ASSESSING INSECT-BASED PRODUCTS AS FEED INGREDIENTS FOR AQUACULTURE

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

Emilie Danielle Paule Devic

June 2016

UNIVERSITY of STIRLING

Faculty of Natural Sciences

Institute of Aquaculture

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.

Name: Emilie Danielle Paule Devic

Sign:

Date:

Primary supervisor: Professor David C. Little

Sign:

Date:

Acknowledgements

Firstly I would like to express my gratitude to Prof. Dave Little, Dr. Matthew Sprague, Dr. Francis Murray and Dr. Kim Jauncey who contributed to my supervision. A particular thanks to Will Leschen too for his great support to achieve the project and encouragements during these last years.

My work was part of the EU FP7 project PROteINSECT (Grant Agreement n°312084) and I would like to acknowledge all the partners involved, in particular Dr. Marc Kenis, Geert Bruggeman, Martin Roffeis and Dr. Elaine Fitches. I would like to acknowledge all the persons who contributed to this project and who helped me over the last years to achieve this work. I am particularly thankful to those who helped me during my field work overseas. In Ghana: Mark Amechi, Berzak Raanan, Jacques Magnee and my dear friend Mr. Charles Adeku; in Thailand: Warren Turner and all the staff from Nam Sai Farms, in particular, Jeab and Aem. I am also grateful to those who generously supported this project with advices, materials and some of their precious time: Dr. Richard Newton, Alastair McPhee and Rob Murray (Buckieburn); Vasilis Karalazos and Daniel Leeming (BioMar Ltd); Erik-Jan Lock and his colleagues Rune Waagbø, Marit Espe, Nini Sissener (NIFES); James Dick, Fiona Strachan, Graeme McWhinnie, Billy Struthers (University of Stirling NAS technical staff); Franck Ducharne and Frederic Viala (Entofood, Malaysia). I am thankful to Dr. Richard Quilliam (BES, University of Stirling) who help me to set up and conduct the agronomy trial.

Thanks to all my friends, near or far, for all the good moments and support during these last years (Nico, Alex, Fanette, A-L, Odile, Saliha, Marie, Sean, Xris, Muni, Janielle, POM, etc.). I must sincerely thank Saurin Hem and Dr. Domenico Caruso, my mentors, who transferred me their passion and determination.

A ma famille, mes parents, mes sœurs (Laura, Chrystel, Moon), Papy et Patrick, merci pour votre soutient, vos précieux mots d'encouragement et votre amour sans conditions et aussi pour vos visites tout autour du monde. Roberto, pour avoir partagé ces années avec moi, pour ton amour à toute épreuve, les nombreux sacrifices, les mots et le soutient dans les moments de doute, grazie.

Abstract

Research has been actively looking for alternative feed ingredients to reduce the reliance of the aquafeed industry on marine ingredients, namely fish meal (FM) and fish oil (FO). In this context, insects, in particular housefly (*Musca domestica*) and black soldier fly (BSF, *Hermetia illucens*) larvae, have been identified as promising candidates. Although a global insect farming industry is emerging, it is for now constrained by regulatory and technical bottlenecks that raise the question 'where and how insect-based products could be integrated into aquaculture'. The literature indicated a high interspecies variability of the results when replacing FM with insect meals in fish diets and previous work failed to consider the existing challenges related to the insect production to demonstrate commercial relevance and applicability. In this thesis, maggot meals (MM) and frass (insect digestate) were assessed as strategic feed ingredients for two commercially important farmed species: Atlantic salmon, (*Salmo salar*) and Nile tilapia (*Oreochromis niloticus*), in their relevant contexts. Case studies showed that both housefly and BSF MM are high quality feed ingredients and suitable alternative to FM. Specifically, dietary inclusions of up to 200 g/kg of crude or defatted housefly larvae meal did not compromised the feed digestibility and utilisation and the growth performance and body composition of salmon parr (freshwater stage), compared to a FM-based control diet. Hormone (17α-methyltestosterone) treated diets containing between 250 and 1000 g/kg BSF or housefly meal were found as effective as a commonly used pure hormone-treated FM in sex-reversal process leading to 99.8 to 100% males, high survival and evenness of the fish produced. In a commercial diet for advanced nursing of Nile tilapia fingerlings, up to 80 g/kg BSF meal was included without impairing the fish performance and body composition; dietary inclusion was limited by the lipid content of the crude MM. Finally, BSF frass derived from brewery spent grains or processed food wastes were found more effective when used as soil bio-fertilisers with minimum application rate of 10.0 tonnes/ha or 5.0 tonnes/ha, respectively (for a spring onion culture), rather than supplemental feeds for tilapia farmed in semi-intensive conditions (fertilised pond). The study also indicated that site-specific conditions should be accounted to support appropriate and sustainable use of insect-based products but in any case, juvenile fish should be strategically targeted given their requirements. It is expected that this approach, could support the sustainable intensification of aquaculture and contribute more broadly to food security whilst contributing to the development of a circular economy.

List of abbreviations

AA	Amino Acid(s)
ADC	Apparent Digestibility Coefficient(s)
ASF	Animal Source Food
BSF	Black Soldier Fly
BW	Brewery Wastes
CEF	Controlled Environment Facility
CSC	Critical Standing Crop
DDGS	Distiller's Dried Grains with Solubles
DEFRA	Department for Environment, Food and Rural Affairs
DHA	Docosahexaenoic Acid (22:6n-3)
DM	Dry Matter
DO	Dissolved Oxygen
EAA	Essential Amino Acid(s)
ECR	Economic Conversion Ratio
EFA	Essential Fatty Acid(s)
EPA	Eicosapentaenoic Acid (20:5n-3)
FA	Fatty Acid(s)
FCR	Feed Conversion Ratio
FM	Fish Meal
FO	Fish Oil
FW	Food wastes
HGV	Heavy Goods Vehicle
IBS	Integrated Bio-System
IPIFF	International Platform of Insects for Food and Feed
Lc PUFA	Long-chain Polyunsaturated Fatty Acid(s)
LIDC	Low-Income Developing Country(ies)
MM	Maggot Meal(s)
N	Nitrogen
n-3	Omega-3 Fatty Acid(s)
n-6	Omega-6 Fatty Acid(s)
NFE	Nitrogen Free Extract
OM	Organic Matter
PAP	Processed Animal Protein(s)
PI	Profit Index
RB	Rice Bran
RFID	Radio Frequency Identification
SF	Supplementary Feed(s)
SGR	Specific Growth Rate
UK	United Kingdom
USA	United States of America
USD	United States Dollar(s)

