.3 | 14 |
| Cow manure[2] | 1 | 20 .7 | H | No (with straws) | C | 10 803 | 20.7 | L | L | M | 71.3 | 4.76 | 0.13 | 12.15 | 3.62 | 15 |
| Pig manure[2] | 1 | 20 .7 | H | No (with straws) | C | 76.64 | 20.7 | L | L | M | 57.45 | 7.38 | 0.1 | 13.47 | 12.51 | 15 |
| **Agricultural and Industrial by-products** | | | | | | | | | | | | | | | | |
| Cow blood[3] | 18 | 20 .7 | E | Yes | C | 0.7 | 0 | L | L | H | - | - | - | - | - | 14 |
| Abattoir waste[3] | 18 | 20 .7 | E | Yes | C | 1 300 | 0 | L | L | H | - | - | - | - | - | 12 |
| Rumen waste[3] | 18 | 20 .7 | E | Yes | C | 0.32 | 0 | L | L | H | - | - | - | - | - | 14 |
| Milk waste[2] | 1.5 | 20 .7 | E | Yes | C | 0.075 | 0 | L | L | M | - | - | - | - | - | 13 |
| Brewery waste[4] | 24 | 20 .7 | E | Yes | C | 50 | 62.1 | L | L | L | - | - | - | - | - | 13 |
| Wheat bran [2] | 16 | 20 .7 | E | Yes | C | 0.1 | 142.2 | L | L | L | - | - | - | - | - | 13 |
| **Large scale agrobuisness** | | | | | | | | | | | | | | | | |
| Golden Exotic fruit waste[5] | 94 | 62.1 | E | Yes | C | 28.7 | - | L | H | L | - | - | - | - | - | 16 |
| Blue Skies Fruit waste[6] | 87 | 62.1 | E | Yes | C | 20 | - | L | H | L | - | - | - | - | - | 16 |
| **"Organic waste"** | | | | | | | | | | | | | | | | |
| Municipal waste[7] | 7 | 20 .7 | E | No | C | 12 710 | - | H | H | H | - | - | - | - | - | 18 |
| Market waste[8] | 7 | 20 .7 | E | Yes | C | 1 | 2.1 | M | M | M | 73.4 | 11.41 | 12.5 | 6.16 | 12.85 | 14 |

[1]Prices converted from Ghana Cedi to US$ , based on the exchange rate of 1 US$=4.83 GHS; [2]Source: Animal Research Institute, 2016; [3]J'Famco Abbatoir, Madina, Greater Accra Region, July 2015; [4]Accra Brewery Limited, 2015; [5]Golden Exotic Report, 2012; [6]Blue Skies Holding report, 2012; [7]Miezah *et al.*, 2015; [8]Market Owner, Fruits and vegetable waste, Key informant interview, 2016. Abbreviations: C: Constant; S: Seasonal; L: Low; M: Medium; H: High.E : Easy.Scoring system works as following : H=3, M=2, L=1.

Once the substrate was identified, and the system implemented, the production process was working as follow: an input of 500 kg market waste (comprising by weight 60% watermelon, 20% avocado and 20% of either papaya or mango according to the season) seeded with 28.5g of BSF eggs, resulted in 175.5 Kg of larvae (fresh weight) and 114.74kg of biofertiliser (see Figure 3.3).

The substrate mix was chosen for its practicality, as this combination (with the sole exception of papaya or mango) was constant and available all year round allowing to ensure a consistency in the substrate quality. As a matter of viability the spoiled fruits were fed whole to the larvae.

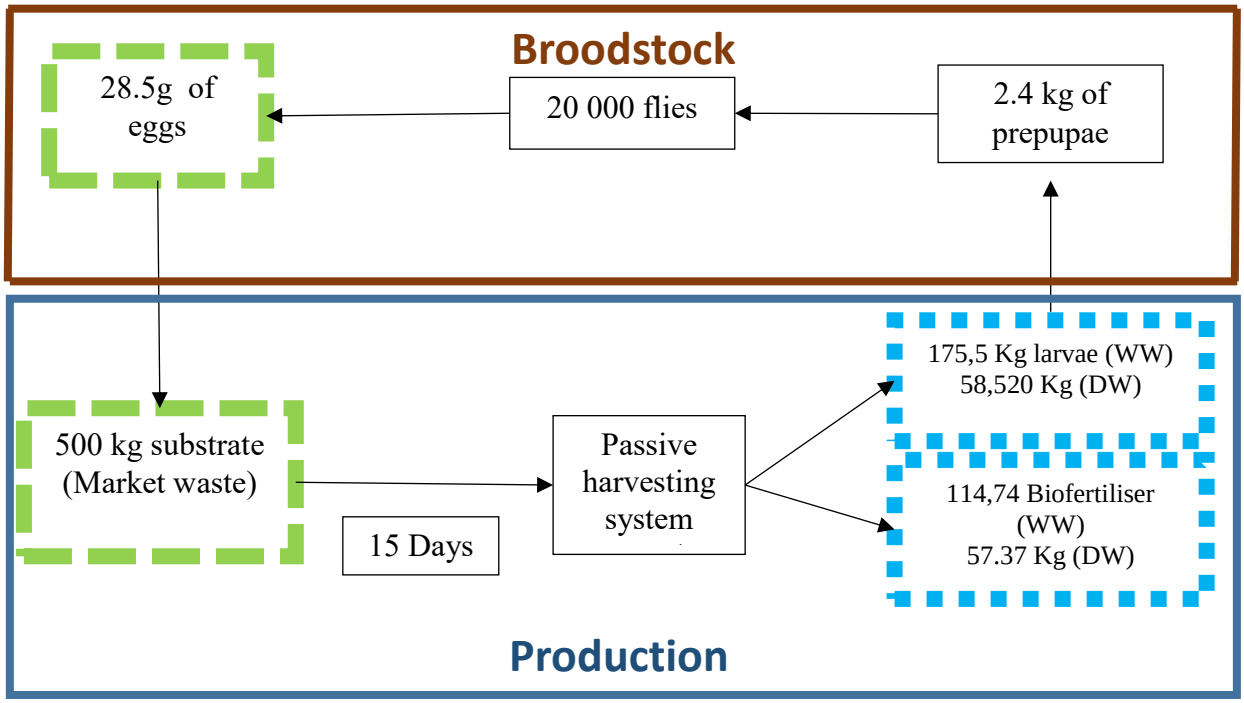



**Figure 3.3**: Schematic representation of the BSF bioconversion system used in Ghana. Green boxes: inputs, blue boxes: outputs.

## 3.4 Discussion

Of the waste streams identified in the surroundings of Accra, (Greater Accra Region, Ghana), 15 potential sources were found and investigated. Amongst these ones, 2 were excluded from our study (Golden Exotic fruit waste, and BlueSkies fruit waste) as they were too far from the production site (near 100km) and their by-products were already up-cycled locally.

Golden Exotic is a company established in 2013 producing fresh fruits for export. They produce around 28,700 kg of waste per day (Golden Exotic report, 2012), but compost them on-site to produce compost for their own plants. Blueskies is a British company started in 1997 specialised in fruit juice, mainly for export, but also for local sell. Similarly, they produce around 20,000 kg of waste per day, but also use them for composting on-site. Therefore as these substrate were not directly available for our system, they were not implemented.

However, on a more general rule, a special care should be given for the use of fruit waste produced in industrial quantites. In the greater Accra Region, several studies demonstrated that pesticides, such as lindan, endosulphane, lambda cyalothrin, chloropyriphos, and DDT were present –and common- on fruits peels, above accepted levels (Amoah, 2008; Amoah *et al.,* 2006; Asante *et al.,* 2009; Amoah, 2012). While some recent evidences might suggest that the BSF might be able to break-down some pesticides (azoxystrobin and propiconazole, according to Lalander *et al.,* 2016), the risk of bioaccumulation could still exist in the larvae.

The products from abattoir (cow blood, rumen waste, abattoir waste), despite being close to the site, present in large quantities and free, were excluded as potential substrates. The risk of bacterial contamination being too high: bovine tuberculosis is still very present in Ghana, ranked 19$^{th}$ in Africa for the highest estimated number of new cases of tuberculosis per year (Ghana Health Service, 2014). In 2007 only 47,632 new tuberculosis cases were recorded. While the tuberculosis strain involved in Bovin (*Mycobacterium bovis*) and Human (*M. tuberculosis*) is slightly different, it can still be transmissible, as contamination may occur through direct inhalation from animals, consumption of uncooked infected meat or infected unpasteurized milk (Ayele *et al*., 2004; Acquash *et al.,* 2012). In the North Tongu District (Volta Region) the prevalence rate of *M. bovis* if

up to 19% in the cattle population. Therefore, being a major sanitation problem this substrate was not implemented in our system.
Another product of animal origin was analysed: milk waste, from a nearby milk processing plant. However, while the protein content is generally high, the very low quantities (75 kg per day) prevent its use in a BSF plant.

The brewery waste are usually depicted to be an excellent substrate for producing the larvae (Kalová & Borkovcová, 2013) and their outputs from beer companies is large in Ghana. In the case of Accra Brewery Limited, located in the center of Accra, up to 50 tons are produced daily. However, as this by-product is usually fiber and protein rich, it makes an excellent feed for livestock. Therefore –in our case study- it was not given for free, but on the contrary sold mostly to pig farmers at a price rate of 62.1 US$ per ton. The same case applies for wheat bran, a by-product from the flour industry, rich in fiber, lipid and protein, already used for livestock feed at a rate of 142.2 US$. In these two cases, the use of these products for animal feeds makes them too expensive (and irrelevant as they are already up-cycled) for a BSF bioconversion.

Another type of waste is produced in large quantites: manures. The animal intensive farming, has developed rapidly due to the rise in the population and generates large amounts of manure that have to be disposed (Tao *et al.*, 2016). However decomposing manure is not necessarily easy and can impact the larvae development. For instance, similarly to our study where BSF larvae developed poorer on manures than fruit waste, Nguyen *et al.*, (2013) demonstrated that the lowest growth rate obtained by BSF larvae in their experiment, was when they were fed with pig manure, compared to other kinds of organic waste. Tomberlin *et al.*, (2002) found that the growth of larvae (up to the prepupal stage) was slower on cow manure (near 2 months) than poultry manure (4 weeks) or pig manure (6 weeks). One of the arguments proposed for this, is the high fiber content of the substrate. On a general rule, the lower the fiber content in the feed substrate, the better the digestion and assimilation by the larvae, and consequently, the shorter the bioconversion period (Ushakova *et al.*, 2018). When exceeding 7% (on a DM basis) it will affect the bioconversion, giving lighter larvae (Li *et al.*, 2015, Pelaez-Samaniego *et al.*, 2017). For example, it was shown that the bio-utilisation by larvae reached 65% for kitchen waste, and 40% for the poultry litter (from layers). The latter is very problematic as it is in fact a mix of manure and wood dust, or wood shavings. Although

the litter composition indicates it is nutrient rich and full of energy, the mass fraction of digestible proteins is only low, as it contains a lot of fibers and a relatively small fraction of digestible carbohydrates. The wood dust, mixed with the manure can contain up to 15-35% of poorly digestible lignin and 20-35% of hemicellulose, decreasing the nutritional value of the substrate, leading to a low efficiency utilisation by the larvae. Therefore using poultry litter from layers (therefore having saw dust) to grow larvae does not perform well. In our case it even prohibited the growth of the larvae, and therefore was not a suitable substrate at all. The same case arise with cow manure, having a high fiber content with hemicellulose and lignin, making it difficult for the larvae to process (Ur Rehman *et al.*, 2017). However, it was demonstrated, that when using a co-digestion method by mixing cow manure (rich in fiber), and poultry manure (from broilers, therefore with a lower fiber content) the digestibility of the substrate was significantly improved. According to the published data, the co-digestion of such waste combination at a ratio of 40:60 provided a synergetic effect and increased cellulose conversion by 22.6%, hemicellulose conversion by 6.9% and lignin conversion by 32.3% (Ur Rehman *et al.*, 2017). These co-digestion methods are known to enhance the nutrient absorption by the larvae (Abudi *et al.*, 2016, Chen *et al.*, 2016, Razaviarani *et al.*, 2013). Similarly, Ushakova *et al.* (2018) demonstrated that when developing BSF larvae on a substrate containing animal excrements and pre-seeded with gut bacteria, it decreased the release of gas, while increasing the larval growth, and consequently the bioconversion process. Therefore, *H. illucens* can have the potential to degrade high-fiber manures, decreasing the risk of pollution, *if* the substrate is seeded with a proper strain of bacteria allowing a first degradation of the fiberous structures (such as lignin or hemicellulose) pollution by co-digestion. Beside the fiber content, the freshness of the manure is also to take in consideration. Tomberlin *et al.* (2002) demonstrated that BSF fed 5 day old hen manure grew half the rate of those fed with 18h-old manure. Similarly, Oonicx *et al.* (2015) reported that BSF fed with rehumidified dry manures reached the prepupal stage with extended period of time: it took 144 days when grown on pig manure or poultry (broilers, without saw dust), and up to 215 days when fed with cow manure. This is most likely due to a loss of protein in dry manure (Sheppard, 1983). Beside these development consideration, the collectability has to be taken into account. While the manure production is large in the Greater Accra Region, it is usually a small scale production system. Cows are grazing in peri-urban areas, therefore their faeces are not easily collectible easily. Pigs are usually kept in pens by half a dozen, making it hard to collect

enough feces to run properly a BSF system. For poultry manure, when they are produced as layers, the faeces drop into a saw dust-covered floor, creating problems highlighted above. Otherwise, when they are collected in gutters –in the case of intensive broiler production- the faeces are rarely fresh, as they are collected in batches when the trough is full, therefore not useable. The growth rate of the larvae grown on manures is highlighted in the Figure 3.2, and performed the lowest growth recorded in our trial. Also, a part of manures are already used as fertiliser by farmers, which explain the cost of these feeding substrates (20.7 US$/ton).

Currently, about 12 710 tonnes of municipal waste are generated in the Greater Accra Region (Miezah *et al.*, 2015) making it a potential rearing substrate for the BSF industry. In Accra only 67% of the total waste is collected due to logistics problems. The remaining part being left in the open environment causing severe hygienic and health problems (Government of Ghana, 2008; Mwesigye *et al.*, 2009; Annepu & Themelis, 2013; MOH, 2000). According to Valkenburg *et al.*, (2008), waste composition from these sources are highly heterogeneous in nature (Table 3.3) and have highly variable physical characteristics depending on their sources. If not collected and properly treated, the organic solid waste fraction can be a catalyst in spreading diseases by sheltering and feeding various diseases vectors (Sharholy *et al.*, 2007; Ali *et al.*, 2012; Hossain *et al.*, 2014) and has other negative environmental impacts posing serious health risk. At the moment, most of the collected waste are being landfilled and/or burned. As there is no primary sorting, the heterogeneity of the generated waste is a major hindrance in its utilization as a raw material in a BSF production system. In addition, as all kind of waste are mixed, the risk of heavy metal contamination through battery leakages, electronic equipment *etc.*, is a significant constrain. However, some entrepreneurs are starting to address this situation: Accra Compost & recycling plant limited, a company opened in 2012, is aiming to treat a part of the municipal waste generated in the Greater Accra Region. However –while being still in improvement phase- their waste separation is mostly manual, and the extraction of metallic pieces (such as batteries) is only efficient at 70%, meaning that 30% of metallic object still falls into their compost, rending it unusable for any future utilisation. Source sorting and separation of waste is one of the traditional methods and fundamental steps in an integrated waste management system providing good quality starting material for recycling. However, the success of such techniques depends largely on the active participation of the waste generators and how

they comply with the principles of sorting and separation of the waste. Therefore, as long as no primary sorting of waste, despite being produced in large quantities, done for the organic fraction, this source of inputs is unusable in our context.

**Table 3.4:** Composition of the Accra Municipal Waste (according Miezah *et al.*, 2015)

| | composition (% of overall volume) |
|---|---|
| Organic | 61 |
| Plastic | 14 |
| Inert | 6 |
| Paper | 5 |
| Miscellaneous | 5 |
| Metal | 3 |
| Glass | 3 |
| Textile | 2 |
| Leather & Rubber | 1 |

A last type of waste generated in large amounts was investigated: market refuse, and more specifically fruits refuse. The Madina market, located near our site (7 km), hosts up to a thousand sellers daily. Most of them sell fruits and vegetables, either cultivated on peri-urban sites, or sometimes coming from more distant regions (like the Volta or Northern Region according to the type of products). Such transports generate wastage, and unsold fruits are usually thrown away if they are not sold within 2 days of arrival. The market is locally owned, as the waste disposal bin. Up to a ton of fruit and vegetable waste are generated every day, and are costly to dispose for the market owner (up to 10 US$ every week). Therefore, most of the waste is already sorted (as a bin is held specifically for the market sellers) and easily collectible.

The nutrient profile is good for BSF development, although the moisture content is high. Larvae tend to prefer homogeneous substrates with a water content of about 60-70% (Lardé, 1990). When fed a substrate containing more than 90% moisture, the larvae grow, but at a lower performance. It causes also an issue when it comes to transportation: the drier the substrate is, the better it is to move it from a point A to a point B, reducing the weight, and volume.

In our case, the moisture level was around 70%, and when it was too high (*i.e.* the fruits being too fresh) the raw fruits were placed (after transportation to the production site) onto a tray to dewater first for a few days, before being incorporated into the mix. The

transportation constrain was mitigated by the fact that the market was located nearby, reducing the transport costs.

At the moment, very few studies have been published about the nutritional requirements of the BSF larvae, and researches are just scrapping the surface. Some authors (Tomberlin & Sheppard, 2002; Myers *et al.*, 2008) recommend the use of a feeding substrate containing at least 15% protein to ensure a correct growth of the BSF larvae. However, a satisfactory growth can be achieved with a substrate displaying a lower protein content (like in our case: the fruit waste mix, displayed protein levels of 11.41%), as long as the proteins are digestible, and as lipids and carbohydrates are accessible, and easily degradable (Ushakova *et al.*, 2018).

Interestingly enough, while the nutritional profile of the BSF larvae varies greatly with the type of substrate they were fed on, the protein and lipid content are not necessarily correlated (Oonincx *et al.*, 2015). Only the presence of starch is known to affect the mass fraction of protein and lipid in the larvae, facilitating their storage. For instance, it was demonstrated by Ushakova *et al.* (2018) that when larvae were fed with corn meal (having 10% protein, and 70% carbohydrates, with starch comprising two-third of it) they contained 36.5% protein and 45.5% lipid. On the contrary, when larvae were fed distiller's grains (rich in proteins, with a lower composition in fiber) they contained 48.3% protein and 20.6% lipid.

A special care should be done when manipulating fruit waste, as fecal coliform bacteria are usually present -above accepted levels- on the skin of the fruits (Amoah, 2012). Similarly, as heavy metals can be stored in vegetables, it is an issue in Ghana, especially peri-urban agriculture, were fields can be heavily contaminated (Anim-Gyampo *et al.*, 2012; Addo *et al.*, 2012; Asante & Ntow, 2009). The most famous one –Agbogbloshie- being located in the vicinity of Accra (about 30 km from the market), is the second biggest E-waste dumping site of the world. Electronical waste are burned to extract metals, later melted down and resold as raw materials. Consequently, the fields nearby –where vegetable are grown- and the water streams are heavily polluted. Studies have shown that Lead (Pb) Arsenic (As) Mercury (Hg) Iron (FE), arsenic (As) and Antimoine (Sb) were (way) above accepted levels, both in the field and human urine (Asante *et al.* 2012; Kawaguchi *et al.*, 2012). The Korle Lagoon, deserving a large portion of irrigation on the nearby fields, being considered as the most polluted water body on earth (Nixon *et al.*, 2007). As mentioned before, this issue can be more generalizable as there is no efficient

waste disposal system, and most of the waste generated ends up burned in houses vicinity or production fields (Miezah *et al.*, 2015). Therefore, for a commercial-scale plant, it is adviseable, when using agricultural by-products, to test regularly for heavy metal contamination. As the market vegetable waste were available all year round, at an acceptable price (2.1 US$/ton), at a rate of 1 ton per day, displayed a good nutrient profile, and provided a good growth rate of the larvae, the substrate was therefore selected for our production system.

In our production system, in the optic to produce a quality-reliable and constant maggot meal, only four fruits were isolated. Watermelons were used in a proportion of 60% (by weight) of our initial substrate mix. They are produced all year round from the Northern region, and usually available on large quantity as the transportation produce a lot of wastage. Then, avocados, produced all year round from the Volta Region, composed 20% of our substrate mix. The final ingredient being either mangoes or papaya, and composed the last 20% of our mix. These two fruits are produced in the Koforidua region, but were subjected to seasonality, therefore mangoes were used during the raining season and papaya, during the dry season. The system runned for 18 months, and is resumed in the Figure 3.3.

Out of 500 Kg of substrate entering, and using 28.5 g of BSF eggs, 175.5 kg of larvae were harvested (resulting in 58.5 Kg when dried) and about 114 Kg of biofertiliser. The substrate is usually mechanically mixed by larval movements facilitating its aeration (Ushakova *et al.*, 2018). However if the layer is too thick, the larval food intake will be reduced, impacting their growth (Gobbi, 2012; Gobbi *et al.*, 2013; Nguyen *et al.*, 2013). A thickness of 15 cm seems optimal for the larvae growth, above this threshold, the risks of an anaerobic degradation are great, potentially wiping out the breeding overnight (Maquart 2019, pers. com.). In order to avoid this, the substrate in our system -consisting of whole decaying fruits- was turned daily to ensure a proper oxygenation of the mix.
The harvesting process was done using a passive sieving system. Briefly, the substrate containing the larvae was placed on top of a sieve (mesh of 0.5cm), and let overnight. The larvae crawling from the surface to the bottom of the tray felt into a bucket placed underneath. To ensure a consistent production of flies (and consequently eggs) 2.4 kg of larvae had to be kept to continue their growth as prepupae, then self-harvest using a tray containing a 30° slope to be later harvested in the trough. The prepupae were then kept

into a box containing saw dust until they hatched as flies. The maggot meal obtained was then used for the experiment highlighted in the Chapter 5.

According Diener *et al.* (2015), with a production around 100 Kg of dry maggot meal per month, our system can be classified as a small scale operation plant. Such system, while producing very few maggot meal, and biofertiliser, is far from being economically viable, however, this is just a first step in increasing the production, and mastering the technology involved. While large scale, centralised, BSF factories (treating up to 200 tonnes of waste per day, according to Diener *et al.*, 2015) can produce a steady quality and production of maggot meal and disserve feed to international feed companies, they require large investments, and have a lower flexibility in adaptation if a waste source (or market) changes. A small to medium operation process can, on the contrary, deal with local waste management issues, be more adaptable, provide local employment, and can deserve local feed and fertiliser markets. However, it has a lower degree of efficiency, cannot guarantee the consistence of its products (for quality and quantity), and cannot –on a regular basis- test for the presence of contaminants due to the high cost of these tests. Both type of systems are interesting: while on (the large scale operation) can provide large quantities of feed for an international market, the second one is a more *local* scale, potentially addressing issues on waste management strategies, and local valorisation and circulation of both waste and by-products.

# CHAPTER 4: OPTIMISING THE NUTRITIONAL VALUE OF THE BLACK SOLDIER FLY MEAL: WHEN IS THE BEST TIME TO HARVEST THE LARVAE?

# 4.1 Introduction

The need for alternative protein sources is rising since fish meal production, perceived as the most limited and environmentally damaging (Naylor *et al.,* 2009; Ziegler *et al.,* 2016) protein source, has been in decline since its peak of production in the early 1990s (Shepherd & Jackson, 2013) and relies on low stocks of wild fish species that have since become fully exploited. Soybean became rapidly a protein-source alternative to fishmeal use in animal diet. It is now the most important protein-rich ingredient for terrestrial animal feeds (Van Krimpen *et al.,* 2013). However, its production is criticised for its environmental impact: it requires high water consumption (Steinfeld *et al.* 2006), the use of pesticides and fertiliser (Carvalho, 1999) and cause significant environmental deterioration with deforestation in areas with great biodiversity importance (Carvalho, 1999; Osava, 1999; FAO 2006; Foley *et al.*, 2011; Robinson *et al.,* 2011; Munkung *et al.*, 2013; Tritsch & Arvor, 2016; Da Costa *et al.,* 2017). Beside this negative environmental impact, conventional proteins resources are becoming less favourable from an economic point of view. Feed costs represent 60 to 70% of total production costs (Van Huis *et al.,* 2013). Therefore the need for alternative protein sources for livestock is becoming increasingly urgent.

Insect meal has been identified as one such ingredient (Rumpold & Schlüter 2013; Van der Spiegel *et al.,* 2013; Van Huis, 2013, Makkar *et al.,* 2014; Barroso *et al.,* 2015). Rearing insects on un-used organic waste and to use them as feed can also improve the environmental footprint of vertebrate production (Makkar *et al.,* 2014; Tomberlin *et al.,* 2015; Singh-Ackbarali & Maharaj, 2017). Due to their high protein rates, Barroso *et al.,* (2014) estimated that the Diptera order is the most susceptible insect group in terms of nutritive value, to substitute fish meal in the animal diet. The Black Soldier Fly –BSF- (*Hermetia illucens*) appears to be one of the best candidate, for farmed insect as feed (Barroso *et al.,* 2015; Oonincx *et al.,* 2015). The fly is considered as a non-pest species (Sheppard *et al.,* 1994; Diener *et al.,* 2009), does not feed at the adult stage, relying on the energy stored during the larval stage, and cannot transmit nor carry diseases. Its larvae are involved with up-cycling organic matter (Lardé, 1990) and can feed on a wide range of substrates from manures to food waste ( Diener, *et al.,* 2011, Lalander *et al.,* 2014; Oonicx, 2015; Barragan-Fonseca *et al.,* 2017). They conduct to an excellent bioconversion rate of organic matter (Oonincx *et al.,* 2015) with usually a reduction of

the waste load by 70% (Diener *et al.*, 2011) and therefore, can be used as a waste remediation system (Newton *et al.*, 1997). In addition, while occupying a substrate, the larvae aerate and dry it, reducing odors (Coulibaly *et al.*, 2004). Maggots modify the microflora of the substrate, and reduce drastically the levels of pathogenic bacteria (Erickson, *et al.*, 2004; Liu *et al.* 2008; Lalander *et al.*, 2014). Their presence also inhibit the oviposition of houseflies (Furman *et al.*, 1959; Sheppard, 1983; Bradley & Sheppard, 1984). This particularity is especially interesting in the lights of public health, environmental, biosecurity and logistical challenges of waste management in both developed and developing world.

The protein and fat composition of the BSF larvae are highly influenced by the substrate they are fed on (Sheppard *et al.*, 1994; Newton *et al.*¸2005; St-Hilaire *et al.*, 2007; Diener *et al.*, 2009; Spranghers *et al.*, 2017; Wang & Shelomi, 2017). However, the effects are not always linear: in Oonincx *et al.* (2015), the larvae were bred on different substrates where the proteins and fat contents were known. While, the larvae bred on a protein-rich substrate were more proteinaceous than the others, the percentage of fat in the substrate was not correlated with the larval fat percentage. In Spranghers *et al.* (2017) a similar experiment was performed, but this time, there was no significant correlation between protein of the substrates and those of the prepupae. However a high correlation was observed between the lipid content of the prepupae and the non-fiber carbohydrate content of the substrate.

The quality and origin of the strain can influence the phenotypic plasticity (development and waste conversion) of the larvae (Zhu *et al.*, 2013), and therefore modify its nutrient composition. As highlighted in Wang & Shelomi (2017) natural variation among individuals and batches can vary significantly: commercially available black soldier fly larvae from a same company based in Germany, and fed with the same substrate, showed values ranging from 31.7% to 47.6% crude protein and 11.8–34.3% fat in different studies (Kroeckel *et al.*, 2012; De Marco *et al.*, 2015)

Significant variations, especially in proteins and lipids levels occur during the different developmental stages of the larvae (Liu *et al.*, 2017). The BSF larval development goes through 5 instars called “white larvae”, where the larvae is extremely photophobic (Everest Canary, 2009) and prefers to stay within the substrate. Its harvest is therefore rather difficult. The $6^{th}$ instar is often referred as “prepupae”, corresponding to the pupariation stage, when the larvae cease feeding to complete immobilisation and reduction in length. The reduction of mobility and retraction of the segment is a gradual

process. The cuticle becoming progressively more pigmented (Barros-Cordeiro *et al.*, 2014). On its early stage the prepupae enters into a migratory phase, looking for a shady and dry place to pupate. This change of behaviour makes them easy to harvest; a 30° slope system on the edges of the production bays is enough to 'self-harvest' the larvae, if their substrate is moist enough. Unfortunately, when the larvae turn to prepupae, their cuticle is thicker. Cuticle weight can reach about 24% of the whole dry mass basis (Sheppard *et al.* 2008). The cuticle is mainly composed of chitin, an n-acetylated polysaccharide that forms various complexes with proteins and other carbohydrates. Its presence can affect the digestibility of other nutrients (i.e. as acting as an anti-nutritional factor, "ANF"), including proteins (Longvah *et al.,* 2011) or lipids (Kroeckel *et al.,* 2012), leading to a reduction in growth if fed to livestock.

The evolution of the nutritional composition of the Black Soldier Fly through its transformation from white larvae and prepupae, is still poorly understood, Hence determination of the optimal harvest stage at which nutritional quality and yields are maximised is a critical to successful commercialisation. This study aim to asses changes in the proximate nutritional profile, and yield of BSFL within later development stages. The potential for use of morphological indicators as determinants of development stage was also assessed.

## 4.2 Material and Methods

### 4.2.1 Source of the fly and breeding

The strain of flies was sourced from Hermetia Futtermittel GbR, Baruth/Mark (Germany), and breed locally at the University of Stirling in a controlled environment cabinet (CEF: Microclimat 1750E and -15°c Model Option. Manufactured by Snijders Scientific B.V. located in Tilburg, The Netherlands) at 30°C and 65% relative humidity.

The larvae were bred into a plastic box, of 30x30x30 cm containing 2 Kg of substrate constituted of 60% banana, 25% de-stoned mangoes and 15% de-stoned spoiled-avocado. The nutritional composition of this feed is listed in table 4.1. All the fruits were previously blended and thoroughly mixed prior to inoculation of the larvae. The homogenised substrate ensured a uniform nutritional profile was available to all larvae. The breeding

boxes were incubated in the same cabinet, with the same constant temperature and relative humidity. All containers were randomly placed inside the cabinet.The experiment was done in quadriplicates.

**Table 4.2**: Nutritional composition of the ingredients constituting the substrate, and the substrate itself (values expressed in % on a dry matter basis, presented 'as is').

| Ingredient | Moisture | Crude Protein | Lipid | Ash |
|---|---|---|---|---|
| Avocado | 67.4 | 8.1 | 62.2 | 6.0 |
| Banana | 86.6 | 5.3 | 1.6 | 4.9 |
| Mango | 86.2 | 4.7 | 0.6 | 2.9 |
| Substrate mix | 83.6 | 5.5 | 10.4 | 4.5 |

### 4.2.2 Sampling procedure

**Egg sampling:** BSF eggs were collected daily at the same time (to ensure age consistency) from colony cages using blocks of corrugated cardboard (4 cm X 9 cm) placed on top of a plastic containers containing moist breeding substrate mixed with 50% insect frass. Corrugated cardboards were dissected to remove all the egg mass out. Once the egg clusters were harvested, 0.02 g ±0.0004 g of eggs where placed into a moist petri dish. 2 day-old egg clusters were observed under a microscop to look at the coloration of the ocelli (see Figure 4.1) as described in May (1961) to assess their viability. When a minimum 90% of eggs were "eyed" the batch was considered viable, and used for the experiment or otherwise discarded.

**Feeding stage dynamic sampling:** the substrate was seeded with eyed-eggs, a day prior to their hatching. Mean larval growth rate was assessed by daily collection and bulk weighing of 100 larvae for each of four replicates. This assessment started at 150 degree-days ("DD" i.e 5 day old larvae cultured at 30°C) to limit the risk of injury by handling too small larvae, up to 720 DD. Starting from 300 DD, 10 g of larvae were collected every two days washed from fruit residue, surface dried with a paper towel and frost-killed for proximate analysis of four pooled replicates. Another batch of 10 larvae were kept in absolute ethanol for morphological analysis. This process was repeated every two days from 300 DD to 720 DD, when all the larvae had turned into pupae.

**Figure 4.2**: Details of the eggs. Left: day-old eggs, right: "eyed-eggs" after 2 days at 30°C.

**Morphological assessment:** The larvae were analysed under a microscope (Olympus SZ-PT X10), and photographed using an Olympus SC 100 GMBH camera. Drawings were done using Photoshop CS6.

### 4.2.3 Proximate analysis

**Dry matter (DM):** the larvae were dried overnight, until constant weight in a freeze drier to prevent any adulteration of the protein or lipid content. The dry matter was calculated by weighing the samples before and after.

**Ash** content was assessed by weighing 1g of dried sample into a crucible and placed into a muffle furnace at 600°C for 12h. The ash content was calculated by weighing the end-sample.

**Crude protein content (CP)** was assessed using the Kjedahl method. Briefly, proteins in the sample were digested under catalytic heating, and released ammonia when reacted with sulfuric acid. The result was ammonium sulfate. Then an alkaline distillation was applied to free ammonia which was subsequently absorbed by boric acid and further titrated with hypochloric acid titrant. CP content was calculated according to the acid consumption multiplied by the conversion factor (*i.e.* X 6.25). However, since the exoskeleton of insect contains chitin, a nitrogen-based polysaccharide, this could lead to an overestimation of the true protein content.

**Chitin content** was measured according to the procedure described in Liu *et al.* (2012). Briefly, the samples were freeze dried, and then grinded. 5 g were submersed into a 1 M HCl solution (250 mL) at 100 °C for 30 min to remove minerals and catechols. Then the sample was rinsed with distilled water until neutrality was reached. Deproteinization was performed with an alkaline treatment using 1 M of NaOH (250 mL) solution at 80 °C for 24 h. The obtained product was washed with distilled water until the pH became neutral. The precipitate was further treated with 1% potassium permanganate solution (100 mL) for 1 h. Finally, the chitin was washed with distilled water and oven-dried at 50 °C. After having estimated the chitin content in the sample, a correction factor was applied to estimate the true protein content.

**The crude fat content** was estimated using the non-destructive Folch method (Folch *et al.,* 1957). Briefly, total lipids were extracted from 0.5 g of sample, by homogenising in 20 volumes of ice-cold chloroform/methanol (2:1 v/v) using an ultra-turrax tissue disruptor (Fisher Scientific, Loughborough, U.K.) and determined gravimetrically after an overnight dessication under vaccum.

### 4.2.4 Statistical analysis

Statistical analysis was performed by SPSS V21(SPSS Inc., Chicago, IL, USA). Growth, DM, CP, protein corrected, fat content, ash and chitin levels at the different stages of the larval cycle were analysed by one-way analysis of variance (ANOVA) followed by a Tuckey's HSD for post-hoc testing to compare the significance between the means of different life cycle stages. $p<0.05$ was considered to be a significant difference between the values compared. All results, are expressed as mean±SE.

## 4.3 Results

This study presented the change in morphological and nutritional composition of the Black Soldier fly larvae during its transition between the white larvae stage ($5^{th}$ instar) to the prepupal stage ($6^{th}$ instar). The time frame selected was from 300 degree days (DD) to 720 DD, corresponding to day 10 up to day 24 at 30°C. At 300 DD the larvae are

starting their 5th instar, while around 360 DD they enter into the 6th instar, and starting to pupate around 720 DD. The process involves both deep morphological and nutritional changes. This variations are presented in the table 4.2.

**Morphology:**

Beside the change of coloration which appear progressively, all the other features appears –or disappears- after the 5th molt.

On the ventral side of the white larvae, on every segment beside the last abdominal one (*i.e.* anal segment), a row of crawling setae is observed (Fig. 4.3A & 4.4). Typically, there is one row per segment, located distally, and bearing between 16 to 18 setae. Each setae is about 0.1 mm long, and the row extends for 3.3 mm. This feature disappears on the 6th instar larvae. On the prepupae only, ventrally, on the 6th abdominal segment, a distinct scar can be observed, measuring about a millimeter long for 0.15 mm width (Fig 4.3B & 4.4).

Along with these changes, the head is subjected to deep morphological changes: For the white larvae (5th instar) the labrum, lingual and labium are all membranous and finely pubescent, the maxillae is also pubescent and bear a strongly chitinsed two-hooked process. The palps are tubular, the mandibles are fused to the genae, and bear finely pubescent lips extending into a curved finger-like process (Fig. 4.3C). After the 5th molt, for the prepupal stage, the cuticle become progressively darker and the pubescence is longer and coarser. At this stage, the head is strongly chitinised and the ocelli become more prominent. The mouthpart are reduced and completely fused. A hook appears frontally, and can be clearly felt when rubbing a fingertip to it (Fig. 4.3D).

**Growth:**

The larvae grew rapidly between 300 DD to 720 DD (Figure 4.2), however, when the larvae reached the 5th instar, the growth was steady, with no significant variation (Anova, Post hoc Turkey, $p>0.05$). This plateauing continued during the prepupal stage.