Table of contents

List of tables

List of figures

Chapter 1. General introduction

1.1 Introduction

In this introductory chapter, a short overview of the current state of the aquaculture and aquafeed industries, including the challenges related to the reliance of the sector on finite resources such as marine ingredients, used at unsustainable rates, will firstly frame the context of this study. In the literature, the use of alternative feed ingredients was suggested as one of the solutions to overcome the issue. Thus, following a description of the criteria leading to the selection of suitable alternative sources of protein especially, current conventional and unconventional feedstuffs will be reviewed. Then, the particular case of insects, identified as promising candidates in the global assessment of potential feedstuffs, will be approached from the nutritional, production, and regulatory aspects. Finally, focus will be made on dipterans (flies) by reviewing the reasons for the recent rising interest from the aquaculture and aquafeed industries, the outcomes of the previous research studies on different farmed fish species and the remaining bottlenecks and limitations that hamper the full development of this emerging industry. From the foregoing discussion, knowledge gaps being identified, research hypotheses and objectives of the present study will be presented.

1.2 Aquaculture and aquafeeds

1.2.1 Generalities

Aquaculture is a fast growing industry contributing globally to the food security. Food fish production, for instance, has increased at an annual average rate of 6.2% between 2000 and 2012 (FAO, 2014). The increasing population worldwide, expected to reach 9.7 billion individuals by 2050 (United Nations Department of Economic and Social Affairs, 2015), is driving up the demand for animal source food (ASF) and aquatic food in particular (Speedy, 2003; Troell *et al.*, 2014). Wild fish captures have remained stable for the last 20 years and are not expected to increase considering the current state of the natural stocks; therefore, the demand for food fish is more likely to be supplied by aquaculture, through the development, the intensification and the diversification of the sector. In 2012, aquaculture contributed to almost the half of the global food fish supply and models predicted a contribution of 62% by 2030 (World Bank, 2013; FAO, 2014). Thanks to technological progress and markets improvements, culture of low-trophic species such as tilapia, carps and *Pangasius*/catfish are expecting to expand at a

faster rate than high-value species, such as salmon, for which the industry have already been through substantial advancements over the last years. Therefore, while salmon production is predicted to increase slightly, tilapia production is more likely be multiplied by two between 2008 and 2030 (World Bank, 2013).

Not different from terrestrial livestock, exogenous feeding methods are commonly used in aquaculture to support fish growth and economic performance of the farming systems. In 2014, a total one billion tonne of animal feed was produced among which aquafeeds represented only 4.0 % remaining, therefore, a minor user of exogenous feeds (IFIF, 2014). However, between 2010 and 2012, non-fed aquaculture species production decreased from 33.5 to 30.8% of the total global food fish production, indicating a substantial growth in the farming of fed species (FAO, 2014). Fed-aquaculture is represented by more than 200 fish and crustacean species among which eight species or groups account for 62.2% of the total feed used: carps (*Ctenopharyngodon idellus*; *Cyprinus carpio* and *Carassius carassius*), Nile tilapia (*Oreochromis niloticus*), catla (*Catla catla*), whiteleg shrimp (*Litopenaeus vannamei*), Atlantic salmon (*Salmo salar*), pangasiid catfishes (*Pangasianodon hypophthalmus* and *Pangasius bocourti*), and rohu (*Labeo rohita*) (Tacon *et al*., 2011; FAO, 2012). In 2008, 29.2 million tonnes of aquafeeds were produced and shared among 11 groups of farmed species as represented in Figure 1.1.

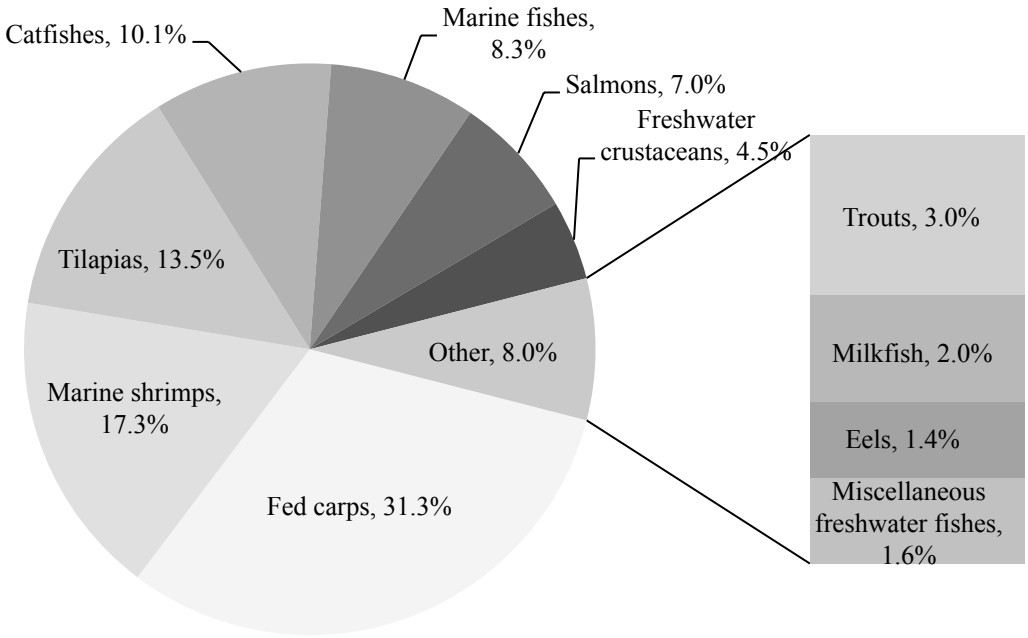

Figure 1.1 Share of the total global production of commercial aquafeeds between the main farmed group aquatic species of in 2008 (adapted from FAO, 2014)

Feeds and feeding practices are various and depend on multiple factors such as the purpose of the farming activity (market / profit or home / local consumption), the financial resources and/or the availability of the feeds or feedstuffs (price, quantity, quality, etc.). Intensive and semi-intensive aquaculture systems rely on the use of exogenous feeds that can be formulated commercial diets, farm-made feed mixtures or even single feedstuffs. Complete formulated feeds, covering specific nutritional requirements, are commonly used to ensure high performances of intensive systems; recent advancements and research have led to the development of effective feeds improving nutrients utilisation, conversion ratios and production costs. For instance, carnivorous species and species cultured in flowing water are being fed with highly nutritious extruded pellets whereas species grown in nutrients-rich water (ponds, fertilised water bodies), at low density, are traditionally fed with supplementary feeds, usually fresh feeds (single feedstuff), farm-made mixes or cheap commercial diet (Hardy, 2006; Hasan *et al.*, 2007; Tacon *et al.*, 2011).