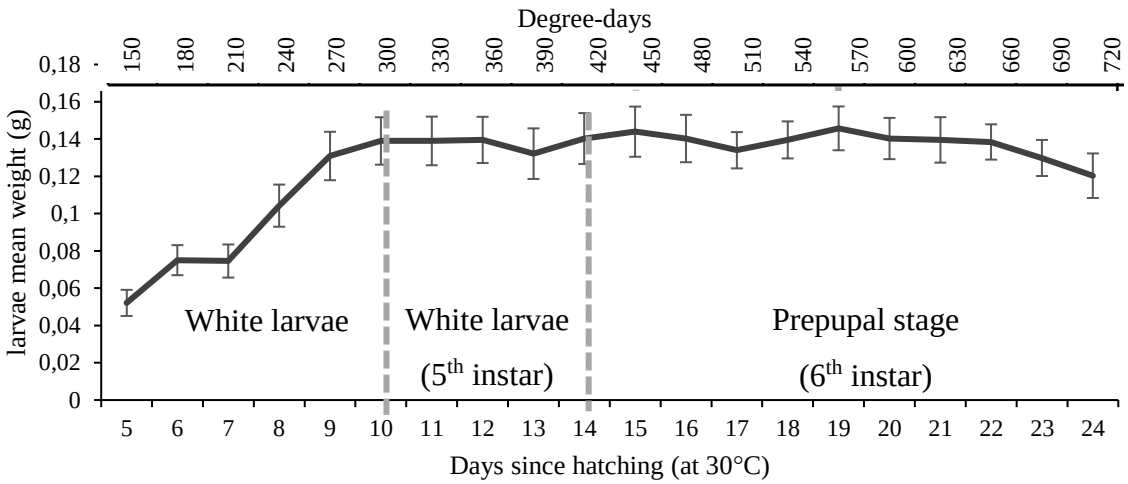



**Figure 4.3**: Growth rate of the larvae, from day 5 to day 24 –post hatching (30°C). SE indicated by error bars.

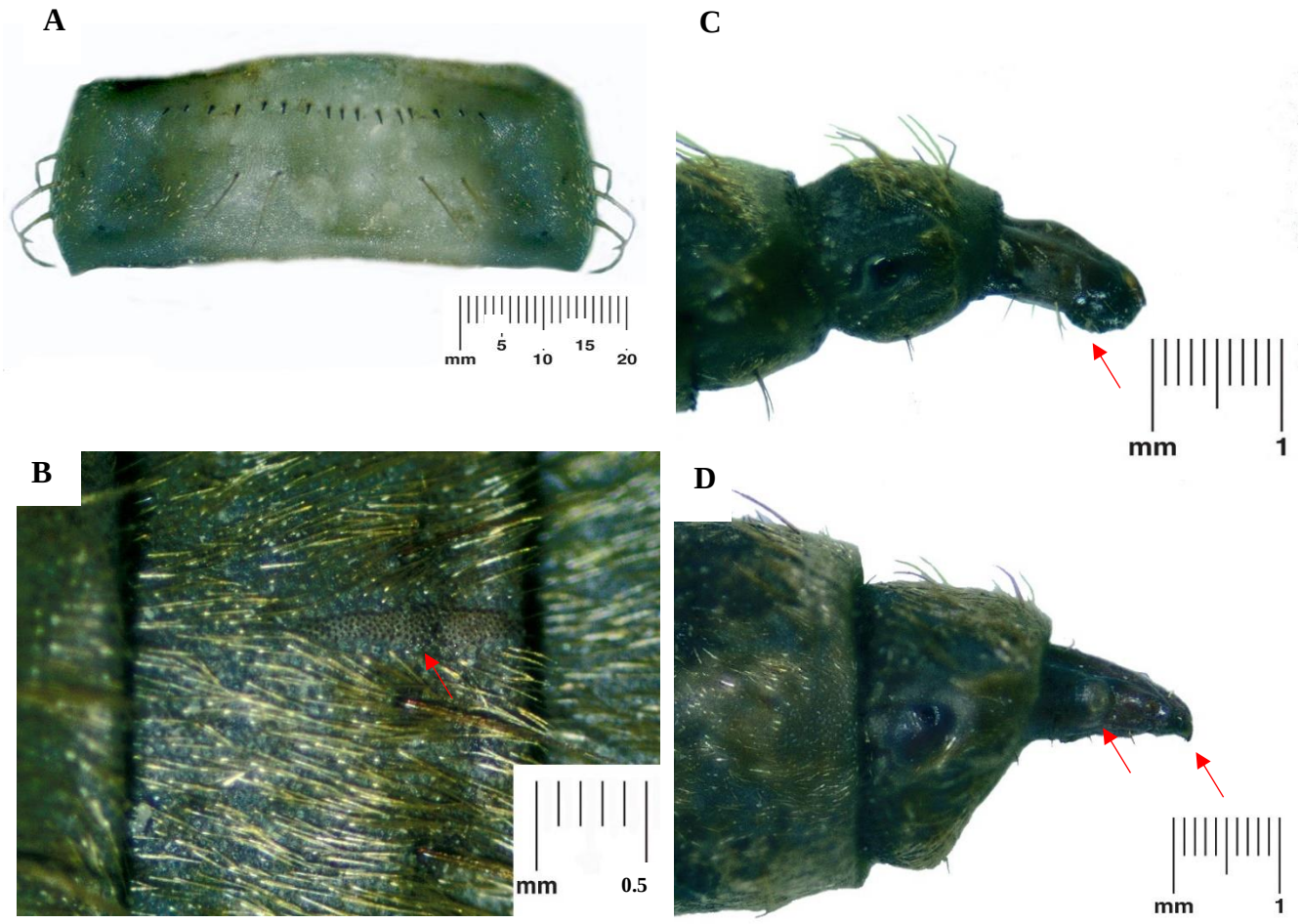



**Figure 4.3**: Morphological features of Black Soldier Fly larvae. A: details of the crawling setae on the ventral side of the white larvae; B: details of the scar in the ventral side of the prepupae, located on the 6th abdominal segment; C: Side view of the white larvae head; D: Side view of the prepupae head, where the hook-like structure is clearly visible, and the eyes become prominent.

**Proximate composition:**

The dry matter, along with the ash content remains stable through the development, with no significative differences ($p>0.05$). The CP content, along with the corrected protein is stable with no significant differences, beside for the 720 DD old prepupae (Anova, post hoc Turkey, $p<0.05$). The crude fat content increased toward the end of the white larvae stage (300-360DD i.e. 10-12 days of culture and 5$^{th}$ instar) (Anova, Post hoc Tukey, $p<0.05$) and then remains stable throughout the prepual stage with no significant statistical differences observed. However chitin is not-detectable in the 5$^{th}$ instar, being synthetized only during the prepupal stage, increasing rapidly from 420-480 DD the proportion is not significantly different ($p>0.05$), but increased significantly for the late prepupae (600-720 DD) (Anova, Post hoc Turkey, $p<0.05$). All these results are summarised in Table 4.2.

**Table 4.3**: Nutritional, morphological and behavioral changes of the BSF larvae

| **Time (days) post hatching** | **10** | **12** | **14** | **16** | **18** | **20** | **24** |
|---|---|---|---|---|---|---|---|
| **Degree days (at 30°C)** | **300** | **360** | **420** | **480** | **540** | **600** | **720** |
| **Designation** | **White larvae (5th instar)** | | **Prepupae (6th instar)** | | | | |
| **Characteristic** | The larvae are fattening, have a whitish-ivory coloration. | | The larvae stop feeding, its cuticle get thicker and darker, and enter into a wandering phase, commonly designated as "self-harvesting". On its late days, it will enter into a quiescence phase. | | | | |
| **Behaviour** | Mobile, very photophobic, hard to harvest. | | Extremely mobile, even under daylight, Wandering phase for the first few days, then quiescence around day 24 | | | | |
| **Appearance** |  | |  | | | | |
| **Chitin level (g/Kg)** | Nd[1] | Nd[1] | 78.25±3.92 **a** | 82.43±1.69 **a** | 87.41±5.45 **a** | 134.20±4.14 **b** | 139.09±4.27 **b** |
| **Proximate analysis (%)** | | | | | | | |
| Dry matter | 38.78±1.40 | 39.21±1.71 | 38.22±1.37 | 39.51±0.76 | 40.13±0.88 | 38.76±1.21 | 39.70±1.21 |
| Crude protein | 26.21±3.52 | 21.38±3.84 | 26.91±1.58 | 27.10±1.54 | 30.79±2.14 | 33.93±1.80 | 38.75±1.96 **a** |
| Corrected protein | Nd[1] | Nd[1] | 23.98±1.46 | 24.01±1.47 | 27.51±2.22 | 28.90±1.89 | 33.54±1.94 **a** |
| Crude lipid | 15.26±0.76 **a** | 17.74±0.33 **b** | 19.11±0.14 **c** | 19.62±0.57 **c** | 18.39±1.95 **c** | 17.96±1.21 **c** | 18.60±0.89 **c** |
| Ash | 4.26±0.48 | 4.32±0.35 | 4.68±0.32 | 4.92±0.27 | 4.94±0.07 | 4.67±0.27 | 4.95±0.37 |

Values are based on duplicated analysis on each of the four replicates. They are presented as the mean±SE, a,b,c, referring to significant differences (Anova, Post hoc Tukey, $p<0.05$). Nd[1]: the chitin values were undetectable.

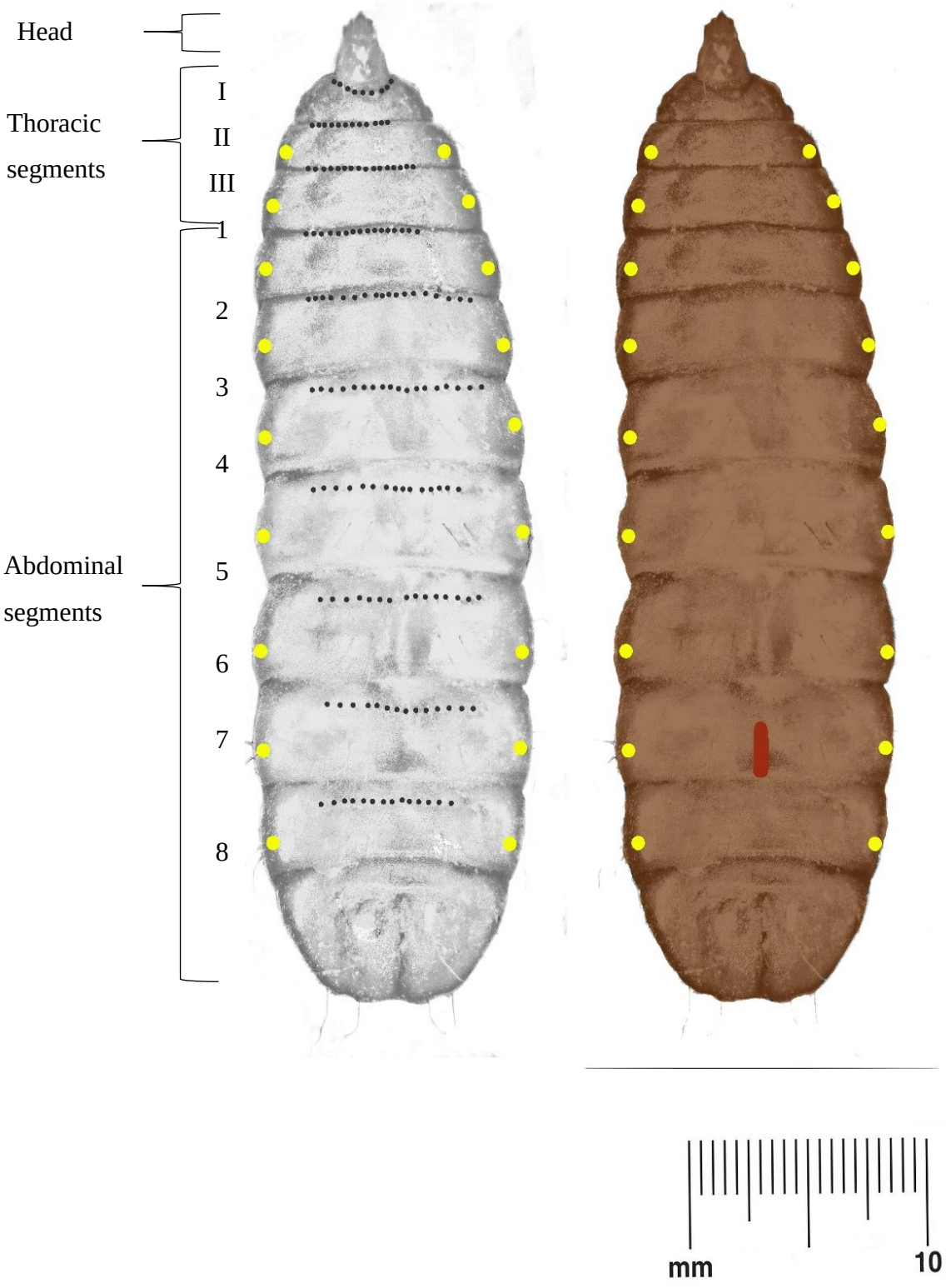



**Figure 4.3**: Morphological differences on the ventral side between the white larvae (left) and the prepuae (right). Yellow dots indicate the stigmates, black dots represents the crawling setae, red notch indicate the scar present in the prepupae.

## 4.4 Discussion

The concept of prepupae, applied to the Black Soldier Fly 6th instars, was borrowed from Coleoptera biology (Costa & Vanin, 1984). Heslop-Harrison (1958) defined it as the "*very distinctive pharate phase that exists between the outset of quiescence in the last larval stage and the moult which reveals the external form of the pupae*". For *Hermetia illucens*, after the 5th molt, at the prepupal stage, the cuticle become progressively darker and thicker and the pubescence is longer and coarser. This is the best external indicator that the pupation process is about to start. In Stratiomyidae and Xylomyidae, the pupae is formed within the last larval skin, which is used as a hard cocoon (called "puparium"), highly chitineous, and impregnated with plates of calcium carbonate crystals (Woodley, 1989).

At the prepupal stage, the head is strongly chitinised and the ocelli become more prominent. The mouthpart are reduced and fused preventing the larvae to feed (Schreemer, 1982). Therefore the growth stops, and the larvae weight starts plateauing. The weight, however, start to decrease when the larvae is entering into the pupal stage: the reserves are therefore used partially for the metamorphosis. After the pupation process, the adult only weight half of the pupae weight (Liu *et al.*, 2017). A distinct hook appears frontally before the head, and can be clearly felt when rubbing the fingertip on it. This hook-like structure is used by the prepupae to extract itself from the substrate and have a grip on a coarse surface.

The ventral setae, or "crawling setae", described in May (1961) as "small, black, flattened, tooth-like processes" are located in the ventral side of the larvae. Usually there is one row per segment, located distally, and bearing between 16 to 18 setae. This row extends up to 3.3 mm horizontally. Each setae is about 0.1 mm long. The crawling setae are lacking on the anal segment of the white larvae. They seems to have an ectodermic origin as they can be observed on the exuvias of the larvae. They are used by the white larvae to increase their adherence to the substrate and enable them to move quickly inside it. They disappear when the larvae turned into the prepupal stage.

In the prepupae, a distinct scar, of about a millimeter long, located distally on the 6th abdominal segment (Fig. 4.3 & 4.4) appears. It is present in all larvae and does not hold a sexual purpose, like the Herold's organ in Coleopteras or Lepidoptera (Herold, 1815; Hinks & Byers, 1973). The fact that it is located at the tip of where the pupae will be

located inside the puparium suggest that it is used by the larvae as a gripping point inside the puparium to "fix" the pupae, and after the imaginal molt, to help the fly to extract from the puparium. At the end of the prepupal stage, the larvae will start to enter into quiescence, but prior the pupation process, the prepupae will arrange itself vertically –if possible- in the substrate with the head protruding above the surface (May, 1961) to facilitate the extraction of the adult.

From a nutritional point of view, the dry matter remains stable ranging from 38.76% to 40.13%, with no significant differences between the stages. The dry matter content of fresh larvae is usually comprised between 20-41% (Diener *et al.*, 2009; Finke, 2013; Nguyen *et al.*, 2015; Oonicx *et al.*, 2015b; Sheppard *et al.*, 2008; Barragan-Fonseca *et al.*, 2017: Spranghers *et al.*, 2017). It can be influenced by the diet and larval stages, being usually higher for the later stages (L5-L6) (Rachmawati *et al.*, 2010). The ash content remains stable between white larvae and prepupal stages with no significant differences. This results is in accordance with Liu *et al.* (2017) observation where the ash content of their larvae was stable from the 4$^{th}$ day-old larvae to late-prepupal stage. However their values were higher, ranging from 7.8% to 10.3%, while ours are within 4.26 - 4.95%, this difference can be explained by a difference of substrate used: in their case, they used commercial broiler chicken feed which ash content was about 7.9%, while in our experiment the ash content of the substrate was only 4.5% (table 4.1). The fat content is significantly higher for prepupae (19.11% for 420 DD prepupae), compared to white larvae, and remain stable during this stage. These results are coherent with Liu *et al.*¸(2017) where about 22.6% where detected for 12 day-old white larvae, and increased to 28% for early prepupae. However, in their experiment, the lipid content was significantly reduced to 24.2% for the late prepupae, which was not the case in our experiment. The CP content, remained stable from the 5$^{th}$ instar larvae (26.21-21.38% for 300-360 DD respectively) but increased during the prepupal stage, peaking significantly at 38.75% for late prepupae (720 DD old). These results are coherent with the study of Liu *et al.* (2017). However, in their case, they expressed the protein content solely as "Crude Protein" only. This is frequent in most publications. The method recognised by the AOAC (1990) to test for CP content is the Kjedahl method. The samples are digested into boiling sulphuric acid (at about 400°C) for an hour, then the nitrogen content is determined by titration. The protein content is then estimated by multiplying this result by 6.25. According to Finke (2007) this factor is acceptable for estimating the true protein content of most insect species. However, since the exoskeleton

of insects contains chitin, a nitrogen-containing polysaccharide, this may lead to an over-estimation of the crude protein content (Diener *et al.*, 2009).

Using the corrected protein level, the levels were lowered by 10.89-13.45% for 420-720 DD old larvae respectively. The corrected values for prepupae were therefore ranging from 23.98-33.54%. In Spranghers *et al.,* (2017) their values were higher, ranging from 37.7-40.7%. This difference can be explained by the poor protein content of our feeding substrate (5.5% CP), the substrate composition being very important for the larval nutrient composition Sheppard *et al.,* 1994; St-Hilaire *et al.,* 2007; Diener *et al.,* 2009; Newton *et al.*¸2005; Spranghers *et al.,* 2017; Wang & Shelomi, 2017).

Beside misrepresenting the actual protein content, the presence of chitin is largely considered to be anti-nutritional factor (Sanchez-Muros et al 2015). Chitin is an n-acetylated polysaccharide and a major component of the insect cuticle, which is always covalently bound to catechol compounds and sclerotin-like proteins (Majtan *et al.*, 2007). Chitin is accounting for 5-8% of the total nitrogen in insects. (Kumar, 2000). The chitin level varies between species (Vetter, 2007 on fungus; Barroso, 2014 on insects) and within stages. While it is undetectable for the white larvae stage, as soon as the larvae enters into the prepupal stage its proportion increases drastically from 7.82% at 420 DD, to 13.42% at 600DD, and then plateaued around 13.91% at 720 DD, just before the pupation starts. The increase of chitin in the larval skin, coupled with deposits of calcium carbonate plates or crystal (Woodley, 1989), will increase the rigidity and resistance of the skin, which will form the puparium, inside which, the larvae will starts the pupation process. A similar observation was made on *Musca domestica* by Dwivedi & Agrawal (1995), where within 2 days, the chitin content rose from 6.7 mg to 10 mg in the larvae. The chitin contents of the prepupae in this study are higher than the contents reported in the literature for Black Soldier Fly, ranging from 6.1% (Spranghers *et al.,* 2017) 7.5 % (Finke, 2007) up to 8.7% DM (Diener *et al.,* 2009). This could be explained by the age of the prepupae, which at its early stage (in the "wandering stage", around 420-480 DD) have a lower chitin level (7.82%), which might have been used in these studies, while the late prepupae (600-720 DD) have a higher content. With a caloric content of 17.1 $KJ.g^{-1}$, chitin could be a source of carbohydrates, and it could also constitute a substancial percentage of the total energy intake. However, the β1,4 bond in chitin is indigestible for several fish species (Rust, 2002 *in* Sanchez-Murros *et al.,* 2015) and, in some studies, its presence might affect the growth performance by influencing the feed intake, availability and digestibility of nutrients, including proteins (Longvah *et al.,* 2011) or lipids (Kroeckel

*et al.*, 2012), leading to a reduction in growth. The hydrolysis of chitin requires the involvement of chitinase and chiobiase enzymes. High digestive chitinase levels were observed in cobia (*Rachycentron canadum* L.) (Fines & Holt, 2010) and in a broad range of marine teleost fish, (Fange *et al.*, 1976; Danulat & Kausch, 1984). On the other hand, chinolytic activity has also been described in gut bacteria, which may play a role in its hydrolysis and the apparent absorption of carbohydrates derived from chitin (Sugita *et al.*, 1999).

Therefore, while the late prepupae (around 720 DD) are significantly more proteinaceous (33.54%), than the white larvae (21.38%), the presence of chitin can potentially decrease the digestibility of the MM produced by the fish, and this has to be taken in consideration during the selection of the lifecycle to harvest.

The removal of chitin during meal manufacturing should be investigated. Chitosan can be extracted (Guangdong Entomological Institute, in a patent developed in 2015) or alternatively chitin could be degraded by enzymatic methods before being added to diets as product of hydrolysis (*i.e.* Chito-oligosaccharides, acetylglucosamine or chitosan) or via an alkaline extraction (DeFoliart *et al.*, 1982; Belluco *et al.*, 2013; Shiau & Yu, 1999; Se-Kwon & Niranjan, 2005; Lin *et al.*, 2012a,b). The removal can also be done using mechanical methods, such as removing the skin of the larvae by pressing them. The addition of hydrolytic enzymes to diets is a global practice in animal feed production. The addition of chitinase and its effects on digestibility has not been studied, but could increase chitin digestibility. However, if properly removed and purified, chitin could potentially become a high value by-product. Chitin and chitosan can be used as antioxidant, anti-inflammatory, drug delivery-media and plastic (Park & Kim, 2010). Several recent studies, highlight the fact that despite being an antinutritional factor, chitin may have a positive effect on the functions of the immune system. The chitin is cited as having antimicrobial properties (Rinaudo, 2006), and can act as an immunostimulator effective on a short term basis (Mastan, 2015). A study done on chickens, showed that poultry fed with a commercial broiler diet containing 20% dried whey and 0.5% to 2% chitin had significantly improved weight compared to controls, switching the feed efficiency ratio from 2.5 to 2.38. This improvement was attributed to a change in the intestinal microflora brought by the chitinous supplement (Ravi Kumar, 2000). Another study by Vahedi & Ghodratizadeh (2011), showed that rainbow trout immune activity was enhanced by the presence of chitin (10, 25 and 50 mg/kg) in their diet. A similar improvement of the immune system was described by Esteban *et al.* (2000) on gilthead

seabream. As in-feed antibiotics are banned in the EU since January 2006 (regulation EC/1831/2003) there is an increasing need for reliable in-feed antibiotics alternatives and immunostimulant (Spranghers *et al.*, 2017). For this purpose, the use of insect-meal, and their inherent chitin, may diminish the use of antibiotics in the animal-production industry (Van Huis *et al.*, 2013).

As the removal of the chitin is a rather complex thing, and as high chitin values can affect the digestibility of the meal, we suggest to target the harvesting of the white larvae stage. There is no differences in biomass between the 2 stages, as the growth as stopped, suggesting that the quantity of insect meal produced will be the same, and therefore will not affect the economics of a large scale production. However the fact the larvae are highly photophobic at this stage renders the separation process (larval frass from undigested material) complex, as no triggers for self-harvesting have been identified yet. This is one of the most cited issued for upscaling (Pastor *et al.*, 2015; Rumpold & Schlüter, 2013), yet to overcome. Several companies have specialised in BSF production as animal feed, but their methods are patented and proprietary, and thus unavailable to academics. Therefore, the quantity of BSFM producted is still low, thus a strategic use of this protein source should be done to maximise its potential.

# CHAPTER 5: SUBSTITUTION OF NURSERY FEED BY LOCALLY-PRODUCED MAGGOT MEAL ON SEX-REVERSAL DIETS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

# 5.1 Introduction

With a production estimated at 28,000 tonnes in 2012 (Asiedu *et al.,* 2015) Tilapia is the most farmed fish in Ghana. Most of its production (85%) is realised from cage production (Asiedu *et al.,* 2015). All-male tilapia culture is often preferred in grow out systems as its leading to more uniform marketable fish produced at a faster rate due to significant differences in growth between males and females (Mair & Little, 1991; Little & Hulata, 2000; Phelps & Popman, 2000). Also, by growing all male fry, energy that would be required for reproductive activities such as nest building, guarding and release of sperm and ova is saved. Some earlier methods suggested manual sexing at 6 weeks, and discarding the females after being manually sexed, but this method despite being labour-intensive, means that half of the fish are lost (Bhujel, 2014). Hormonal sex-reversal is therefore preferred. Oral administration of 17α-methyltestosterone (MT) via the feed is the most frequent method, as it is the most effective and economically feasible method (Phelps, 2006; El-Greisy & El-Gamal, 2012; Mensah *et al.*, 2013).

However to obtain a good sex-reversal rate, the feed –acting as a way to carry the hormone- has to be very palatable. To achieve this, usually high levels of fish meal are used. However, fish meal quality in Ghana can be fluctuating, as it is becoming a scarce resource, its rising value stimulates a potential adulteration. Moreover the price of the starter feed is an issue leading farmers to use lower quality feed. While a lower sex-reversal rate can be acceptable for the production in cages, it can impact severely the overall growth of the batch when the production is in ponds (Pullin & Lowe-McConnell, 1982; Vera Cruz & Mair, 1994; Little, 1989). New feed sources acting as a suitable carrier for the hormone have therefore to be found.

Insect meal is considered to be such ingredient (Rumpold & Schlüter 2013; Van der Spiegel *et al.,* 2013; Van Huis, 2013; Barroso *et al.* 2015, Makkar *et al.,* 2014). Food containing insect meal have been studied in African catfish (*Clarias gariepinus*; Fasakin *et al.,* 2003), turbot (*Psetta maxima*; Kroeckel *et al.* 2012), Nile tilapia (*Oreochromis niloticus*; Ogunji *et al.,* 2007, 2008) and rainbow trout (*Oncorhynchus mykiss*; St-Hilaire *et al*., 2007a, b).

The Black Soldier Fly (*Hermetia illucens*) appears to be one of the best candidate, due to its high conversion rate (Oonincx *et al*., 2015; Barroso *et al.*, 2015). The fly does not feed at the adult stage, and cannot transmit nor carry diseases, it has an excellent bioconversion

of the substrate (Oonincx *et al.,* 2015) and can be used as a waste remediation system (Newton *et al.,* 1997). In addition its fatty acid profile can be modulated with the substrate the larvae were fed on, as shown by Saint-Hilaire *et al.* (2007) where larvae fed on fish offals produced a foodstuff high in Omega-3.

With an estimated production of 1.3 billion tons each year, food waste are valued at 680 billions US$ in industrialised countries and 310 billions US$ in developing countries (FAO, 2011; The Economist, 2014). Acting as a waste remediation service, the Black Soldier Fly can contribute to organic waste remediation, poverty alleviation –by creating local employment- and create a local, soon-to-be cheap feedstuff for Aquaculture.

Devic *et al*., (2013) estimated that if the maggot meal substituted 30% of the fish meal used in the feed of a farm producing 6 000 metric tons of tilapia in Ghana, it would require 1.4 MT, 60.8 MT and 175.5 MT of dry maggot meal to produce the broodstock, the juveniles and the grow-out respectively. However, while the technology is still in development, scaling up the production is still rather difficult. The main constraint is addressed on the harvesting of the larvae from the substrate (Pastor *et al.,* 2015; Rumpold & Schlüter, 2013). Therefore there are still limitations for the scaling-up production of this high quality feed.

A strategic use of this feed ingredient in the Ghanaian context could be to substitute the low quality and expensive FM traditionnaly required to such process, by producing locally a quality assured BSFM for juvenile tilapia sex-reversal process.

The aim of the current study is to evaluate the performance of the maggot meal (MM) as a substitute of the classical starter feed and the pure fishmeal used commonly for the sex-reversal of tilapia fry in pond happa-systems in Ghana. Key outcome measures include sex-reversal efficiency, growth and survival rates.

# 5.2 Material and Methods

## 5.2.1 Culture technique

The experiment was performed in a large local commercial tilapia hatchery in Asetsuare (Eastern Region) in Ghana, between the 14/01/2016 to the 20/03/2016. Day–old *O. niloticus* swim-up fry were harvested using a downwelling system.

## 5.2.2 Hormone – feed preparation

Hormone-treated feed was prepared as described by Killian & Kohler (1991). A stock solution of 17α-methyltestosterone (MT) was made by dissolving 60mg of hormone in 1 L of pure ethanol (El-Greisy & El-Gamal, 2012; Popma & Green, 1990; Celik *et al.* 2011). This solution was evenly poured onto 1 kg of the diet and mixed. The mixing process was repeated several time to ensure an even distribution of the MT throughout the feed. Treated diets were fan-dried in shade at 30 ºC for 24 hours then kept in the shade and used within 5 days (Barry *et al.*, 2007).

A commercial nutritionally-complete formulated feed obtained from Ranaan fish feed company© in Tema, Ghana ('Super Start Feed' ('SS0')) is commonly used in Ghana for the sex-reversal process – and was used as a positive control in this trial. The fishmeal was collected in Ranaan fish feed company©. It was composed of processed by-products from the tuna industry (from the *Petit Navire*© industry in Tema, Ghana. Maggot Meal was obtained from 450 degree-days old old-Black Soldier Fly (*Hermetia illucens*) larvae fed on fruit waste (composed of 60% watermelon, 20% papaya, 20% avocado) and produced at the Animal Research Institute in Frafraha road, in Ghana. The larvae were harvested and placed into a bowl with saw dust to make them empty their gut overnight, and then dried into an electrical oven at 55°C during 2 days. The dried larvae were then grinded using a grinder (Binatone Blender BLG-699) until it was flour-size particles. All feed was sieved using a fine mesh to homogenize the size of the feed by discarding large particles. The different treatments were prepared as following Table 5.1:

**Table 5.2**: Composition of the different treatments (in % as fed basis)

| Ingredients | Treatment 1 (positive control) | Treatment 2 | Treatment 3 | Treatment 4 | Treatment 5 (Negative control) |
|---|---|---|---|---|---|
| Fish meal | 0 | 0 | 0 | 100 | 100 |
| Commercial feed | 100 | 50 | 0 | 0 | 0 |
| Maggot meal | 0 | 50 | 100 | 0 | 0 |
| Methyltestosterone | 60mg/kg | 60mg/kg | 60mg/kg | 60mg/kg | 0 |

## 5.2.3 Experimental setup

The pond volume was 4968 $m^3$ and was filled with the Volta river water 3 weeks prior to the experiment. The experiment started once the pond experienced an algae bloom and turned green. Treatments (Table 5.1) were randomly assigned to hapas equally distributed across the pond. All fry used during the experiment were pooled into the same container, and then, 4500 fry / hapas were randomly assigned to each of four replicates per treatment. Each hapas size was sieved manually and adjusted to allow the correct density of the fish among them. The size was 100x100x40 cm resulting in a stocking density of 11.25 fish/litre.

Fry were fed 5 times a day (at 8:00, 10:00, 12:00, 14:00 and 16:00h). The amount of feed given to the fry daily was calculated over the total biomass of the pond. The amount of feed given daily is resumed in Table 5.2. A sampling was done every 5 days to re-adjust the quantity of feed given accordingly.

**Table 5.2**: Quantity of feed given to the fry, according to their total biomass

| Days | % of total biomass |
|---|---|
| 0-5 | 30 |
| 6-10 | 30 |
| 11-15 | 20 |
| 16-25 | 20 |

Every five days, 50 fishes were collected within each hapas to record the average weight. Morts were collected and recorded at every feeding session. At the end of the 26 days period, 50 fishes were sacrificed using clove oil (Ross & Ross, 2008) to be individually weighted and measured. After the first 25 days, fish were placed into larger hapas (3x5m) and were all fed with standard commercial diet ("SS1" and then "SS2") regardless of their treatment for a further 6 weeks.

### 5.2.4 Sexing of the fish

After 6 weeks post sex-reversal process, 50 fishes were randomly selected in each hapa, euthanized using clove oil (Ross & Ross, 2008) and dissected under binocular following the procedure described in both Guerrero & Shelton (1974) and Wassermann & Afonso (2002). The sex was assessed using Guerrero & Shelton (1974) and Guerrero (1975) papers.

### 5.2.5 Water parameters

The Ph, Oxygen dissolved in water and temperature were recorded inside the hapas 5 times a day prior to each feeding session. Hardness, alkalinity, Ammonium, Nitrates and nitrites were recorded every 5 days using a spectrophotometer. The water parameters were recorded to ensure that the quality conditions would not adversely influence the trial results.

### 5.2.6 Proximate analysis

All feedstuff were analysed using the standard methods of AOAC (1990) for moisture, protein, fat and ash. Moisture content was estimated by heating samples in an oven at 105°C overnight, and weighing the samples before and after. Nitrogen content was measured using a micro-kjeldahl apparatus and crude protein was estimated by multiplying nitrogen content by 6.25. Total lipids content was determined by ether extraction for 16 h. and ash was determined by combusting samples in a muff furnace at 550 ºC for 6h. The chitin level was assessed using the methodology presented in (Black & Schwartz, 1950). The fatty acid profile was done following Folch *et al.* (1957).

**Amino-Acid analysis:**

The amino acid composition of feedstuffs and diets were determined by ALS Food and Pharmaceutical (Cambridgeshire, UK) and Eurofin Food and Feed testings (Moss, Norway) using High-Performance Liquid Chromatography (HPLC) methods according to their respective commercial procedures.

### 5.2.7 Statistical Analysis

All statistical calculations were carried out using IBM SPSS Statistics (Version 21). A significance level of 5% was chosen for all analyses. Normal distribution of the data sets was verified using Shapiro-Wilk test and homogeneity of the variance was tested with Levene's test. Significant differences between treatments ($p<0.05$) were assessed using one-way analysis of variance (ANOVA) parametric test or Kruskal-Wallis non-parametric test when preliminary assumptions were violated. In the case of significant differences, Tukey's HSD post-hoc test was then applied to rank the groups.

## 5.3 Results

### 5.3.1 Water parameters

Water temperature and dissolved oxygen varied slightly during the course of the experiment and the diurnal periods with an average of 29.4±1.62°C and 8.46±3.91 mg/L respectively. Water PH (8.61±0.70) remained stable during the 25 day experimental period. The levels of nitrites, ammonia and alkalinity levels remains stable during the course of the experiment with about 0.6±01 mg/L, 0.9±0.5mg/L and 66.1±7.2mg/L respectively. Nevertheless all values were within the tolerance limits for Nile tilapia (Beveridge & McAndrew, 2000; El-Sayed, 2006).

### 5.3.2 Feed composition

The protein content was significantly higher in the commercial formulated diet than fishmeal and maggot meal (64.31±0.27) (Anova, Post hoc Tukey, $p<0.01$).The lowest protein rate was recorded in the maggot meal which contained 43.19±0.28% crude proteins. The crude lipid level was also significantly higher (17.31±0.36%) in the formulated diet (Anova, Post hoc Tukey, $p<0.01$). The maggot meal also contained chitin (5.16±0.45%). The gross energy was significantly higher (20.97±0.01) for the commercial diet (Anova, Post hoc Tukey, $p<0.01$). The maggot meal contained

significantly more saturated fatty acid (5.81± 0.04%) than the other feedstuff, but also contained significantly lower polyunsaturated fatty acids (1.36±0.05%) (Anova, Post hoc Tukey, $p<0.01$). All the results are summarized in the table 5.3.

**Table 5.3**: Nutritional profile of the different types of feed used during the sex-reversal experiment.

| | **Commercial diet (SS0)** | **Fish meal (FM)** | **Maggot meal (MM)** |
|---|---|---|---|
| **Proximate composition (%)** | | | |
| Dry matter | 93.04± 0.55 | 92.85± 0.06 | 93.45± 0.14 |
| Crude protein | 64.31± 0.27 **a** | 53.11± 0.12 **b** | 43.19± 0.28 **c** |
| Crude lipid | 17.31±0.36 **a** | 10.67±0.15 **c** | 15.45±0.39 **b** |
| Ash | 17.34± 0.51 | 10.95 ± 0.05 | 15.95± 0.38 |
| Chitin | ND[1] | ND[1] | 5.16± 0.45 |
| Gross Energy (MJ/kg) | 20.97± 0.01 **a** | 18.9± 0.02 | 18.98± 0.14 |
| **Essential Amino Acid composition (g/100g xN)** | | | |
| Valine | 2.76± 0.02 | 2.94± 0.21 | 2.69± 0.06 |
| Iso-Leucine | 2.19± 0.01 | 2.48± 0.18 | 1.85± 0.04 |
| Leucine | 4.16± 0.01 | 4.36± 0.31 | 2.99± 0.07 |
| Phenylalanine | 2.28± 0.01 | 2.46± 0.17 | 1.88± 0.03 |
| Histidine | 1.02± 0.00 | 1.96± 0.12 | 1.33± 0.04 |
| Lysine | 3.16± 0.02 | 4.29± 0.29 | 2.75± 0.04 |
| Arginine | 3.00± 0.03 | 3.36± 0.21 | 2.06± 0.05 |
| Methionine | 1.24± 0.02 | 1.59± 0.07 | 0.78± 0.03 |
| Threonine | 2.21± 0.00 | 2.58± 0.17 | 1.75± 0.04 |

Values are based on duplicate analysis of four samples of each type of feed. Values are presented as the mean±standard error, a, b, c, referring to significant differences ($p<0.01$). [1]Abbreviation: ND: Non detectable.

| | Commercial diet (SS0) | Fish meal (FM) | Maggot meal (MM) |
|---|---|---|---|
| **Fatty Acid Profile (g/100g of % total fatty acid)** | | | |
| 12 :0 | N.D. | N.D. | 2.00± 0.02 |
| 14 :0 | 0.37± 0.08 | 0.35± 0.04 | 0.57± 0.04 |
| 16 :0 | 1.81± 0.05 | 2.39± 0.03 | 2.35± 0.03 |
| 18 :0 | 0.42± 0.01 | 0.90± 0.01 | 0.32± 0.04 |
| 20 :0 | 0.02± 0.00 | 0.02± 0.01 | 0.01± 0.01 |
| **Total saturated[1] :** | **2.68± 0.08 c** | **3.84± 0.04 b** | **5.81± 0.04 a** |
| 16 :1n-7 | 0.53± 0.01 | 0.48± 0.05 | 0.47± 0.02 |
| 18 :1n-9 | 2.50± 0.08 | 1.55± 0.19 | 1.66± 0.03 |
| 18 :1n-7 | 0.30± 0.01 | 0.30± 0.07 | 0.46± 0.02 |
| 20 :1n-9 | 0.42± 0.01 | 0.10± 0.06 | 0.00 |
| 22 :1n-11 | 0.50± 0.02 | 0.03± 0.01 | 0.01± 0.02 |
| **Total monounsaturated[2] :** | **4.37± 0.14** | **2.60± 0.04** | **2.82± 0.01** |
| 18 :2n-6 | 1.27± 0.04 | 0.15± 0.03 | 1.18± 0.03 |
| 20 :2n-6 | 0.03± 0.01 | 0.03± 0.01 | N.D. |
| 20 :4n-6 | 0.05± 0.02 | 0.22± 0.04 | N.D. |
| 22 :5n-6 | 0.01± 0.00 | 0.13± 0.03 | N.D. |
| ***Total n-6 PUFA[3]*:** | ***1.41± 0.05*** | ***0.59± 0.05*** | ***1.19± 0.03*** |
| 18 :3n-3 | 0.13± 0.10 | 0.05± 0.02 | 0.09± 0.01 |
| 18 :4n-3 | 0.17± 0.00 | 0.05± 0.01 | 0.06 |
| 20 :4n-3 | 0.04± 0.00 | 0.04± 0.01 | N.D. |
| 20 :5n-3 | 0.54± 0.01 | 0.52± 0.01 | 0.01 |
| 22 :5n-3 | 0.04± 0.03 | 0.09± 0.06 | N.D. |
| 22 :6n-3 | 0.44± 0.38 | 2.02± 0.05 | N.D. |
| ***Total n-3 PUFA[4]*** | ***1.39± 0.27*** | ***2.82± 0.25*** | ***0.16± 0.04*** |
| **Total PUFA[5]** | **2.93± 0.23 b** | **3.54± 0.06 a** | **1.36± 0.05 c** |

Values are based on duplicate analysis of four samples of each type of feed. Values are presented as the mean±standard error, a, b, c, referring to significant differences ($p<0.01$).[1]includes 15:0; 17:0 ; 20:00; 22:0 and 24:0; [2] Includes 14:1; 16 :1n-9 ; 17 :1 ; 20 :1n-11 ; 20 :1n-7; 22 :1n-9cis; 24 :1-9; [3] Includes: 18:3n-6; 20:3n-6; 22:4n-6; [4] Includes 20:3n-3; [5] Includes 16:2; 16:3; 16:4. N.D.: not detected.

### 5.3.3 Sex-reversal rate

The proportion of males after the experiment was not significantly affected ($p>0.05$) by the diet containing the 17α-methyltestosterone. The only significative difference (Anova, Post hoc Turkey, $p<0.01$) was observed for the negative control, containing no hormone, where a lower proportion of males was detected (64%) (Table 5.4).

Survival rates ranged between 98.98±5.29 and 84.28±5.29% and were acceptable (table 5.4). There was no significant difference amongst the hormone-treated treatments ($p>0.05$), while the only significant difference was observed for the feed without hormone, where the survival was significantly better (Anova, Post hoc Tukey, $p<0.01$).

**Table 5.3**: Percentage of phenotypic males after 6 weeks post-experiment and survival amongst the different treatments during the sex-reversal period. Superscript *** referring to a significant difference with the control ($p<0.01$).

| Treatments | Males (%) | Survival (%) |
|---|---|---|
| 100 CM + MT | 99±1.15 | 84.28±5.29 |
| 50/50% CM & MM+ MT | 98±1.63 | 89.17±3.21 |
| 100% MM+ MT | 98±1.91 | 86.28±2.08 |
| 100% FM+ MT | 95±3.41 | 87±2.44 |
| 100% FM | 64±6.92 *** | 98.98±5.29 *** |

Abbreviations: CM: Commercial feed, MM: Maggot meal, FM: Fish meal, MT: Methyltestosterone (60mg/kg).

### 5.3.4 Growth rate

The growth rate is significantly greater when the commercial feed is used (Anova, Tuckey post-hoc test $p<0.05$). The other treatments achieving broadly similar growth rates with no significant differences (Figure 5.1)

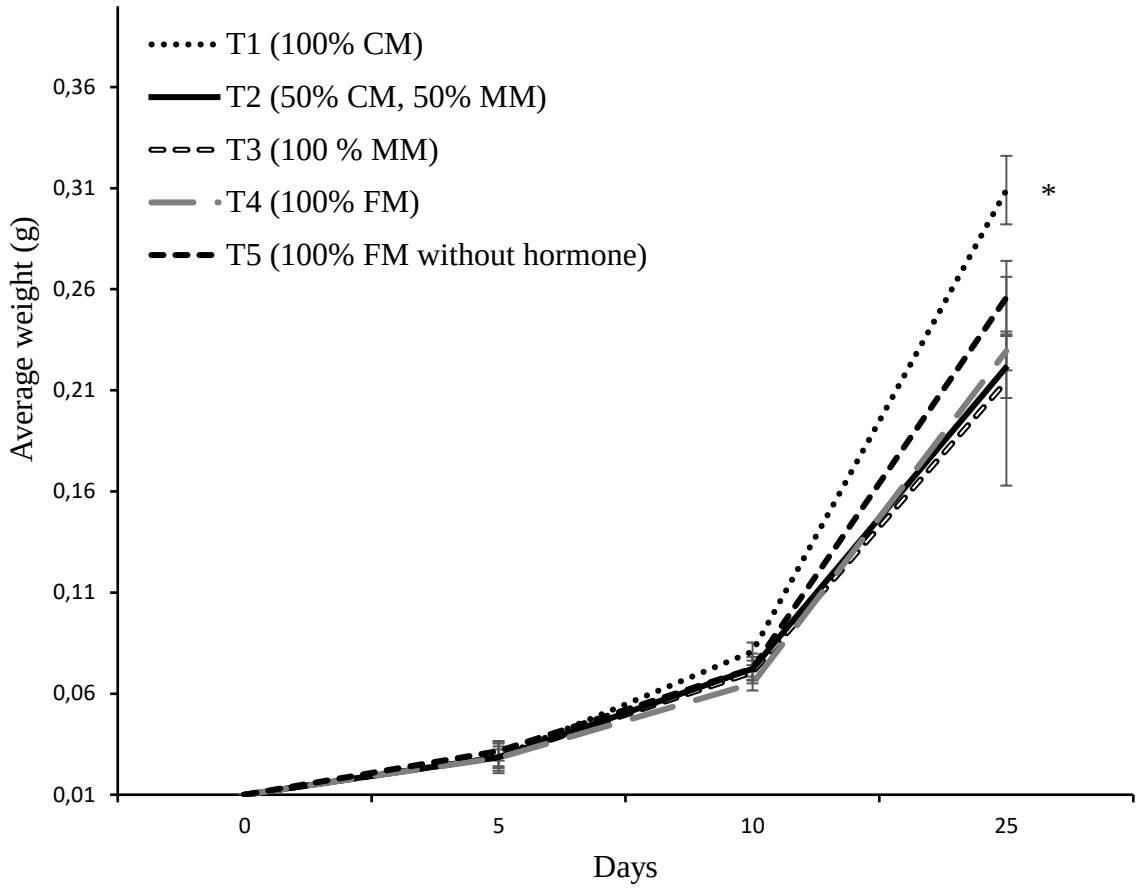



**Figure 5.3**: Growth rate of the fish during the sex-reversal period. Superscript * referring to a significant difference ($p<0.05$). Abbreviations: CM: Commercial feed, MM: Maggot meal, FM: Fish meal, MT: Methyltestosterone (60mg/kg).

## 5.4 Discussion

Successful sex-reversion of tilapia fry relies on a combination of good farming and feeding practices. Quality and palatability of the hormone-treated feed are the main drivers to ensure a maximized daily intake of hormone, although pure FM is preferred to produce monosex-tilapia (Bhujel, 1997), commercial feed (using high levels of FM) formulated for monosex-tilapia are also widely used (Phelps, 2006). However, as the fishmeal is becoming a scarce resource, its value is rising stimulating a potential adulteration. This highly unreliable quality, and price increase stresses the importance to identifying potential cost-effective alternatives.

The maggot meal presented a good floatability, ensuring a good feed intake by the fry on the surface. The low level of lipids in the maggot meal (15.45%) did not induce clots in

the feed which could have alterated the feed intake. It is also interesting to notice that the commercial-formulated diet was within this range (17.31%). The maggot meal meets all requirements in essential amino acid for Nile tilapia fry (Ogunji *et al.* 2005; Diogenes *et al.* 2016). The fatty acid profile is strongly linked with the substrate the larvae were fed on (Oonicx *et al.,* 2015), and could be enriched in Omega-3, and other polyunsaturated fatty acids if the insects were fed using marine products (Saint-Hilaire *et al.* 2007). This particularity could be useful if the feedwaste from the fish-feed factories could be used as a substrate, enhancing the final insect product.

Although few females have been identified, the sex-reversal process was very efficient across treatments with high proportion of males recorded (98-99%). This result suggests that the fry received a sufficient dose of MT during the 25 days process and that the quality nor the type of feed compromised the feeding. Sex-reversal is considered effective in cage-production systems when at least 96% males are produced (Mair & Little, 1991). According to Vera Cruz & Mair (1994) to avoid significant impacts on grow out fish crops in ponds, sex-reversed population counting more than 98% are recommended. Indeed, even a small proportion of females can lead to a substantial recruitment and to heterogenous growth resulting in non-uniform sized fish. The purchase of fry is a major cost for farmers and investment in monosex seed, which is more expensive than mixed-sex fry, contributes to better performance during grow out, thus the importance of a high percentage of males (Little, 1989).

However it is interesting to notice that the percentage of males in the negative control treatment without hormone, was higher (64%) than the 50% expected in a normal population. Despite a continuous flow and a complete change of the water every week, some hormone residue circulated inside the pond affecting the control. This marginal phenomenon was also observed by Abucay & Mair (1997). This potential impact must be taken into account when designing sex reversal experiments to avoid any hormone-contamination of controls and incorrect interpretation of results.

Survival is also a key parameter to consider, firstly for obvious economic reasons, but also because during the sex-reversal process, a high density of fish is necessary for efficient reversal. It is a factor that directly influences the success of the process. The high density allows a crowding effect which ensure a very active feeding response (Phelps & Popma, 2002), it also reduces hierarchical interaction among fish thereby resulting in a more uniform population, and therefore a more uniform hormone intake by the fish (Little, 1991 *in* Vera Cruz & Mair (1994). In this study, the survival rate was high, and

not significantly different across the hormone-treated treatments, those rates were higher (89.14% in average) than those observed by Vera Cruz & Mair (1994) when fish were stocked at 6,000 fish/$m^3$ (76.1%). However in the non-treated feed, the survival rate was significantly higher than the others (98.98±5.29%) suggesting that the hormone stresses the fry and increase mortality. This results was also observed by Buddle (1984b).

To conclude, the presence of BSFM did not affect the main parameters of the experiment (being the sex reversal rate, and the survival) and can be considered as a viable protein source for sex-reversal feed. The only significant impact is on the growth of the fish. However this aspect can be considered negligeable since the fish could be later grown back to normal using an appropriately-formulated feed.

Productions of MM being still at their infancy, the low quantities produced can be used in highly specialised diets such as sex-reversal feed. Insect industry is on its way to the industrialisation of the farming process and has restored the hope for a use of this emerging feedstuff for aquaculture as the quality, the quantity and the price would not be a limiting factor anymore. Economies of scale should allow the industrialisation of insect farming and development of markets with more competitive prices. When the price point will be achieved, making the insect meal cheaper, or at least as expensive as the fish meal, hatcheries could therefore buy insect meal from a local producer or even invest in the development of their own insect production system to reduce their cost.

# CHAPTER 6: *IN VIVO* DIGESTIBILITY OF THE WHOLE MEAL AND DEFATTED BLACK SOLDIER FLY MEAL IN NILE TILAPIA FRIES DIET (*OREOCHROMIS NILOTICUS*)

# 6.1 Introduction

A current research priority in animal nutrition is the active search for sustainable feedstuffs to supply protein and energy to reduce the dependence on marine ingredients such as FM (Fish Meal) or FO (Fish Oil) (Glencross *et al.*, 2007). Aquaculture being one of the fastest growing animal food production sector, is presently the main consumer of FM and FO in its feed formulations (Tacon & Metian, 2008; FAO, 2016). However, the global rise of commodity prices and especially those of FM and FO, along with concerns on their sustainability have led to exploration and identification of alternative feedstuffs that could contribute to a decrease in their use, as a proportion of total ingredients (Naylor *et al.*, 2009, Olsen & Hasan, 2012; Oliva-Teles *et al.*, 2015, Tacon *et al.*, 2011).

Plant-based proteins such as soybean offer an alternative, but are also heavily criticised for their environmental impact, usually that associated with habitat loss in South America (Carvalho, 1999; Osava, 1999; FAO, 2006; Robinson *et al.*, 2011; Tritsch & Arvor, 2016; Da Costa *et al.*, 2017), the use of enormous quantities of freshwater (Steinfeld *et al.*, 2006), pesticide and inorganic fertiliser utilisation (Carvalho, 1999) that cause significant environmental degradation overall (Osava, 1999). Additional to these issues, their inclusion in formulated feeds is also somewhat limited by their nutritional characteristics, usually poor digestibility resulting in fish growth performance, health and welfare (Francis *et al.*, 2009; Pratoomyot *et al.*, 2011).

One of the most-often cited issues regarding the development of aquaculture in Thailand is the unstable supply and variation of the quality of raw materials used by the commercial aquaculture feed manufacturing plants (FAO, 2018). While large scale facilities can assess by themselves the quality and vary their suppliers of their raw material, this is less so the case for small, or medium scale producers (FAO, 2018). The quality of FM is particularly of concern in the tilapia hatchery sector, as it is heavily relied upon during the sex-reversal process. Because of its high palatability FM maximises the hormone intake during the sex-reversal process in hatcheries, and helps to achieve a near 100% all male sex-reversal. However, other nutrient-rich and high quality reliable sources of protein need to be investigated to overcome this reliance and potentially replace, or at least reduce the use of FM.

The possibility to produce a FM-free, or at least reduce the FM quantity, in the diets of Nile tilapia based on a multi-ingredient formulation requires the determination of

apparent digestibility coefficients of protein (ADCp), and energy (ADCe) of the different feedstuffs. This enables a more refined diet formulation to be applied specific to each fish species and their various life-stages. There is considerable variation in the ADC profiles of feed ingredients for fish and these values need to be fully verified prior the formulation of a complete diet.

In other words, while a feed ingredient may appear from its chemical composition to be an excellent source of nutrients it could be of little actual nutritional value unless it can be digested and absorbed by the target species. To know the nutrient digestibility of the various feed ingredients used in formulating fish feeds is desirable so that the effective substitution of one ingredient for another can be achieved. Therefore, the nutritive value of mixed-feed relies on the nutrient composition of each individual feedstuff components and the ability of the animal to digest and absorb the nutrients from these (Moreau, 1996, Watanabe *et al*., 1996, Degani *et al.*, 1997, Falaye & Jauncey, 1999, Riche *et al.*, 2001). Knowing the digestibility (*in vitro* or *in vivo*) is a necessary step to predict accurately the protein quality of ingredients for animal diets (Barragan-Fonseca *et al.*, 2017).

Knowing the nutrient digestibility of feed ingredients is also important for the potential interchangeability of ingredients in case of stock shortage or sudden price increase, without reducing animal performance, allowing flexibility by the farmer to adapt to changes in the feed market situation. In combination, chemical analysis and apparent digestibility coefficient (ADC) determination allow the formulator to precisely estimate not only the contribution of a particular protein source to a complete fish feed, but also how much feed wastes and undigested nutrient (faeces) will potentially accumulate and pollute the fish ponds, therefore, maximising the feed efficiency (Furuya *et al.*, 2001; Köprücü & Özdemir 2005).

Recent studies have highlighted the potential of insects for fish and livestock feed in the global assessment of potential feedstuffs (Makkar *et al.*, 2014; Sánchez-Muros *et al.*, 2014). In addition to their fatty acid composition, usually low in omega-3 FA, the BSF meal has a strong potential for fish due to a macronutrient nutritional profile similar to FM (Barroso *et al.*, 2014). The black soldier fly larvae can contain between 38% to 55% protein (Mutefela, 2015; Wang & Shelomi, 2017) and between 12% to 42% lipid (Kroeckel *et al.*, 2012; Mutafela, 2015).

Although insect meals are not yet produced in sufficient volumes to be used in commercial fish feed production, they do show great promise as sustainable ingredients for future aquafeed production (Makkar *et al.*, 2014; Tran *et al.*, 2015). We have

previously shown (Chapter 5) that a direct substitution of FM and commercial meal by BSFM was possible, but that this affected some fish performance parameters. Therefore in order to optimise its use, it is important to assess its digestibility coefficients prior formulating a diet for Tilapia fry during their sex-reversal period (which will be the aim in Chapter 7).

## 6.2 Material and Methods

### 6.2.1 Ingredient and diet development

The experimental design was based on a diet formulation strategy allowing the use of the diet-substitution digestibility method (Aknes *et al.,* 1996). To do so, a basal diet was formulated, based on the manufacturer's nutritional composition of the feedstuffs, and prepared following the requirements for the breeding of tilapia fry (according to Jauncey, 2002) using the Winfeed™ software. In order to limit the inclusion of FM, which was tested for its digestibility during this experiment, its inclusion level was kept low in the basal diet and was replaced by pork meal. The composition and nutritional value of the basal diet is specified in Table 6.1 and 6.2. An inert dietary marker – Yttrium oxide- was included in the feed at a level of 0.1%. A basal mash was prepared and thoroughly mixed, forming the basis for all experimental diets in this study. The ingredients of study for each tested diets were added at a 30% inclusion to a sub-sample of the basal mash (Table 6.3). The test ingredients for apparent digestibility were soybean, fishmeal, Black Soldier Fly Meal Full fat (BSF WM) and Black Soldier Fly Meal Defatted (BSF DF). All type of tested feed consisted of finely-grinded flour, previously sieved through a mesh (Ø<1mm) to remove the larger particles. Due to the size of the fry, each flour had to be dispersed using a spoon on the surface of water gently, to maximise its absorption by the fish.

**Table 6.1:** Formulation of the basal diet.

| | Inclusion rate (%) as-fed basis |
|---|---|
| Pork meal[1] | 55.5 |
| Fish meal[2] | 5.2 |
| Rice bran[3] | 12.3 |
| Corn[4] | 6.8 |
| Soybean[5] | 19.6 |
| Vitamins premix[6] | 0.5 |
| Yttrium oxyde | 0.1 |

[1] Pork meal: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[2] Fish meal: Tuna factory by-products, T.C. Union Agrotech Co.Ltd. 68 moo 8, Rama 1 road, Bangkrachao, Samutsakorn, Puhket, Thailand

[3] Rice Bran: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[4] Corn: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[5] Soybean: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[6] Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-a-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

**Table 6.2:** Nutritional value of the Basal diet.

| Dry matter content | Ash (%DM) | Protein (%DM) | Lipid (%DM) | Carbohydrates (%DM) |
|---|---|---|---|---|
| 96.26±2.22 | 11.71±0.26 | 40.14±1.3 | 7.90±0.20 | 36.50±1.43 |

**Table 6.3:** Composition of the different tested diets (expressed as % inclusion, as-fed basis).

| | Diet 1 | Diet 2 – Fish meal[1] | Diet 3- Soybean[2] | Diet 4 - BSFMFF[3] | Diet 5 – BSFMDF[4] |
|---|---|---|---|---|---|
| Basal diet (with yttrium oxide) | **100** | **70** | **70** | **70** | **70** |
| Fish meal[1] | 0 | **30** | 0 | 0 | 0 |
| Soybean[2] | 0 | 0 | **30** | 0 | 0 |
| BSF WM[3] | 0 | 0 | 0 | **30** | 0 |
| BSF DF[4] | 0 | 0 | 0 | 0 | **30** |

[1] Fish meal: Tuna factory by-products, T.C. Union Agrotech Co.Ltd. 68 moo 8, Rama 1 road, Bangkrachao, Samutsakorn, Puhket, Thailand

[2] Soybean: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[3] BSF WM: Maggot meal full fat, white larvae stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia

[4] BSF DF: Maggot meal defatted, white larvae stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia

### 6.2.2 Fish handling and faecal collection.

The experiment was conducted at Nam Sai Farms, Prachinburi, Thailand, and approved byt the Ethical Committee (Reference: AWERB/1617/202/New Non ASPA, granted on 12/05/2017). Sex-reversed, hatchery-reared, Nile tilapia (*Oreochromis niloticus*) were transfered from hapas into experimental tanks (50 L). Each of the tanks were stocked with 400 fry of 0.250±0.08g (mean±SD; *n*=50) and kept continuously aerated to maintain a DO above 5mg/L. Freshwater at 27.8°C±2.3 was continuously, except during feeding and 1h prior to faeces collection, supplied at a flow rate of about 4L/min from a recirculation system. The tanks were located in an outdoor facility and exposed to a photoperiod of about 12:12 (light:dark). Treatments were randomly assigned among 20 tanks, with each treatment having 4 replicates. Fish were hand-fed 5 times a day (8, 10, 12, 14, 16h) daily to 20% of their body weight (Jauncey, 2002). The fish were allowed to acclimatise to their allocated dietary treatments for 7 days before faecal collection commenced (Wybourne & Carter, 1999). The fish were fed normally during the day, and the tanks were thoroughly cleaned one hour after the last feeding session. The faeces were collected overnight using the settlement methods based on those reported by Cho & Kaushik (1990). After collection, the faecal sample was quickly sun-dried and placed in a small 50 ml Falcon tube™ and stored at -20°C. The faeces from each tank were collected and pooled over a 10 day period.

### 6.2.3 Chemical and digestibility analysis

All chemical analyses were done at the University of Stirling. Diet and faecal samples were analysed for dry matter, yttrium, ash, phosphorous, nitrogen and gross energy content. Dry matter was calculated by gravimetric analysis following oven drying at 110°C for 24h. Total yttrium and phosphorus concentrations were determined after mixed acid digestion using inductively coupled plasma atomic emission spectrophotometry (ICP-AES) based on the method described by Hillebrand *et al.* (1953). Protein levels were calculated from the determination of total nitrogen by LECO auto-analyser, based on N x 6.25. Crude fat content of the diet was determined gravimetrically following extraction of the lipids according to the Soxhlet method. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace

at 550°C for 12h. Gross energy was determined by adiabatic bomb calorimetry. Differences in the ratios of the parameters of dry matter, protein, gross energy to yttrium in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficient ($ADC_{diet}$) for each of the nutritional parameters examined in each diet based on the following formula (Maynard & Loosli, 1979)

$$ADCdiet = 1 - (\frac{Y\ diet\ \times Parameter\ Faeces}{Y\ faeces\ \times Parameter\ diet})$$

Where $Y_{diet}$ and $Y_{faeces}$ represent the yttrium content of the diet and faeces, respectively, and $Parameters_{diet}$ and $Parameters_{faeces}$ represent the nutritional parameters of concern (dry matter, protein or energy) content of the diet and faeces, respectively. The digestibility value for each of the test ingredients in the tested diets examined in this study were calculated according to the formula:

$$\text{Nutr. ADingredient} = \frac{(ADtest\ \times Nutrtest\ - (ADbasal\ \times Nutrbasal\ \times 0.7))}{(O.3\ \times Nutringredient)}$$

Where $Nutr.AD_{ingredient}$ is the digestibility of a given nutrient from the test ingredient included in the test diet at 30%. $AD_{test}$ is the apparent digestibility of the test diet. $AD_{basal}$ is the apparent digestibility of the basal diet, which makes up 70% of the test diet. $Nutr_{ingredient}$, $Nutr_{test}$ and $Nutr_{basal}$ are the level of the nutrient of interest in the ingredient, test diet and basal diet respectively (Sugiura *et al.,* 1998).

Digestibility values greater than 100% were not corrected because we consider that this data could potentially indicate a synergistic effect between the diet and test ingredient and should be stipulated as determined. However, for reasons of practicality, the total level of digestible nutrients/energy were only calculated assuming a maximum digestibility of 100% or a minimum of 0%.

### 6.2.4 Water parameters

The pH, dissolved oxygen and temperature in the water were recorded inside the tanks 5 times a day prior to feeding. As the water was kept within a recirculation system, the parameters were tested in one tank randomly selected. Hardness, alkalinity, ammonium, nitrates and nitrites were recorded every 5 days using a spectrophotometer. The water parameters were recorded to ensure that the quality conditions would not adversely influence the trial results.

### 6.2.5 Statistical analysis

All values are means unless stated otherwise. Data were analysed for homogeneity using Cochran's test. Effect of ingredient on digestibility of organic matter, nitrogen, phosphorous and gross energy in each of the diets were examined by 2-way ANOVA (or 1-way ANOVA when necessary). Limits for all statistical ranges were set at $P<0.05$.

## 6.3 Results

### 6.3.1 Water parameters

Water temperature and dissolved oxygen varied slightly during the course of the experiment and with diurnal periods with an average of 29.9±1.27°C and 8.46±3.91 mg/L respectively. Water pH (7.42±0.34) remained stable during the 10 day experimental period. The levels of $NH_4/NH_3$, $NO_2$ and alkalinity levels remained stable during the course of the experiment with values of 0.5±0.3 mg/L, 0.5±0.2mg/L and 100.05±25.41mg/L respectively. All values were within the tolerance limits for Nile tilapia (Beveridge & McAndrew, 2000; Xu *et al.*, 2005; El-Sayed, 2006).

### 6.3.2 Feed composition

The dry matter content was fairly constant amongst the different feedstuffs, however, the rice bran and the Black Soldier fly meal full fat had a significantly lower dry matter content (88.95 ±1.54% and 91.73 ±1.80% respectively) than the other ingredients (Anova, LSD test, $p<0.05$) (Table 6.4).

The fish meal and Black Soldier fly defatted meal were the most proteinaceous feedstuffs (with protein levels of 60.27±0.15% and 57.09±0.44% respectively) (Anova, LSD test, $p<0.01$) followed by pork meal (48.04±0.14%), soybean (49.27±0.37%) and Black Soldier fly whole meal (47.77±0.23%). The least proteinaceous feedstuffs were rice bran and corn (14.92±0.14% and 8.42±1.12% respectively) (Table 6.4). The lipid content of

the soybean meal and corn meal (2.82±0.42% and 4.22±0.15% respectively) were the lowest, and significantly lower than the other ingredients, including, pork meal and fish meal (8.46±0.65% and 8.49±0.23% respectively). Each of these had significantly lower levels of lipid than rice bran (15.8±0.08%) and the Black Soldier fly full fat meal (21.76±0.18%) (Table 6.4). The ash content of each sample was significantly different from each other, except the 2 types of BSF meals, which displayed similar ash levels (9.83±0.7% and 10.16±0.59% for the BSF WM and BSF DF respectively). The lowest ash content being for corn (1.70±0.03%) and the highest for fish meal (24.61±0.33%) (Table 6.4). Except for the pork meal and rice bran, which had similar levels of phosphorus (1005.74±59.6 mg/kg and 928.40±63.47 mg/kg respectively), the rest were significantly different among each other. There were no significant differences among the energy content of each of the samples.

### 6.3.3 Diet and ingredient digestibility

The apparent digestibility of the diets were used as the basis for the calculation of the ingredient digestibilities. However, when the yttrium levels were analysed, it appeared that they varied from the ratio initially expected (each diet was formulated to have 0.7% of yttrium oxide, originating from the basal diet). Instead, slightly variable results were obtained among each of the test diets, allowing us to use two different calculation techniques: the first one, assuming that the ratio 70%: 30% (basal diet: ingredient tested) should be used, while the second one took in consideration this variability and used the actual yttrium values to calculate the ingredient digestibility values, leading to more conservative results.

**Table 6.4:** Nutritional composition of the experimental ingredients

| | Dry matter content (%) | Crude protein (%DW) | Crude Lipid (%DW) | Ash (% DW) | Phosphorous (mg/kg) | Energy content (MJ/ kg DM) |
|---|---|---|---|---|---|---|
| Pork meal[1] | 97.12±1.29 [a] | 48.04±0.14 [a] | 8.46±0.65 [a] | 14.26±0.13 [a] | 1005.74±59.6 [a] | 19.3±2.21 |
| Fish meal[2] | 98.88±1.34 [a] | 60.27±0.15 [b] | 8.49±0.23 [a] | 24.61±0.33 [b] | 2074.72±164.9 [b] | 18.5±1.45 |
| Rice bran[3] | 88.95±1.54 [b] | 14.92±0.14 [c] | 15.8±0.085 [b] | 8.91±0.13 [c] | 928.40±63.47 [a] | 18.2±2.13 |
| Corn[4] | 97.49±0.43 [a] | 8.42±1.12 [c] | 4.22±0.15 [c] | 1.70±0.03 [d] | 107.06±31.82 [c] | 18±0.89 |
| Soybean[5] | 98.05±0.20 [a] | 49.27±0.37 [a] | 2.82±0.42 [c] | 7.45±0.04 [e] | 314.23±2.14 [d] | 19.4±1.43 |
| BSF WM[6] | 91.73±1.80 [b] | 47.77±0.23 [a] | 21.76±0.18 [d] | 9.83±0.70 [f] | 525.04±13.39 [e] | 22.2±1.9 |
| BSF DF[7] | 97.63±1.71 [a] | 57.09±0.44 [b] | 12.21±0.10 [b] | 10.16±0.59 [f] | 621.70±47.26 [f] | 21.5±1.45 |

[1] Pork meal: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[2] Fish meal: Tuna factory by-products, T.C. Union Agrotech Co.Ltd. 68 moo 8, Rama 1 road, Bangkrachao, Samutsakorn, Puhket, Thailand.

[3] Rice Bran: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[4] Corn: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[5] Soybean: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.

[6] BSF WM: Maggot meal full fat, white larve stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia

[7] BSF DF: Maggot meal defatted, white larve stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia

Average of triplicates ± SE; similar superscript letters in the column do not differ (P <0.05).

**Table 6.5:** Apparent digestibility (ADCs) of the ingredients based on the initial premise that the yttrium oxide was present at 0.7% in each of the test diets.

| | **Fish** | **Soy** | **BSF WM** | **BSF DF** |
|---|---|---|---|---|
| Basal diet ratio | 70% | 70% | 70% | 70% |
| Ingredient ratio | 30% | 30% | 30% | 30% |
| **Ingredient digestibility coefficients** | | | | |
| Dry matter | 0.60±0.04 [a] | 1.23±0.03 [b] | 1.18±0.01 [b] | 1.07±0.04 [b] |
| Lipids | 1.05±0.04 | 1.76±0.21 | 1.25±0.33 | 1.12±0.18 |
| Protein | 0.72±0.01 [a] | 1.06±0.12 [b] | 0.95±0.02 [b] | 0.95±0.04 [b] |

Average of triplicates ± SE; similar superscript letters in the column do not differ (P <0.05).

When considering that the yttrium oxide proportion was 0.7% of the diet, the apparent digestibility coefficient of the ingredients can be calculated as displayed in Table 6.5: the dry matter digestibility was excellent for the Soybean meal, Black Soldier fly whole meal and BSF defatted meal (values >1), with the sole exception of fishmeal displaying an

ADC of 0.60±0.04. The ash ADC values are not considered, as were the carbohydrate ADC values (calculated using the ash values) as they were clearly erroneous, displaying either negative or values substantially largely above 1.00. The ADC of lipids was also excellent, being highly digestible (> 1.00) from each ingredient. The protein digestibilities were significantly lower for the fishmeal (72±1%), while those of the soybean and both BSF meals displayed excellent values, near to 100% digestibility).

**Table 6.6:** Apparent digestibility (ADCs) of the ingredients based on the measured yttrium values from the diets.

| | **Fish** | **Soy** | **BSF WM** | **BSF DF** |
|---|---|---|---|---|
| Basal diet ratio* | 69% | 57% | 57% | 55% |
| Ingredient ratio * | 31% | 43% | 43% | 45% |
| **Ingredient digestibility coefficient** | | | | |
| Dry matter | 0.59±0.05 [a] | 0.98±0.02 [b] | 0.95±0.01 [b] | 0.85±0.03 [b] |
| Lipids | 1.04±0.08 | 1.88±0.43 | 0.95±0.03 | 0.91±0.08 |
| Protein | 0.71±0.03 [a] | 0.91±0.04 [b] | 0.84±0.04 [b] | 0.80±0.02 [b] |

Average of triplicates ± SE; similar superscript letters in the column do not differ (P <0.05).
** Values corrected using the actual yttrium concentrations in the different diets.*

When assessing the digestibility coefficients of the ingredients using the actual yttrium proportion in the samples, the ADCs were substantially lower. The results are presented in Table 6.6: the dry matter digestibilities were excellent for Soybean meal, Black Soldier fly whole meal and BSF Defatted meal (values close to 1.00), with the sole exception of fishmeal displaying an ADC of 0.60±0.04. The ash values are not considered, like the carbohydrates values (calculated using the ash values) as they were considered erroneous, displaying either negative or values considerably above 1.00. The ADC of lipids was very high (1.04, 1.88, 0.95, 0.91 for fishmeal, soybean, BSF whole meal and BSF defatted meal respectively). The protein digestibility was significantly lower for the fishmeal (0.71±0.03) while the soybean and BSF meals had excellent values (0.91, 0.84 and 0.80 respectively).

## 6.4 Discussion

The nutritional composition of the Black Soldier fly full fat meal (BSF WM), grown on kitchen waste, displayed a protein level lower than that of the FM (47.77% compared to

60.27% for FM). The defatting of the BSF WM, allowed a concentration of the protein (increased from 47.77% to 57.09%) concomitant with a decrease of the lipids (from 21.76% to 12.21%) producing a meal more similar to the FM profile (containing 60.27% protein and 8.49% lipid). Regarding the ash content, the FM displayed higher rates (24.61%) than the BSF meals (9.83% for the whole meal and 10.16% for the defatted meal).

It was interesting to note that both protein and lipid levels of the FM were lower than expected, according to the manufacturer's label. The FM was expected to be 68.7% protein and 10.5% lipid (Nam Sai Farms, personal communication, 2017). The ash level however, was higher (24.61%) than expected (20.3%). Upon investigation, using a microscope, the presence of vegetable material, probably soybean or fine saw dust, was detected, which was attributed to decreasing the protein level, and also decreasing the digestibility of the feedstuff. This adulteration illustrates, and stresses, the importance of the need to look for alternative protein feedstuffs.

The basis of diet formulation depends on having accurate and reliable data on the digestible nutrient of raw materials used to make those diets (reviewed by Glencross *et al.*, 2007). The determination of the digestible nutrient and energy value of feedstuffs depends on having a viable method to measure the digestibility of these parameters from the diets (Choubert *et al.*, 1982; Sugiura *et al.*, 1998; Weatherup & McCracken 1998).

As a preliminary step, it is very important to allow the fish to adapt to a new diet before the commencement of any faecal sampling, or digestibility assessment. This allows the fish to adapt to the chemical composition of the new diet and establish an equilibrium in their gut with a new microflora and digestive processes, to stabilise the absorption efficiencies. The calculated ADCs typically fluctuated for the first 3 days of collection before their variability diminished (Blyth *et al.*, 2014). In temperate and tropical species, it is advised to allow the fish to acclimatise with a new diet during a period of 4 to 14 days for a range of fish species before starting the collection of any faecal material (Glencross *et al.*, 2005; Gaylord & Barrows 2008; Glencross *et al.*, 2012; Blyth *et al.*, 2014). In this experiment we decided to wait for 7 days before starting the experiment as advised in Glencross *et al.* (2012) which allowed a low variability in the ADCs coefficient.

During an *in vivo* digestibility experiment, it is essential to define a proper faecal collection method, as this can greatly influence the digestibility assessment of a diet (Windell *et al.*, 1978; Weatherup & McCracken, 1998; Vandenberg & De la Noue, 2001).

The faecal collection method is a vital component of the process as levels of the inert dietary marker (in the case of this study: yttrium oxide) and the nutritional values of the feed and in the digested excreta, will be assessed. For these reasons, the accuracy, and techniques used for sample collection have a significant effect on the determination of the digestibility values of diets (Windell *et al.,* 1978; Weatherup & McCracken 1998; Vandenberg & De la Noue 2001; Glencross *et al.,* 2005). Two main methods can be used: the collection of un-defecated digesta directly from the fish gut (either by intestinal dissection, suction or stripping) (according to Austreng, 1978; Vandenberg & De la Noue 2001; Glencross *et al.,* 2005; Aslaksen *et al.,* 2007) and the collection of faeces settled at the bottom of the tank. The later can involve either a siphoning of the faeces from the bottom of the tank, the collection of decanted faeces or a continuous collection (Choubert *et al.,* 1982; Cho & Kaushik 1990; Vandenberg & De la Noue 2001; Glencross *et al.,* 2005). While the collection of undefecated digesta is the most conservative method to evaluate the ADCs (Vandenberg & de la Noue 2001; Glencross *et al.,* 2005), it is more labour intensive than collecting faeces from the water column and is constrained by fish size (*i.e.* if the fish are too small to be handled). Moreover, as the faeces were not yet expelled, samples were therefore collected at one time point providing a snapshot of the ADC. In addition, as it can be a destructive method for the fish (if dissection is used), the amount of sample collected can be limiting. In contrast, the collection of faeces from the water column is typically less labour intensive, can be applied to fish of any size and does not inflict stress on the animals (reviewed by Glencross *et al.,* 2007). However, due to the passive nature of this method, there is a risk for the samples to be contaminated with scales, mucus or any other exogenous material, but also to be overestimate true digestibility values if nutrients are leaching through the water column (Glencross *et al.,* 2007).

Considering the stage of the fish in this experiment, (*i.e.* post sex-reversed fry of 0.25g) the faecal settlement collection method had to be used. The diet-substitution digestibility method was used according Aknes *et al.,* 1996, but due to the size of the fish some issues arose. Some values were clearly erroneous, like the ash content digestibility values and the carbohydrates values (calculated using the ash content), and were not useable. This is most likely due to the low nutrient content allowing it potentially to leach out of the feces when they were expelled into the tank, making it hard to detect during the analysis. Despite multiple, independent analyses, these values were not useable.

Secondly, upon testing, the yttrium concentration in the different diets were not exactly 70% as planned in the protocol. This is most likely due to a problem in the mixing. The yttrium oxide proportions were very low, and therefore due to the ambient humidity in Thailand, may have slightly aggregated in the diets and did not mix perfectly. Therefore two ways of calculations were possible, and both we consider correct for different reasons, one using the 70%:30% basal diet to tested ingredient ratio, and the second using the actual ratios given with the yttrium values (between 69%:31% for the fish meal to 55%:45% for the Black Soldier Fly defatted meal). The second method giving more conservative values.

In both scenarios, some apparent digestibility values can be higher than a 100% digestibility. This is due to the fact that either the nutrient levels were very low, therefore could introduce and exacerbate a measurement error and making a potential dilution of the nutrient in the tank prior its collection more likely, or could be caused by interaction effects between ingredients for the values slightly above 100%. In the case of soybean the lipid level was very low (2.82%) causing apparent digestibility values for lipid to be way above 100% (176% and 188% for the two scenarios respectively). A similar case was observed by Glencross *et al.* (2007) were a lipid ADC (for soybean as well) was recorded at 400%. Similarly, the lipid levels were very low causing this error.

For FM, and the two BSF meals (BSFM) the lipid digestibility was very good (being 105,125, and 112% in the first scenario for FM, BSF WM and BSF DF respectively and 104, 95, 91% in the second scenario). The near 100% apparent digestibility values indicate synergetic effects in the feed, potentially enhancing the digestibility of other lipid sources in the diet.

Regarding the protein digestibility, for all ingredients, it was variable with the lowest observable value for the FM (72 and 71% in each scenario). This was lower than the usual estimates (around 80% usually) and further suggests the possible adulteration of this feedstuff in our study.

Regarding the 2 BSFM's it is interesting to notice that the protein digestibility was very similar, and in both cases excellent (95% in the first scenario, and 84-80 for BSF WM and BSF DF in the second scenario).

The ADC of the dry matter was lower for the FM (60% in both scenarios) than for the BSFM and the soybean meal (118, 107 and 123% respectively for the first scenario and 95, 85, 98% for the second scenario).

To date, most of the digestibility investigations on BSFM have been done either on chickens (broilers and layers) (Cullere *et al.*, 2016; Schiavone *et al.*, 2017) or quails and guinea fowls (Wallace *et al.*, 2017; Wallace *et al.*, 2018). Presently, no *in vivo* digestibility measurements have been reported on Tilapia fry, however, the ADC for dry matter are within the range published from previous *in vitro* experiments, where the BSF larvae digestibility was estimated around 82-90% (Arango Gutiérrez *et al.*, 2004; Bosh *et al.*, 2014). It was interesting to notice however, that when pre-pupae were used, the digestibility drops to 77.7% (Bosh *et al.*, 2014) most likely due to the higher chitin content (around 15% DM at this stage according Bosh *et al.*, 2014). A similar observation was made during an *in vivo* digestibility experiment on turbot (*Psetta maxima*) (Kroeckel *et al.*, 2012). Based on undefectated digesta, the ADCs coefficient of BSFM were lower for organic matter (71%), crude protein (63.1%), crude lipid (78%), and gross energy (54.4%) than FM (83.2, 89.1, 98.7, 84.9% respectively) (Kroeckel *et al.*, 2012). The author attributed it to the absence of chitinase activity or chitinolytic activity in the mid gut of turbot. As they used defatted prepupae, the presence of chitin might have influenced the feed intake, availability, and digestibility of the nutrients and therefore growth performance. The processing of the meal could be important, as it would have been possible that the defatting process might have caused an integration of the lipids into the chitin structure, lowering their availability and therefore decreasing their digestibility (Kroeckel *et al.*, 2012). In our case, only white larvae were used, which might explain the comparatively good digestibility of the lipids. These results are in line with Shiau & Yu (1999) who demonstrated that lipid digestion in diets for tilapia supplemented with chitin was significantly decreased. Similar observations were done on mice (Han *et al.*, 1999) and broiler chickens (Razdan & Pettersson, 1994), where the presence of dietary structural polysaccharides, like chitin, was reported to inhibit nutrient absorption from intestinal tract and thereby reducing fat absorption.