1.2.2 Challenges

Feeds generally account for 40-60 % of the production costs in intensive and semi-intensive aquaculture suggesting that volatility of the feeds and feed ingredients prices can jeopardised business profitability (Hasan *et al.*, 2007; Rana *et al.*, 2009). The animal feed industry is undeniably dependent on resources available and their respective market prices, which vary according to the rules of supply and demand. Given the expected growth of the worldwide population, the needs for food, water, space and energy will continue to pull prices of the commodities upwards. The aquafeed industry is highly dependent on fish meal (FM) and fish oil (FO) as high-quality sources of protein, energy and essential fatty acids (EFA) meeting the requirements of the juvenile stages (fry, fingerlings) of several fish species and of carnivorous species. Marine ingredients are mainly derived from natural pelagic stocks now being over-exploited, by-catches fisheries of fish trimmings or offal processing from the food industry (Tacon *et al.*, 2006; IFFO, 2013). It was estimated that in 2008, aquaculture was the main consumer of marine ingredients with 60.8% of the global FM production (the rest being shared with the pig and poultry farming mainly) and 73.8% of the global FO production (the rest being used for human food and supplements mainly) going to aquafeeds, contributing significantly to the continuous depletion of the

natural resources becoming scarce and expensive (Hardy and Tacon, 2002; New and Wijkström, 2002; Merino *et al.*, 2010; Tacon *et al.*, 2011).

In the aquafeed sector, high-value carnivorous finfish species such as salmonids and crustaceans are highly dependent on FM and FO (Figure 1.2). To a lesser extent, compound feeds for omnivorous and herbivorous farmed finfish species (carps, tilapias and catfishes) often contain FM and FO as a secondary source of nutrients and energy or to improve feed palatability (Tacon *et al.*, 2006). Nevertheless, steadily declining inclusions levels of FM and FO are reported in most aquatic species compound feeds, in response to increasing price (Tacon and Metian, 2008).

Indeed, similarly to other commodities, FM and FO prices are continuously rising, owing to the increasing competition and demand, the limited supply related to the over-exploitation of finite resources and some environmental aspects (El-Niño) and the increasing costs of energy and transportation. According to recent trends, it is expected that between 2010 and 2030, FM and FO prices will rise by 90 and 70 %, respectively (World Bank, 2013). Pressure on natural resources can also raise questions about sustainability, however with proper management and responsible sourcing, current production levels could be maintained without affecting further fish stocks (Jackson, 2012; IFFO, 2013; World Bank, 2013). Nonetheless, given the rapid expansion of aquaculture, demand for feed and feed ingredients will certainly increase further. Therefore, it is becoming essential to further reduce the aquaculture reliance to marine ingredients by reducing the dietary levels in most farmed fish.

In addition, because the development of the aquaculture production is more likely to be in favour of non-carnivorous species, the sustainable supply of animal and plant proteins, lipids and carbohydrates sources other than FM and FO will also become essential given the multiple uses of these resources (livestock feeding, biofuels, human nutrition, etc.) (Tacon *et al.*, 2011). Although dietary requirements differ inter and intra-species (depending on their life stage), the dispensation of feeds that meet the fish specific requirements is essential for optimal growth and health. The challenge of the aquafeed industry is, therefore, to identify sustainable, consistent, and cost-efficient feed ingredients to meet the high nutritional requirements of each aquatic species.