In European seabass (*Dicentrarchus labrax*), the ADCs of the diets, and digestive enzymes were not affected by an dietary inclusion of BSF prepupae meal at 19.5% (Magalhães *et al.*, 2017). This suggests that even in fishes used to a chitin-free meal and therefore not adapted to its ingestion, it is possible to use low amounts of BSF meal to lower the fraction of FM used in the diet without affecting the overall digestibility of the meal.

While the presence of chitin potentially lowered the digestibility of the Black Soldier fly meal, it is still possible to use it as a feed ingredient when prepupae are used, by keeping

the dietary inclusion low, or potentially with the addition of chitinases or probiotic bacteria to enhance the digestibility of the feedstuff. This highlights the importance of the use of white larvae instead of the prepupae when producing a BSF meal, as they are more digestible at this stage.

In the present case, the BSF meal, either full fat or defatted was proven to be a good feed ingredient for tilapia fry, even showing in our case, better digestibility values for proteins than fishmeal, and could be used as a substitute of fish meal in their sex-reversal diet, as part of a fully formulated feed.

# CHAPTER 7: BLACK SOLDIER FLY FORMULATED FEED USED AS HORMONE CARRIER FOR SEX-REVERSAL PROCESS IN TILAPIA FRY (*OREOCHROMIS NILOTICUS*)

# 7.1 Introduction

Aquaculture in Thailand started in 1922 in the vicinity of Bangkok based on imported Chinese carps. In 1951 the Department of Fisheries started to promote aquaculture production (FAO, 2018). Presently, more than 50 aquatic species are cultivated throughout the country. Thailand is one of the top aquaculture producing nations in the world. In 2016, the aquaculture production was estimated to be around 962,571 metric tons, employing more than 660,000 people in fish farms and their related industries (World Bank, 2016). In terms of annual (inland) production the five most important species, are Nile tilapia (*Oreochromis niloticus*), hybrid catfish (*Clarias sp*), silver barb (*Barbodes goniotus*), snakeskin gourami (*Trichogaster pectoralis*) and the giant river prawn (*Macrobranchium rosenbergii)*. Combined, these species contribute to almost 95% in quantity and 92% in value of the total aquaculture production in Thailand (FAO, 2018). Tilapia production contributes close to 30% (83,780 tonnes) of the total freshwater aquaculture production (FAO, 2018). As the sector is rapidly growing, there is a trend towards standardization of product size, feeds, production systems, quality control and marketing in the supermarket chains. Consequently, the main cultured type of tilapia are hormonal sex reversed fish.

The sub-optimal growth and low or variable size (and market value) from mixed-sex populations of tilapia has been a notable constraint to the commercial development of the species. This led to research efforts in the 1970's to develop technology to produce all-male fry in order to circumvent this problem. With the development of the technology, production of all-male tilapia on a cost-effective and commercially viable scale emerged in the mid 1980's. Following this breakthrough, since the late 1990's the number of monosex tilapia hatcheries in Thailand has increased drastically as the demand for sex-reversed fry increased. The ability to produce all-male tilapia fry has largely revolutionised the profile of the industry in Thailand over the past 15 years, and consequently produced a major shift in productivity and profitability (FAO, 2018).

All-male culture of tilapia is preferred in grow-out systems as they better tolerate supplementary feed, can be raised in higher densities, and this leads to a more uniform marketable product, produced at a faster rate due to a substantially better growth rate of males and relative to females (Mair & Little, 1991; Little & Hulata, 2000; Phelps & Popman, 2000). Typically, all-male tilapia are harvested when they reach 400 g – 1000 g,

which compares to mix-sex tilapia usually harvested around 250-350 g after the same culture duration (Dan & Little, 2000). This has made their farming a much more profitable business. The faster growth of monosex tilapia is related to the fact that fish do not have to invest energy in reproductive development and behaviour such as producing egg, maternal care (mouth brooding by females, or digging a nest in the pond damaging it) and energy expenditure on courtship by males (Macintosh & Little, 1995). Also, production of monosex fry reduces problems associated with growth stunting caused by competition for food that otherwise occurs between recruits and stocked fish in pond production (Dan & Little, 2000). With improved farming practices commercial hatcheries have increased their reversal rates and quality of monosex tilapia fry. The quality of seed, environmental factors and husbandry are very important factors to consider, but feed quality and its management are essential for its success (Popma & Green, 1990; Phelps, 2006; Little & Hulata, 2000). While several methods exist to produce all-male fry (see introduction in Chapter 5) the preferred commercial method is the addition of 17α-methyltestosterone (MT) hormone into the feed during the first 21 days. The daily intake of feed (meeting the fish early stages dietary requirements), and uniform concentration of methyl-testosterone hormone at a level of 60 mg per kg of feed, needs to start prior the gonad differentiation, therefore right after the newly hatched fish resorb their yolk-sack (D'Cotta *et al.*, 2001). The development of hapa-based broodstock management, was essential in allowing the collection of same-age tilapia eggs and yolk-sack larvae to be later fed with methyl-testosterone based feed (Haitook *et al.*, 1999; Little *et al.*, 1995).

The sex-reversal is considered effective in cage-production systems when at least 96% males are produced (Mair & Little, 1991) and according to Vera Cruz & Mair (1994) to avoid significant impacts on grow out fish crops in ponds, sex-reversed population counting more than 98% males are recommended. Therefore, in order to maximise the hormone intake, the feed needs to meet the fishs dietary requirements, have a good buoyancy, and very importantly, good palatability. In Thailand, and in most of large-scale tilapia producing countries, pure fish meal (FM) is used as a feed for the early feeding larvae as it contains high levels of protein, and has an excellent palatability (Phelps, 2006; NRC, 2011).

In recent years, the global rise of FM prices has led to an adulteration of this feedstuff, often mixed with lower-quality products (such as soybean meal or saw dust as seen in Chapter 6). However, high quality FM is crucial for the tilapia sex-reversal process, as the pond production requires a nearly 100% success of this process. The purchase of fry

is a major cost for farmers and investment in monosex seed, which is more expensive than mixed-sex fry, contributes to better performance during grow out, thus the importance of a high percentage of males (Little, 1989). If a percentage close to 100% males is not attained, it might depreciate the reputation of the hatchery, and could harm the business' economic viability. Currently, only large-scale hatcheries can afford to test on a regular basis the nutritional value of their FM prior its use for tilapia fry, to ensure the consistency of this key-product, while small to medium scale hatcheries relies only on the manufacturer declarations.

In this context, locally produced Black Soldier Fly Meal (BSFM) could be used as a protein substitute for FM in a fully formulated feed. We have demonstrated previously (see Chapter 5) that the BSFM could be used as a direct substitute of FM or commercial feed during the sex-reversal phase, allowing acceptable results in male production percentage, and survival. However, the percentage of males resulting from this was lower that the norm accepted in Thailand (*i.e.* > 99%), and as the economy of scale is yet to be achieved in insect production, an even more strategic use of this meal is required to optimise its use. Building on the digestibility data from the previous chapter, the aim of this study, conducted in a commercial hatchery in Thailand, was to investigate the efficiency of a fully-formulated isoenergetic and isoproteic diet, using both full fat maggot meal and defatted maggot meal as protein substitutes for FM. Indicators on the viability of this substitution would include; (i) percentage of males, (ii) overall survival, (iii) growth, and (iv) size homogeneity of the fish. At the end of the sex-reversal period, a stress test was applied to estimate the resilience of the fish to transportation-related stress.

# 7.2 Material and Methods

## 7.2.1 Culture technique

The experiment was performed in Nam Sai Farms (Ban Sang, Prachinburi province, Thailand) between the 18/02/2018 and the 19/04/2018. The experiment was approved by the Ethical Committee (Ref: AWERB/1718/060 New Non ASPA 05/01/2018). One day – old *O. niloticus* swim-up fry were harvested using a downwelling system.

### 7.2.2 Water parameters

The Ph, Oxygen dissolved in water and temperature were recorded inside the hapas 5 times a day prior to each feeding session. Hardness, alkalinity, Ammonium, Nitrates and nitrites were recorded every 5 days using a spectrophotometer. The water parameters were recorded to ensure that the quality conditions would not adversely influence the trial results.

### 7.2.3 Hormone – feed preparation

Hormone-treated feed was prepared as described by (Killian & Kohler, 1991). A stock solution of 17α-methyltestosterone (MT) was made by dissolving 60mg of hormone in 1 L of pure ethanol (El-Greisy & El-Gamal, 2012; Popma & Green, 1990; Celik *et al.,* 2011) for 1 kg of feed. The solution was then evenly poured onto the diet and mixed. The mixing process was repeated several time to ensure an even distribution of the MT throughout the feed. Diets were fan-dried in the shade at 30 ºC for 24 hours before being kept refrigerated at -20°C prior to use (Barry *et al.,* 2007). The maggot meal was sourced from EntoFood in Kuala Lumpur (Malaysia; details in table 7.1). The different ingredients were finely grinded, and sieved (Ø<1mm) to remove the larger particles, prior being mixed to produced the formulated feed. As a quality insurance, once each experimental diet was completed, the mix was re-sieved ensure that it was flour-like and homogeneous.

### 7.2.4 Experimental setup

An earthen pond area (2000 $m^2$, water depth: 1.2 metre) located at Nam Aig site (13°58’35.29’’N; 101°14’50.74”E, WGS84) was used for the experiment. The pond was drained, and sun dried for a week. It was then filled with predator-free water, screened through a fine mesh from an on-farm reservoir. The experiment started once the pond had developed an algae bloom and turned green. 48 hapas ($5m^2$) were randomly placed inside the pond.

To perform this experiment, 12 iso-proteic and iso-energetic, formulated diets with different inclusion of maggot meal (defatted and full fat larvae) were tested (details in Table 7.1). These were formulated based on the protein, and lipid values for fish meal,

soybean, maggot meal (either full fat and defatted) obtained using the most conservative values (from the second calculation method described in chapter 6) gathered from the previous digestibility experiment, and the diets were formulated using the Winfeed™ software. The nutritional values of each feedstuff is presented in Table 7.2. All the diet formulations were based on the diet requirements for tilapia fry production (according to Jauncey, 1998 and Hayashi *et al.*, 2002). Each treatment was replicated 4 times. In each hapa, 28,000 one-day-old tilapia fry were stocked to achieve a density of 12 fish of 0.015 g per litre.

**Table 7.1: Composition of the different treatments (% as fed basis):**

| Ingredients/Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish meal [1] | 96 | 96 | 25 | 19 | 15 | 7 | 0 | 45 | 40 | 36 | 31 | 26 |
| Soybean [2] | 0 | 0 | 14 | 10 | 7 | 5 | 0 | 30 | 25 | 20 | 14 | 12 |
| BSF WM [3] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 30 | 36 | 45 | 49 |
| BSF DF [4] | 0 | 0 | 52 | 62 | 70 | 80 | 91 | 0 | 0 | 0 | 0 | 0 |
| Rice bran [5] | 0 | 0 | 2.5 | 3.5 | 3 | 3 | 4 | 0.5 | 1.5 | 3.5 | 5 | 6.5 |
| Corn [6] | 0 | 0 | 3 | 2.5 | 2.5 | 3 | 4 | 0.5 | 1.5 | 3.5 | 5 | 6.5 |
| Soybean oil [7] | 4 | 4 | 3.5 | 3 | 2.5 | 2 | 1 | 4 | 2 | 1 | 0 | 0 |
| Methyltestosterone (mg/kg) | 60 | 0 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| **Protein (% DM)** | **40.91** | **40.91** | **41.25** | **41.56** | **42.07** | **42.36** | **42.37** | **40.82** | **40.68** | **39.6** | **38.74** | **37.66** |
| **Lipid (% DM)** | **12.15** | **12.15** | **12.31** | **12.44** | **12.32** | **12.22** | **11.90** | **12.92** | **12.63** | **12.63** | **13.36** | **14.01** |

[1] Fish meal: Tuna factory by-products, T.C. Union Agrotech Co.Ltd. 68 moo 8, Rama 1 road, Bangkrachao, Samutsakorn, Puhket, Thailand.
[2] Soybean: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.
[3] BSF WM: Maggot meal whole meal (full fat), white larve stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia.
[4] BSF DF: Maggot meal defatted, white larve stage, bred on kitchen waste, EntoFood, Kuala Lumpur Malaysia.
[5] Rice Bran: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.
[6] Corn: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.
[7] Soybean oil: Khok Kruad Karnkaset, mueang, Nakhon Ratchasima, Thailand.
Treatment 1: Positive control, Treatment 2: Negative control.

**Table 7.2**: Nutritional composition of the Black Soldier Fly meal defatted, whole meal, and fishmeal.

| | BSF defatted meal | BSF whole meal | Fishmeal |
|---|---|---|---|
| **Proximate composition (%)** | | | |
| Dry matter | 97.63±1.71 | 91.73±1.80 | 98.88±1.34 |
| Crude protein | 57.09±0.44 | 47.77±0.23 | 60.27±0.15 |
| Crude lipid | 12.21±0.10 | 21.76±0.18 | 8.49±0.23 |
| Ash | 10.16±0.59 | 9.83±0.70 | 24.61±0.33 |
| Gross energy (MJ/Kg) | 21.5±1.45 | 22.2±1.9 | 18.5±1.45 |
| **Apparent digestible values[1]** | | | |
| Crude protein | 45.53 | 40.13 | 42.61 |
| Crude lipid | 11.10 | 20.75 | 8.49 |
| **Essential amino acids (g/100g xN)** | | | |
| Histidine | 2.13±0.24 | 1.21±0.25 | 1.63±0.08 |
| Threonine | 2.43±0.19 | 1.52±0.11 | 2.45±0.09 |
| Arginine | 3.25±0.08 | 2.34±0.08 | 3.34±0.13 |
| Valine | 3.89±0.27 | 2.51±0.16 | 2.78±0.17 |
| Methionine | 1.21±0.04 | 0.75±0.07 | 1.45±0.08 |
| Lysine | 3.62±0.34 | 2.19±0.25 | 3.96±0.22 |
| Iso-leucine | 2.93±0.18 | 1.85±0.18 | 2.32±0.17 |
| Leucine | 4.02±0.24 | 2.83±0.19 | 3.89±0.12 |
| Phenylalanine | 2.62±0.12 | 1.73±0.11 | 2.19±0.03 |
| **Fatty acid composition (g/100 of total % fatty acid)** | | | |
| 12:00 | 3.23±0.0.3 | 6.8±0.19 | N.D. |
| 14:0 | 0.89±0.04 | 2.08±0.08 | 0.41±0.03 |
| 16:0 | 2.53±0.21 | 5.84±0.96 | 1.78±0.14 |
| 18:0 | 0.34±0.03 | 0.76±0.08 | 0.46±0.09 |
| ***Total saturated[1]*** | **7.45±0.51** | **16.45±1.32** | **2.89±0.34** |
| 16:1n-7 | 0.29±0.02 | 0.83±0.08 | 0.52±0.03 |
| 18:1n-9 | 2.45±0.19 | 5.32±0.84 | 1.19±0.02 |
| 18:1n-7 | N.D. | N.D. | 0.13±0.03 |
| ***Total monounsaturated[2]*** | **2.78±0.27** | **6.64±0.76** | **2.32±0.37** |
| 18:2n-6 | 1.29±0.08 | 2.43±0.39 | 0.21±0.02 |
| 18:3n-6 | 0.06±0.01 | N.D. | 0.09±0.01 |
| ***Total n-6 PUFA[3]*** | **1.26±0.07** | **2.56±0.23** | **0.53±0.01** |
| 18:3n-3 | 0.09±0.02 | 0.17±0.03 | 0.09±0.00 |
| 18:4n-3 | 0.01±0.01 | 0.08±0.02 | 0.04±0.01 |
| 20:5n-3 | 0.09±0.01 | 0.12±0.01 | 0.53±0.07 |
| 22:5n-3 | N.D. | N.D. | 0.09±0.03 |
| 22:6n-3 | N.D. | 0.03±0.00 | 1.12±0.13 |
| ***Total n-3 PUFA[4]*** | **0.21±0.03** | **0.42±0.03** | **1.72±0.18** |
| ***Total PUFA[5]*** | **1.43±0.15** | **3.04±0.12** | **2.32±0.76** |
| ***Total fatty acids*** | **11.23±0.98** | **26.78±1.28** | **7.43±2.12** |

Values are based on duplicate analysis of four samples of each type of feed. Values are presented as the mean±standard error [1]includes 15:0; 17:0 ; 20:00; 22:0 and 24:0; [2] Includes 14:1; 16 :1n-9 ; 17 :1 ; 20 :1n-11 ; 20 :1n-7; 22 :1n-9cis; 22:1n-9; 24 :1-9; [3] Includes: 18:3n-6; 20:3n-6; 22:4n-6; [4] Includes 20:3n-3; [5] Includes 16:2; 16:3; 16:4. N.D.: not detected.

The fish were fed 5 times a day, over 21 days with a hormone impregnated diet (17α-methyltestosterone used at 60mg/Kg of feed).

During this period, the fish were weighed every 10 days to assess their growth rate. To do so, about 100 fishs were randomly caught from each hapa, counted and bulk weighed. Any obvious mortalities were recorded at every feeding session, and also at the end of the 21 day period when all the fish were harvested. At the end of the 21-day period, fish were graded into 3 sizes using a hand grader with mesh of 7.5 and 9 mm to assess the uniformity of the fish.

For each hapa, 300 fish were retained for a further 6-weeks post-sex-reversal treatment, to assess their sex. The rest of the fish returned to the farms commercial system. At the end of the 6-week period, 100 fish were sacrificed by an overdose of clove oil and dissected to extract the gonads for sex-testing.

The fish size distribution was assessed using 2 hand graders. The fish below 7.5mm, the first mesh, were considered “small”, the “medium” were sized between 7.5 and 9mm (the second mesh) and all fish above this were considered “large”.

### 7.2.5 Sexing of the fish

A total of 100 fish were randomly selected from each hapa, euthanized using clove oil (Ross & Ross, 2008) and dissected under a binocular microscope following the procedure described in both Guerrero & Shelton (1974) and Wassermann & Afonso (2002). The sex was assessed using Guerrero & Shelton (1974) and Guerrero (1975) papers.

### 7.2.6 Fish feeding ration

Fry were fed 5 times a day (at 8:00, 09:30, 11:30, 13:30 and 16:00h). Initially, the amount of feed given to the fry daily was calculated as a function of the total biomass of all the hapas in the pond. The daily feed rate was 30% body weight until day 5, recalculated until day 10 and 20% to day 21. Fish were sampled every 5 days to estimate the total biomass and adjust the feed ration of individual hapas accordingly.

### 7.2.7 Assessing the stress resistance of the fish

During the sex-reversal stage, the final weight, survival and percentage of males are the main quality indicators of feeding management. Resistance to stress is also essential from a production point of view, as stress impacts the commercial value of the fish. Acute or chronic stress can impact welfare and can predispose fish to disease (Eddie & Norman, 2008). Transport and handling can be considered as acute forms of stress during the production and distribution of the sex-reversed fry.

Therefore, a stress test is an important tool to evaluate the quality of larvae and fingerling production (Dhert *et al.*, 1992). Amongst the various stress tests applied, air exposure is commonly used for several species (Sakakura *et al.*, 1998, on yellowtail fingerlings; Arends *et al.*, 1999, Koven *et al.*, 2001 and Van Anholt *et al.*, 2004; in gilthead sea bream; Benfey & Biron, 2000, on rainbow and brook trout; Martins *et al.*, 2000, on piaractus; Luz & Portella, 2005 on *Hoplias lacerdae*; Trushenski *et al.*, 2010 on cobias, Macniven, 1999, Luz *et al.*, 2012 on Nile tilapia). It is described as an efficient evaluation of feed quality on the stress resistance of the fish seeds (Ako *et al.*, 1994; Kanazawa, 1997; Luz, 2007, Luz *et al.*, 2012) and the quality of the fish lot based on diet quality (Luz *et al.*, 2012).

At the end of the sex-reversal experiment, 100 fish were collected and placed in 1m² hapas containing an air blower, to ensure an adequate oxygenation of the water. Then the 21-day old sex-reversed fry were exposed to air for 7-minutes on a sieve, before being placed back into the water. As the stress hormone –cortisol- peaks between 2.5–4.5 h post stress exposure (Cnaani *et al.*, 2004), it is advised (Luz *et al.*, 2012) to let the fish rest for 24h before measuring the survival. A day later the fish were counted, avoiding any additional stress, and kept for a further 6-days in the hapas, in case the level of mortality increased due to the occurrence of opportunistic diseases. The hapas were emptied 6-days later to assess final level of mortality and the fish were returned back into the commercial production.

### 7.2.8 Statistical Analysis

All statistical calculations were carried out using IBM SPSS Statistics (Version 21). A significance level of 5% was chosen for all analyses. Normal distribution of the data sets

was verified using Shapiro-Wilk test and homogeneity of the variance was tested with Levene's test. Significant differences with the control ($p<0.05$) were assessed using a Chi-two parametric test.

## 7.3 Results

### 7.3.1 Water parameters

Over the course of the trial, water temperature and dissolved oxygen, ranged from 27.9 to 33.8°C and 2.6 to 7.1g/L respectively. Water pH remained stable during the experiment, (7.9±0.3; mean±SD), while nitrites, ammonia and alkalinity levels decreased during the first 10 days before stabilising around 0.5±0.2 mg/L, 0.8±0.3 mg/L and 69.3±5.1 mg/L respectively. All values were within the tolerance range of Nile tilapia (Beveridge & McAndrew, 2000; Xu *et al.*, 2005; El-Sayed, 2006).

### 7.3.2 Sex reversal success and survival

No significant differences in sex-reversal rate were observed between hormone treated diets ($p>0.05$) with values ranging from 98.41 to 100% (Table 7.3). In addition to females some inter-sex fish (displaying both females and male characteristics) were observed. The only significant difference was, as expected, between the negative control (fish meal diet with no methyl-testosterone hormone) and all the other treatments ($p<0.05$) with a mean reversal rate of 92.17%.

The survival, was not affected by the different diets (Table 7.3). Ranging from 82.69 to 91.57% it did not significantly differ ($p>0.05$) for the different dietary treatments with the control.

**Table 7.3**: Percentage of phenotypic males, and survival after 6 weeks post-experiment.

| Inclusion of BSFM | Treatment | Males (%) | Survival rate (%) |
|---|---|---|---|
| Positive control | 1 | 99.20±0.99 | 87.00±6.18 |
| Negative control | 2 | 92.17±4.23 *** | 82.59±7.29 |
| BSF DF: 50% | 3 | 99.70±0.6 | 84.43±2.65 |
| BSF DF: 60% | 4 | 99.17±1.67 | 84.05±1.16 |
| BSF DF: 70% | 5 | 99.69±0.63 | 88.80±6.13 |
| BSF DF: 80% | 6 | 100.00 ±0.0 | 91.57±2.03 |
| BSF DF: 90% | 7 | 99.74±0.51 | 89.90±2.55 |
| BSF WM: 20% | 8 | 98.43±1.06 | 82.92±3.83 |
| BSF WM: 30% | 9 | 99.75±0.5 | 82.69±4.56 |
| BSF WM: 35% | 10 | 98.41±0.66 | 83.69±6.56 |
| BSF WM: 45% | 11 | 99.00±1.41 | 83.27±9.76 |
| BSF WM: 50% | 12 | 99.24±0.96 | 86.41±8.92 |

Abbreviations: BSF DF: Black Soldier Fly meal defatted, BSF WM: Black soldier Fly whole meal, the following number referring to its proportion in the fry's diet, based on a dry matter basis. Superscript *** Mean±SE (n=4) referring to a significant difference with the positive control (Diet 1) ($p<0.01$).

### 7.3.3 Growth and weight heterogeneity

The only significant difference in final mean weight ($p<0.05$) was between the positive control and treatments BSF defatted meal at an inclusion of 70-80 and 90%, (0.13g compared to 0.19g for the positive control). This result is translated in the final harvest fish weight, where the total weight of the fish is therefore significantly affected ($p<0.05$), displaying harvests of 2 831, 2 948 and 3 314g respectively (for the BSF DF treatments of 70, 80, 90% respectively), while the control's harvest was 4 938g (See Table 7.4).

**Table 7.3**: Average weight of the fish at the end of the sex-reversal process, and total biomass.

| Inclusion of BSFM | Treatment | Average fish weight at the end of the sex-reversal process (g) | Total biomass at the end of the sex-reversal period (g) |
|---|---|---|---|
| Positive control | 1 | 0.19±0.03 | 4 938.2±264.3 |
| Negative control | 2 | 0.16±0.03 | 4 427.7±641.4 |
| BSF DF: 50% | 3 | 0.17±0.02 | 3 852.0±667.6 |
| BSF DF: 60% | 4 | 0.16±0.02 | 3 453.5±720.2 |
| BSF DF: 70% | 5 | 0.13±0.02 * | 2 831.0±656.1 * |
| BSF DF: 80% | 6 | 0.13±0.02 * | 2 948.5±452.0 * |
| BSF DF: 90% | 7 | 0.13±0.02 * | 3 314.7±581.9 * |
| BSF WM: 20% | 8 | 0.25±0.04 | 5 369.5±304.9 |
| BSF WM: 30% | 9 | 0.23±0.02 | 5 070.7±153.1 |
| BSF WM: 35% | 10 | 0.22±0.03 | 4 683.2±384.6 |
| BSF WM: 45% | 11 | 0.21±0.04 | 4 863.0±253.8 |
| BSF WM: 50% | 12 | 0.21±0.03 | 4 894.7±407.1 |

Abbreviations: BSF DF: Black Soldier Fly meal defatted, BSF WM: Black soldier Fly whole meal. , the following number referring to its proportion in the fry's diet, based on a dry matter basis. Superscript * Mean±SE (n=4) referring to a significant difference with the positive control (Diet 1) ($p<0.05$).

Regarding the size distribution of the fish, the control displayed a class distribution of 41.73% small fish (<7.5mm), 45.12% medium (between 7.5mm and 9 mm) and 13.13% large fish (>9 mm). The results are displayed in the Figure 7.1.

No significant differences ($p>0.05$) were observed relative to the negative control (displaying for small, medium and large fish respectively: 42.45; 37.68; 19.86%). However, the proportion of the different fish class, at the end of the sex-reversal period, were affected by the black soldier fly defatted (BSF DF) meal treatments, displaying much more smaller fish (ranging from 66.53 to 87.47%, compared to the control having only 41.73%), and fewer medium and large-size fish than the control (11.78-31.33% for the medium,0.56-1.37% for the large, while the control displayed respectively 45.12 and 13.13% for these two fish classes). The diets 3 and 4 containing respectively 50 and 60% of BSF DF meal displayed only a significant difference ($p<0.01$) with the control for the large fish (1.37 and 0.94%) compared with the control having 13.13% of large fish in the total harvested population. The diets 5, 6 and 7 showed much more variation and were mostly composed of small fish. In these treatments, each fish class (small, medium, large) was significantly different with the control's ($p<0.01$) and displayed for –small, medium and large fish respectively- 84.47, 14.95, 0.56% for diet 5 (containing 70% of BSF DF as

a protein substitute); 84.55, 14.71, 0.72% for diet 6 (containing 80% of BSF DF as a protein substitute); 87.47, 11.78, 0.73% for diet 7 (containing 90% of BSF DF as a protein substitute). The dietary treatments containing BSF whole meal (*i.e.* diets 8, 9, 10, 11, 12) showed no significant differences ($p>0.05$) for any fish class with the control.

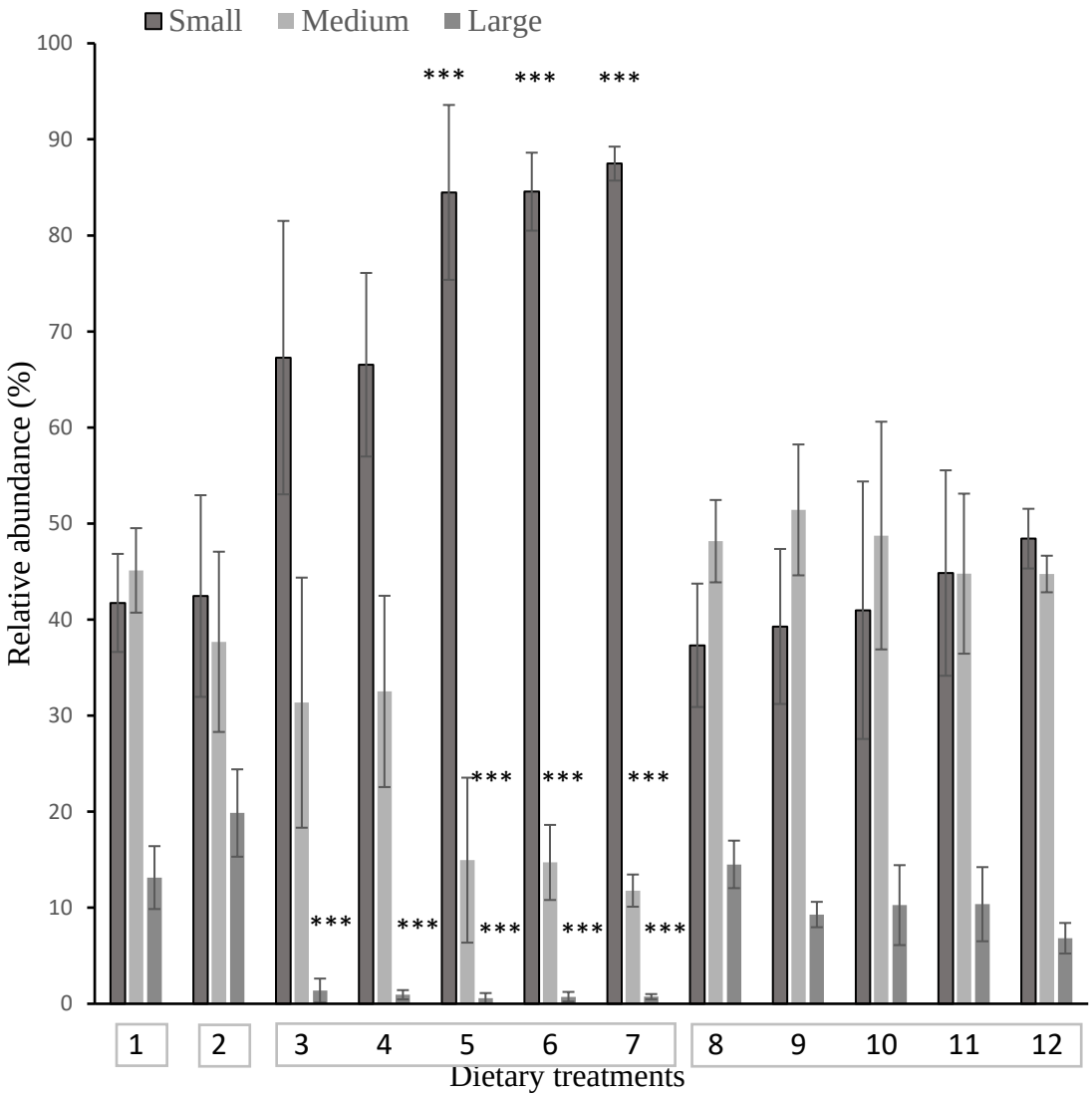



**Figure 7.3** : Relative abundance (in % of the total population) of small, medium and large fry at the end of the 21 day experimental period. For comparable columns (class size), means±SE (n=4).

Superscript *** referring to a significant difference with the positive control (Diet 1) ($p<0.01$); Superscript * referring to a significant difference with the positive control (Diet 1) ($p<0.05$). Abbreviations: Dietary treatment 1: Control (100% fish meal –FM- as a protein source with methyltestosterone –MT-); Dietary treatment 2:negative control (100% FM with no MT); Dietary treatments from 3 to 7 respectively: Black Soldier Fly defatted meal as a main source of protein at an inclusion of 50, 60, 70, 80 and 90% respectively with MT; Dietary treatments from 8 to 12 respectively: Black Soldier Fly whole meal as a main source of protein at an inclusion of 20, 30, 35, 45 and 50% respectively with MT.

### 7.3.4 Stress resistance

With respect to stress test outcomes after 24hrs, the BSF Whole Meal diets performed as well as the positive control (Table 7.5), with the exception of the 50% inclusion which performed significantly better (94%, $p<0.05$) . The negative control (*i.e.* with no hormone added to the mix) performed significantly poorer (50.75%, $p<0.01$) than the control (83%). For the diets containing defatted maggot meal, beside the 60% and 80% inclusion, they were significantly lower than the control. The treatments with defatted maggot meal inclusions of 60%,70% and 80% gave 24h survival rates of 57.5%, 51% and 67.25% respectively, significantly lower than the positive control ($p <0.01$ for BSF DF 60-70% and $p<0.05$ for BSF DF 80%). The results of the stress test conducted 7 days post-trauma where almost identical to the ones obtained 24h post-trauma.

**Table 7.3**: Fish survival after being subjected to a 7 min air exposure stress test, after their sex-reversal process.

| Inclusion of BSFM | Treatment | Survival after 24h | Survival after 7 days |
|---|---|---|---|
| Positive control | 1 | 83±7.16 | 82.50±7.14 |
| Negative control | 2 | 50.75±4.11 *** | 49.75±4.57 *** |
| BSF DF: 50% | 3 | 85.50±3.42 | 84.50±3.87 |
| BSF DF: 60% | 4 | 57.50±6.45 *** | 56.50±7.19 *** |
| BSF DF: 70% | 5 | 51.00±2.94 *** | 50.75±2.63 *** |
| BSF DF: 80% | 6 | 67.25±4.50 * | 66.75±4.99 * |
| BSF DF: 90% | 7 | 81.25±4.99 | 80.50±4.36 |
| BSF WM: 20% | 8 | 77.50±6.35 | 77.00±6.16 |
| BSF WM: 30% | 9 | 82.75±3.86 | 82.75±3.86 |
| BSF WM: 35% | 10 | 82.25±3.10 | 82.00±2.94 |
| BSF WM: 45% | 11 | 84.50±3.87 | 84.50±3.87 |
| BSF WM: 50% | 12 | 94.00±1.16 * | 94.00±1.16 * |

Abbreviations: BSF DF: Black Soldier Fly meal defatted, BSF WM: Black soldier Fly whole meal. , the following number referring to its proportion in the fry's diet, based on a dry matter basis Superscript *** Mean±SE (n=4) referring to a significant difference with the positive control (Diet 1) ($p<0.01$). Superscript * Mean±SE (n=4) referring to a significant difference with the positive control (Diet 1) ($p<0.05$).

## 7.4 Discussion

Sex reversal remains the industry standard for reproduction control in tilapia, but poor-quality seeds are often identified as a significant constraint on the development of tilapia aquaculture in Asia (Little *et al.*, 1999). Not only does it discourage new entrants or poor small-holders to enter into the business, but a low sex-reversal success or poor stress resistance affects directly the reputation of commercial hatcheries. As stated previously, FM is becoming a scarce resource, resulting in price increases, which further results in its potential adulteration. A lower quality FM dramatically affects the efficiency of the treatment, and harms the business reputation, consequently affecting the business viability. Therefore, finding new alternatives to replace it, or at least, reduce reliance on it, are critical in such a competitive market. In this study, Black Soldier Fly Meal (BSFM) was considered as an alternative proteinaceous feedstuff, and not as a pure substitution product as in chapter 5, allowing a reduction in fish meal inclusion in a formulated diet.

In order to create isonitrogenous and isoenergetic diets, satisfying the nutritional requirements of the tilapia fry (NRC, 2011; Jauncey, 1998; Hayashi *et al.*, 2002) the formulation of the different dietary treatments had to use different inclusion rates of defatted and whole meal BSFM. The lipid content of the whole BSF meal (21.76% on a dry matter basis) was a constraint limiting its inclusion rate, consequently, levels above 50% were not possible (otherwise the lipid content of the overall diet would have been too high), consequently, as the defatted meal had a lower protein content (12.21% on a dry matter basis) it allowed a greater inclusion (from 50 to 90% substitution with FM). The fact that the lipid content of each dietary treatments was similar –kept at 12% on a dry matter basis- helped to ensure a similar floatability between dietary treatments. In a similar study conducted by Devic (2016) the high lipid content of the BSF whole meal used (41.5% DM basis) resulted in a coagulated diet, quickly sinking into the water, therefore affecting directly the feed intake by the fish (as they feed on the surface).

Very few females were identified, indicating that the sex-reversal process was generally very efficient across treatments, with high proportion of males recorded (98.41-100%). No significant differences for the hormone-treated diets and the positive control were observed. These results suggest that the fry received a sufficient dose of MT during the 21-day process and that neither the quality nor the type of feed used compromised the feeding. The sex-reversal process is considered effective for cage-production systems

when at least 96% males are produced (Mair & Little, 1991), and 98% in pond production (Vera Cruz & Mair, 1994). In Thailand due to a competitive market, the aim is to produce at least 99% males, and results below this are considered as low quality (Bhujel, 2014; Turner, 2015). In our case, the percentage of males obtained in the control was 99.2%, and none of the other dietary treatments significantly affected the percentage obtained. It can be concluded that they all satisfied the required quality for commercial operations. The only significant difference was for the negative control (with no hormone added to the feed), were the proportion of males was 92.17%. This result is interesting, because a normal proportion of males around 50% was expected. Similar, to the previous experiment (chapter 5) where 64% males were observed in the control, this was most likely due to residual hormone circulating in the pond. While this skewed result was expected, it was not possible, as a matter of consistency and rigor, but also technically (which is part of the constraint inherent to working on a commercial fish farm) to specifically isolate this treatment in another pond: the water quality, temperature or other exogenous factors could have influenced the result. The use of androgenic hormone in the feed, and the residual effect in the water was tested by several authors. In an outdoor tank, Phelps *et al.* (1992) used small hapas (separated by 30 cm apart) in static water in a 20 $m^2$ tank containing fish given a MT (or fluoxymesterone) treated-feed or a non-treated feed. The treatments were randomly assigned within the tank but there was no evidence of hormone leaching affecting the sex ratio of non-treated fish. Soto (1992) used a similar setup, with eighteen 0.12 $m^2$ hapas distributed in a 20$m^2$ tank to evaluate the androgenic potential of mestanolone to sex reverse Nile tilapia fry. No evidence of non-hormone treated fish having a skewed sex ratio (even though fish were surrounded by hapas containing fish being fed a hormone treated feed) was observed. However, Abucay *et al.* (1997) found that reusing water that had held tilapia fry during a 25-day MT treatment could alter sex ratios. It was later highlighted when a second group of fish were stocked into the same water and given a non-hormone treated feed, that the sex ratio was skewed. They also found that when "all" female fry were stocked into a cage in an aquarium and when MT treated feed was added to the bottom of the aquarium, where the fish had no access to it, the sex ratio became skewed to males. Therefore, it is plausible that unmetabolized MT and its remains, through uneaten feed, can accumulate in the water of recirculating systems. The degree of accumulation appears to depend on the frequency and dose of MT administered to the target fish. Effects of the excreted metabolites and

unmetabolized MT on non-target fish held in the same system could range from elevated serum MT levels to altered sex ratios (Abucay *et al.*, 1997).

The survival of the fish was not significantly affected by the different dietary treatments. It was a key factor, for obvious economic reasons, but also because during the sex-reversal process, a high density of fish is essential as it creates a crowding effect, triggering an active feeding response (Phelps & Popma, 2000). Also, it reduces hierarchical interactions between fishes, resulting in a more uniform population, and therefore, resulting in a more uniform hormone intake for the fry (Little, 1991; Vera-Cruz & Mair, 1994). The survival rates were better than those reported in the literature where, for fry stocked at the same density (*i.e.* 6 fish/litre), Vera Cruz & Mair (1994) reported a survival of 76.1%. This is most likely due to improved fry-breeding techniques. This could explain why the negative control (dietary treatment containing no hormone) displays no significant difference with the hormone treated diets. The presence of the hormone usually stresses the fish physiology, and increase mortality (Buddle, 1984), therefore, rates around 75-80% survival are usually expected. Over the years, hatcheries have drastically improved their production process, decreasing fry mortality. Additionally, to decrease any potential source of stress, fish handling was kept to a minimum, therefore batch sampling was not done every five days (like in Chapter 5), to assess the growth rate of the whole batch (as in Chapter 5) but only at day 10 and day 21 (end of the experiment).

The average fish weight was within the normal range usually accepted at the end of a sex-reversal process, between 100 to 500 mg (Popma & Green, 1990; Popma & Lovshin, 1995). The fish fed a black soldier fly whole meal treatment, and the negative control (pure FM without MT), displayed no significant differences to the positive control (0.19g). The final fry weight was comprised between 0.16 to 0.25g. The only significant differences were observed for the fish fed black soldier fly defatted meal treatment at an inclusion rate of 70-80-90% which displayed a lower average weight of 0.13g. This difference in average final weight for the fry was therefore noticeable in the final harvest, where only these treatments were significantly different with the control, displaying final harvest of 2 831, 2 948 and 3 314g respectively. The whole meal BSF treatments, the controls, and the BSF DF (of 50 and 60%) displayed ranges between 4 683.25g and 5 369.50g.

Consequently, these observations reflect differences in mean growth rates (& the linked attribute of size heterogeneity). The grading process showed disparities for the BSF DF

treatments, displaying mostly small fish, and very low medium/large fish proportions. While it was significantly different for the large fish for all BSF DF treatments, only the inclusion rates of 70-80-90% showed significantly different rates for small and medium fish with the control. Therefore, as the proportion of smaller fish was significantly higher for the BSF DF inclusions at 70-80-90%, the average weight of the fish was smaller, and so was the final harvest weight. The relative abundance of small, medium and large fish is an important factor to consider, as larger fish tend to display more aggressive behaviour toward smaller fish, which can lead to opportunistic cannibalism and consequently increased levels of mortalities (Dambo & Rana, 1992). In addition to this, they also require more MT-treated feed (as they are larger, they will therefore consume more feed) which is an expensive input considering the price of the hormone (around 2.8 USD/g) and the quality of the feed. The use of a green water in the pond usually helps to prevent such aggressive behaviour, which usually worsens when the water is clear (Devic, 2016). As all treatments, regardless of the dietary allocation, displayed a majority of small (for the BSF DF treatments) or medium (all other treatments) fish, this size hierarchy problem did not impact the survival rates.

In Thailand, tilapia fry of 250 mg are considered to be the commercial standard in commercial hatcheries, and most desired market size because of a lower farm-gate price, and a better tolerance to transportation (Nasr-Allah *et al.*, 2014; Turner, 2015) compared to larger fry. During the sex reversal process, factors such as high density and restricted feed rations are usually applied to control the growth of the fish, leading to more uniform distribution, and evenly distributed small/medium size proportion. The fish can still later, if necessary, be grown to market size during additional nursing phases using a cheaper feed (Little & Hulata, 2000).

At the end of the sex-reversed fry production, when sold, the fish are subjected to handling, transportation and transfer to other ponds. This creates a major source of stress, which can be sometimes translated in a high mortality rate. Resistance to stress is therefore important, and stress tests can be a useful tool to evaluate the quality of larvae and fingerlings production (Dhert *et al.*, 1992). Challenge tests are often conducted on-farm to assess the quality of the fry, ensuring that the fry are good quality and therefore good value for money (Macniven, 1999). According to Koven *et al.* (2001), feeding is crucial at this stage, since a better quality diet may impact fish survival rates after their transfer, reducing the stress effects caused by this handling. The assessment of the stress

resistance after only 24h, was proven sufficient in this experiment, as only very slight variations occurs after 7 days.

While the control diet displayed an acceptable result to the 7-minute air-exposure stress test with 83% survival, the negative control (without MT) shows a significantly lower survival, dropping to 50.75%. While is known that the presence of MT hormone stresses the fish physiology (Buddle, 1984), it is possible that the animal could get accustomed to a stressful environment, and shows an enhanced resistance toward future sources of stresses.

One of the emerging issues about BSF meal is the fact that the chitin and some specific fatty acids (C12:0) might provide a protective and immunostimulant effect, increasing the resistance of the fed-animals (Ravi Kumar, 2000; Mastan, 2015; Spranghers *et al.*, 2017; Gasco *et al.*, 2018). With respect to the chitin, while the quantities were extremely low (as the larvae were harvested at the white larvae stage – see chapter 4) it has been reported to have a positive effect (even in very low quantities) on the functions of the immune system, and contains antimicrobial properties (Rinaudo, 2006) and acts as a short term immunostimulant (Ravi Kumar, 2000; Mastan, 2015). Similarly, the high levels of C12:0 medium-chain fatty acid, lauric acid (Spranghers *et al.*, 2017) contained in the BSF fat, are known to have strong antiviral and antibacterial effect (Devi & Kim, 2014; Gasco *et al.*, 2018) and several authors suggested that it may have probiotic effects on the microbiota of fish and antibiotic effects on gastrointestinal disease-causing bacteria (Skrivanova *et al.*, 2006; Spranghers *et al.*, 2017; Gasco *et al.*, 2018), improving the resistance of the fed-animal.

Both of these effects combined may have helped to boost the fish immune system and increase their resistance toward stress. In our experiment, the BSF WM treatments display similar stress resistance rates than the control, with the sole exception of the last treatment (BSF WM 50%), which showed a significantly higher resistance to stress (94% instead of 83% for the control). This result suggests that it is plausible that the antimicrobial effects of the fat contained in the larvae (for both types of meal, the larvae were harvested at the same time, hence they had a similar chitin content) may have conferred a protective effect to the fish, giving a neat advantage for the use of BSF WM as a protein substitute in tilapia fry diets. When using defatted meal, for the BSF treatments with an inclusion of 60%, 70% and 80%, they displayed significantly lower results (57.50, 51, 67.25% respectively) the control. The BSF DF 50% and BSF DF 90% showed no difference with the control. For the lowest BSF DF inclusion (BSF DF 50%), this stress resistance might

be due to the presence of FM, still present in large quantities, while for the BSF DF 90% a protective effect might have been conferred by the sufficient presence of chitin and BSF fat, in the BSF DF feed. The 3 treatments between (60-70-80%) could therefore be a transition in resistance between *appreciable* quantities of fishmeal (BSF DF 50%) and a *large enough* quantity of BSF DF (BSF DF 90%) to potentially confer a protective effect. Combining several feed ingredients into a formulated diet, in addition to allowing a greater flexibility in terms of feed formulation and adaptation to market volatility, leads to better performance than any single source due to an improved nutrient balance provided by a synergistic effect (NRC, 2011; Parker, 2011). It can also reduce the effects of a poorer quality product. In the present experiment, the BSF WM, displayed better results than the BSF DF meal in terms of size, proportion of fish class, and stress resistance. Besides being quicker to produce, it is also cheaper than producing a defatted meal due to all additional industrial costs involved. It is generally acknowledged that defatted products can be stored for longer periods than whole meals, which can deteriorate rapidly under tropical conditions. This constraint to the shelf life of these products is currently being investigated and further developed at Entofood in Malaysia (Emilie Devic, Pers. Comm., 2018). However, the possible antimicrobial effects of fat (C12:0) contained in the BSF whole meal, still needs to be investigated thoroughly, but could be an important value-added factor for this type of feedstuff, not only in tilapia fry, but in animal feeds in general (Spranghers *et al.*, 2017; Gasco *et al.*, 2018).

Larviculture is usually considered to be a technical bottleneck phase in the production of fish species due to a lack of technical development and lack of information regarding the best handling procedures to be used (Santos & Luz, 2009). One of the main advantages that Nile tilapia has over other fish species, is that it can be fed with a formulated diet from first feeding (Sanches & Hayashi, 1999; Toyama *et al.*, 2000; Hayashi *et al.*, 2002; Meurer *et al.*, 2005; Boscolo *et al.*, 2008), thus facilitating its early rearing. While the poor-quality seed has been identified as a very significant constraint on the development of the freshwater fish culture industry in Asia (Little *et al.*, 1999), improvements made by commercial hatcheries enabled its continued development.

While, the hatchery technology used in Nam Sai farms and in several hatcheries worldwide is economically viable, it is dependent on the quality of their main feed product: fishmeal. With prices rising and increasing risks of adulteration, hatcheries are being forced to look for viable alternative feedstuffs, allowing them to be more flexible and react quickly to market prices as a consequence. The Black Soldier Fly meal can be

a potential alternative for use in a sex-reversal diet, without affecting the percentage of males, survival, growth, total harvest or fish class size. Beside improving local sanitation, creating local employment, this new feed product would led to the creation of a quality consistent product, potentially cheaper than the imported FM, while improving the quality of the feed during the sex reversal process, ensuring that the quality of their fry is consistent. However, considering the production capacity at Nam Sai Farms, and the use of the diet using 50% BSF WM (which displayed the most promising results) a quantity of about 9 tons would be required monthly. The availability of this product is still limited and is far from matching the demand yet (Bhujel, 2013). While some progress has recently been made in the insect industry, this is not yet a credible alternative for a large scale hatchery but might become it in the near future when the economy forecast will allow, and when its production technology will have improved.

# CHAPTER 8: GENERAL DISCUSSION AND FUTURE PERSPECTIVES

## 8.1 General

In the present work, we assessed in *on field* researchs the potential of using the Black Soldier Fly (BSF) larvae produced from a sustainable feed source in pilot-scale operation system in tropical countries as a strategic feed ingredient for tilapia fry. Based on a contextualised research in Ghana (Greater Accra Region), a substrate sustainability index was developed to help selecting a breeding substrate for the BSF larvae, meeting sustainable, safe, and cost-effective criteria (Chapter 3). Then the trade-off between nutritional quality and yield characteristic of BSF larvae at different life stages (white larvae or pre-pupae) was studied to select the optimal harvesting time to produce the maggot meal (MM) (Chapter 4). While it is clear that MM currently has a very limited capacity to meet the ever growing demand for aquafeed ingredients, there is clear need for more targeted, strategic use of this new feed source. In this realm the potential of MM as nursery feed for Tilapia fry (*Oreochromis niloticus*) during their sex reversal period was assessed in two contexts. The first one, issued in Ghana, tested the effect of a basic substitution of both commercial feed and fishmeal (FM) by increasing inclusion level of MM (Chapter 5). Following this, a digestibility study was conducted in Thailand, on the fry, to investigate the apparent digestibility coefficient of two types of MM produced in Malaysia, from kitchen waste: a whole MM and a defatted version (Chapter 6). Built upon the obtained data, isoenergetic and isoproteic diets were formulated using both types of MM to assess its efficiency as a protein replacement of FM for tilapia fry during their sex-reversal period.

## 8.2 Substrate selection and potential waste remediation service

The selection of the substrate is a critical step toward the economic viability and safety of a Black Soldier Fly production system. As highlighted in Chapter 3, several key variables have to be taken into account during the selection process of a potential substrate: its co-location with the production site, quantity, seasonality, purchasing cost, risks involved and its impact on larvae growth. One of the arguments highlighted in the

Chapter 3 was how to choose, and what is considered a *real waste*. In the realm of a sustainable service, it only make sense to feed the BSF larvae with unused organic waste. The feeding substrate is one of the main constraints for development of the BSF industry in the Western world. The regulation for the use of potential substrates is yet too strict: at the moment, only agricultural by-products, or refused human food, can be used. However, these waste streams are already used in the pig industry as feed (even if it is under strict controls following the last foot and mouth outbreak). While being non sustainable, as the initial substrate is already used, entering in this competition with another livestock sector means that the purchasing price of the substrate would be probably too high to compete with. Consequently, the Black Soldier Fly bioconversion may be more interesting in Muslim countries as there is no –or few- pig industries. This lack of concurrence, allows to free these waste streams, rending them available for the production of maggots.

One of the most common argument against the BSF conversion is the lack of logic behind feeding the larvae with 'feed' that can otherwise be used to feed directly livestock. While it is true in most cases –stressing the need to identify properly a potential substrate- for some the BSF bioconversion could still be of interest. For example, if the feeding substrate, does not display a specific amino -or fatty- acid profile, or have a high fiber content, the larvae bioconversion can act as a nutritional 'make-up' and turn this unsuitable feedstuff into a more nutritionally-favourable feed for livestock. The BSF larvae can also concentrate proteins and lipids from the feed source, and convert a heterogenous substrate into larvae, a more *homogenous, nutrient-dense*, product rendering it more easily usable as a feed ingredient. Finally, it can be of interest to 'recycle' the nutrients of highly perishable substrates, by converting them into stable and storable maggot meal.

BSF bioconversion can also reduce the costs of disposal of organic wastes and improve sanitation by the provision of a waste remediation service while producing both a feed ingredient and high quality organic fertiliser. No other insect comes close to closing so many material flow loops and creating a self-sustaining food system as the BSF.

## 8.3 Maggot meal quality

Once a proper substrate was identified to produce the larvae, the next main research question was when to harvest the larvae ? The BSF larval goes through 5 instars which

can be referred as “white larvae”. This usually last for 10-12 days (at 30°C). At this stage the larvae is extremely photophobic (Everest Canary, 2009) and prefers to stay within the substrate rendering its harvest difficult. The last instar ($6^{th}$) is often referred as “pre-pupae”, corresponding to the pupariation stage, when the larvae cease feeding to complete immobilisation and reduction in length. With this physiological change, a behavioural change arise: the larvae escape the substrate, looking for a suitable, dry and shady place to safely pupate. At this stage, it is very easy to collect the larvae, as a simple 30° angled slope, ending in a trough is enough to collect the insects. Understanding the evolution of the nutritional composition of the BSF during these two stages (white larvae vs pre-pupae) is essential for a successful commercialisation. In Chapter 4, we demonstrated that as the weight plateaued during these 2 stages, the biomass produced for either “white larvae” or “pre-pupae” was similar. Then, while the pre-pupae stage was more proteinaceous (33.54% at 720 degree day old, dry matter basis, with corrected levels of proteins) than the white larvae (26.21% at 300 degree day old), the level of chitin (139.09g/Kg) was too high for the utilisation of the meal as feed. The β1,4 bond in chitin is indigestible by most fish species (Rust, 2002 *in* Sanchez-Murros *et al.*, 2015) and, could even reduce growth performance by influencing the feed intake, availability and digestibility of nutrients, including proteins (Longvah *et al.*, 2011) or lipids (Kroeckel *et al.*, 2012). The presence of chitin in the pre-pupae is therefore a major hindrance for its use. While some studies suggest that the chitin might act as short-term immunostimulant, and can display probiotic effects, this effect is only demonstrated at very low quantities (Ravi Kumar, 2000; Esteban *et al.*, 2000; Vahedi & Ghodratizadeh, 2011; Van Huis *et al.*, 2013; Mastan, 2015; Spranghers *et al.*, 2017), with a magnitude order of dozens of milligrams per kilogram of feed.

Some solutions could be found to *later* remove the chitin, purify it and use it for its antioxidant, anti-inflammatory properties, or as novel drug delivery-media and plastic source (Park & Kim, 2010) but these solutions are costly, still at the development stage, and yet to be up-scaled. The removal, without any further purification goal, could be achieved, by using mechanical methods such as cold-pressing (as the chitin is mainly deposited in the outer skin of the larvae) or chemically, with the addition of chitinase in the final meal, helping to improve its digestibility.

Therefore, as a matter of efficiency, for the moment only the white larvae stage should be used for commercial purposes. While it is hard to harvest the larvae, several systems have emerged in the evolving large-scale commercial sector such as rotating drums,

vibrating screening mesh, or the use of a specially formulated feed, allowing an easier separation of the substrate and the larvae by physical screening.

As the quantities produced are low, a strategic use of this meal has to be found to maximise its efficiency. In the present study, the maggot meal (MM) was used as a fish meal substitute in tilapia fry diets during their sex-reversal time. In chapter 5 the MM was used as a direct substitution product to FM and commercial feed ("super starter feed") in the context of Ghana, while in Chapters 6-7 it was used as a protein alternative source to FM in fully-formulated diets. In the present Thesis, 3 different types of MM were assessed as potential feed ingredients for tilapia fry.

The nutritional profile of each type of MM is presented in the Table 8.1. As stated before, the lipid content and fatty acid profile were strongly influenced by the substrate used to grow the larvae, but remained acceptable for tilapia fry (Jauncey, 1998). In addition, the relatively low levels of lipid in the BSF whole meals did not affect the flotability of the meal (Chapters 5 & 7), as reported by Devic (2016) where the resulted feed coagulated on the surface of water before sinking rapidly. In line with Barroso *et al.* (2014) the protein content of the BSF meals were satisfactory (above 40%, and even reaching 57.09% for the defatted meal) and displayed a similar amino acid profile to FM (Chapters 5,6,7).

**Table 8.1:** Nutritional composition of the BSF meal used in this study, compared to fishmeal.

| | **Maggot meal (Ghana)** | **BSF whole meal (Malaysia)** | **BSF defatted meal (Malaysia)** | **Fishmeal (Ghana)** | **Fishmeal (Thailand)** |
|---|---|---|---|---|---|
| **Substrate** | Fruit Waste | Kitchen waste | Kitchen waste | - | - |
| **Proximate composition (%)** | | | | | |
| Dry matter | 92.45 | 91.73 | 97.63 | 92.85 | 98.88 |
| Crude protein | 43.19 | 47.77 | 57.09 | 53.11 | 60.27 |
| Crude lipid | 15.45 | 21.76 | 12.21 | 10.67 | 8.49 |
| Ash | 15.95 | 9.83 | 10.16 | 10.95 | 24.61 |
| Gross energy (MJ/Kg) | 18.98 | 22.2 | 21.5 | 18.9 | 18.5 |
| **Essential amino acids (g/100g xN)** | | | | | |
| Histidine | 1.33 | 1.21 | 2.13 | 1.96 | 1.63 |
| Threonine | 1.75 | 1.52 | 2.43 | 2.58 | 2.45 |
| Arginine | 2.06 | 2.34 | 3.25 | 3.36 | 3.34 |
| Valine | 2.69 | 2.51 | 3.89 | 2.94 | 2.78 |
| Methionine | 0.78 | 0.75 | 1.21 | 1.59 | 1.45 |
| Lysine | 2.75 | 2.19 | 3.62 | 4.29 | 3.96 |
| Iso-leucine | 1.85 | 1.85 | 2.93 | 2.48 | 2.32 |
| Leucine | 2.99 | 2.83 | 4.02 | 4.36 | 3.89 |
| Phenylalanine | 1.88 | 1.73 | 2.62 | 2.46 | 2.19 |
| **Fatty acid composition (g/100g of total % fatty acid)** | | | | | |
| 12:00 | 2.00 | 6.8 | 3.23 | N.D. | N.D. |
| 14:0 | 0.57 | 2.08 | 0.89 | 0.35 | 0.41 |
| 16:0 | 2.35 | 5.84 | 2.53 | 2.39 | 1.78 |
| 18:0 | 0.32 | 0.76 | 0.34 | 0.90 | 0.46 |
| ***Total saturated*[1]** | **5.81** | **16.45** | **7.45** | **3.84** | **2.89** |
| 16:1n-7 | 0.47 | 0.83 | 0.29 | 0.48 | 0.52 |
| 18:1n-9 | 1.66 | 5.32 | 2.45 | 1.55 | 1.19 |
| 18:1n-7 | 0.46 | N.D. | N.D. | 0.30 | 0.13 |
| ***Total monounsaturated*[2]** | **2.82** | **6.64** | **2.78** | **2.60** | **2.32** |
| 18:2n-6 | 1.18 | 2.43 | 1.29 | 0.15 | 0.21 |
| ***Total n-6 PUFA*[3]** | **1.19** | **2.56** | **1.26** | **0.59** | **0.53** |
| 18:3n-3 | 0.09 | 0.17 | 0.09 | 0.05 | 0.09 |
| 18:4n-3 | 0.06 | 0.08 | 0.01 | 0.05 | 0.04 |
| 20:5n-3 | 0.01 | 0.12 | 0.09 | 0.52 | 0.53 |
| 22:5n-3 | N.D. | N.D. | N.D. | 0.09 | 0.09 |
| 22:6n-3 | N.D. | 0.03 | 0.00 | 2.02 | 1.12 |
| ***Total n-3 PUFA*[4]** | **0.16** | **0.42** | **0.21** | **2.82** | **1.72** |
| ***Total PUFA*[5]** | **1.36** | **3.04** | **1.43** | **3.54** | **2.32** |

Values are presented 'as is'. [1]includes 15:0; 17:0 ; 20:00; 22:0 and 24:0; [2] Includes 14:1; 16 :1n-9 ; 17 :1 ; 20 :1n-11 ; 20 :1n-7; 22 :1n-9cis; 24 :1-9; [3] Includes: 18:3n-6; 20:3n-6; 22:4n-6; [4] Includes 20:3n-3; [5] Includes 16:2; 16:3; 16:4. N.D.: not detected.

In our system (see Chapter 3, Fgure 3.3) the production of the fresh maggots represented about 35% of the initial weight of the substrate, and contributed to more than half of the bioconversion outputs. Together –*fresh*- larvae and compost, contributed to a reduction of the initial substrate by 41.95%. The larvae contained around 60% moisture, and

therefore when dried, out of 500 Kg of substrate, only about 70Kg of (dried) MM was produced. As yet no viable large-scale production systems have been commercialised, it is necessary to have a strategic use of this new feedstuff. Numerous studies (see Chapter 1.4.5.1) have demonstrated the suitability of BSF meal as feed ingredient for fish. Nevertheless the interspecies and life-stage variability of the results make a generalisation difficult. Low-trophic and herbivorous species like Nile tilapia (*Oreochromis niloticus*) are more flexible in terms of feed ingredients and require less FM than carnivorous fish species (Tacon & Metian, 2015). Nevertheless FM is usually included in tilapia fry diets because it is considered as an excellent source of essential nutrients and improve the feeding response. In order to optimise the feed efficiency, we decided to focus our experiments on tilapia fry during their sex-reversal period. Tilapia fry are considered being a critical stage in the intensive farming process, and their nutritional requirements are usually high and specific. The development of hapa-based broodstock management, allowed the collection of same-age tilapia eggs and yolk-sack larvae, to enable their transformation into males through a feed impregnated with Methyl-testosterone (Little *et al.*, 1995). The successful sex-reversion of tilapia fry relies on a combination of good farming and feeding practices. Quality and palatability of the hormone-treated feed are the main quality to ensure a maximized daily intake of hormone, although pure FM is preferred to produce monosex-tilapia (Bhujel, 1997), commercial feed (using high levels of FM) formulated for monosex-tilapia are also widely used (Phelps, 2006).

Building on this, it was assumed that MM could reduce the reliance on marine products, usually of high cost (like in Ghana, Chapter 5) or potentially being adulterated (like in Thailand, Chapters 6 &7). In this optic the MM could be considered as a locally sourced, high quality feedstuff for tilapia fry during this key-period. All experiments were conducted on-farm to demonstrate the commercial relevance of the results in two different production contexts:

**In Ghana**, tilapia are mostly produced in cages (85% of total production according Asiedu *et al.*, 2015) near the Volta region. While monosex tilapia is widely used, 96% males after a sex reversal process is considered to be an acceptable rate. Tilapia hatcheries are constrained by expensive and poor quality fish meal or locally-sourced commercial feed (more widely used in local hatcheries). Therefore the MM could be a valuable alternative feedstuff, being locally produced out of an unused waste stream and provide a quality reliable, and potentially cheap feedstuff. Instead of having to formulate a diet, it could be just used to substitute FM or expensive commercial feed. Combining several

feed ingredients into a diet usually leads to better performance than any single source due to an improved nutrient balance provided by a synergic effect (NRC, 2011; Parker, 2011), and could subdue the effects of a lower quality product. In this scenario, as the quantity requirements are low for a fry production, a small to medium-scale MM factory would be sufficient. The MM produced from the system highlighted in chapter 3 met all requirements in terms of protein, lipids, amino acid and fatty acid profile for Nile tilapia fry (Ogunji *et al.* 2005; Diogenes *et al.,* 2016). Therefore, while the diets were not necessarily isoenergetic nor isoproteic, they were sufficient to cover the nutritional requirements of the fry stage. The proportion of males nor the survival were affected by the dietary treatments. This result suggests that the fry received a sufficient dose of MT during the 25 days process, meaning that the quality nor the type of feed compromised the feeding. The only significant difference observed was for the growth rate of the commercial-feed fed fish, being better. This result can be explained by the higher protein content of the commercial feed compared to the MM or the FM, but it is not an issue as the fish can be easily grown to market size later using a proper –and cheaper- feed at the fingerling stage (Little & Hulata, 2000). As we highlighted, tilapia fry can be fed with diets containing either half commercial feed and MM or 100% MM without affecting the percentage nor survival of males obtained. The results obtained are within the acceptable range in Ghana (*i.e.* at least 96% males) for cage production (Mair & Little, 1991), but would not be acceptable for a pond-production system, like in Thailand, were at least 98% would be required, (Vera Cruz & Mair, 1994).

**In Thailand**, the sex-reversal industry is now the standard for reproduction control in tilapias, and poor-quality juveniles affect directly the hatchery reputation, and consequently its economic viability. As the tilapia production is done mostly in ponds, the presence of females can impact severely the fish population in the pond (contrary to a cage-based production where fry and fingerlings could escape through the mesh lowering this risk). Their presence leads to stunted growth of the stocked fish, and smaller and inconsistent size of fish at harvest. Consequently, in addition to the fact that monosex quality seeds are expensive, near 100% males is expected at the end of the sex-reversal process. Therefore, in this scenario the price of the FM is not so much an issue by itself (as long as it is kept under an acceptable threshold price) but its adulteration can lead to dramatic effects: lowering the palatability of the feed, decreasing its intake by the fish, thus decreasing the hormone absorption and lowering the percentage of males obtained.

In Chapter 6 we highlighted that the quality of the FM used in our experiment was lower than expected. Upon investigations, it appeared that it was adulterated with probably soybean or saw dust, lowering its digestibility, and effectiveness during a sex-reversal process. While large-scale hatcheries can test regularly the quality of their feedstuff, it is not the case for small to medium businesses' relying only the manufacturer's guidance. In both case, they could gain from using MM: either to obtain a locally source protein source with a constant quality, or to decrease the proportion of FM in their sex-reversal diets, being replaced by a more quality-constant product. In this optic we decided to test the inclusion of BSF meal as a protein substitute to FM. As the basis of diet formulation resides in having reliable and accurate digestibility measurements, they were tested *in vivo* on the fry. In chapter 6 the apparent digestibility coefficient (ADC) of the different feedstuffs highlighted that both BSF defatted meal (BSF DF) and whole meal (BSF WM) were highly digestible (85%, 95% respectively), even more than FM (59%) regarding the $ADC_{dry\ matter}$ and $ADC_{proteins}$ (80,84 & 71% respectively). While the experiment was the first of its kind on tilapia fry, the obtained values were within the range from *in vitro* studies (Gutiérrez *et al.*, 2004; Bosh *et al.*, 2014). Built upon these data 10 isoproteic and isoenergetic diets were formulated using either incremental levels of either BSF DF or BSF WM to substitute FM. This allows a greater flexibility in terms of feed formulation while decreasing the quantity of MM required: as it happens to be, instead of focusing on the quantity, the accent was placed on the quality of the feedstuff, lowering the proportion of potentially poor quality FM. As all diets were isoenergetic, the lipid content of the feedstuff was a significant constraint in the design: the BSF WM containing high lipid levels (21.76% DM basis) its inclusion into the different formulations had to be lower than the inclusion of BSF DF. Consequently, the incremental protein replacement for BSF DF went from 50 to 90% replacement of FM, contrary to the diets made with BSF WM ranging from 20-50%. As we discovered, no difference on the percentage of male obtained was detectable, indicating that all fish, regardless their diets, received a sufficient dose of methyl-testosterone. Similarly the survival rate was not affected. However, the average final weight of the fish, total harvested weight at the end of the process, and relative fish abundance (skewed in favour of smaller fish) showed that the BSF DF dietary treatments at 70-80 and 90% protein replacement affected these parameters. The relative abundance of small, medium and large fish is an important factor to consider, as larger fish tends to display aggressive behaviour toward smaller ones, which can lead to opportunistic cannibalism and consequently increase mortalities in the

hapa (Dambo & Rana, 1992). The medium size fish are preferred, as in Thailand tilapia fry of 250 mg are considered to be the commercial standard in commercial hatcheries, and most desired market size because of a lower farm-gate price, and a better tolerance to transportation (Nasr-Allah *et al.*, 2014; Turner, 2015) compared to larger fry. The fish can still later –if necessary- be grown to market size during additional nursing phases using a cheaper feed (Little & Hulata, 2000).

The last important take-up point from this experiment is regarding the stress resistance of the fish. It is significantly improved when the fish were fed a diet containing 50% BSF WM as a protein source, and lowered significantly when fed diets containing 60, 70 and 80% BSF DF. Regarding the BSF DF inclusion, the stress resistance is only affected for the medium values (diets containing 60, 70 and 80%) but not for the low value (50% inclusion) nor the high one (90% inclusion). This can be explained by the fact that for the lower ones, the presence of FM is still present in large quantities in the mix (around 25% inclusion) and gain from a protective effect for the 90% treatment probably by the sufficient presence of chitin and BSF fat, in the BSF DF feed. The 3 medium treatments (60-70-80%) could therefore be a transition resistance between a still large quantity of fishmeal (BSF DF 50%) and a large enough quantity of BSF DF (BSF DF 90%) conferring a protective effect. The presence of both chitin and specific saturated fatty acids (C12:0) in sufficient quantity in the MM, might have helped to boost the stress resistance of the fish. The chitin is known to confer a positive effect on the functions of the immune system, and displays antimicrobial properties (Rinaudo, 2006) acting as a short-term immunostimulator (Ravi Kumar, 2000; Mastan, 2015). Saturated fatty acids, especially lauric acid (Spranghers *et al.*, 2017), are known to have strong antiviral and antibacterial effects (Devi & Kim, 2014; Gasco *et al.*, 2018), acting as a probiotic on the microbiota of the fry and displaying antibiotic effects on gastrointestinal disease-causing bacteria (Skrivanova *et al.*, 2006). Both of these effects combined would have helped to boost the fish immune system and increase its resistance toward stress. Research is preliminary in this field, but if these hypothesis are confirmed, it would give a strong advantage for the use of insect meal, potentially reducing the necessity to use in-feed antibiotics (Van Huis *et al.*, 2013) in livestock feed. Moreover, increasing stress resistance of the fish has obvious economic consequences for a hatchery.

Upon these observations, it is therefore plausible to use BSF meal as a protein replacement to FM in tilapia fry hatcheries. By using it in a fully-formulated diet, it increases flexibility toward market price volatility while lowering its quantity. Therefore,

it enables the hatchery to play on the quality of the MM rather than its quantity, maximising its potential. Considering the production capacity of tilapia fry in Nam Sai Farms, and the use of the diet using 50% BSF WM (which displayed the most promising results) a quantity of about 9 tons of BSF WM would be required monthly to perform the sex-reversal process. This quantity is fairly low and while it is yet to be achieved today, it is a plausible alternative in the near future for medium to large scale operation systems.

## 8.4 Biofertiliser production

Another by-product generated from the bioconversion process is the frass. It consists of undigested substrates residues thoroughly mixed with insect excreta. Compared to the initial substrate offered to the larvae, the frass is odorless, homogenous, friable and its moisture content is generally reduced by half (Čičková *et al.,* 2012c; Zhu *et al.,* 2012; Wang *et al.,* 2013). Dry matter and volume reductions of between 50-80% have been reported for animal manures, organic waste, faecal sludge or agricultural by-products treated with larvae, leading to a material containing soluble and available nutrients such as nitrogen, phosphorous, potassium and calcium available for the plants (Calvert, 1979; Newton *et al.,* 2005b; Myers *et al.,* 2008; Diener *et al.,* 2011a; Čičková *et al.,* 2012c; Gobbi *et al.*, 2013; Lalander *et al.*, 2013; Wang *et al.*, 2013; Caruso *et al.*, 2014). In our system, the quantity produced was only limited (22.7% of the initial substrate quantity, on a wet weight basis, or 85.3% on a dry matter basis) representing less than half of the total (fresh) outputs (39.5%). This was only due to the substrate used –fruit waste- containing a high moisture content (73.4%). In other systems it can go up to 80 to 95% of the total fresh outputs (*i.e.* larvae + frass) (Calvert, 1979; Čičková *et al.,* 2012b; Wang *et al.*, 2013; Caruso *et al.,* 2014). Therefore it is important to find a suitable application for this non-negligible by-product (Van Zanten *et al.*, 2015).

In most studies, frass composition was comparable to "classical" organic fertilisers owing to their optimal levels of N,P,K (Choi *et al.,* 2009; Zhu *et al.,* 2012; Wang *et al.,* 2013; Lalander *et al.,* 2014). The results of the agronomy trials conducted by Devic (2016) showed the positive effects on the use of frass as a biofertiliser, for plant growth and soil fertility. Not only the plants performed better, but the structure of the soil was improved, being more aerated compared to the unamended soil or soils improved with NPK inorganic fertilisers. This is a characteristic of organic soil conditioners that usually

encourages the development of a root system and therefore the growth of the plants (Singer *et al.*, 1998). However, like any other organic fertilizer, the release of nutrients is a slow process, and consequently its use is not suitable for a newly treated soil or for fast-growing crops such as maize.

In addition to improving the soil structure, BSF frass seems to display a protective effect against chitin-based organisms such as insects, fungus or nematodes (Vickerson *et al*., 2015). This characteristic, demonstrated on the first time with BSF frass- is attributable to the low quantity of chitin remaining in the frass. During their growth, the larvae molts several times, leaving a chitin-rich exuvia in the substrate. The addition of chitin in an organic fertilizer has been studied before, but only on little quantities, either pure or from lobster shells (for Ilangumaran *et al.*, 2017). Fragments of chitin or chitosan have an eliciting activity with the plant, leading to a variety of defense response in host plant in response to a 'potential' microbial infection, like the accumulation of phytoalexins, pathogens related proteins, proteinase inhibitors, lignin synthesis and callose formation (El Hadrami *et al.*, 2010). Therefore, based on this properties, BSF frass could provide a synergetic effect, by conferring a protective effect strengthening plant defense, reduce the impact of disease on the yields and quality of the crops (Ramirez *et al.*, 2010; Sharp, 2013; Deborde *et al.*, 2016; Orzali *et al.*, 2017).

While reducing the composting time from almost 4-6 months to 15 days, decreasing the green house gases emissions by 47 times (Mertenat *et al.*, 2019) and considering the evolution of inorganic and organic fertilisers, it is likely that BSF frass will be competitive with the locally used organic fertilisers. In a study published in 2017 by Aggrey, it was demonstrated that local farmers in Ghana, would be willing to pay the same price for this new organic fertiliser than for the traditional manures (20.7 US$/ton). However, if these protective effects are confirmed by further studies, the advantage conferred could therefore increase the value of the frass, making it a very valuable co-product of this bioconversion.

## 8.5 Circular economy

The Black Soldier Fly technology addresses three key problems: by providing a waste remediation service and source of high quality feed for livestock as well as organic fertiliser for horticulture. This ability makes it interesting in the realm of a circular economy. While (yet) no products have entered commercial feed and fertiliser markets, this will come in the near future. In our case, the BSF operations implemented in Ghana had an investment cost (buildings & equipment) of about 8 000 US$, and had a low efficiency, producing only 100 kilogram of dried maggot meal per month. While it obviously required improvement to be upscaled, it is qualified as a small scale system. Several large-scale companies, aiming to treat up to 200 tonnes of substrate per day are starting to emerge. The most high profile enterprises include Protix (in the Netherland), Agriprotein (South-Africa), Innovafeed (France) and Entohack (Indonesia) that are aiming to release their products on international markets in the near future.

From the size disparity of these two production systems, two scenarios can be formulated, each addressing separates problems:

i) Large-scale producers will trade on international markets, providing ingredients to large-scale feed producers. They will tend to use a high quality substrate, to ensure the production of steady quality and quantity of larvae and compost. The production will be highly automatized, and will use environment-controlled cabinets to maximise the fly production. However, their conception requires large investments funds, allow a narrow flexibility if the substrate changes (or price increases) and usually enters in direct competition to other livestock sector, as they are using the same feeding substrate.

ii) On the contrary, small to medium scale BSF systems can address more local problems. Due to the greater plasticity in source and amounts of waste possible to treat, it can serve and benefit a wide range of entrepreneurs from a public toilet owner in the urban centre of an African

city, a medium-scale fish-producer or an organic waste manager in an Asian food market (Diener *et al.*, 2015). Therefore they can act as *real* waste remediation systems, potentially acquiring their substrate for free, selling their by-products locally, and can still be upscaled. By providing local employment, they could contribute to community development. However, their waste streams usually require a primary sorting, cannot provide a quality-reliable feedstuff or compost, and cannot test on a regular basis their products to ensure that no bioaccumulation has occurred. In addition, the fly-colony will have to cope with environmental conditions (fluctuation in temperature, humidity etc.) and will require a significant labour source to work properly, reducing its economic returns but potentially enhancing its social outcomes.

By addressing two different issues, one on *a local level*, and the other on *the international level*, these systems can have a synergistic effect, assuming that they would be economically viable. At the moment, too little information is available to make such assumptions.

## 8.6 Constrains & limitations

For the development and implementation of this bioconversion system, some constraints are yet to be overcome. The most obvious one being its economic viability. It is the cornerstone of every system, and unfortunately, yet no studies can answer properly this question, as no market price has been given on the MM nor the compost. The economic aspect of the MM production, as an alternative feedstuff, will be the key factor to consider its implementation into the feed sector (Rust *et al.*, 2003).