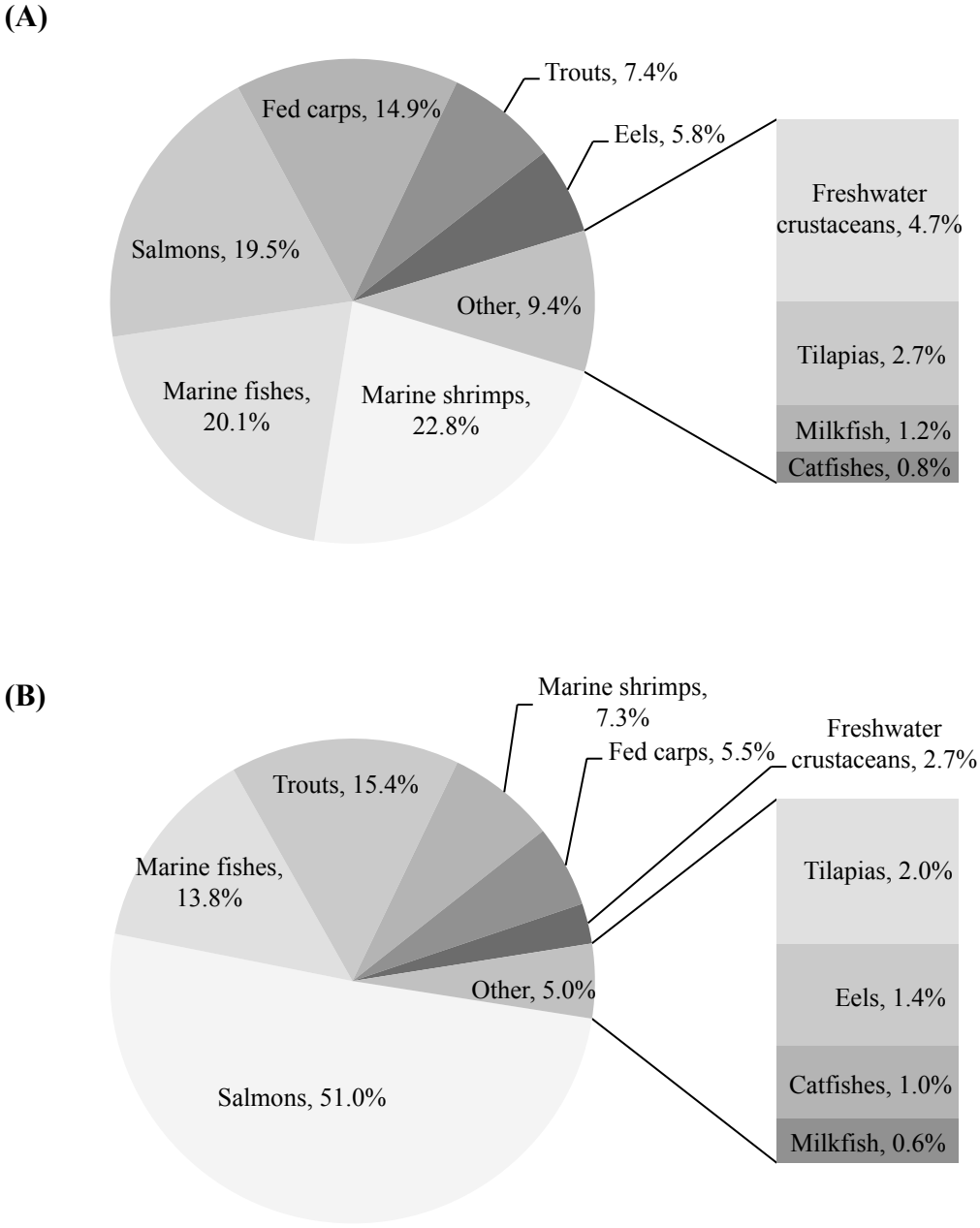

Figure 1.2 Estimated global use of (A) fish meal and (B) fish oil (percentage of as fed basis) within compound aquafeeds in 2003 by major species (source: Tacon *et al.,* 2006)

1.2.3 Solutions and future prospects

Feed volumes and therefore, expensive feed ingredients such as FM and FO, used in aquaculture can be reduced by improving the feed efficiency, and more specifically, the feeding practices and feed conversion ratios (FCR). Further reductions of marine ingredients dietary inclusion levels should ultimately lead to a more efficient use (Naylor *et al.*, 2009). FO is an almost unique and the most economically viable dietary source of EFA and especially omega 3 long-chain polyunsaturated fatty acids (n-3 LcPUFA), potential alternatives will not be further discussed here. Nutrients originating from FM (amino acids, minerals, etc.) could be obtained by a balanced combination of other feed ingredients and although this is challenging it is not impossible as proved by the significant progress made over the last decades (Tacon *et al*., 2011; FAO, 2014). Although FM and FO dietary inclusions have already been drastically reduced for several finfish and crustaceans cultured species, it is expected that these ingredients become more strategically used, at critical stages of the production systems to meet high and specific requirements (Tacon and Metian, 2008; Tacon *et al*., 2011; Jackson, 2012).

Over the last years, intensive research has contributed to identify various alternative ingredients from different origins (animal, plant or single-cell) which have contributed to the decreased use of FM in aquafeeds. Olsen and Hasan (2012) reported a reduction in the estimated proportion of FM used in salmon feed from 45 to 22 % between 1995 and 2010 and a projected further decrease from 22 to 12 % between 2010 and 2020. Among non-fish alternatives, soybean meal and its derived concentrates have become a common protein supplement in aquafeeds; in 2007, it represented 25 % (in weight) of the total compound aquafeeds produced (Gatlin *et al.*, 2007). In 2008, it was reported that feeds for herbivorous and omnivorous fish and crustaceans contained in average 25 % soybean meal (Tacon *et al.*, 2011). In feeds for carnivorous species, especially salmonids, inclusion levels are however limited due to nutritional characteristics that can negatively impact fish performance (Pratoomyot *et al.*, 2011). Nevertheless, given the environmental and socioeconomic impacts related to the production of crop-based ingredients such as soy (deforestation, use of agro-chemicals, social displacement, geographic sourcing, etc.), concerns about the increasing use for aquaculture are also arising (WWF, 2014; Fry *et al*., 2016).

More research is needed to continue identifying high-quality, nutritionally competent and safe feed ingredients, that are also produced or sourced sustainably (Tacon *et al.*, 2011). Complete evaluation including not only the nutritional aspects but also the whole value chain (production, processing and costs) and the environmental and human health implications of the potential alternatives should be addressed. In order to avoid repeating the errors committed in the past, a sustainable management of the resources used should also be seriously considered.

1.3 Alternative sources of proteins

1.3.1 Selection criteria

To define the suitability of an alternative feed ingredient, a range of aspects must be considered such as nutritional and physical characteristics, functionality, availability, sustainability or market price (Glencross *et al.*, 2007; NRC, 2011). Although several protein sources might have a nutritional profile similar to FM, it will never be completely identical; for instance, plant protein sources present high similarities with FM in terms of apparent protein digestibility, however their amino acid (AA) composition is limiting and do not match fish dietary requirements. In addition, effects on the fish performance are also key criteria; alternative feedstuffs can reduce the palatability of a feed or cause heath issues (enteritis for example), subsequently resulting in a reduction of the feed intake which compromise the growth (Hardy, 2006). Although the nutrient composition is important, so it is the identification of anti-nutritional factors that can affect the fish physiology on various aspects (Francis *et al.*, 2001).

Obviously, if an alternative feedstuff is more cost-effective than FM and does not have adverse effects on the fish performance, it is almost directly accepted by feed manufacturers. Effectiveness can be manipulated and improved by various processing methods, but this often results in overpriced products (protein concentrates, synthetic AA, etc.) affecting competitiveness and consequently, leading to more expensive diets to manufacture (NRC, 2011; Rust *et al.*, 2011).

Several alternatives to FM have already been identified and are widely used in aquafeeds, but some challenges remain (see 0 above) and there is still a window for new markets and novel products, which should be assessed in independent contexts.

Identification, evaluation and use of locally available products such as unconventional and underutilised protein sources are anticipated (Tacon *et al.*, 2011).

1.3.2 Alternative sources of protein

1.3.2.1 Plant protein sources

So far, plant protein sources have been the main choice to replace FM in fish diets; mostly used are oilseed meals (soybean, rapeseed, sunflower and cottonseed), grain meals (wheat and corn glutens) and legumes (peas, beans, peanuts and lupin). High substitution levels of marine ingredients with plants were achieved for herbivorous and omnivorous farmed species whereas, for carnivorous species, it was limited for the various reasons stated (see 1.3.1: nutritional deficiencies, reduced digestibility, enteritis, effects on growth), even if the nutritional quality has been improved by further processing (heating, defatting), pre-treating (enzyme), or by adding dietary supplements (AA, antioxidants) (Francis *et al.*, 2001; Hardy, 2006; Gatlin *et al.*, 2007; Olsen and Hasan, 2012).

1.3.2.2 Animal protein sources

Processed animal protein ingredients, principally terrestrial animal by-products such as meat and bone meals, blood meals and poultry by-products, are more comparable to FM than plants in terms of AA composition, however, the nutritional composition is highly variable depending on the product (NRC, 2011). Despite the relaxation of the European restrictions implemented after the outbreak of bovine spongiform encephalopathy in the United Kingdom (UK) in the 1980's, to use animal products in aquaculture and the evidence suggesting that risks of contamination through fish is close to zero, there is still a continuing mistrust in the sector that limits usage in Europe (Ingrosso *et al.*, 2006; Dalla Valle *et al.*, 2008; Naylor *et al.*, 2009).