In a study done by IPIFF in 2018, the legal framework was identified as one of the main factor impacting the growth of the insect sector. Food and feed safety is essential for the insect industry, and like any food or feed company in Europe, insect producers have to follow principles established under the European Feed Safety risk management policy. Responsibility for the safety of feed/food placed on the market relies on the individual feed/food business operators. Although the traceability of products must be ensured. In the EU today, the opportunities for using and feeding insects are still quite limited. Insects are for example not allowed to be used as feed for poultry and pigs and may not be fed

with former foodstuff containing meat, fish or food losses originating from restaurants or catering establishments. These restrict greatly the market and efforts are ongoing to broaden the opportunities available. Consequently, the breeding substrates available in Europe are not yet sustainable –as they could be used already as feed- rending the BSF breeding irrelevant at the moment. This issue is however usually not present in LIDC, on a general basis more flexible toward the type of substrate to be used to breed the larvae. However, the saprophagy of the insect, and the idea that larvae fed on "waste" are used to feed fish can still be considered too much of a taboo. However, surely, when the price point has been achieved, it is more likely that minds and investors wallets will open to this idea.

Another constraint comes with the domestication of the BSF. Being now a cosmopolitan species, the phenotypic plasticity of the fly is great, and affect the global production system. It has been demonstrated by Zhu *et al.* (2013) that three strains of BSF (from Texas, Guangzhou, and Wuhan, China) displayed very different profiles in their development time and bioconversion efficiency. The Wuhan strain appeared to be fitter, reached the prepupal stage 17.7% faster than the one from Guangzhou and 29.9% than the Texan one. They were also heavier (14.4–37.0% more than those from Guanghzhou or Texas respectively). Consequently the bioconversion rates were affected, with that same strain reducing the dry matter of the substrate by 46.0% (swine), 40.1% (dairy), and 48.4% (chicken) more than the Guangzhou strain and 6.9, 7.2, and 7.9% more than the Texas strain. This strain differences are crucial into a large scale BSF production plant, as they directly affect the quality and quantity of the by-products obtained. Therefore, no general numbers can be extrapolated for a "classical BSF production", hampering the generalisation of a cost analysis, as it is site and strain specific. Similarly to livestock domestication by humans, occured some 10,500 years ago for cows, the 'domestication' of the BSF will be required. The artificial selection of specific phenotypic-trait could help to maximise the potential of the larvae, and target several key production stages (*i.e.* maximising egg laying, bioconversion etc…). As an example of such issue, the egg production is very variable amongst different location: in our site in Ghana the oviposition rate was very low, while in Tanzania, Indonesia or Malaysia, under the same breeding condition, the egg production was not an issue (Jesse Willems, Max Breinsteiner, Emilie Devic, Pers. Comm., 2018). This particularity is most likely associated with a phenotypical trait of the strain.

The upscaling is also difficult for the harvesting process. As demonstrated in Chapter 4 the white larvae is the ideal larval instar for MM production, however at this stage they tend to stay within the substrate, rending their harvesting challenging. Specific machineries have to be designed and built to enable the mass production of the BSF larvae. But, being only technical problems, it is only a matter of time before already-existing machineries are converted to sort the larvae from the substrate with an automatic process.

As we highlighted in chapter 3, the substrate has to be selected very carefully: mirroring what they eat, the larvae can become formidable bioaccumulators of heavy metals or pesticides. Having a high tolerance to heavy metals (Cai *et al.,* 2017) it stresses even more the problem as no external warning signs could be detectable. The only way to prevent it remains by ensuring the quality of the feeding substrates, and by testing on a regular basis the MM (Van Der Spiegel *et al.*, 2013). This problematic is especially true for small to medium scale production system, having no easy nor cheap techniques to test for this type of contaminants.

In a nutshell, to have a successful implementation in the near future, the BSF industry should meet an industrialization of its farming process, be economically viable and cost-competitive, while at least meeting or exceeding already existing systems producing conventional protein sources for aquaculture. This will be achieved through optimizing the production process, using economically competitive and sustainable substrate resources, closing a nutrient loop, as in a circular economic system (Rumpold & Schlüter, 2013; Van Huis *et al.*, 2013; Pastor *et al.*, 2015).

## 8.7 Future perspectives

This Thesis highlighted the high potential of BSF meal as a high quality ingredient in diets for highly targeted specific fish stages where low amounts, but high quality feed, are required. Accounting for location-specific and environmental condition, the production of MM could aim to maximise its sustainability and quality based on a circular economy strategy, where unused waste streams would be upcycled into valuable co-products. It could support a sustainable development of aquaculture in low income and developing countries. In addition while producing maggots, the frass being a non-

negligible by-product, could improve the sustainability and profitability of surrounding crop culture while decreasing pesticide and fertiliser use.

Further investigations are required on the potential ability of the MM to enhance the immune system and the resistance to stress, through the presence of chitin or lauric acid. The outcomes of this Thesis shows that fish fed with diets containing whole BSF meal have greater performances than defatted MM suggesting that fat has an important role to play. Consequently, the potential role of BSF meal as a substitution to "in feed antibiotic" should be investigated. If this role is proven, it will increase drastically the value of this new feedstuff.

Obviously, studies on the lifecycle of the BSF should be continued in order to provide a better understanding of its biology, enabling its commercialisation on a large scale basis. Similarly to cow or sheep, the domestication process of this "new" species is crucial in order to up-scale its production.

Finally, although Nile tilapia is an economically relevant, and sustainable species in aquaculture, future researches on BSF should focus on invertebrates such as prawns or shrimps, being intensively farmed. Similarly, outside aquaculture, their use as feed for the poultry sector has to be investigated, as they could be fed live, and present a very high digestibility, maximising their potential in this sector.

As a conclusion, the BSF bioconversion process is threefold: waste remediation service, larvae and compost production. If this "*triumvirat*" is achieved, and not only focused on one of these aspects, the BSF industry could meet the sustainability and economic criteria it was looking for. Upon which it would have the potential to expand and be profitable to both developed and LIDC.

# LITERATURE


Abowei J.F.N. & Ekubo A.T., 2011. A review of conventional and unconventional feeds in fish nutrition. *British Journal of Pharmacology and Toxicology.* 2(4): 179-191.

Abucay J.S. & Mair G.C., 1997. Hormonal sex reversal of tilapias: implication of hormone treatment application in closed water systems. *Aquaculture Research*. 28: 841-845.

Abudi Z.N., Hu Z., Xiao B., Abood A.R., Rajaa N., Laghari M., 2016. Effects of pretreatments on thickness waste activated sludge and rice straw co-digestion: experimental and modelling study. *Journal of Environmental Management.* 177: 213-222.

Acquah S.E.K., Quaye L., Ziem J.B., Kuugbee E.D., Iddrisu A.Y., Sagoe K., 2012. Prevalence of smear positive tuberculosis among outpatient attendees, the case of the Tamale Teaching Hospital. *Journal of Medical and Biomedical Sciences* (2012) 1(4): 34-41

Adarme-Vega T., Lim D.K., Timmins M., Vernen F., Li Y., Schenk P., 2012. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. *Microbial Cell Factories*. 11(1): 96.

Addo M.A., Darko E.O., Gordon C., Nyarko B.J.B., Gbadago J.K., Nyarko E., Affum H.A., Botwe B.O., 2012. Evaluation of heavy metals contamination of soil and vegetation in the vicinity of a cement factory in the Volta Region, Ghana. *International Journal of Science and Technology*. 2. 1: 40-50.

Aggrey J.K., 2017. Feasibility of "Black Soldier Fly larvae" composting and its utilization for vegetable farming in Greater Accra, Ghana. *MPhil thesis.* University of Legon, Ghana. 139p.

Ajani E.K., Nwanna L.C., Musa B.O., 2004. Replacement of fishmeal with maggot meal in the diets of Nile tilapia, *Oreochromis niloticus. World Aquaculture.* 35(1): 52-54.

Ako A., Tamaru C.S., Bass P., 1994. Enhancing the resistance to physical stress in larvae of mugil cephalus by feeding of enriched artemia nauplii. *Aquaculture*. 122: 81-90.

Aksnes A., Hjertnes T., Opstvedt J., 1996. Comparison of two assay methods for determination of nutrient and energy digestibility in fish. *Aquaculture*. 140: 343–359.

Alegbeleye W.O., Obasa S.O., Olude O.O., Otubu K., Jimoh W., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus*

*variegatus* L.) for African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings. *Aquaculture Research.* 43: 412-420.

Ali G., Nitivattananon V., Abbas S., Sabir M., 2012. Green waste to biogas: Renewable energy possibilities for Thailand's green markets. *Renewable and Sustainable Energy Reviews*. 16(7): 5423-5429.

Alvarez L., 2012. The role of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) in sustainable waste management in northern climates. *Ph.D. thesis, University of Windsor.* 171p.

Alves A.V., Sanjinez-Argandõna E.J., Linzmeier A.M., Cardoso C.A.L., Macedo M.L.R., 2016. Foodvalue of mealworm grown on *Acrocomia aculeata* pulp flour. *PlosOne.* 11: e0151275.

Annepu R. & Themelis N. J., 2013. Analysis of Waste Management in Accra, Ghana and Recommendations for further Improvements. *Advancing Sustainable Waste Management.* Earth Engineering Center, Columbia University.

Amoah P., Dreschel P., Abaidoo R.C., Ntow W.J., 2006. Pesticide and Pathogen Contamination of Vegetables in Ghana's Urban Markets. *Archives of Environmental Contamination and Toxicology.* 50 (1): 1-6.

Amoah P., 2008. Wastewater Irrigated Vegetable Production: Contamination pathway for health risk reduction in Accra, Kumasi and Tamale – Ghana. PhD Thesis. Kwame Nkrumah University of Science and Technology (KNUST).

Amoah P., 2012. An analysis of the Quality of wastewater used to irrigate Vegetables in Accra, Kumasi and Tamale, Ghana. *Agriculture in Urban planning: Generating livehoods and food security.* Mark Rewood Publisher.272p.

Amompah B., 2013. Water Ressources Commission. Water Use Register. Akotsombo. Ghana. 10p.

Aneme A., Afam-Anene O., Ike K., Ekekwe N., 2013. Preliminary investigation on quantity and proximate quality of maggots produced from four different sources of livestock wastes. *Journal of Research in Biology*. 6(3): 1060-1065.

Aniebo A.O., Erondu E.S., Owen O.J., 2009. Replacement of fish meal with maggot meal in African catfish (*Clarias gariepinus*) diets. *Revista UDO Agricola*. 9(3): 666-671.

Aniebo A.O., Odukwe C.A., Ebenebe C.I., Ajuogu P.K., Owen O.J., Onu P.N., 2011. Effect of housefly larvae (*Musca domestica*) meal on the carcass and sensor qualities of the mud catfish (*Clarias gariepinus*). *Advances in food and energy security*. 1: 24-28.

Anim-Gyampo M., Ntiforo A., Kumi M., 2012. Assessment of Heavy Metals in the waste-water irrigated lettuce in Ghana: the case of Tamale municipality. *Journal of sustainable development.* 5(11): 94-102.

AOAC (Association of Official Analytical Chemists), 1980. Official methods of analysis. Washington, D.C., USA.

AOAC, 1990. Official methods of Analysis, 15th ed. Association of Official Analytical Chemist, Inc., Washington D.C., USA.

Arends R.J., Mancera J.M., Muñoz J.L., 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. *Journal of endocrinology*. 163: 149-157.

Asante K. A., Ntow W.J., 2009. Status of Environmental Contamination in Ghana: the perspective of a research scientist. *Interdisciplinary studies on Environmental Chemistry*. 14: 253-260.

Asante K.A., Charles T. A., Biney A., Agyekum W. A., Bello M., Otsuka M., Itai T., Takahashi S., Tanabe S., 2012. Multi-trace element levels and arsenic speciation in urine of e-waste recycling workers from Agbogbloshie, Accra in Ghana. *Science of the Total Environment*. 424: 63-73.

Asiedu B., Failler P., Beyens Y., 2015. Enhancing aquaculture development: mapping the tilapia aquaculture value chain in Ghana. *Reviews in Aquaculture*. 7:1-9.

Aslaksen M.A., Kraugerud O.F., Penn M., Svihus B., Denstadli V., Jorgensen H.Y., Hillestad M., Krogdahl A., Storebakken, T., 2007. Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. *Aquaculture.* 272: 541–555.

Asmah R., 2008. Development potential and financial viability of fish farming in Ghana. *Ph.D. thesis*. University of Stirling. 289p.

Austreng E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of different segments of the gastrointestinal tract. *Aquaculture*. 13: 265–272.

Ayele W.Y., Neill S.D., Zinsstag J., Weiss G. M., Pavlik I., 2004. BTB: an old disease but a new threat to Africa. *The International Journal of Tuberculosis and Lung* Disease. 8: 924-937.

Badenhorst R., 2017. Genetic Diversity in a Commercial Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomydiae), population. *Master thesis.* Stellenbosh University. 134p.

Banks I.J., Gibson W.T., Cameron M.M., 2014. Growth rates of Black Soldier Fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical medicine and International health.* 19: 14-22.

Barragan-Fonsecra K.B., Dicke M., Van Loon J.J.A., 2017. Nutritional value of the Black Soldier Fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. *Journal of Insects as food and feed*. DOI: 10.3920/JIFF2016.0055.

Barros-Cordeiro K.B., Bào S.N., Pujol-Luz J.R., 2014. Intra-puparial development of the black soldier-fly, *Hermetia illucens*. *Journal of Insect Science*. 14 (83): 1-10.

Barroso F.G., De Haro C., Sanchez-Muros M.J., Venegas E., Martinez-Sanchez A., Perez-Banon C., 2014. The potential of various insect species for use as food for fish. *Aquaculture*. 422-423: 193-201p.

Barry T., 2004. Evaluation of the economic, social, and biological feasibility of bioconverting food wastes with the black soldier fly (*Hermetia illucens*). PhD Dissertation, University of Texas, August 2004, 176 p.

Barry T.P., Marwah A., Marwah P., 2007. Stability of 17α-methyl testosterone in fish feed. *Aquaculture*. 271: 523–529.

Beal C.M., Gerber L.N., Sills D.L., Huntley M.E., Machesky S.C., Walsh M.J., 2015. Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment. *Algal Research*. 10: 266–79.

Becker W., 2003. Microalgae in Human and Animal Nutrition. *In: Handbook of microalgae culture: biotechnology and applied phycology.* Doi:10.1002/9780470995280.ch18.

Beschovski V.L. & Manassieva E.P., 1996. Contribution to the study of the Stratiomyidae species in the Balkan peninsula, with description of *Nemotelus rumelicus* spec. nov. (Insecta: Diptera). *Reinchenbachia.* 31(39) : 217-223.

Begun N.N., Chakraborthy S.C., Zaher M., Abdul M.M., Gupta M.V., 1994. Replacement of fish meal by low cost animal protein as a quality fish feed ingredients for the Indian major carp, *Labeo rohita*. *Journal of the Science of food and agriculture.* 64: 191-197.

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

Belton B., Bush S.R., Little D.C., 2018. Not just for the wealthy: rethinking farmed fish consumption in the Global South. *Global Food Security.* 16: 85-92.

Belton B., Little D.C., 2008. The Development of Aquaculture in Central Thailand: Domestic Demand versus Export-Led Production. *Journal of Agrarian Change*. 8(1): 123-143.

Benelli G., Canale A., Raspi A., Fornaciari G., 2014. The death scenario of an Italian Renaissance princes scan shed light on a zoological dilemma: did the black soldier fly reach Europe with Columbus. *Journal of Archaeological Science.* 49: 203-205.

Benfey T.J. & Biron M., 2000. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). *Aquaculture*. 184: 167-176.

Beveridge M.C.M & McAndrew B.J., 2000. Tilapias: Biology and exploitation. Kluwer Academic Publisher.

Bhujet R.C., 2014. A manual for tilapia business management. *CAB International Publisher.* 199p.

Biancarossa I., 2016. Bioconversion of brown seaweed by black soldier fly larvae, effects on nutritional composition and feed safety consideration. *Poster. Conference Feed2016*- Geel. Belgium.

Bishop C.D., Angus R.A., Watts S.A., 1995. The use of feather meal as a replacement for fish meal in the diet of *Oreochromis niloticus* fry. *Bioresource Technology*. 54(3): 291-295.

Black M.M. & Schwartz H.M., 1950. The estimation of chitin and chitin nitrogen in crawfish waste and derived products. *Analyst*. 75: 185-189.

Blue Skies. 2012. Making fruit happy. *Blue Skies Sustainability Report.* 29p.

Bodri M. & Cole E.R., 2007. Black Soldier Fly (*Hermetia illucens* Linnaeus) as Feed for the American Alligator (*Alligator mississippiensis* Daudin). *Georgia Journal of Science.* 65(2): 82-85.

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

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

Booth D.C., & Sheppard D.C., 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. *Environmental Entomology*. 13: 421-423.

Borgmeier T., 1930. Uber das vorkommen der larven von *Hermetia illucens* L. (Dipt., Stratiomyidae) in den nestern von Meliponiden. Zoologica Anz . 90: 225-230.

Borrelli L., Coretti L., Dipineto L., Bovera F., Menna F., Chiariotti L., Nizza A., Lembo F., Fioretti A., 2017. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. *Scientific reports.* 7: 16269. Doi:10.1038/s41598-017-16560-6.

Borthakur S. & Sarma K., 1998. Effects of some non-conventionnal fish meal replacers on the growth, feed conversion and body composition of *Clarias batrachus* (Linn.) fingerlings. *Journal of Environmental Ecology.* 16: 694-698.

Bosch G., Zhang S., Oonincx D.G.A.B., Hendriks W.H., 2014. Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science.* 29(3): 1-4.

Boscolo W.R., Hayashi C., Meurer F., 2001. Fish, meat and bone, poultry by-products and silkworms meals as an attractive in diets for Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Revista brasileira de zootecnia.* 30: 1397-1402.

Boscolo W.R., Signor A., Signor A.A., 2008. A. Inclusão de amido em dietas para larvas de tilápia-do-nilo. *Revista Brasileira de Zootecnia.* 37 : 177-180.

Bouafou K.G.M., 2011. Revue bibliographique sur les asticots et leur emploi dans l'alimentation animale. *Journal of Animal & Plant Science*. 12(2): 1543-1551.

Borowska J., Sulima B., Nilinska M., Pyza E., 2004. Heavy metal accumulation and its effects on development, survival and immuno-competent cells of the housefly Musca domestica from closed laboratory populations as model organism. *Fresenius Environmental Bulletin.* 13(12): 1402-1409.

Borowska J. & Pyza E., 2011. Effects of heavy metals on insect immunocompetent cells. *Journal of Insect Physiology.* 57(6): 760-770.

Borowitzka M.A., 1997. Algae for aquaculture: Opportunities and constrains. *Journal of Applied Phycology*. 9: 393-401.

Bradley G.H., 1930. Hermetia illucens L. A pest in sanitary privies in Louisiana. *Journal of Economic Entomology.* 23: 1012-1013.

Bradley S.W., Booth D.C., Sheppard D.C., 1984. Parasitism of the Black Soldier Fly by *Trichopia sp.* (Hymenoptera: Diapriidae) in poultry houses. *Environmental entomology.* 13: 451-454.

Bradley S.W. & Sheppard D.C., 1984. House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. *Journal of Chemical Ecology.* 10: 853-859.

Buddle R., 1984. Monosex tilapia fry production. ICLARM Newsletter. 7:4-6.

Buddle R., 1984b. Androgen-induced sex inversion of Oreochromis (Trewavas) hybrid fry stocked in cages standing in an earthen pond. *Aquaculture*. 40: 233-239.

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

Burtle G., Newton G.L., Sheppard D.C., 2012. Mass production of Black Soldier Fly prepupae for Aquaculture diets. *Aquaculture international*. University of Georgia, Tifton Campus. Georgia, USA. 6p.

Bussler S., Rumpold B.A., Jander E., Rawel H.M., Schluter O.K., 2016. Recovery and techno-functionality of flours and proteins from two edible insect species: Meal

worm (*Tenebrio molitor*) and black soldier fly (*Hermetia illucens*) larvae. Heliyon. 2, DOI: e00218.

Cai J. & Leung P.S., 2017. Short-term projection of global fish demand and supply gaps. *FAO Fisheries and Aquaculture technical paper.* 607. Rome, Italy. 114p

Cai M., Ma S., Hu R., Tomberlin J.K., Yu C., Huang Y., Zhan S., Li W., Zheng L., Yu Z., Zhang J., 2018a. Systematic characterization and proposed pathway of tetracycline degradation in solid waste treatment by *Hermetia illucens* with intestinal microbiota. *Environmental pollution.* 242(A): 634-642.

Cai M., Ma S., Hu R., Tomberlin J.K., Thomashow L.S., Zheng L., Li W., Yu Z., Zhang J., 2018b. Rapidly mitigating antibiotic resistant risks in chicken manure by *Hermetia illucens* bioconversion with intestinal microflora. *Environmental Microbiology.* 20(11): 4051-4062. DOI: 10.1111/1462-2920.14450.

Calder P.C., 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochemistry Society Transactions.* 45(5): 1105-1115.

Calvert C.C., Martin R.D., Morgan N.O., 1969. House fly pupae as food for poultry. *Journal of Economical Entomology.* 62: 938-939.

Carl Von Linnée, 1758. Systema Naturae. *Leide University.* Accessible at: https://gallica.bnf.fr/scripts/ConsultationTout.exe?O=n096634

Caruso D., Devic E., Subamia I.W., Talamond P., Baras E., 2013. Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae. IRD Edition. 159p.

Carvalho R., 1999. A Amazônia rumo ao 'ciclo da soja. *Amazônia papers, programa Amazônia, Amigos da terra, Saõ Paulo, Brazil.* 1:2.

Catts E.P. & Heskell N.H., 1990. Entomology and death: A procedural guide. Joyce's Print Shop.

Cauchie H.M., Murugan G., Thomé J.P., Dumont H.J., 1997. Intra- and interspecific variation in the chitin content of some anstacans. *Hydrobiologia*. 359: 223-228.

Celik I., Guner Y., Celik P., 2011. Effect of Orally-Administered 17α-Methyltestosterone at Different Doses on the Sex Reversal of the Nile Tilapia (*Oreochromis niloticus*, Linneaus 1758). *Journal of Animal and Veterinary Advances.* 10: 853-857.

Čičková H., Kozánek M., Moravek I., Takáč P., 2012a. A behavioral method for separation of House fly (Diptera: Muscidae). *Journal of Economical Entomology.* 105: 62-66.

Čičková H., Kozánek M., Takáč P., 2012b. Improvement of survival of the house fly (*Musca domestica* L.) larvae under mass-rearing conditions. *Bulletin of Entomological Researches. 103: 119-125.*

Čičková H., Newton G.L., Lacy R.C., Kozanek M., 2015. The use of fly larvae for organic waste treatment. *Waste Management*. 35: 68-80.

Chakraborty S.B., Mazumdar D., Banerjee S., 2010. Determination of ideal stocking density for cage culture of monosex Nile Tilapia (*Oreochromis niloticus*) in India. *Proceed of the Zoological Society*. 63(1): 53-59.

Charlton A.J., Dickison M., Wakefield M.E., Fitches E., Kenis M., Han R., Zhu F., Kone N., Grant M., Devic E., Bruggemen G., Prior R., Smith R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. *Journal of Insects as food and Feed.* 1: 16-17.

Charles H., Godfray J., Beddington J.R., Crute I.R., Haddad L., Lawrence D., Muir J.F., Pretty J., Robinson S., Thomas S.M., Toulmin C., 2010.Food security: the challenge of feeding 9 billion people. *Science.* 327: 812-818.

Chavez F.P., Ryan J., Lluch-Cota S.E., Niquen C.M., 2003. From anchovies to sardines and back: multidecadal change in the Pacific Ocean. *Science.* 299(5604): 217-221.

Chen G., Liu G., Yan B., Shan R., Wang J., Li T., Xu W., 2016. Experimental study of co-digestion of food waste and tall fescue for bio-gas production. *Renowable Energy.* 88: 273-279.

Cheng Y.K.J., Chiu S.L.H., Lo I.M.C., 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. *Waste Management*. 67: 315-323.

Chevin H., 1986. Présence d'*Hermetia illucens* (L.) en Charente Maritime (Dipt. Stratiomyidae). *L'Entomologiste*. 42:76.

Cho C.Y., Kaushik S.J., 1990. Nutritional energetics in fish: energy and protein utilisation in rainbow trout (*Salmo gairdnerii*). *World Review of Nutrition and Dietetics*. 61: 132–172.

Choi W.H., Yun J.H., Chu J.P., Chu K.B., 2012. Antibacterial effects of extracts of *Hermetia illucens* (Diptera: Stratiomyidae) larvae against Gram-negative bacteria. *Entomological Research.* 42(5). Doi:10.1111/j.1748-5967.2012.00465.x

Choi W.H., Jiang M., 2014. Evaluation of antibacterial activity of hexanedioic acid isolated from *Hermetia illucens* larvae. *Journal of applied biomedecine.* 12 : 179-189.

Choubert G., De la Noue J., Luquet P., 1982. Digestibility in fish: improved device for the automatic collection of feces. *Aquaculture*. 29: 185–189.

Choudhury A.R., Natarajan A.K., Kesavarapu S., Veeraraghavan A., Dugyala S.K., Rao K., Thota K.R., 2018. Technical feasibility of *Hermetia illucens* in integrated

waste management, renovated with sewage water, an overview. *Scientific Research.* 5(4): 1-27.

Chor W.K., Lim L.S., Shapawi R., 2013. Evaluation of Feather Meal as a Dietary Protein Source for African Catfish Fry, *Clarias gariepinus*. *Journal of Fisheries and Aquatic Science, 8: 697-705.*

Christie W.W., 1993. Lipid Analysis-Isolation, Separation, Identification and Structural Analysis of Lipids, 3rd Ed. The Oily Press.

Clemens H.P. & Inslee T., 1968. The production of unisexual broods of Tilapia mossambica sex reversed with methyltestosterone. *Transactions of the American Fisheries Societies*. 97: 18-21.

Cnaani A., Tinman S., Avidar Y., Ron M., Hulata G., 2004. Comparative study of biochemical parameters in response to stress in *Oreochromis aureus, O. mossambicus* and two strains of *O. niloticus. Aquaculture Research.* 35(15): 1434-1440.

Costa C. & Vanin S.A., 1984. On the concept of the "pre-pupae", with special reference to the Coleoptera. *Revista Brasileira de Zoologia*. 2(6): 339-345.

Coulibaly M., Sheppard D.C., Rains G.C., 2004. Odor control and elimination by using Black Soldier Fly to digest swine and chicken manure. *University of Georgia internal report*. 4p.

Copello A., 1926. Biologia de *Hermetia illucens. Revista de la Sociedad Entomologia Argentina.* 1: 23-27.

Cullere M., Tasioniero G., Giaccone V., Miotti-Scapin R., Claeys E., De Smet S., Dalle Zotte A., 2016. Black soldier fly as dietary protein source for broiler quails: Apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. *Animals.* 1(12): 1-8.

D'Errico F., Villa G., Fornaciari C., 1988. Dental esthetics of an Italian Renaissance noble-women, Isabella d'Aragona. A case of chronic mercury intoxication. *Ossa.* 13: 233-254.

Da Zhu P., Asnani H., Zurbrugg C., Anapolsky S., Mani, S., 2008. Improving Municipal Solid Waste Management in India, A Source Book for Policy Makers and Practitioners.Washington, D.C.: World Bank.

Dan N.C. & Little D.C., 2000. The culture performance of monosex and mix-sex new-season and overwintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in northern Vietnam. *Aquaculture.* 184(3): 221-231.

Daniel N., Sivaramakrishnan T., Saravanan K., Shalini B., Baruah A., Kiruba Sankar R., Roy D., 2016. A review on microalgae as potential fish feed ingredient. *Journal of the Andaman Science Association.* 21(1): 140-144.

Danieli P.P., Ronchi B., Speranza S., 2011. Alternative animal protein sources for aquaculture: a preliminary study on nutritional traits of Mediterranean brocade (*Spodoptera littoralis* Boisduval) larvae. *Italian Journal of animal sciences*. 10(1): 109.

Danulat E., & Kausch H., 1984. Chitinase activity in the digestive tract of the cod, *Gadus morhua* (L.). *Fish Biology.* 24(2): 125-133.

Dauphin P., 2003. Présence de *Hermetia illucens* (Linné, 1758) dans le sud-ouest de la France (diptère Stratiomyidae). *Bulletin de la Société linnéenne de Bordeaux*. 31 (4): 229-230.

Davies R.H. & Wray C., 1993. Use of larvae of *Lucilia serricata* in colonisation and invasion studies of *Salmonella enteritidis* in poultry. *Proceedings of the Flair Workshop*. No. 6, 10/13 December 1992, Southampton, UK.

De Jesùs A.J., Olsen A.R., Bryce J.R., Whiting R.C., 2004. Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae). *International Journal of food microbiology*. 93(2): 259-262.

De Marco M., Martinez S., Hernandez F., Madrid J., Gai F., Rotolo L., Belforti M., Bergero D., Katz H., Dabbou S., Kovitvadhi A., Zoccarato I., Gasco L., Schiavone A., 2015. Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology*. 209: 211-218.

De Foliart G.R., Finke M.D., Sunde M.L., 1982. Potential value of the Mormon cricket (Orthoptera: Tettigonidae) harvested as a high-protein feed for poultry. *Journal of Entomology.* 75: 848-852.

Debode J., De Tender C., Soltaninejad S., Van Malderghem C., Haegeman A., Van Der Linden I., Cottyn B., Heyndrickx M., Maes M., 2016. Chitin mixed in potting soil alters lettuce growth, the survival of zoonotic bacteria on the leaves and associated rhizosphere microbiology. *Frontiers in Microbiology.* 7(565): 1- 15.

Degani G., Viola S., Yehuda Y., 1997. Apparent digestibility of protein and carbohydrate in feed ingredients for adult tilapia (*Oreochromis aureus* X *O. niloticus*). 49: 115-123.

Devi S.M. & Kim I.H., 2014. Effects of medium chain fatty acids (mcfa) and probiotic (*Enteroccocus faecium*) supplementation on the growth performance, digestibility and blood profiles in weanling pigs. *Veterinary Medicine.* 59: 527-535.

Devic E., 2016. Assessing insect-based products as feed ingredients for aquaculture. *PhD thesis.* University of Stirling. 215p.

Devic E., Leschen W., Murray F.J., Little D.C., 2017. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (Oreochromis niloticus) fed diets containing Black Soldier Fly (Hermetia illucens) larvae meal. *Aquaculture nutrition.* DOI 10.1111/anu.12573.

Devic E., Little D.C., Leschen W., Jauncey K., 2013. Modeling the substitution of fishmeal by maggot-meal in Tilapia feeds - case of a commercial production farm in West Africa In: Hulata G (ed.) ISTA10 Special Issue, Jerusalem: Society of Israeli Aquaculture. 10th International Symposium on Tilapia in Aquaculture - ISTA10, 6.10.2013 - 10.10.2016, Jerusalem, Israel, Israeli Journal of Aquaculture -Bamidgeh, p. 1054.

Devic E. & Maquart P.O., 2015. *Dirhinius giffardii* (Hymenoptera: Chalcididae), parasitoid affecting Black Soldier Fly Production in West Africa. *Entomologia*. 3: 284: 25-27.

Dhert P.P., Lavens P., Sorgeloos P., 1992. Stress evaluation: a tool for quality control of hatchery-produced shrimp and fish fry. *Aquaculture Europe*. 17: 6-10.

Diener S., Zurbrügg C., Tockner K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste management & Research.* 27(6): 603-610.

Diener, S., Zurbrügg, C., Tockner, K., 2010. The potential use of the black soldier fly, Hermetia illucens, as animal feed: Bioaccumulation of heavy metals and effects on the life cycle. *Journal of Insects as food and feed.* 1(4): 261-270.

Diener S., Studt Solano N.M., Roa Guitiérrez F., Zurbrügg C., Tockner K., 2011. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste biomass valorisation.* 2(4): 357-363.

Diener S., Lalander C., Zurbruegg C., Vinnerås B., 2015. Opportunities and constrains for medium-scale organic waste treatment with fly larvae composting. *Conference paper.* 8p.

Dierenfeld E.S. & King J., 2008. Digestibility and mineral availability of phoenix worms (*Hermetia illucens*) ingested by mountain chicken frogs (*Leptodactylus fallax*). *Journal of Herpetological Medicine and Surgery.* 18: 100-105.

Diogenes A.F., Fernandes J.B.K., Dorigam J.C.P., Sakomura N.K., Rodrigues F.H.F, Lima B.T.M., Gonçales F.H., 2016. Establishing the optimal essential amino acid ratios in the juveniles of Nile tilapia (*Oreochromis niloticus*) by the deletion method. *Aquaculture nutrition*. 22: 435-443.

Djissou A.S.M., Vodounou J.V., Tossavi C.E., Toguyeni A., Fiogbe E.D., 2016. Complete replacement of fish meal by unconventional proteins sources in diet of *Oreochromis niloticus* (L., 1758) fingerlings: growth performance, feed utilization and body composition. *Fisheries and Aquatic studies.* 4(5): 242-247.

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

DOF, 2007. Fisheries Statistics of Thailand 2005. Department of Fisheries, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Dortmans B.M.A., Diener S., Verstappen B.M., Zurbrügg C., 2017. Black Soldier Fly Biowaste Processing - A Step-by-Step Guide. *Eawag: Swiss Federal Institute of Aquatic Science and Technology*, Dübendorf, Switzerland. 100p.

Dresner E., 1954. Observations on the biology and habits of pupal parasites of the oriental fruit fly. In: Proceedings, Hawaiian Entomological Society XV: 299-310.

Drew D.J.W., & Pieterse E., 2015. Markets, money and maggots. *Journal of Insects as Food and Feed.* 1: 227-231.

Dwivedi J. & Agrawal O.P., 1995. Degradation of cuticle during larval-pupal and pupal-adult development of the housefly, *Musca domestica*. *Physiological Entomology*. 20: 318-322.

Ebenso I.E., Udo M.T., 2003. Effects of live maggot on growth of the Nile perch, *Oreochromis niloticus* (*Cichlidae*) in South-Eastern Nigeria. *Global Journal of Agriculture Science.* 2: 72-73.

Eddie E.D. & Norman Y.S., 2008. vol. 19. Springer; Netherlands: (Modulation of Fish Growth Hormone Levels by Salinity, Temperature, Pollutants and Aquaculture Related Stress: A Review). pp. 97–120.

EFSA Scientific Committee, 2015. Scientific Opinion on a risk profile related to production and consumption of insects as food and feed. *EFSA Journal*. 13(10): 4257, 60 pp. doi:10.2903/j.efsa.2015.4257.

Ekop E.A, Udoh A.I, Akpan P.E., 2010. Proximate and anti-nutrient composition of four edible insects in Akwa Ibom State, Nigeria. *World Journal of Applied Science and Technology*. 2 (2):224–231.

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

El-Dahhar A.A. & El-Shazli A., 1993. Effect of essential amino acid (methionine and lysine) and treated oil in fish diet on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus*. *Aquaculture and Fisheries Management*. 24: 731–739.

El-Ebiary E., 2005. Use of soybean meal and/or corn gluten meal as partial substitutes for fish meal in Nile tilapia (*Oreochromis niloticus*) fingerling diets. *Egyptian Journal of Aquatic Research*. 31(2): 432–442.

El-Greisy Z.A. & El-Gamal A.E., 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17-Alpha-methytestosterone with respect to the

degree of sex-stability after one year of treatment. *Egyptian Journal of Aquatic Research*. 38: 59-66.

El-Hadrami A., Adam L.R., El-Hadrami I., Daayf F., 2010. Chitosan in plant protection. *Marine Drugs.*8: 968-987.

El-Saidy D. & Gaber M.M.A., 2002. Complete replacement of fish meal by soybean meal with dietary L-lysine supplementation for Nile tilapia *Oreochromis niloticus* (L.) fingerlings. *Journal of the World Aquaculture Society*. 33: 297–306.

El-Sayed A.F.M., 1998. Total replacement of fishmeal with animal protein sources in Nile Tilapia (Oreochromis niloticus) feeds. *Aquaculture Researches*. 29: 275-280.

El-Sayed A.F.M., 2004. Protein nutrition of farmed tilapia; searching for unconventional sources, *in*: New dimension in farmed Tilapia: *Proceedings of the Sixth International Symposium on tilapia Aquaculture.* Pp: 364-378.

El-Sayed A.F.M., 2006. Tilapia culture, CABI publishing. Wallingford.UK.

El-Sayed A.F.M., Mansour C.R., Ezzat A.A., 2003. Effects of dietary protein level on spawning performance of Nile Tilapia (Oreochromis niloticus) broodstock reared at different water salinities. *Aquaculture*. 220: 619-632.

Elwert C., Knips I., Katz P., 2011. A novel protein source: Maggot meal from *Hermetia illucens*. Poster.

Emehinaiye P.A., 2012. Growth performance of *Oreochromis niloticus* fingerlings fed with varying levels of migratory locuts (*Locusta migratoria*) meal. *Bachelor of Aquaculture and Fisheries Management.* University of Agriculture. Abeokuta, Ogun State.

Erickson M.C., Islam M., Sheppard C., Liao J., Doyle M.P., 2004. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of the black soldier fly. *Journal of Food Protection*. 67: 685-690.

Esteban M.A., Cuesta A., Ortuno J., Meseguer J., 2001. Immunomodulatory effects of dietary intake of chitin on gilthead seabream (Sparus aurata L.) innate immune system. *Fish & Shellfish Immunology*. 11: 303-315.

EU, 2017. L 138/111 available at: http://www.svscr.cz/wp-content/files/obchodovani/Eurlex_2017R0893_osvden_1a_en.pdf

Ezewudo B., Bakare O., Ugwumba A.A.A., 2015. Utilization of *Musca domestica* maggots in the diet of *Oreochromis niloticus* (Linnaeus, 1758) fingerlings. *African Journal of Agricultural Research.* 10(23):2363-2371.

Falaye A.E. & Jauncey K., 1999. Acceptability and digestibility by tilapia *Oreochromis niloticus* of feeds containing cocoa. *Aquaculture Nutrition.* 5: 157-161.

Fange B., Lundblad G., Lindsay J., 1976. Lysozyme and chitinase in blood and Iymphomyeloid tissues of marine fish. *Marine Biology*. 36: 277-282.

FAO, 2007. The State of World Fisheries and Aquaculture. FAO. Rome, 162p.

FAO, 2011. Global food losses and food waste – Extent, causes and prevention. Rome. 37p.

FAO, 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200p.

FAO, 2018. Gender and food loss in sustainable food value chains – A guiding note. Rome, 44p.

FAO, 2018: http://www.fao.org/fishery/facp/THA/en

Fauna Europea, 2017. http://faunaeurop.org

Fasakin E.A., Balogun A.M., Ajayi O.O., 2003. Evaluation of full-cat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlinds. *Aquaculture Research.* 34: 733-738.

Ferrari A., Soares A.T.C., Amorim D.S., Thyssen P.J., Guimarães M.A., 2009. Comparação dos patrõdes de atratividade de *Hermetia illucens* (Diptera: Stratiomyidae) associada a carcaças de *Rattus norvegicus* enterradas e tratadas comp hormônios esteròides. *Revista Brasileira de Entomologia.* 53(4) : 565-569.