It is estimated that the global volume of animal by-products meals available is 2 to 3 times greater than that of FM, resulting in the largest source of animal protein (Tacon *et al.*, 2006). Other animal protein sources that have attracted interest lately are fish hydrolysates (protein concentrates), that can be derived from by-products, improving growth and feed intake of farmed species like the Atlantic salmon (Refstie *et al.*, 2004) and krill meal (zooplankton) that requires more investigation in terms processing but

has been successfully used as a taste enhancer or pigment in fish feeds (Tacon *et al.*, 2006; NRC, 2011).

1.3.2.3 Others

At last, single-cell proteins from bacteria, yeast and micro-algae have been assessed in few studies as FM substitutes for finfish and shrimps. Advantages remain in the high nutritive value and productivity of the systems where cells multiply (up to 10,000 tonnes per year for microalgae for example according to Richmond, 2004) using mostly renewable carbon sources derived from agro-industry waste streams (Tacon *et al.*, 2006). Nevertheless, industrial systems involve high-cost innovative processes resulting in very expensive raw materials, in other words, it may not be a viable solution for aquaculture yet (Tacon *et al.*, 2006; Olsen and Hasan, 2012).

1.3.3 Unconventional and underutilised sources of protein

Undoubtedly, there are still poorly or un-investigated tracks to explore. These are underutilised, not efficiently studied, forgotten or sometimes, endemic resources that are not widely acknowledged or not commercialised also called unconventional sources of nutrients. Being nutrient-rich, a variety of unconventional feedstuffs have been used in fish nutrition and previous studies indicated encouraging results; however, all were associated with issues that prevented them from breaking through (Table 1.1). It is the growing need for ASF in developing countries in Asia and Africa that has revealed the potential of these products, commonly used by small-holder farmers through low-cost, farm-made, feed formulations (Hasan *et al.*, 2007; PAF, 2011); thus, non-conventional feedstuffs have been mainly studied in local context and considered for only low-trophic levels species such as tilapia and catfish (El-Sayed, 2004; Hasan *et al*., 2007; Sogbesan and Ugwumba, 2008a). Given the growing demand for cost-effective feedstuffs, it is becoming critical to develop local opportunities to produce nutritional, safe, sustainable and economically viable alternative ingredients to support the development of the aquaculture sector, in particular under-utilised sources of nutrients (El-Sayed, 2004; Rust *et al.*, 2011; Tacon *et al.*, 2011). In this context, thanks to the development of sustainable novel technologies that could be adapted globally, and depending on the current identified limitations and cost associated, the standardisation and up-scaling production of selected unconventional feedstuffs are probable.

Table 1.1 Some examples of unconventional feedstuffs and the associated issues limiting a conventional use

Unconventional feedstuffs		**Limitations**									
		Availability / consistency related aspects				**Nutritional aspects**			**Cost related aspects**		
Type	Examples	Geographic specificity	Seasonality	Low volume	Inconsistent quality	Low digestibility	Toxic or / anti-nutrients factors	Biological / chemical contamination risks	Pre-treatment / processing	Selection / collection	Sold (not free)
Leaves protein	Cassava, cucumber, squash …	●	●	●		●	●		●		●
Aquatic plants	Duckweed, water hyacinth …	●		●		●		●			
Cakes and pulps	Palm or Jatropha kernel cakes, sugar beet or coffee pulps, olive cake …	●				●	●		●	●	●
Invertebrates	Earthworms, termites, snails, marine polychaetes…	●		●	●					●	
Other animal protein sources	Tadpole and toads, feather meal, fish silage …	●		●	●				●	●	●
Organic wastes and by-products	Table wastes, market wastes, brewery wastes, cereal brans …	●	●		●	●		●		●	●
Farmed insects	Maggots, crickets, silkworm, mealworms …			●					●		●

Based on Spinelli, 1980; Rojas, 2002; Hasan *et al*., 2007; Sogbesan and Ugwumba, 2008; Abowei and Ekubo, 2011; Krome, 2014; PAF, 2011; Tacon *et al*., 2011

1.4 Insects, an emergent feedstuff

Invertebrates, in particular insects, have always been considered as non-conventional feed ingredients because of their limited usage for rural aquaculture, their limited supply (wild capture or small-scale farming) and non-commercialisation (Abowei and Ekubo, 2011). Using insects as a source of nutrients is not a novel concept; edible insect have been part of human diets for centuries (Bukkens, 1997) and first investigations on insects as potential feedstuffs for animals started in the 1970's mainly for poultry and pigs (Teotia and Miller, 1974; Phelps *et al*., 1975; Newton *et al*., 1977; Calvert, 1979); later in the 1980's research started to investigate the potential of invertebrate for fish (Bondari and Sheppard, 1981, 1987; Tacon *et al*., 1983; Stafford and Tacon, 1984). Since the beginning of the 2000's, a renewed interest in insects has risen, motivated by the global need for alternative feed resources and a diversification of the waste management strategies.

1.4.1 Nutritional aspects

Numerous studies have demonstrated the suitability of insects as a source of nutrients for animals. Moreover, terrestrial and aquatic insects or other invertebrates are part of several fish species natural diets stressing their potential as a feed ingredient for farmed fish (Henry *et al.*, 2015). Nutritional composition varies widely among species and depends also on the life stage and the rearing conditions (Sánchez-Muros *et al.*, 2014). Barroso *et al.* (2014) selected 16 species among the orders Coleoptera, Diptera and Orthoptera and compared the analysed nutritional compositions to FM and soybean meal, the most commonly used ingredients in aquafeeds. Compared to FM, these insects had lower protein contents (40-60 %, on a dry matter basis) and higher lipid levels (20 % in average). AA composition was mainly related to the taxonomic groups and it appeared clearly that orthopteran and coleopteran essential amino acid (EAA) profiles were similar to soybean meal whereas dipteran EAA profiles were better balanced than soybean meal and highly similar to FM. The study has also highlighted the influence of the insect diets (rearing substrate) on their fatty acid (FA) profile ; this was also suggested by St-Hilaire *et al*. (2007a) who managed to enrich BSF larvae with n-3 LcPUFA, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, through feeding on fish offal. The possibility to manipulate insects FA composition is a great

advantage to overcome the lack of LcPUFA, essential for carnivorous and marine fish species. At the opposite, defatting process was also suggested to improve the quality of insect meals to remove unnecessary lipids and FA not matching with the fish dietary requirements and concentrate proteins and AA (Fasakin *et al*., 2003; Kroeckel *et al*., 2012; Henry *et al*., 2015).

Although no anti-nutrient factor has been identified in insects, chitin, the main constituent of insect cuticles and indigestible polysaccharide for monogastric animals including fish (Rust, 2002), may impair the fish performance (Shiau and Yu, 1999; Olsen *et al*., 2006). However, the contradictory results of numerous studies reviewed by Henry *et al*. (2015) suggest that more investigations are required to clarify this aspect. In addition, although chitin removal might improve protein quality and feed digestibility (Newton *et al.*, 2005; Sheppard *et al.*, 2007; Rumpold and Schlüter, 2013), technical and economic feasibility of chitin extraction have yet to be determined (Diener *et al.*, 2011).

Finally, antifungal activity and antibacterial peptides have been detected in numerous insect species, potentially improving the shelf-life of insect-based feeds (Ravi *et al.*, 2011; Zhao *et al.*, 2010).

1.4.2 Insect farming

Mass production of insect is fundamental to supply the growing demand for proteins in both human and animal nutrition, avoiding thereby, the over-exploitation of wild resources (Sánchez-Muros *et al.*, 2014). Insect farming is not a new practice as commercial mass production systems already existed in sericulture (silkworms), apiculture (bees) or to support the integrated and biological control of pests in agriculture (Rumpold and Schlüter, 2013; Morales-Ramos *et al.*, 2014); this knowledge was the basis for the development of mass rearing of edible insects. Insect farming is essential to ensure quality, safety, traceability and consistency of the insect-based products (Rumpold and Schlüter, 2013) and it is not very different than conventional livestock in its principle since it is a process converting a source of nutrients (feed) into biomass (Defoliart, 1995; Paoletti and Dufour, 2002). Locust and crickets (Orthoptera), mealworms (Coleoptera) and black soldier fly (BSF) and common housefly larvae (Diptera) are now successfully farmed globally and might be key insects to consider for

animal and fish nutrition (van Huis *et al.*, 2013; Barroso *et al.*, 2014; Drew and Pieterse, 2015).

The production of a kilogramme of insect biomass result in less greenhouse gas emissions and requires less land than conventional livestock production, thereby resulting in a smaller ecological footprint (Oonincx *et al*., 2010; Oonincx and de Boer, 2012). Insects are highly prolific organisms that grow fast relying on various organic substrates. Highly efficient converters, it is suggested that insects fed on low or no-value organic wastes can reduce the environmental impact of the livestock sector (Oonincx *et al*., 2015; van Zanten *et al*., 2015).

1.4.3 Waste remediation and circular economy

Global concerns are rising toward the amount of waste generated by human activities nowadays, in particular organic wastes which include animal manures, crop residues, food processing wastes, municipal biosolids and wastes from some industries (Westerman and Bicudo, 2005). With 30 to 40% of the food produced globally currently wasted or lost (FAO, 2011) and the intensification of the livestock industry expecting to generate twice more manures by 2050 (FAO, 2006), sustainable solutions are most wanted. The use of waste streams or mass flows of no or low economic value not yet harnessed in other value chains (low competition) is the most favourable option to consider to farm insects cost-efficiently and sustainably (PROteINSECT, 2016a) and thanks to their ability to feed on and benefit from nutrient-poor substrates, insects are usually cultured on organic wastes or by-products from the food and agro-industry (van Huis *et al.*, 2013). Therefore, mass-rearing systems are developed following the integrated bio-systems (IBS) approach (Warburton *et al.*, 2002) where products or mass flows of no or low economic value, that could impair the environment if not appropriately treated or disposed, are used to generate valuable products in an environmentally friendly manner; the latter becoming subsequently inputs of another system, thereby closing the loop for nutrients and materials flows, resulting in zero wastes (Figure 1.3).

Although challenges remain, related to selection, transportation or risks of contamination, there is a waste remediation opportunity associated with the production of insect biomass and by-products (residues or frass), that can be further processed by

separating various fraction (oil, protein extract, chitin…), depending on the degree of technology, and ultimately used as biofuels, feed ingredients / additives or bio-fertilisers for crops (Das *et al.*, 2010; Li *et al.*, 2011a; van Huis *et al.*, 2013). The value chain that is developing around the emerging insect farming industry is circular and based on the relationships between the environment (ecological impacts and benefits) and the market demand for alternative feedstuffs (economic activities). Thus, the efficient use of insects can close the nutrients loop applying the principles of circular economy (Veldkamp *et al*., 2012; van Huis *et al*., 2013).

In Europe, the development of a Circular Economy Strategy has been initiated (EC, 2016a) and it includes the revision of the regulations concerning waste management and recycling strategies and the market for secondary raw materials. This initiative would probably contribute to develop further the insect farming industry by integrating it in the sustainable European economic growth.