Fines B.C., & Holt G.J., 2010. Chitinase and apparent digestibility of chitin in the digestive tract of juvenile cobia, *Rachycentron canadum. Aquaculture.* 303(1): 34-39.

Finke M., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoological Biology*. 21: 269-285.

Finke M., 2007. Estimate of chitin in raw whole insects. *Zoo biology*. 26: 105-115.

Finke M., 2013. Complete nutrient content of four species of feeder insects. *Zoology and Biology.* 32: 27-36.

Finke M., & Oonincx D.G.A.B., 2014. Insects as food for insectivores. In book: *Mass Production of Beneficial Organisms*. Chapter 17. Elsevier. 52p.

Folch J., Lees M., Sloane Stanley G.H., 1957. A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biology and Chemistry.* 226: 497-509.

Foley J.A., Ramankutty N., Brauman K.A., Cassidy E.S., Gerber J.S., Johnston M., Mueller N.D., O'Connel C., Ray D.K., West P.C., Balzer C., Bennett E.M., Carpenter S.R., Hill J., Monfreda C., Polasky S., Rockström J., Sheehan J., Siebert S., Tilman D., Zaks D.P.M., 2011. Solution for a cultivated planet. *Nature*. 478: 337-342.

Fornaciari G., 2006. The Aragonese mummies of the Basilica of Saint Domenico maggiore in Naples. *Journal of History of Medecine*. 18:843-864.

Franco M., 2013. Updated Italian Checklist of Soldier Flies (Diptera, Stratiomyidae). *Zookeys*. 336:61-78.

Friedman M., 2013. Nutritional Improvement of Food and Feed Proteins. *Advances in Experimental Medicine and Biology*. 105. 882p.

Fry J.P., Love D.C., MacDonald G.K., West P.C., Engstrom P.M., Nachman K.E., Lawrence R.S., 2016. Environmental health impacts of feeding crops to farmed fish. *Environment International.* 91: 201-214.

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

Furuya W.M., Pezzato L.E., Pezzato A.C., Barros M.M., Miranda E.C., 2001. Coeficiente de digestibilidade e valores de aminoácidos digestìveis de alguns ingredientes para tilápia do Nilo (*Oreochromis niloticus*). *Revista Brasiliana de Zootechnia*. 30(4): 1143–1149.

Gabler F. & Vinnerås B., 2014. Using Black Soldier Fly for Waste Recycling and Effective Salmonella spp. Reduction. Swedish University of Agricultural Sciences; Uppsala, Sweden: 2014.

Gatlin D.M., Barrows F.T., Brown P., Dabrowski K., Gaylord T.G., Hardy R.W., Herman E., Hu G., Krogdahl A., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D., Souza E., Stone D., Wilson R., Wurtele E., 2007. Expanding the utilization of sustainable plant products in aquafeed: a review. *Aquaculture Research.* 38(6): 551-579.

Gasco L., Finke M., Van Huis A., 2018. Can diets containing insects promote animal health? *Journal of Insects as Food and Feed.* 4(1):1-4.

Gaylord T.G. & Barrows F.T., 2008. Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for Rainbow trout. *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society.*39: 827–834.

Gerber L.N., Tester J.W., Beal C.M., Huntley M.E., Sills D.L., 2016. Target Cultivation and Financing Parameters for Sustainable Production of Fuel and Feed from Microalgae. *Environmental Science & Technology*. 50 (7): 3333–3341.

Ghana Health Service, 2014. Available at: http://www.ghanahealthservice.org/articles.php?nd=112&tt=Easter%20revellers%20asked%20to%20help%20curb%20the%20spread%20of%20TB. Accessed: 07th November, 2016.

Glencross B. D., Evans D., Dods K., McCafferty P., Hawkins W., Maas R., Sipsas S., 2005. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, Oncorhynchus mykiss, using either stripping or settlement faecal collection methods. *Aquaculture*. 245: 211–220.

Glencross B.D., Blyth D., Tabrett S.J., Bourne N., Irvin S., Fox-Smith T., Smullen R.P., 2012. An examination of digestibility and technical qualities of a range of cereal grains when fed to juvenile barramundi (*Lates calcarifer*) in extruded diets. *Aquaculture Nutrition*. 18: 388–399.

Glencross B.D., Booth M., Allan G.L., 2007. A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquaculture Nutrition. 13: 17–34.

Gobbi F.P., 2012. Biología reproductiva y caracterización morfológica de los estadios larvarios de *Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae). Bases para su producción masiva en Europa. *PhD Thesis*, Universidad de Alicante.

Gobbi F.P., Martìnez-Sànchez A., Rojo S., 2013. The effects of larval diet on Adult life-history traits of the black Soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *European Journal of Entomology.* 110(3): 461-468.

Golden Exotics Limited, 2012. Activity report. 75p.

Gopalakannan A. & Arul V., 2006. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of Cyprinus carpio and the control of Aeromonas hydrophila infection in ponds. *Aquaculture*. 255: 179-187.

Government of Ghana, 2008. National Environmental Sanitation Strategy and Action Plan (NESSAP). Government of Ghana, Ministry of Local Government, Rural Development and Environment, Accra, p. 95.

Guangdong Entomological Institute. 2015. Preparation method for extracting chitosan from Hermetia illucens. *Patent N*°CN102585035A.

Guerrero R.D., 1975. Use of Androgens for the production of all male *Tilapia aurea* (Setindachner). *Transaction of the American Fisheries Society*. 104(2): 342-348.

Guerrero R. D. & Shelton W.L., 1974. An aceto-Carmine squash method for sexing juvenile fishes. *The progressive fish-culturist*. 36(1): 55-56.

Gujarathi G. & Pejaver M., 2013. Occurrence of Black Soldier Fly *Hermetia illucens* (Diptera: Stratiomyidae) in biocompost. *Research Journal of Recent Sciences.* 2(4): 65-66.

Gutierrez G. P., Vergara Ruiz R. A., Mejia Velez H., 2004. Compositional, microbiological and protein digestibility analysis of larval meal of *Hermetia illucens* (*Diptera:Stratiomyidae*) at Angelopolis-Antioquia, Colombia. Revista - Facultad Nacional de Agronomia Medellin, 57 (2): 2491-2499

Habib M.A.B., Hasan M.R., Akand A.M., Siddiqua A., 1994. Evaluation of silkworm pupae meal as a dietary protein source for *Clarias batrachus* fingerlings. 124: 62.

Hait S., Tare V., 2011. Vermistabilization of primary sewage sludge. *Bioressources Technology.* 102: 2812-28120.

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

Halloran A., & Münke C., 2014. Discussion paper: Regulatory frameworks influencing insects as food and feed. 25p.

Halloran A., Roos N., Eilenberg J., Cerutti A., Bruun S., 2016. Life cycle assessment of edible insects for food proteins: a review. *Agronomy Sustainable Development.* 36: 57.

Han L.K., Kimura Y., Okuda H., 1999. Reduction in fat storage during chitin–chitosan treatment in mice fed a high-fat diet. *International Journal of Obesity and Related Metabolic Disorders.* 23: 174–179.

Hannesson R., Barrange M., Herrick S.J., 2006. Climate change and the Economics of the world's fisheries. *Edwar Elgar Publishing Limited, Cheltenham* U.K.

Hardy R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquaculture Research*. 41: 770–776.

Hasan M.R., Hecht T., De Silva S.S., Tacon A.G.J., 2007. Study and analysis of feeds and fertilizers for sustainable aquaculture development. *FAO Fisheries Technical Paper N°497*. FAO, Rome. 531p.

Hasim N.A., Shamsul Bahri A.R., Basari N., Sharudin N.H., 2017. Mass infestation of black soldier fly Hermetia illucens (Diptera: Stratiomyidae) on colonies of the Indo-Malayan stingless bees Geniotrigona thoracica and Heterotrigona itama. *Journal of Biodiversity and Environmental Sciences.* 11(2): 2222-3045.

Hayashi C., Boscolo W.R., Soares C.M., Meurer F., 2002. Exigência de proteína digestível para larvas de tilápia do Nilo (*Oreochromis niloticus*), durante a reversão sexual. *Revista Brasileira de Zootecnia*. 31 : 823-828.

He J.Y., Tian L.X., Lemme A., Gao W., Yang H.J., Niu J., Liu Y.J., 2013. Methionine and lysine requirements for maintenance and efficiency of utilization for growth of two sizes of tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition*.19: 629-640.

He X., Zhang Y., Shen M., Zeng M., Zhou M., Li M., 2016. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. *Bioressources Technology.* 218: 867-873.

Hem S., Toure S., Sagbla C., Legendre M., 2008. Bioconversion of palm kernel meal for aquaculture: experiences from the forest region (Republic of Guinea). *African Journal of Biotechnology.* 7: 1192-1198.

Henry M., Gasco L., Piccolo G., Fountoulaki E., 2015. Review on the use of insects in the diet of farmed fish: past and future. *Animal Feed Science and Technology*. 203: 1-22.

Herold J.M.D., 1815. Entwicklungsgeschichte der Schmetterlinge anatomisch und physiologisch bear-beitet. *Cassel und Marburg*. 167p.

Hesolp-Harrison G., 1958. On the origin and function of the pupal stadia in holometabolous Insecta. *Proceedings of the University of Durham Philosophical Society*. 13(8): 59-79.

Heussler C.D., Walter A., Oberkofler H., Insam H., Arthofer W., Schlick-Steiner B.C., Steiner F.M., 2018. Influence of three artificial light sources on oviposition and half-life of the Black Soldier Fly, Hermetia illucens (Diptera: Stratiomyidae): Improving small-scale indoor rearing. *PLoS ONE*. 13(5): e0197896.

Heuzé V. & Tran G., 2013. Locust meal, locust, grasshoppers and Crickets. *Feedipedia. ORG*.

Hilborn R., Banobi J., Hall S.J., Pucylowski T., Wasworth T.E., 2018. The environmental cost of animal source foods. *Ecology Society of America.* 16(6): 329-335.

Hillebrand W.F., Lundell G.E.F., Bright H.A., Hoffman J.I., 1953. Applied Inorganic Analysis. Wiley, New York, USA. 217p.

Hinks C.F. & Byers J.R., 1973. Characters for determining the sex of cutworms and other noctuid larvae (Lepidoptera: Noctuidae). *Revue Canadienne de Zoologie*. 51(12): 1235-1241.

HLPE, 2014. Food losses and waste in the context of sustainable food systems. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security. Rome. Available at: www.fao.org/3/a-i3901e.pdf

Holland J., 2016. Algae becoming increasingly relevant due to soaring fishmeal and fish oil demand, prices. *Seafood Source*. 17 March 2016. https://www.seafoodsource.com/news/supply-trade/algae-becoming-increasingly-relevant-due-to-soaring-fishmeal-and-fish-oil-demand-prices

Holmes L., Vanlaerhoven S.L., Tomberlin J.K., 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*. 41(4): 971-978.

Holmes L., Vanlaerhoven S.L., Tomberlin J.K., 2013. Substrate effects on pupation and adult emergence of Hermetia illucens (Diptera: Stratiomyidae). *Environmental Entomology*. 42(2): 370-374.

Holt P.S., Geden C.J., Moore R.W., Gast R.K., 2007. Isolation of *Salmonella enterica* serovar Enteritidis from houseflies (*Musca domestica*) found in rooms containing *Salmonella* serovar Enteritidis-challenged hens. *Applied and Environmental Microbiology.* DOI: 10.1128/AEM.00803-07.

Hossain M.A., Islam M.N., Alim M.A., 1993. Evaluation of silkworm pupae meal as dietary protein source for catfish (*Heteropneustes fossilis* Bloch). *Fish Nutrition in Practice: Fourth International Symposium of Fish Nutrition and Feeding,* Biarritz, France, pp: 785-791.

Hossain M.A., Nahar N., Kamal M., 1997. Nutrient digestibility coefficient of some plant and animal poteins for rohu (*Labeo rohita*). *Aquaculture.* 151: 37-45.

Hossain H. Z., Hossain Q. H., Monir M. M. U., Ahmed M. T., 2014. Municipal solid waste (MSW) as a source of renewable energy in Bangladesh: revisited. *Renewable and Sustainable Energy Reviews*. 39: 35-41.

Hwangbo J., Hong E-C., Jang A., Kang A., Oh J.S., Kim B.W., Park B.S., 2009. Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. *Journal of Environmental Biology.* 30(4): 609-614.

INPN, https://inpn.mnhn.fr/espece/cd_nom/217341. Last access: 23/03/2017.

Ingrosso L., Novoa B., Valle A.Z.D., Cardone F., Aranguren R., Sbriccoli M., 2006. Scrapie infectivity is quickly cleared in tissues of orally-infected farmed fish. *Veterinary Research.* 2, 21.

Ilangumaran G., Stratton G., Ravichandran S., Shukla P.S., Potin P., Asiedu S., Prithiviraj B., 2017. Microbial degradation of Lobster shells to extract chitin derivatives for plant disease management. *Frontiers in microbiology.* 8 (781): 1-14.

Ishaaya I., Casida J.E., 1974. Dietary TH6040 Alters Composition and Enzyme Activity of Housefly larval cuticle. *Pesticide Biochemistry and Physiology*. 4: 484-490.

ISSER. 2016. Annual report. 38p. Available at: http://www.isser.edu.gh/images/Publication_files/ISSER%20ANNUAL%20REPORT%202015-2016.pdf

Iwalewa Megbowon and T.O. Mojekwu, 2014. Tilapia Sex Reversal Using Methyl Testosterone (MT) and its Effect on Fish, Man and Environment. *Biotechnology*. 13: 213-216.

Jabir M.D.A.R.J., Razak S.A., Vikineswary S., 2012. Nutritive potential and utilization of superworms (*Zophobas morio*) meal in the diet of Nile Tilapia (*Oreochromis niloticus*) juvenile. *African Journal of Biotechnology.* 11: 6592-6598.

Jackson A., 2016. Fishmeal and Fish oil its role in sustainable aquaculture. *Symposium on perspectives for fishmeal and fishoil*. Hirtshals, Denmark. 29-30 august 2016.

Jauncey K., & Ross B., 1982. A guide to tilapia feeds and feeding. Institute of Aquaculture, University of Stirling, Stirling. 250p.

Jauncey K., 1998. Tilapia feeds and feeding. *Pices Press LTD.* 241p.

Jeon H., Park S., Choi J., Jeong G., Lee S-B. Choi Y., Lee S-J., 2011. The intestinal bacterial community in the food waste reducing larvae of *Hermetia illucens*. *Current Microbiology*. 62: 1390-1399.

Jintasatapom O., Chumkam S., Jintasatapom O., 2011. Substitution of silkworm pupae (*Bombyx mori*) for fishmeal in broodstock diets for snakeskin gourami (*Trichogaster pectoralis*). *Journal of Agricultural Sciences and Technology.* 1: 1341-1344.

Jongema Y., 2017. List of edible insects of the world. Available at: www.wur.nl/en/Expertise-Services/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm (accessed 10 October, 2018)

Julita U., Suryani Y., Kinasih I., Yuliawati A., Cahyanto T., Maryeti Y., Permana A.D., Fitri L.L., 2018. Growth performance and nutritional composition of black soldier fly, *Hermetia illucens* (L.), (Diptera: Stratiomyidae) reared on horse and sheep manure. *IOP Conference Series: Earth and Environmental Science*. 187: 012071.

Kalová M., Borkovcová M., 2013. Voracious larvae *Hermetia illucens* and treatment of selected types of biodegradable waste. *Acta Universitatis Agriculturae et Silvicuturae Mendelianae Brunensis.* 1: 77-83.

Kanazawa A., 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture*. 155: 129-134.

Karapanagiotidis I.T., Daskalopoulou I., Vogiatzis C., Rumbos E., Mente C., Athanassiou C., 2014. Substitution of fishmeal by fly *Hermetia illucens* prepupae meal in the diet of gilthead seabream (*Sparus aurata*). *Hydromedit*. 45: 110-114.

Karapanagiotidis I.T., Bell M.V., Little D.C., Yakupitiyage A., Rakshit S.K., 2006. Polyunsaturated fatty acid content of wild and farmed tilapias in Thailand: effect of aquaculture practices and implications for human nutrition. *Journal of Agricultural and Food Chemistry*. 54: 4304–4310. DOI: 16756360.

Kareem A.O., Ogunremi J.B., 2012. Growth performance of *Clarias gariepinus* fed compounded rations and maggots. *Journal of Environmental Issues and Agriculture in Developing Countries.* 4(1): 1-5.

Kassam L. & Dorward A., 2016a. A comparative review of direct and indirect poverty impacts of small scale pond aquaculture and small and medium enterprise cage aquaculture in Ghana. *Aquaculture*. *In press.* 59p.

Kassam L. & Dorward A., 2016b. A new institutional economic analysis of pond and cage aquaculture value chains in Ghana. *Aquaculture*. *In press.* 65p.

Katayama N., Yamashita M., Wada H., Mitsuhashi J., Force S.A.T. 2005. Entomophagy as part of a space diet for habitation on Mars. *Journal of Space Technology and Sciences*. 21: 27–38.

Kawaguchi M., Misaki K., Sato H., Yokokawa T., Itai T., Nguyen T. M., Ono J., 2012. Trace Element Contamination around the E-waste Recycling Site at Agbogbloshie, Accra City, Ghana. *Environmental Pollution and Ecotoxicology*. 76: 161-167.

Kenis M., Koné N., Chrysostome C.A.A.M., Devic E., Koko G.K.D., Clottey V.A., Nacambo S., Mensah G.A., 2014. Insects used for animal feed in West Africa. *Entomologia*. 218(2): 107-114.

Killian H. & Kohler C., 1991. Influence of 17-α-methyltestosterone on red tilapia under two thermal regimes. *Journal of the Aquaculture society*. 22(2): 83-94.

Kim J.I., 1997. Newly recording two exotic insects species from Korea. Journal of Korean Biota. 2: 223-225

Kim J.G., Choi Y.C., Choi J.Y., Kim W.T., Jeong G.S., Park K.H., Hwang S.J., 2008. Ecology of the Black Soldier Fly, *Hermetia illucens* (Diptera: Stratiomyidae) in Korea. *Korean journal of applied entomology.* 47(4): 337-343.

Kim H.W., Bae S., Park K., Lee S., Choi Y., Han S., Koh Y., 2011. Biochemical characterization of digestive enzymes in the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology.* 14: 11-14.

Kim H.W., Setyabrata S., Lee Y.J., Jones O.G., Kim Y.H.B., 2016. Pre-treated mealworm larvae and silkworm pupae as a novel protein ingredient in emulsion sausages. *Innovation in Food Science and Emerging technologies.* 38: 116-123.

Kim S.Y., Lee K.Y., Kim N.J., 2016. Effects of agricultural byproducts, DDG and MSG, on the larval development of mealworms. *International Journal of Indian Entomology.* 32: 69-79.

Kimura S., 1973. The control of chitin deposition by ecdysterone in larvae of Bombyx morio. *Journal of Insect Physiology*. 19: 2177-2181.

Köprücü K. & Özdemir Y., 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (Oreochromis niloticus). *Aquaculture*. 250: 308–316.

Koven W.M., Barry Y., Lutsky S., 2001.The effect of dietaryarachidonic acid (20:4 *n* 6) on growth, survival and resistance to handling stress in gilthead seabream (sparus aurata) larvae. *Aquaculture*. 193: 107-122.

Kroeckel S., Harjes A.G.E., Roth I., Katz H., Wuertz S., Susenbeth A., Schulz C., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute –growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*. 364: 345-352.

Krogdahl A., Henre G.I., Mommsen T.P., 2005. Carbonhydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquaculture Nutrition*. 11: 103-122.

Ladròn De Guevara O., Padilla P., Garcìa L., Pino J.M., Ramos-Elorduy J., 1995. Amino acid determination in some edible Mexican insects. *Amino Acids.* 9(2): 161-173.

Lalander C.H., Senecal J., Gros Calvo M., Ahrens L., Josefsson S., Wiberg K., Vinnerås B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. *Science of the Total Environment.* 565: 279-286.

Lalander C.H., Fidjeland J., Diener S., Eriksson S., Vinnerås B., 2014. High waste-to-biomass conversion and efficient Salmonella spp. Reduction using black soldier fly for waste recycling. *Agronomy for Sustainable development*. 35 (1). DOI: 10.1007/s13593-014-0235-4.

Langeland M., Werner Hallgren S., Huyben D., Kiessling A., Vidakovic A., 2017. Comparative digestibility of 3 Black Soldier Fly products in rainbow trout. *Conference poster presented in the European Aquaculture Society Conference.* 17-20 October 2017. Dubrovnik, Croatia.

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

Lardé G., 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Biological wastes.* 33: 307-310.

Li Q.L., Zheng L., Qiu N., Cai H., Tomberlin J.K., Yu Z., 2011. Bioconversion of dairy manure by black soldier fl y (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management Research.* 31:1316-20.

Li W., Li M., Zheng L., Liu Y., Zhang Y., Yu Z., Ma Z., Li Q., 2015. Simultaneous utilisation of glucose and xylose for lipid accumulation in black soldier fly. *Biotechnology for Biofuels.* 8: 117.

Li L., Michael Stasiak L.L., Xie B., Fu Y., Gidzinski D., Dixon M., Liu H., 2016. Rearing *Tenebrio molitor* in BLSS: dietary fiber affects larval growth, development, and respiration characteristics. *Acta Astronautica.* 118: 130-136.

Leclercq M., 1997. A propos d'*Hermetia illucens* (Linnaeus, 1758) (« soldier fly ») (Diptera *Stratiomyidae : Hermetiinae*). *Bulletin et Annales de la Société royale belge d'Entomologie*. 133: 275-282.

Leong S.Y., Kutty S.R.M., Malakahmad A., Tan C.K., 2016. Feasibility study of biodiesel production using lipids of *Hermetia illucens* larvae fed with organic waste. *Waste Management.* 47: 84-90.

Lin S., Mao S., Guan Y., Luo L., Pan Y., 2012a. Dietary administration of chito oligosaccharides to enhance growth, innateimmunite response and disease resistance of *Trachinotus ovatus. Fish and Shellfish Immunology.* 32: 909-913.

Lin S., Mao S., Guan Y., Luo L., Pan Y., 2012b. Effects of dietary chitosan, oligosaccharides and *Bacillus coagulans* on the growth, innateimmunity and resistance of koi (*Cyprinus carpio koi*). *Aquaculture.* 342: 36-41.

Lindner E., 1936. Die amerikanische *Hermetia illucens* im Mittelmeergebiet (Stratiomyiidae, Dipt.). *Zoologica Anzeiger*.113: 335-336

Little D.C., 1989. An evaluation of strategies for production of Nile tilapia (*Oreochromis niloticus* L.) fry suitable for hormonal treatment. University of Stirling.

Little D.C., Satapornvanit A.; Edwards P., 2002. Freshwater fish seed quality in Asia. In: Edwards, P.; Demaine, H.; Little, D.C. (Eds.), Rural Aquaculture. CAB International, Wallingford, UK: 185-196. ISBN 9780851995656.

Little D.C. & Hulata G., 2000. Strategies for tilapia seed production, in Beveridge M.C.M., McAndrew,B.J. Tilapias: biology and exploitation. Kluwr Academic Publisher. pp: 267-326.

Liu Q., Tomberlin J.K., Brady J.A., Sanford M.S., Yu Z., 2008. Black Soldier Fly (Diptera : Stratiomyidae) larvae reduce *Escherichia coli* in dairy manure. *Pest Management*. 37(6): 1525-1530.

Liu S., Sun J., Yu L., Zhang C., Bi J., Zhu F., Qu M., Jiang C., Yang Q., 2012. Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky. *Molecules*. 17: 4604-4611.

Liu X., Chen X., Wang H., Yang Q., Rehman K.U., Li W., Cai M., Li Q., Mazza L., Zhang J., Yu Z., Zheng L., 2017. Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PlosOne*. 12 (8): e0182601.

Lock E.J., Arsiwalla T., Waagbø R., 2013. Insect larval meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) post-molt. *Aquaculture nutrition*. 43: 156-162.

Lock E.J., Arsiwalla T., Waagbo R., 2014. Insect meal: a promising source of nutrients in the diet of Atlantic salmon (*Salmo salar*), in: Vanthomme P., Munke C., Van Huis A., (Eds.), First international conference "Insects to feed the world", Wageningen university, Ede-Wageningen, The Netherlands, 74.

Lock E.J., Arsiwalla T., Waagbo R., 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postmolt. *Aquaculture nutrition.* 22: 1202-1213.

Longvah T., Mangthya K., Ramulu P., 2011. Nutrient composition and protein quality evaluation of eri silkworms (*Samia ricinii*) prepupae and pupae. *Food Chemistry.* 128(2): 400-403.

Lomas N., 2012. Comparison and selection of saprophagous Diptera species for poultry manure conversion. *M.Sc thesis.* University of Gelph. 137p.

Lord W.D., Goff M.L., Adkins T.R., Haskell N.H., 1994. The black soldier fly Hermetia illucens (Diptera: Stratiomyidae) as a potential measure of human postmortem interval: observations and case histories. *Journal of Forensic Sciences.* 39: 215-222.

Luz R.K., 2007. Resistência ao estresse e crescimento de larvas de peixes neotropicais alimentados com diferentes dietas. *Pesquisa agropecuária brasileira.* 42: 65-72.

Luz R.K. & Portella M.C., 2005. Tolerance to the air exposition test of *hoplias lacerdae* larvae and juvenile during its initial development. *Brazilian archives of biology and technology*. 48: 567-573.

Luz R.K., Ribeiro P.A.P., Ikeda A.L., Santos A.E.H., Filho R.M., Turra E.M., Teixeira E., De A., 2012. Performance and stress resistance of nile tilapias fed different crude protein levels. *Revista brasileira de zootecnia.* 41(2): 457-461.

McCallan E., 1974. *Hermetia illucens* (L.) (Diptera: Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. *Entomologist's Monthly Magazine*. 109: 232-234.

Macintosh D.J., & Little D.C., 1995. Nile tilapia (*Oreochromis niloticus*). In: Bromage, N.R., Roberts, R.J. (Eds.), *Broodstock Management and Egg and Larval Quality. Blackwell Scientific* Ltd., USA, pp. 277–320 (Chapter 12).

Macniven A.M., 1999. Development and evaluation of a stress challenge testing methodology for assessment of Nile tilapia (*Oreochromis niloticus*, Linn.) fry quality. *Master thesis.* University of Stirling. 49p.

Macniven A., Little D.C., 2001. Development and evaluation of a stress challenge testing methodology for assessment of Nile tilapia (*Oreochromis niloticus*, Linn.) fry quality. *Aquaculture research.* 32 (9): 671-679.

Madu C.T., Ufodike E.B.C., 2003. Growth and survival of catfish (*Clarias anguillaris*) juveniles fed live tilapia and maggots as unconventional diets. *Journal of Aquatic Science.* 18: 47-52.

Magalhães R., Sanchez-Lopez A., Leal R. S., Martınez-Llorens S., OlivaTeles A., Peres H., 2017. Black soldier fly (*Hermetia illucens*) prepupae meal as a fish meal replacement in diets for European seabass (*Dicentrarchus labrax*). *Aquaculture*. 476: 79-85.

Mair G.C. & Little D.C., 2001. Population control in farmed tilapia. NAGA, ICLARM Quaterly. 8-13.

Majtan J., Bilikova K., Markovic O., Grof J., Kogan G., Simuth J., 2007. Isolation and characterization of chitin from bumblebee (*Bombus terrestris*). International Journal of Biological Macromolecules. 40 (3): 237-241.

Makkar H.P.S., Tran G., Heuzé V., Ankers P., 2014. State-of-the-art of insects as animal feed. *Animal Feed Science and Technology*. 197: 1-33.

Malloch J.R., 1917. A preliminary classification of Diptera, exclusive of pupipara, based upon larval and pupal characters, with keys to imagines in certain Families. Part I. Bulletin of the Illinois State Laboratory of Natural History. 12: 323-324.

Maquart P.O., Murray F.J., Newton R.W., Leschen W.A., Little D.C. Potential for commercial scale insect-based transformation of organic waste for aquafeed and crop production in Ghana. *PhD conference.* University of Stirling, Scotland. 22 February 2015. Poster.

Manurung R., Supriatna A., Esyanthi R.R., Putra R.E., 2016. Bioconversion of rice straw waste by black soldier fly larvae (*Hermetia illucens* L.): optimal feed rate for biomass production. *Journal of Entomology and Zoological Studies.* 4(4): 1036-1041.

Manzano-Agugliaro F., Sanchez-Muros M.J., Barroso F.G., Martínez-Sánchez A., Rojo S., Pérez-Bañón C., 2012. Insects for biodiesel production. *Renewable and Sustainable Energy Reviews*. 16 (6): 3744-3753.

Marshall S.A., Woodley N.E., Hauser M., 2015. The historical spread of the Black Soldier Fly, *Hermetia illucens* (L.) (Diptera Stratiomydae, Hermetiinae), and its establishment in Canada. *Journal of Entomological Society of Ontario.* 146:51-54.

Martìnez-Sanchez A., Magaña C., Saloña M., Rojo S., 2011. First record of *Hermetia illucens* (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. *Forensic Science International*. 206: 76-78.

Martins M.L., Moraes F.R., Moraes J.R.E., 2000. Falha na resposta do cortisol ao estresse por captura e por carragenina em piaractus mesopotamicus holmberg, 1887 (osteichthyes: characidae). *Acta scientiarum*. 22: 545-552.

Mastan S.A., 2015. Use of immunostimulant in aquaculture disease management. *International Journal of Fisheries and Aquatic Studies*. 2(4): 277-280.

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

Maynard L.A. & Loosli J.K., 1979. Animal Nutrition, 6th edition. McGraw-Hill Book, New York, NY.Osava.

Meneguz M., Schiavone A., Gai F., Dama A., Lussiana C.,Renna M., Gasco L., 2018. Effects of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. *Journal of Science in Food and Agriculture.* Doi.org/10.1002/jsfa.9127. 9p.

Mensah E., Attipoe F., Ashun-Johnson M., 2013. Effect of different stocking densitities on growth performance and profitability of *Oreochromis niloticus* fry reared in hapas-in-pond system. *International Journal of Fisheries and Aquaculture*. 5(8): 204-209.

Merino G., Barange M., Mullon C., Rodwell L., 2010. Impacts of global environmental change and aquaculture expansion on marine ecosystems. *Global environmental change.* 20(4): 586-596.

Meurer F., Hayashi C., Boscolo W.R., 2005. Fontes protéicas suplementadas com aminoácidos e minerais para tilápia do Nilo durante a reversão sexual. *Revista Brasileira de Zootecnia*. 34: 1-6.

Mertenat A., Diener S., Zurbrügg C., 2019. Black Soldier Fly biowaste treatment – assessment of global warming potential. *Waste management.* 84: 173-181.

Miezah K., Obiri-Danso K., Kádár Z., Fei-Baffoe B., Mensah M. Y., 2015. Municipal solid waste characterization and quantification as a measure towards effective waste management in Ghana. *Waste Management*. 46: 15-27.

MoH, 2000. Prevalence of Diseases in Greater Accra Region. Ministry of Health, Ghana. Ministry of Works and Housing Korle Lagoon Ecological Restoration Project-EIA Report, Accra.

Morales-Ramos J.A., Rojas M.G., Shapiro-Ilan D.I., Tedders, W.L., 2011a, Self-selection of two diet components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae and its impact on fitness. *Environmental Entomology*. 40: 1285-1294.

Morales-Ramos J.A., Rojas M.G., Shapiro-Ilan D.I., Tedders, W.L., 2011b. Automated insect separation system. US Patent No. US 8,025,027 B1.

Moreau Y., 1996. Measurement of the apparent digestibility coefficients for *Oreochromis niloticus* of agro-industrial by-products available in Cote d'Ivoire. *Third International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings.* 14: 204-210.

Moreno-Garcia F., Adjallé K., Barnabé S., Raghavan G.S.V., 2017. Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. *Renewable & Sustainable Energy Reviews*. 76: 493–506.

Morimoto N., & Kiritani K., 1995. Fauna of exotic insects in japan. *Bulletin of the National institute of Agro-environmental sciences*. 12: 87-120.

Moula N., Scippo M.L., Douny C., Degand G., Dawans E., Cabaraux J.F., Medigo R.C., Leroy P., Francis F., Detilleux J., 2018. Performances of local poultry breed fed black soldier fly larvae reared on horse manure. *Animal Nutrition.* 1(4): 73-78.

Mourya V.K. & Inamdar N.N., 2008. Chitosan-modification and applications: opportunites galore. *Reactive & functional polymers*. 68: 1013-1051.

Muin H., Nurul Nadwa A.F., Razak S.A., 2012. Proximate composition, amino acid and fatty acid profile of the prepupae Black Soldier Fly (Hermetia illucens). International Seminar on Marine Science and Aquaculture, Univeristi Malaysia Sabah, Kota Kinabalu. 12-15 March 2012.

Muin H., Taufek N.M., Yusuf H.M., Razak S.A., 2014. Nutrient composition and apparent digestibility of Black Soldier Fly meal fed to Tilapia. *Conference paper.* Conference: 18th Biological Sciences Graduate Congress, At Univesity of Malaya, Kuala Lumpur, Malaysia, Volume: PT1-21.

Mutafela R.N., 2015. High value organic waste treatment via Black Soldier Fly bioconversion: onsite pilot study. Master's thesis, KHT Royal Institute of Technology. *Stockholm, Sweden.* 97p.

Myers H.M., Tomberlin J.K., Lambert B.D., Kattes D., 2008. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. *Environmental Entomology*. 37:11-15.

Mwesigye P., Mbogoma J., Nyakongo J., Idan I.A., Kapindula D., Hassan S., Berkel R. V., 2009. Intergrated assessment of present status of environmentally-sound management of wastes in Africa. United Nations Industrial Development Organisation.

Naylor R.L., Hardy R.W.Bureau D.P., Chiu A., Elliott M., Farrell A. P., Forster I., Gatlin D.M., Goldburg R.J., Hua K., Nichols P. D., 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*. 106: 15103-15110.

Naylor R.L., Goldburg R.J., Primavera J.H., Kautsky N., Beveridge M.C.M., Clay J., Folke C., Lubchenco J., Mooney H., Troell M., 2000. Effect of aquaculture on world fish supplies. *Nature*. 405(6790): 1017, DOI: 10.1038/35016500.

Nell J.A., O'Connor, W.A., 1991. The evaluation of fresh algae and stored algal concentrates as a food source for Sydney Rock Oyster, *Saccostrea commercialis* (Iredale and Roughley), larvae. *Aquaculture*. 99: 277–28.

Newby R., 1997. Use of soldier fly larvae in organic waste management. *Proceedings of the "Compost 97" Conference, 14-15 July, Griffith University, Brisbane, Australia*.

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

Newton L., Sheppard C., Watson D.W., Burtle G., Dove R., 2005. Using the Black Soldier Fly, *Hermetia Illucens*, as a Value-added Tool for the Management of Swine Manure. *Animal Poultry Waste Management Center*. North Carolina State University Raleigh. NC 17.

Nixon S. W., Buckley B. A., Granger S. L., Entsua-Mensah M., Ansa-Asare O., White M. J., Mckinney R. A., Mensah E., 2007. Anthropogenic enrichment and nutrients in some tropical lagoons of Ghana, West Africa. *Ecological Applications*. 17: 144–164.

Ng W.K., Liew F.L., Wong K.W., 2001. Potential of mealworms (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus. Aquaculture research*. 32(1): 273-280.

Nguyen T.T.X., Tomberlin J.K., Vanlaerhoven S., 2013. Influence of ressources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. *Journal of Medical Entomology*. 50(4): 898-906.

Nguyen T.T.X., Tomberlin J.K., Vanlaerhoven S., 2015. Ability of Black Soldier Fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental Entomology*. 44: 406-410.

Nogales-Mérida S., Gobbi P., Jòzefiak D., Mazurkiewicz J., Dudek K., Rawski M., Kieronczyk B., Jozefiak A., 2018. Insect meals in fish nutrition. *Reviews in Aquaculture.* DOI: *10.1111/raq.12281:* 1-24.

NRC, 2011. Nutrient requirements of fishs and shrimps. *National Research Council, National Academy Press*. Washington D.C.

Nuov S., Little D.C., Yakuptitiyage A., 1995. Nutritent flows in an integrated pig, maggot and fish production system. *Aquaculture research*. 26: 601-606.

OECD-FAO, 2011. OECD-FAO Agricultural outlook 2011-2020. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2011-en).

OECD-FAO, 2012. OECD-FAO Agricultural outlook 2012-2021. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2012-en).

OECD-FAO, 2013. OECD-FAO Agricultural outlook 2013-2022. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2013-en).

OECD-FAO, 2014. OECD-FAO Agricultural outlook 2014-2023. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2014-en).

OECD-FAO, 2015. OECD-FAO Agricultural outlook 2015-2024. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2015-en).

OECD-FAO, 2016. OECD-FAO Agricultural outlook 2016-2025. *OECD Publishing and FAO.* Available at: http://dx.doi.org/10.1787/agr_outlook-2016-en).

Ogunji J.O., Wirth M., Rennert B., 2002. Assessing the dietary amino acid requirements of tilapia, *Oreochromis niloticus* fingerlings. Deutscher Tropentag, 9-11 October 2002. Witzenhausen. 9p.

Ogunji J.O., Kloas W., Wirth M., Neumann N., Pietsch C., 2008a. Effects of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of *Oreochromis niloticus* fingerlings. *Journal of Animal Physiology and Nutrition.* 92: 511-518.

Ogunji J.O., Kloas W., Wirth M., Schultz C., Rennert B., 2008b, Effect of housefly (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). *Asian fisheries Sciences.* 21: 319-331.

Ogunji J.O., Toor R.S., Schulz C., Kloas W., 2008c. Growth Performance, Nutrient utilization of Nile Tilapia *Oreochromis niloticus* fed housefly maggot meal (Magmeal) diets. *Turkish Journal of Fisheries and Aquatic Sciences.* 8: 141-147.

Ogunji J.O., Toor R., Schultz C., Kloas W., 2008d. Growth performance, nutrient utilisation of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. *Turkish Journal of Fisheries and Aquaculture Science.* 8: 141-147.

Oliva C.F., Jacquet M., Gilles J., Lemperiere G., Maquart P.O., Quilici S., Schooneman F., Vreysen J.B., Boyer S., 2012. The sterile insect technique for controlling populations of *Aedes albopictus* (Diptera: Culicidae) on Reunion Island: mating vigour of sterilised males. *PlosOne.* 7(11): e49414. doi:10.1371/journal.pone.0049414.

Oliveira F., Doelle K., List R., O'Reilly J.R., 2015. Assessment of Diptera: Stratiomyidae, genus *Hermetia illucens* (L., 1758) using electron microscopy. *Journal of Entomology and Zoology Studies.* 3(5):147-152.

Olivier P.A., 2004. Disposal apparatus and method for efficiently bioconverting putrescent wastes. *Patent: US 6780637*.

Olivier P.A., & Hyman T., 2011. Making Waste our Greatest Resource - The Small-Scale Production of Food, Fuel, Feed and Fertilizer. 15p.

Oluokun J.A., 2000. Upgrading the nutritive value of full-fat soyabeans meal for broiler production with either fishmeal or black soldier fly larvae meal (Hermetia illucens). *Nigerian Journal of Animal Science.* 3(2): 35-45.