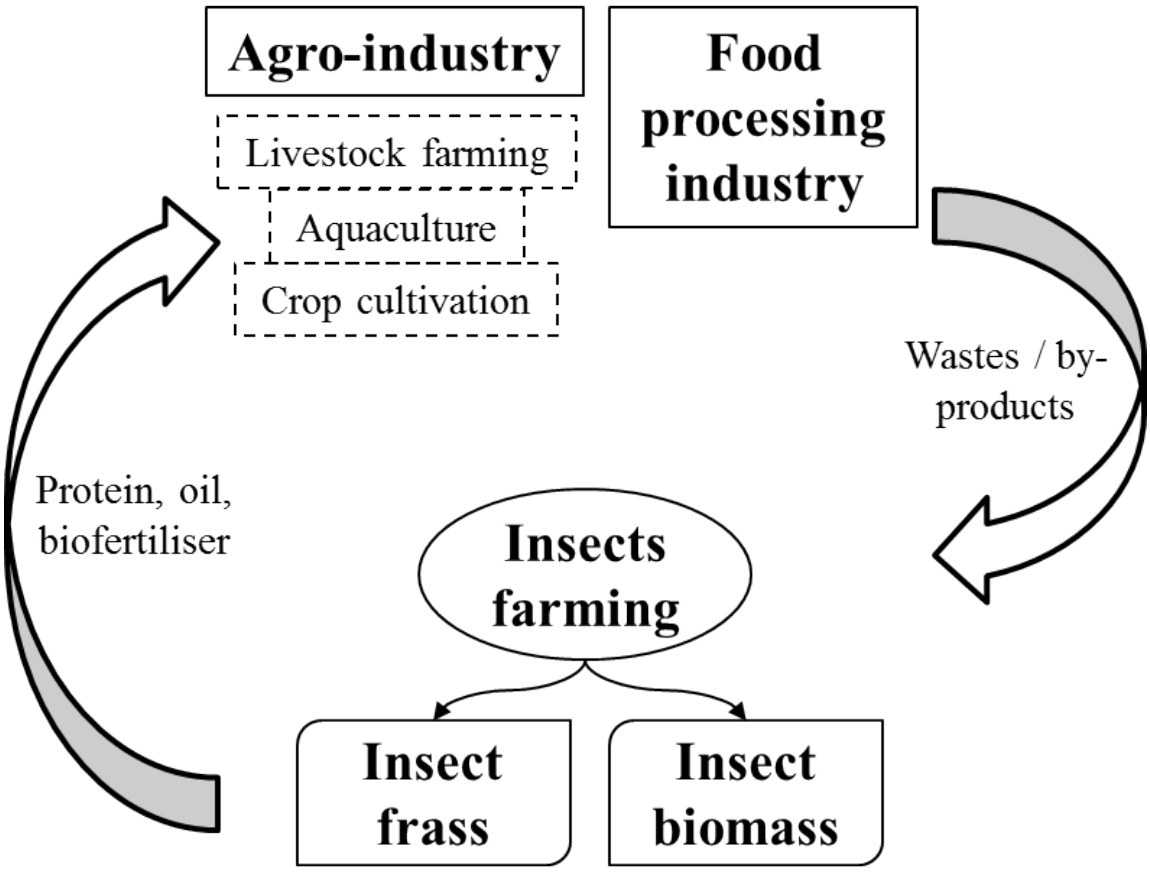

Figure 1.3 Schematic representation of the insect-substrate-products integrated bio-systems (IBS)

1.4.4 Regulatory frameworks

In Europe, since June 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 aquatic species, feeds. As farmed insects are non-pathogenic invertebrates species to human or animals, they are considered as Category 3 material (EC regulation 1069/2009). Re-authorised PAP (EU regulation 142/2011) are derived-Category 3 materials and must answer to stringent requirements during collection, transport and processing, including slaughter in certified abattoirs which is not applicable to insects (Halloran and Münke, 2014). In addition, under the EC regulation 1069/2009, farmed animals intended for animal feeding can only be reared on authorised substrates which do not include animal manures, treated wood and kitchen and table wastes. This last condition restricts considerably the list of substrates that can be used to produce insects and excludes most evident wastes streams. To prevent cross-contamination risks, the EC regulation 1069/2009 also requires that animal by-products and derived PAP imported from non-member countries follow conditions as strict as those applicable to the European Community.

Finally, only hydrolysed insect PAP and insect oil (derived insect fat) can be used in aquafeeds if the insects have been reared on 100 % vegetables and/or eggs and dairy products which are not restricted substrates (EC regulation 999/2001; EC regulation 1069/2009; EU regulation 142/2011).

In a recent scientific opinion report, EFSA Scientific Committee (2015) highlighted that risks of contamination (both biological and chemical) are still high within the whole value chain (from the farming to the processed product) and need to be assessed. Given the lack of knowledge identified, it was suggested that more studies should evaluate the occurrence of biological and chemical hazards when insects are used as food and feed.

In other countries, such as the USA, Canada, Australia or New-Zealand, insects are not listed as acceptable and safe feed ingredients (Halloran and Münke, 2014). In Ghana, Mali, Kenya and Uganda there is no legislation that prevents or authorises the use of insect in animal feeds whereas in China insect meal and defatted insect powder are both listed in the Feed Materials Catalogue as suitable animal feed ingredients (Halloran and Münke, 2014; PROteINSECT, 2013).

1.5 The particular case of flies larvae (Diptera, Insecta)

1.5.1 Interest and benefits

Flies belong to the large order Diptera (from the Greek *'di'*: two, and *'ptera'*: wings) and are holometabolous species meaning that their life cycle is divided into distinct larval and adult life stages (Wiegmann and Yeates, 2007).

1.5.1.1 Farming benefits

With the emergence of an insect industry aiming at producing novel feed ingredients by recycling organic materials fly species, in particular the common housefly (*Musca domestica*) and the Black Soldier Fly (BSF, *Hermetia illucens*) have attracted attention thanks to their numerous attributes. First, they are almost globally distributed, which permits exploitation without species introduction in most part of the world. Being saprophagous, larval stages (or maggots) can fed on various organic resources including low or no-value wastes or by-products; they are considered as great recyclers (Larde, 1990; El Boushy, 1991; Hem *et al*., 2007; Diener, 2010; Čičková *et al*., 2012c; Veldkamp *et al*., 2012; Zhang *et al*., 2012; Wang *et al*., 2013; Nguyen *et al*., 2015; Oonincx *et al*., 2015). The adult housefly feeds on sources of carbohydrate and protein whereas adult BSF relies only on water to survive (Skidmore, 1985; Tomberlin *et al.*, 2002). BSF species is not harmful to human and high-controlled rearing systems for housefly limit considerably the risks of diseases transmission (Bradley and Sheppard, 1984; Leclercq, 1997; Rozendaal, 1997; Sanchez-Arroyo, 2011). Adding to that, the high prolificacy (resulting in a numerous and efficient offspring) and short life cycle (few weeks) of these two species indicate a great potential for mass-rearing.

Maggot farming might have a positive impact on the environment: fly larvae do not compete with human resources, they contribute to waste management or remediation by efficiently converting nutrient-poor, low or no-value substrates or wastes that are costly to treat or dispose into valuable biomass; while feeding on manures, agro-industrial or urban wastes, maggots reduce effectively nutrients, volumes and odours, thereby reducing the 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; Čičková *et al*., 2015; Tomberlin *et al*., 2015; van Zanten *et al*., 2015). In addition, it has

been showed that BSF larvae are able to reduce significantly *E. coli* and *Salmonella* spp in organic wastes (Erickson *et al*., 2004; Liu *et al*., 2008; Lalander *et al*., 2014).