Olsen R.L., Suontama J., Langmyhr E., Mundheim H., Ringo E., Melle E., Malde M., Hmere G., 2006. The replacement of fish meal with Antartic krill *Euphausia superb* in diets for Atlantic salmon *Salmo salar*. *Aquaculture Nutrition*. 12: 280-290.

Olsen R.L., & Hasan M.R., 2012. A limited supply of fishmeal: impact on future increase in global aquaculture production. *Trends in Food Science and Technology.* 27: 120-128.

Omotoso O.T., 2006; Nutritional quality, functional properties and anti-nutrient composition of the larvae of *Cirina forda* (Westwood) (Lepidoptera: Saturniidae). *Journal of Zhejiand University.* 7(1): 51-55.

Oonincx D.G.A.B., De Boer I.J.M., 2012. Environmental impact of the production of mealworms as a protein source for Humans – A life cycle assessment. *PlosOne*. 7. Doi: 10.1371/journal.pone.0051145.

Oonincx D.G.A.B., Van Broekhoven S., Van Huis A., Van Loon J.J.A., 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *Plos One*. 10(12) e0144601.

Oonincx D.G.A.B., Van Keulen P., Finke M.D., Baines F.M., Vermeulen M., Bosch G., 2018. Evidence of vitamin D synthesis in insects exposed to UVb light. Scientific reports. 8: 10807.

Orzali L., Corsi B., Forni C., Riccioni L., 2017. Chitosan in agriculture : a new challenge for managing plant disease. INTECH report. 31 p.

Osava M., 1999. Soy production spreads, threatens Amazon in Brazil. Interpress servic. Available from: http://ipsnews.net/1999/09/environment-brazil-soy-production-spreads-threatens-amazon/

Oyelese O.A., 2007. Utilization of compounded ration and maggots in the diets of *Clarias gariepinus*. *Journal of Applied Science.* 2: 301-306.

PAF, 2011. Partnership of African Fisheries (PAF) Aquaculture working group: Strategic review on aquaculture feeds and feed supply.

Pastor B., Velasquez Y., Gobbi P., Rojo S., 2015. Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. *Journal of Insects as food and feed.* 1(3): 179-193.

Patterson D. & Gatlin D.M. III. 2013. Evaluation of whole and lipid-extracted algae meals in the diets of juvenile red drum (*Sciaenops ocellatus*). *Aquaculture*. 416: 92–98.

Pauly D., Christensen V., Guenette S., Pitcher T.J., Sumaila U.R., Walters C.J., Watson R., Zeller D., 2002. Toward sustainability in world fisheries. *Nature.* 418(6898): 689-695.

Park S.I., Chang B.S., Yoe S.M., 2014. Detection of antimicrobial substances from larvae of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *Entomological Research.* 44(2). Doi: 10.1111/1748-5967.12050.

Park K.P., & Kim M.M., 2010. Application of chitin and its derivatives in biological medicine. *International Journal of Molecular Sciences.* 11(12): 5152-5164.

Pelaez-Samaniego M.R., Hummel R.L., Liao W., Ma J., Jensen J., Kruger C., Frear C., 2017. Approaches for adding value to anaerobically digested dairy fiber. Renewable & Sustainable Energy Reviews. 72: 254-268.

Persson J.A., 2008. Handbook for Kjeldahl Digestion, 4th ed. FOSS, DK-3400, Hilleroed, Denmark.

Phelps R. J., Struthers J. K., Moyo S. J. L., 1975. Investigations into the nutritive value of *Macrotermes falciger* (Isoptera: Termitidae). *Zoologica qfricana* 10, 123-132.

Phelps R.P., 2006. Hormone manipulation of sex, in Lim, C., C.D. (eds.), Tilapia: Biology, culture and nutrition. CRC Press, pp: 211-252.

Phelps R.P. & Popma T.J., 2000. Sex-reversal of Tilapia, in: Costa Pierce, B.A., Rakocy, J.E. (Eds.), Tilapia Aquaculture in the Americas, Vol. 2. The World Aquaculture Society, Baton Rouge, Louisiana, pp: 34-59.

Phoku J.Z., Barnard T.G., Potgieter N., Dutton M.F., 2014. Fungi in housefly (*Musca domestica* L.) as a disease risk indicator –a case study in South-Africa. *Acta Tropica.* 140: 158-165.

Pieterse E., Erasmus S.W., Uushona T., Hoffman L.C., 2018. Black Soldier Fly (*Hermetia illucens*) pre-pupae meal as a dietary protein source for broiler production ensures a tasty chicken with standard meat quality for every pot. *Journal of Science, Food and Agriculture.* DOI*:10.1002/jsfa.9261.*

Pimsler M.L., Crippen T.L., Zheng L., Holmes L., Benbow M.E., Tarone A.M., Ziniu Y., Vanlaerhoven S.L., Tomberlin J., 2011. Bacteria regulate attraction and colonization of a resource by the black soldier fly (Diptera: Stratiomyidae). *Entomological Society of America, Annual meeting. Conference paper.*

Popa R. & Green T.R., 2012. Using black soldier fly larvae for processing organic leachates. *Journal of Economical Entomology.* 105(2): 374-378.

Popma T.J. & Green B.W., 1990. Sex reversal of tilapia in earthen ponds. Research and Development Series. International Center for Aquaculture, Alabama Agriculture Experiment Station, Auburn University, Auburn, AL, p. 15.

Pujol-Luz J.R., Francez P.A.C., Ururahy-Rodrigues A., Constantino R., 2008. The Black Soldier-fly, Hermetia illucens (Diptera, Stratiomyidae), Used to Estimate the Postmortem Interval in Case in Amapá state, Brazil. *Journal Forensic Science.* 53: 476-478.

Pullin R.S.V. & Lowe-McConnell R.H., 1982. The biology and culture of tilapias. *ICLARM Conference proceedings,* 7. International Center For Living Aquatic Ressources Management, Manila, Philippines. 432p.

Pulz O., & Gross W., 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology.* 65: 635-648.

Pratoomyot J., Bendiksen E.Å., Campbell P.J., Jancey K.J., Bell J.G., Tocher D.R., 2011. Effects of different blends of alternative protein sources as alternatives to dietary fishmeal on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.). *Aquaculture.* 316(1): 44-52.

Rachmawati P., 2010. Life history of *Hermetia illucens* (Linnaeus, 1758) (Diptera: Stratiomyidae) on decaying Palm Kernel Meal. Thesis *Sekolah Pascasarjana, Institut Pertanian Bogor*, Bogor, Indonesia, 83 p.

Ramos-Elorduy J., Moreno J., Prado E., 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis.* 10: 142–57.

Ramirez M.I., Rodriguez A.T., Alfonso L., Peniche C., 2010. Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotechnologìa Aplicada.*27: 270-276.

Rana K.J., Siriwardena S., Hasan M.R., 2009. Impact of rising feed ingredient prices on aquafeeds and aquaculture production. In: FAO (Ed.), *Fisheries and Aquaculture Technical Paper*. Food, Agriculture Organization of the United Nations, Rome, Italy, p. 63.

Rao D., Perrino E., Barreras E., 2012. The sustainability of Tilapia farming in Ghana. 20p.

Raubenheimer D. & Rothman J.M., 2011. Nutritional ecology of entomophagy in humans and other primates. *Annual Review of Entomology.* 58: 141-60.

Ravi C., Jeyashree A., Devi K.R., 2011. Antimicrobial peptides from insects: an overview. *Research in biotechnology.* 2(5): 35-46.

Ravi Kumar M.N.V., 2000. A review of chitin and chitosan applications. *Reactive & functional polymers*. 46: 1-27.

Razaviarani V., Buchanan I.D., Malik S., Katalambula H., 2013. Pilot-scale anaerobic co-digestion of municipal wastewater sludge with restaurant grease trap waste. *Journal of Environmental Management.* 123: 26-33.

Razdan A. & Pettersson D., 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. *The British Journal of Nutrition.* 72: 211-288.

Refstie S., Olli J.J. Standal H., 2004. Feed intake, growth, and protein utilization by post-smolt Atlantic salmon (Salmo salar) in response to graded levels of fish protein hydrolysate in the diet. *Aquaculture*. 239: 331–349.

Riche M., Trottier N.L., Ku P.K., Garling D.L., 2001. Apparent digestibility of crude protein and apparent availability of individual amino acids in tilapia (*Oreochromis niloticus*) fed phytase pretreated soybean meal diets. *Fish Physiology and Biochemistry.* 25: 181-194.

Richoux P., 2009. Sur la présence d'*Hermetia illucens* (Linnaeus, 1758) (Diptère Stratiomyidae) dans la région lyonnaise. *Bulletin mensuel de la Société Linnéenne de Lyon.* 78(5-6) :137-138.

Riddick E.W., 2014. Insect protein as a partial replacement for fishmeal in the diets of juvenile fish and crustaceans. *In*: Morales-Ramos J., Guadalupe Rojas M., & Shapiro-Ilan D. (eds.) Mass production of beneficial organisms. *Academic Press*, San Diego, CA, USA, pp. 565-582.

Rinaudo M., 2006. Chitin and chitosan: properties and applications. *Progress in Polymer Science*. 31: 603-632.

Rocha Oliveira F., Doelle K., Smith R.P., 2016. External morphology of Hermetia illucens Stratiomyidae: Diptera (L. 1758) based on electron microscopy. *Annual Research & Review in Biology*.9 (5): 1-10.

Roháček J. & Hora, M. 2013. A northernmost European record of the alien black soldier fly Hermetia illucens (Linnaeus, 1758) (Diptera: Stratiomyidae). Časopis Slezské zemské museum.62: 101–106.

Ross L. & Ross B., 2008. Anaesthetic & sedative techniques for Aquatic animals. 3rd edition. Blackwell publishing. *Stirling (Scotland).* 228p.

Rozendaal J.A., & World Health Organization, 1997. Vector control: methods for use by individuals and communities. World Health Organization eds. 412p.

Rozkošný R., 1983. A biosystematics study of the European *Stratiomyidae* (*Diptera*). II. *Series Entomologica.* 25. 431p.

Rumpold B.A. & Schlüter O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Sciences & Emerging Technologies*. 17: 1-11.

Rumpold B.A. & Schlüter O., 2015. Insect-based protein sources and their potential for human consumption: nutritional composition and processing. *Animal Frontiers.* 5(2):20-24.

Rurangwa E., Agyakwah S.K., Boon H., Bolman B.C., 2015. Development of Aquaculture in Ghana: Analysis of the fish value chain and potential buisness cases. Wageningen report. 58p.

Russin T.A., Boye J.I., Arcand Y., Rajamohamed S.H., 2011. Alternatives techniques for defatting soy: a pratical review. *Food bioprocessing technologies.* 4: 200-223.

Rust M.B., 2003. Nutritional Physiology. Chapter in *fish nutrition.* Doi: 10.1016/B978-012319652-1/50008-2.

Saidin N., Rahman F.A., Abdullah N., Animal feed: Halal perspective. *International conference on humanities, social sciences and education.* London, 20-21 March 2017.

Saint-Hilaire S., Cranfill K., McGuire M.A., Mosley E.E., Tomberlin J.K., Newton L., Sealey W., Sheppard C., Irving S., 2007. Fish offal recycling by the Black Soldier Fly produces a foodstuff high in Omega-3 Fatty acids. *Journal of the world aquaculture society.* 38(2): 309-313.

Sakakura Y., Koshio S., Lida Y., 1998. Dietary vitamin c improves quality of yellowtail (seriola quinqueradiata) seedlings. *Aquaculture.* 161: 427-436.

Sánchez-Arroyo H., 2011. House fly, *Musca domestica* Linnaeus. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Url: http://creatures.ifas.ufl.edu

Sánchez-Muros M.J., Barroso F.G., De Haro C., 2016. Brief summary of insect usage as an industrial animal feed/feed ingredient. *Chapter 10 in Insects as sustainable food ingredients.* Elsvier.

Sánchez-Muros M.J., De Haro C., Sanz A., Trenzado C.E., Villareces S., Barroso F.G., 2015. Nutritional evaluation of Tenebrio molitor meal as fishmeal substitute for Tilapia (*Oreochromis niloticus*) diet. *Aquaculture nutrition*. 22(5). 1-13.

Sanches L.E.F., & Hayash C., 1999. Densidade de estocagem no desempenho de larvas de tilápia-do-nilo (Oreochromis niloticus), durante a reversão sexual. *Acta Scientiarum*. 21: 619-625.

Sarker P.K., Kapuscinski A.R., Bae A.Y., Donaldson E., Sitek A.J., Fitzgerald D.S., Edelson O.F., 2018. Toward sustainable aquafeeds: evaluating substitution of fishmeal with lipid-exctracted microalgal co-product (*nannochloropsis oculata*) in dietsof juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS ONE.* 13(7): e0201315.

Sauter W., 1989. Interessante neue Insektenfunde a us der Schweitz (Dipt., Lep.). *Mittheilungen der Schweizer. Entomologischen Gesellschaft*. 62:147-149.

Sawangkeaw R. & Ngampraserthsith S., 2013. A review of lipid-based biomasses as feedstocks for biofuel production. *Renewable and Sustainable Energy Reviews.* 25: 97-108.

Schiavone A., Cullere M., De Marco M., Meneguz M., Biasato I., Bergagna S., Dezzutto D., Gai F., Dabbou S., Gasco L., Dalle Zotte A., 2016. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: effects on growth performances, feed-choice, blood traits, caracass characteristics and meat quality. *Italian Journal of Animal Science.* DOI: 10.1080/1828051X.2016.1249968.

Schiavone A., De Marco M., Martinez S., Dabbou S., Renna M., Madrid J., Hernandez F., Rotolo L., Costa P., Gai F., Gasco L., 2017. Nutritional value of partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*. 8: 51.

Schremmer V.F., 1986. Die polymetabole larval-entwicklung der waffenfliegenart Hermetia illucens –Ein beitrag zur metamorphose der Stratiomyidae. Naturhistorisches Museum Wien. 88: 405-429.

Schroeder G.L., 1980. Fish farming in manure-loaded ponds. *Technical manual*. 86p.

Schumacher A., Wax C., Gropp J.M., 1997. Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. *Aquaculture*. 151: 15–28.

Sealey W.M., Gaylord T.G., Barrows F.T., Tomberlin J.K., Mcguire M.A., Ross C., St-Hilaire S., 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched Black soldier fly prepupae, *Hermetia illucens*. *Journal of World Aquaculture Society*. 42: 34-45.

Se-Kwon, K. & Niranjan R., 2005. Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review. *Carbohydrates Polymers*. 62: 357-368.

Sharp R.G., 2013. A review of the application of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yield. *Agronomy*. 3: 757-793.

Sharholy M., Ahmad K., Vaishya R. C., Gupta R. D., 2007. Municipal solid waste characteristics and management in Allahabad, India. *Waste management*. 27(4): 490-496.

Shelton W., Rodriguez-Guerrero D., Lopez-Macias J., 1981. Factors affecting androgen sex reversal of *Tilapia aurea*. *Aquaculture*. 25(1): 59-65.

Sheppard D.C., Newton G.L., Thompson S.A., Savage S., 1994. A value added manure management system using the black soldier fly. *Bioresource technology*. 50(3): 275-279.

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

Sheppard D.C., Burtle G., Newton G.L., 2008.”Fishmeal replacement with Black Soldier Fly Prepupae”: A predictive report prepared for the NOAA Aquaculture Programs and the NOAA-USDA Alternative Feeds Initiative. 3p.

Sheppard D.C., Tomberlin J.K., Joyce J.A., Kiser B.C., Sumner S.M., 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*. 39(4): 695-8.

Shiau S.Y. & Yu Y.P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Orechromis niloticus*×*O. aureus*. *Aquaculture.* 179: 439–446.

Singh-Ackbarali D. & Maharaj R., 2017. Mini-livestock ranching: solutions to reducing the carbon footprint and negative environmental impacts of agriculture. In Environmental Sustainability and Climate Change Strategies; Ganpat W., Isaac W-A., Eds.; IGI Global: Hershey, PA, USA; pp:188-212.

Skidmore P., 1985. The biology of the *Muscidae* of the world. *Junk*, Dordrecht, The Netherlands. 550p.

Skrivanova E., Marounek M., Benda V., Brezina P., 2006. Suceptibility of *Eschericha coli, Salmonella sp.* and *Clostridium perfringens* to organic acids and monolaurin. *Veterinary medicine.* 51: 81-88.

Smetana S., Palanisamy M., Mathys A., Heinz V., 2016. Sustainability of insects use for feed and food: life cycle assessment perspective. *Journal of Cleaner Production.* 137: 741-751.

Sogbesan A.O. & Ugwumba A.A.A. 2008. Nutritional values of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. *Turkish Journal of Fish and Aquaculture Sciences.* 164: 159-164.

Speedy A.W., 2003. Global production and consumption of animal source foods. *Journal of Nutrition.* 133(11): 4048S-4053S. Doi: 10.1093/jn/133.11.4048S.

Spinelli J., 1980. Unconventional feed ingredients for fish feed, in: Fish feed technology. Lectures presented at the FAO/UNDP training course in fish feed technology, Held at the college of fisheries, University of Washington, U.S.A., 9 October-15 December 1978. FAO, Rome, pp. 187-214.

Spranghers T., Ottoboni M., Klootwijk C., Ovyn A., Deboosere S., De Meulenaer B., Michiels J., Eekhout M., De Clercq P., De Smet S., 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of Science and Food Agriculture.* 97: 2594-2600.

St-Hilaire S., Cranfill K., McGuire M.A., Mosley E.E., Tomberlin J.K., Newton L., Sealey W., Sheppard C., Irving S., 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *Journal of World Aquaculture Society*.38: 309-313.

Stamer A., 2015. Insect proteins—A new source for animal feed. *EMBO*. 16:676–680. doi: 10.15252/embr.201540528.

Stamer A., Wesselss S., Neidigk R., Hoerstgen-Schwark G., 2014. Black Soldier Fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. *Proceedings of the 4th ISOFAR scientific conference. 'Building Organic Bridges',* at the Organic Wolrd Congress, 13-15 October, Istanbul, Turkey.

Steinfeld H., Gerber P., Wassenaar T., Castel V., Rosales M., De Haan C.P.R.F., 2006. Livestock's long shadow: Environmental Issues and Options. FAO, Rome, Italy.

Stevens J.R., Newton R., Tlutsy M., Little D.C., 2018. The rise of aquaculture by-products: increasing food production value, and sustainability through strategic utilisation. *Marine policy.* Doi: 10.1016/j.marpol.2017.12.027.

Sugita H., Yamada S., Konagaya Y., Deguchi Y., 1999. Production of *β-N*-acetylglucosaminidase and chitinase by *Aeromonas* species isolated from river fish. *Fish Science.* 65: 155–158.

Sugiura S.H., Dong F.M., Rathbone C.K., Hardy R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*. 159: 177– 202.

Surendra K., Olivier R., Tomberlin J.K., Jha R., Khamal S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable energy*. 98: 197-202.

Sutton M., 1995. Archaeological aspects of insect use. *Journal of Archaeological Method and Theory.* 2: 253–98.

Ssepuuya G., Sebatta C., Sikahwa E., Fuuna P., Sengendo M., Mugisha J., Fiaboe K.K.M., Nakimbugwe D., 2019. Perception and awareness of insects as an alternative protein source among fish farmers and fish feed traders. *Journal of Insects as feed and food.* Doi.org/10.3920/JIFF2017.0056. 10p.

Ssymank A. & Doczkal D., 2010. *Hermetia illucens* (Linnaeus, 1758) (Stratiomyidae), a soldier fly new for the German fauna. *Studia dipterologica*. 16:84-86.

Tacon A.G.J., Stafford E.A., Edwards C.A., 1983. A preliminary investigation of the nutritive value of thee terrestrial lumbricid worms for rainbow trout. *Aquaculture*. 35: 187-199.

Tacon A.G.J., De Silva S.S., 1997. Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. *Aquaculture.*151: 379-404.

Tacon A.G.J. & Metian M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*. 285: 146-158.

Tacon A.G.J., & Metian M., 2015. Feed matters: Satisfying the feed demand of Aquaculture. *Reviews in Fisheries Sciences & Aquaculture.* 23: 1-10.

Tao W., Fattah K.P., Huchzermeier M.P., 2016. Struvite recovery from anaerobically digested dairy manure: a review of application potential and hindrance. *Journal of Environmental Management.* 169: 46-57.

Teotia J.S. & Miller B.F., 1973. Fly pupae as a dietary ingredient for starting chicks. *Poultry Sciences.* 52: 1830-1835.

Thackray A.M., Di Y., Zhang C., Wolf H., Pradl L., Vorberg I., Andreoletti O., Bujdoso R., 2014. Prion-induced and spontaneous formation of transmissible toxicity in PrP transgenic Drosophila. *Biochemical Journal*. 463: 31-40.

Thackray A.M., Muhammad F., Zhang C., Denyer M., Spiropoulos J., Crowther D.C., Bujdoso R., 2012. Prion-induced toxicity in PrP transgenic *Drosophila*. *Experimental and Molecular Pathology*. 92: 194-201.

Thackray A.M., Zhang C., Arndt T., Bujdoso R., 2014. Cytosolic PrP can participate in prion-mediated toxicity. *Journal of Virology*. 88: 8129-8138.

The Economist, 2014. Food loss and its intersection with food security. Global food security index 2014: an annual measure of the state of global food security. The Economist Intelligence Unit, London, UK. Available at: http://www.economist.com

Thévenot A., Rivera J.L., Wilfart A., Maillard F., Hassouna M., Senga-Kiesse T., Le Féon S., Aubin J., 2018. Mealworm meal for animal feed: Environmental assessment and sensitivity analysis to guide future prospects. *Journal of cleaner Production.* 170: 1260-1267.

Tilman D., Balzer C., Hill J., Befort B.L., 2011. Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Acadamy of Sciences.* 108: 20260. Doi: 10.1073/pnas.1116437108

Tiu L., 2012. Enhancing sustainability of freshwater prawn production in Ohio. Ohio State University South Centers Newsletter. 11(4): 4.

Tocher D.R. & Harvie D.G., 1988. Fatty acid compositions of the major phosphoglycerides from fish neural tissues; (n-3) and (n-6) polyunsaturated fatty acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and retinas. *Fish Physiology and Biochemistry.* 5: 229-239.

Tòth S., 1994. Angaben zur schweb- un waffenfliegenfauna der insel brissago, Südschweiz (Diptera: Syrphidae, Stratiomyidae). *Entomologische Berichte Luzern*. 32:119-126.

Tomberlin J.K. & Sheppard D.G., 2001. Lekking behaviour of the Black Soldier Fly (Diptera: Stratiomyidae). *Florida Entomologist.* 84(4): 729-730.

Tomberlin J.K., Sheppard D.C., Joyce J.A. 2002: A comparison of selected life history traits of the black soldier fl y (Diptera: Stratiomyidae) when reared on three diets. *Annals of the Entomological Society of America*. 95: 379-387.

Tomberlin J.K., Sheppard C., Joyce J.A., 2004. Black soldier fly (Diptera: Stratiomyidae) colonization of pig carrion in South Georgia. *Journal Forensic Science.* 50: 152-153.

Tomberlin J.K., Van Huis A., Benbow M.E., Jordan H., Astuti D.A., Azzollini D., Banks I., Bava V., Borgemeister C., Cammack J.A., Chapkin R.S., Čičková H., Day A., Dicke M., Drew D.J.W., Emhart C., Epstein M., Finke M., Fischer C.H., Gatlin D., Grabowski N.T., He C., Heckman L., Hubert A., Jacobs J., Josephs J., Khanal S.K., Kleinfinger J.F., Klein G., Leach C., Liu Y., Newton G.L., Olivier R., Pechal J.L., Picard C.J., Rojo S., Roncarati A., Sheppard C., Tarone A.M., Verstappen B., Vickerson A., Yang H., Yen A.L., Yu Z., Zhang J., Zheng L., 2015. Protecting the environment through insect farming as a mean to produce protein for use as livestock, poultry and aquaculture feed. *Journal of insects as food and feed.* 1(4): 307-309.

Troell M., Naylor R.L., Metian M., Beveridge M., Tyedmers P.H., Folke C., Arrow K.J., Barrett S., Crépin A.S., Ehrlich P.R., Gren A., Kautsky N., Levin S.A., Nyborg K., Österblom H., Polasky S., Scheffer M., Walker B.H., Xepapadeas T., De Zeeuw A., 2014. Does aquaculture add resilience to the global food system? *PNAS.* 111(37): 13257-13263.

Toyama G.N., Corrente J.E., Cyrino J.E.P., 2000. Suplementação de vitamina C em rações para reversão sexual da tilápia do Nilo. *Scientia Agrícola.* 57:221-228.

Tritsch I. & Arvor D., 2016. Transition in environmental governance in the Brazilian Amazon: emergence of a new pattern of socio-economic development and deforestation. *Land Use Policy*. 59. 446-455

Trushenski J., Schwarz M., Takeuchi R., 2010. Physiological responses of cobia rachycentron canadum following exposure to low water and air exposure stress challenges. *Aquaculture*. 307: 173-177.

Tsagkarakis A.E., Arapostathi E.I., Strouvalis G.I., 2015. First record of the black soldier fly, *Hermetia illucens*, in Greece. *Entomologia Hellenica.* 24 (2): 27-30.

Tschirner M. & Simon A., 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of insects as food and feed*. DOI: 10.3920/JIFF2014.0008.

Turchetto M., Lafisca S., Costantini G., 2001. Postmortem interval (PMI) determined by study sarcophagous biocenoses: three cases from the province of Venice (Italy). *Forensic Science International*. 120: 28-31.

Tylko G., Banach Z., Borowska J., Niklinska M., Pyza E., 2005. Elemental changes in the brain, muscle, and gut cells of the housefly, *Musca domestica*, exposed to heavy metals. *Microscopy Research and Technique*. 66:239-247.

United Nation Organisation, Department of Economic and Social Affairs, 2017. World population prospects, the 2017 revision. *United Nations. New York.* 53p.

Ur Rehman K., Cai M., Xiao X., Zheng L., Wang H., Soomro A.A., Zhou Y., Li W., Yu Z., Zhang J., 2017. Cellulose decomposition and larval biomass production from the co-digestion of dairy manure and chicken manure by mini-livestock (*Hermetia illucens* L.). *Journal of Environmental Management.* 196: 458-465.

Ushakova N.A., Dontsov A.E., Bastrakov A.I., Garmash N.Y., Pavlov D.S., 2017. Paramagnetics melanin and Mn2+ in black soldier fly Hermetia illucens. *Doklady Biochemistry and Biophysics*. 473(1): 102-105.

Ushakova N.A., Ponomarev S.V., Bakaneva Y.M., Feydorovykh Y.U., Levina A.O., Kotel'nikov A.V., Kotel'nikova S.V., Bastrakov A.I., Kozlova A.A., Pavlov D.S., 2018. Biological efficiency of the prepupae *Hermetia illucens* in the diet of the young Mozambique Tilapia *Oreochromis mossambicus. Biology bulletin.* 45(4): 382-387.

Üstüner T., Hasbenli A., Rozkošný R., 2003. The first record of *Hermetia illucens* (Linnaeus, 1758) (Diptera, Stratiomyidae) from Near East. *Studia dipterologica*. 10(1): 181-185.

Vahedi G. & Ghodratizadeh S., 2011. Effect of chitin supplemented diet on innate immune response of rainbow trout. *World journal of fish & marine science*. 3(6): 509-513.

Valkenburg C., Gerber M. A., Walton C. W., Jones S. B., Thompson B. L., Stevens D. J., 2008. Municipal solid waste (MSW) to liquid fuels synthesis, volume 1. 98p.

Van Anholt R.D., Koven W.M., Lutzky S., 2004. Dietary supplementation arachidonic acid alters the stress response of gilthead seabream (sparus aurata) larvae. *Aquaculture*. 238: 369-383.

Van Broekhoven S., Oonincx D.G.A.B., Van Huis A., Van Loon J.J.A., 2015. Growth performance of feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. *Journal of Insect Physiology.* 73. DOI: 10.1016/j.jinsphys.2014.12.005

Van Der Spiefel M., Noordam M.Y., Van Der Fels-Klerx, 2013. Safety of novel protein sources (Insects, microalgae, seaweed, duckweed and rapeseed) and legislative aspects for their application in food and feed production. *Comprehensive reviews in food science and food safety.* 12(6): 662-678.

Van Huis A., Van Itterbeeck J., Klunder H., Mertens E., Halloran A. Muir G., Vanthomme P., 2013. Edible insects: future prospects for food and feed security. *FAO Forestry paper.* 171: 201p.

Van Itterbeeck J. & Van Huis A., 2012. Environmental manipulation for edible insect procurement: A historical perspective. *Journal of Ethnobiology and Ethnomedicine*. 8: 1-7. Doi: 10.1186/1746-4269-8-3.

Van Krimpen M.M., Bikker P., Van Der Meer I.M., Van Der Peet-Schwering C.M.C., Vereijken J.M., 2013. Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternatives for imported soybean products. Report 662. Wageningen UR Livestock research, Wageningen, Netherlands. 63p.

Van Zanten H.H.E., Mollenhorst H., Oonincx D.G.A.B., Bikker P., Meerburg B.G., De Boer I.J.M., 2015. From environmental nuisance to environmental opportunity: housefly larvae convert waste to livestock feed. *Journal of Cleaner Production.* 102: 362-369.

Vandenberg G.W. & De la Noue J., 2001. Apparent digestibility comparison in rainbow trout (Oncorhynchus mykiss) assessed using three methods of faeces collection and three digestibility markers. Aquaculture Nutrition. 7: 237-245.

Veldkamp T., Van Duinkerken G., Van Huis A., Lakemond C.M.M., Ottevanger E., Bosch G., Van Boekel M.A.J.S., 2012. Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. *Rapport 638 - Wageningen Livestock Research.* 250p.

Vera Cruz E.M. & Mair G.C., 1994. Condition for effective androgen sex-reversal in *Oreochromis niloticus* (L.). *Aquaculture.* 122: 237-248.

Verbeke W., Spranghers T., De Clercq P., De Smet S., Sas B., Eeckhout M., 2015. Insect in animal feed: acceptance and its determinants among farmers, agriculture sector stakeholders and citizens. *Animal feed science and technology*. 16p.

Vetter J., 2007. Chitin content of cultivated mushrooms Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes. *Food Chemistry*. 102: 6-9.

Vickerson A., Radley R., Marchant B., Kaufluss O., Kabaluk T., 2015. Hermetia illucens frass production and use in plant nutrition and pest management. *Patent N° WO 2015013826 A1*.

Wallace P.A., Nyameasem J.K., Adu-Aboagye G.A., Affedzie-Obresi S., Nkegbe K., Murray F., Botchway V., Karbo W., Maquart P.O., Clottey V., 2018. Effects of replacing fishmeal with black soldier fly larval meal in the diets of grower-finishing guinea fowls reared under tropical contritions. *Tropical Animal Health and Production.* 50(9): 1499-1507.

Wallace P.A., Nyameasen J.K., Adu-Aboagye G.A., Affedzie-Obresi S., Nkegbe E.K., Karbo N., Murray F., Leschen W., Maquart P.O., 2017. Impact of black soldier fly larval meal on growth performance, apparent digestibility, haematological and blood chemistry indices of guinea fowl starter keets under tropical conditions. *Tropical Animal Health Production.* 49(6): 1163-1169.

Walsh M.J., Van Doren L.G., Sills D.L., Archibald I., Beal C.M., Lei X.G., 2016. Algal food and fuel coproduction can mitigate greenhouse gas emissions while improving land and water-use efficiency. *Environmental Research Letters*. 11(11): 114006.

Wang H., Zhang Z., Czapar G.F., Winkler M.K.H., Zheng J., 2013. A full scale house fy (Diptera: Muscidae) larvae bioconversion system for value added swine manure reduction. *Waste Management Ressources.* 31: 223-231.

Wang Y.S. & Shelomi M., 2017. Review of Black Soldier Fly (*Hermetia illucens*) as animal feed and human food. *Foods*. 6(10): 1-27.

Wassermann G.J. & Afonso L.O., 2002. Validation of the Aceto-Carmine Technique for evaluating phenotypic sex in Nile Tilapia (*Oreochromis niloticus*) fry. *Ciencia rural santa maria*. 32(1): 133-139.

Watanabe T., Takeuchi S., Satoh S., Kiron V., 1996. Digestible crude protein contents in various feedstuffs determined with four freshwater fish species. *Fisheries Sciences.* 62: 278-282.

Weatherup R.N. & McCracken K.J., 1998. Comparison of the estimation of digestibility of two diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), using two markers and two methods of faeces collection. Aquaculture Ressources. 29: 527-533.

Windell J.T., Foltz J.W., Sarokon J.A., 1978. Methods of fecal collection and nutrient leaching in digestibility studies. *Progress in Fish-Culture*. 40: 51–55.

Woodley N.E., 1989. Phylogeny and classification of the "Orthorrhaphous" Brachycera. In: McAlpine J.F., Wood D.M., Editors. Pp. 1371-1395. Manual of Neartic Diptera. Mongraph 32, Volume 3. Research Branch, Agriculture Canada.

World Bank, 2013. Fish to 2030: prospects for fisheries and aquaculture. *Agriculture and Environmental Services Discussion paper N°3.* Washington D.C., World Bank Group.

W.H.O., 1986. The housefly—biology and control. Training and information guide (advanced level). Geneva, World Health Organization, WHO/VBC/ 86.937. 22p.

Wu S.X., 2009. Studies on optimization of rearing condition and nutriment content of larvae of *Tenebrio molitor* L. *Anhui Agricultural University, Master degree thesis*. 78p.

Wybourne B.A. & Carter C.G., 1999. The effect of plant meal inclusion on feed intake and nutritional adaptation by Atlantic salmon, Salmo salar L. Fishmeal Replacement in Aquaculture Feeds for Atlantic Salmon. Project 93/120. Fisheries Research and Development, Canberra, Australia, pp. 100– 126.

Xiao X., Jin P., Zheng L., Cai M., Yu Z., Yu J., Zhang J., 2018. Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the

growth and immune index of yellow catfish (*Pelteobagus fulvidraco*). *Aquaculture research*. 49: 1569-1577.

Xu J.Y., Miao X.W., Liu Y., Cui S.R., 2005. Behavioral response of tilapia (*Oreochromis niloticus*) to acute ammonia stress monitored by computer vision. *Journal of Zhejiang University Science.* 6(8): 812-816.

Yu G.H., Chen Y.H., Yu Z.N., Cheng P., 2009. Research progress on the larvae and prepupae of black soldier fly *Hermetia illucens* used as animal feedstuff. *Chinese Bulletin of Entomology*. 4-: 41-45.

Yu G.H., Cheng P., Chen Y., Li Y., Yang Z., Chen Y., Tomberlin J., 2011. Inoculating poultry manure with companion bacteria influences growth and development of Black Soldier Fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology.* 40(1): 30-35.

Zarate D.D. & Lovell R.T., 1997. Free lysine (L-lysine. HCL) is utilised for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish. *Aquaculture*. 159: 87-100.

Zhao Y., Park R.D., Muzzarelli R.A., 2010. Chitin deacetylases: properties and applications. *Marine Drugs.* 8(1):24-46.

Zheng L., Crippen T.L., Singh B., Tarone A.M., Dowd S.E., Ziniu Y., Wood T.K., Tomberlin J., 2013. A survey of bacterial diversity from successive life stages of Black Soldier Fly (Diptera: Stratiomyidae) bu using 16S rDNA pyrosequencing. *Journal of Medical Entomology.* 50(3): 647-658.

Zhou F., Tomberlin J.K., Zheng L., Yu Z., Zhang J., 2013. Developmental and waste reduction plasticity of three Black Soldier Fly strains (Diptera: Stratiomyidae) raised on different livestock manures. *Journal of Medical Entomology*. 50(6): 1224-1230.

Ziegler F., Hornborg S., Green B.S., Eigaard O.R., Farmery A.K., Hammar L., Hartmann K., Molander S., Parker R.W., Hognes E.S., Vàzquez-Rowe I., Smith D.M., 2016. Expanding the concept of sustainable seafood using Life Cycle Assessment. *Fish and Fisheries.* 17(4): 1073-1093.

Zurek L., Schal C., Watson D.W., 2000. Diversity and contribution of the intestinal bacterial community to the development of *Musca domestica* (Diptera: Muscidae) larvae. *Entomological Society of America*. 37(6): 924-928.

# APPENDIX. PUBLICATIONS AND PRESENTATIONS FROM THE PROJECT

**In peer-reviewed journals :**

- Wallace P.A., Nyameasem J.K., Adu-Aboagye G.A., Affedzie-Obresi S., Nkegbe E.K., Karbo N., Murray F., Leschen W., **Maquart P.O.**, 2017. Impact of Black Soldier Fly larval meal on growth performance, apparent digestibility, haematological and blood chemistry indices of guinea fowl starter keets under tropical conditions. *Tropical Animal Health Production. DOI 10.1007/s11250-017-1312-x. 7p.*
- Devic E. & **Maquart P.O.**, 2015. *Dirhinus giffardii* (Hymenoptera : Chalcididae), parasitoid affecting Black Soldier Fly production systems in West Africa. *Entomologia.* 3,284: 25-27.
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**Large-audience articles:**

- **Maquart P.O.**, Murray F., Leschen W., Newton R., Little D.C., 2018. Black Soldier Fly: a future for tilapia feed ? *Aquafeed.* 10. 4: 20-22.

**Conference poster:**

- Quilliam R., Nuku Adeku C., **Maquart P.O.**, Newton R., Murray F. Insect frass biofertilisers: a novel soil amendment for resource-poor peri-urban farmers. *Rural transformation and urbanization.* Uppsala, Sweden. 20-21 september 2017. Poster.
- **Maquart P.O.**, Devic E., Murray F.J., Leschen W., Newton R., Raanan B., Turner W., Ducharne F., Viala F., Little D.C. From waste to feed : a novel feed source for monosex tilapia fry. *European Aquaculture Society.* Edinburgh, Scotland. 20-23 September 2016. Poster.
- **Maquart P.O.,** Murray F.J., Newton R.W., Leschen W.A., Little D.C. Potential for commercial scale insect-based transformation of organic waste for aquafeed and crop production in Ghana. *PhD conference.* University of Stirling, Scotland. 22 February 2015. Poster.

**Conference talks:**

- Quilliam R., Nuku Adeku C., **Maquart P.O.**, Newton R., Murray F. Insect frass biofertilisers: a novel soil amendment for resource-poor peri-urban farmers. *Rural transformation and urbanization.* Uppsala, Sweden. 20-21 september 2017. 30 minutes.
- **Maquart P.O.** 26 October 2017. Experience of insects as biorefining factories: The case of the Black Soldier Fly. *International Conference on Natural Product Biotechnology.* Aberdeen, Scotland. 15 minutes

- **Maquart P.O.** 19 November 2016. From waste to feed: the Black Soldier Fly as a novel feed source for monosex tilapia fry. *Lunch Time Seminar*. University of Stirling. Stirling, Scotland. 30 minutes.
- **Maquart P.O.** 4 December 2015. From waste to feed: a novel feed source for Aquaculture. *Farmer's day.* Kpong-Akatamansu, Ghana. 20 minutes.