Substrate residues, or frass, resulting from the larval bioconversion process, consist of undigested substrate residues thoroughly mixed with insect excreta (Alvarez, 2012; Čičková *et al*., 2012a). According to the literature, frass represent 80 to 95 % of the total outputs (i.e. larval biomass + frass; wet weight) of a bioconversion process with fly larvae (Calvert, 1979; Čičková *et al*., 2012b; Wang *et al*., 2013; Caruso *et al*., 2014) and several authors suggested possible valorization routes including hydrolysis into fermentable sugar suitable for the food industry (ethanol fermentation); processing (drying, grinding and packaging) to facilitate handling and storage; further composting (reducing conventional composting duration) or vermicomposting with earthworms (Newton *et al*., 2005b; Li *et al*., 2011c; Čičková *et al*., 2012c; Zhu *et al*., 2012). In most cases, frass chemical composition was comparable to bio-fertilisers with optimal levels of N, P and K to supplement soils (Choi *et al.*, 2009; Zhu *et al.*, 2012; Wang *et al.*, 2013; Lalander *et al.*, 2014); however, limited and unclear results are available from the use of frass as soil amendments or bio-fertiliser (NC State University, 2006; Choi *et al.*, 2009).

1.5.1.2 Nutritional benefits

Dipteran species nutritional attributes indicate several similarities with FM suggesting a high potential as feed ingredients, in particular as a source of protein for aquatic species. Among other insect species, the AA composition of fly larvae meals or "maggot meals" (MM) is the most similar to FM and despite few deficiencies, profiles are well-balanced and superior to soybean meal (Barroso *et al.*, 2014). However, lipid and FA compositions of MM are devoid of EFA (in particular EPA and DHA) which can limit inclusions in marine fish diets. Fortunately, as described in 0 above, FA composition of insects can be manipulated through their diet which is possible under farming conditions (St-Hilaire *et al*., 2007a; Biancarosa *et al*., 2015). It has been demonstrated that the nutritional profile of maggots is not only influenced by the species and its rearing conditions, but also by the life stage at harvest (i.e. larvae, prepupae or pupae) or the post-harvest methods (Henry *et al.*, 2015). Table 1.2 below, adapted from Barroso *et al.* (2014), compares the proximate, AA and FA compositions of BSF larvae, housefly larvae, FM and soybean meal.

1.5.1.3 Emerging industry

Compared to other insects such as crickets or mealworms, maggots are more likely to be used in animal feeds rather than in direct human nutrition, principally for reasons related to the consumer acceptability (Awoniyi, 2007). To date, several commercial pilots for the production of housefly or BSF larvae are being tested in Canada, the USA, South Africa, Spain, the Netherlands, Switzerland, Malaysia, France and China (van Huis *et al*., 2013; Drew and Pieterse, 2015; Henry *et al*., 2015; Pastor *et al*., 2015); novel farming and processing methods are developed to produce a range of products intended for the animal feed industry (crude or defatted MM, protein hydrolysates and oil) and the agriculture/horticulture industry (frass). However, very little information about the current production capacity of the maggot farming industry is available, but it can be expected that if the market demand for insect meals increases, providing that the legislation changes and becomes more flexible towards the use of insect in animal feeds using a broader range of substrates, novel technologies will be developed to improve and upscale the current pilots. In the literature, only a few systems indicated large production capacity because most of the work was conducted on pilot system or in laboratories. Burtle *et al.* (2012) have designed a system in the USA, which could, in theory, produce 3,750 tonnes BSF MM per year using 360 tonnes of daily food leftovers or swine manure. In China, a housefly larvae bioreactor has also shown promising results with a total production of 760-960 tonnes of fresh larvae per year (corresponding to 570-720 tonnes MM/year) using swine manure (Wang *et al.*, 2013). The development and multiplication of this kind of system could contribute significantly to the MM supply globally.

As for now, given the regulatory restrictions (see 1.4.4 above), MM and derivatives are used in pet foods, an industry with a high purchasing power, which has rapidly identified the potential of these products as high-quality feed ingredients. Meanwhile, research keeps investigating the applicability and safeness of MM-based products as feed ingredients for various farmed fish species and livestock in general.

Table 1.2 Proximate, amino acid (AA) and fatty acid (FA) compositions of black soldier fly larvae (BSF) and housefly larvae compared to fish meal (FM) and soybean meal selected and analysed by Barroso *et al.* (2014)

	BSF	**Housefly**	**FM**	**Soybean meal**
Proximate Composition (% Dry Matter)				
Crude protein	36.2±03	46.9±4.1	73.0±0.8	50.4±0.2
Crude fat	18.0±1.6	31.3±1.6	8.2±0.0	3.0±0.0
Ash	9.3±0.3	6.5±1.5	18.0±0.2	7.8±0.0
Nitrogen-free extract	36.5±1.0	15.3±4.0	0.8±0.7	38.8±0.3
Amino Acid (% total AA)				
Arginine	8.24	6.83	7.42	8.03
Histidine	5.29	4.68	7.86	3.28
Isoleucine	5.76	4.89	5.04	5.47
Leucine	6.87	6.75	7.81	8.01
Lysine	7.60	8.36	8.78	6.34
Methionine	1.50	3.00	2.93	1.01
Phenylalanine	6.88	7.01	5.38	5.79
Proline	6.16	5.33	4.76	4.99
Threonine	5.39	4.87	6.26	4.17
Tyrosine	6.35	5.79	3.91	2.93
Valine	6.31	6.08	5.56	5.45
Fatty Acid (% total FA)				
12:0	43.4±0.6	0	0	4.1±1.3
14:0	7.9±0.1	2.4±0.1	7.9±0.4	-
16:0	13.2±0.1	23.1±0.5	23.0±0.6	15.1±0.2
18:0	2.8±0.1	7.2±0.8	5.3±0.1	4.8±0.4
Total Saturated[1]	67.1±0.6	32.6±0.1	36.1±1.1	24.0±1.9
16:1n-7	2.3±0.1	15.1±0.8	7.9±0.3	-
18:1n-7	-	0.3±0.1	4.0±0.7	0.8±0.0
18:1n-9	14.6±0.3	37.1±0.7	8.4±0.1	14.3±0.3
20:1n-9	0	0.2±0.0	0.3±0.4	-
Total monounsaturated[2]	16.9±0.2	52.7±0.2	20.6±0.7	15.1±0.3
18:2n-6	15.2±0.4	6.5±0.0	1.1±0.1	48.5±0.6
18:3n-6	-	0.2±0.0	-	-
20:4n-6	-	0.4±0.0	1.4±0.0	-
Total n-6 PUFA[3]	15.2±0.4	7.1±0.0	2.7±0.2	55.4±0.8
18:3n-3	0.7±0.1	0.3±0.0	0.2±0.3	6.9±0.2
18:4n-3	-	0.1±0.0	1.9±0.0	-
20:5n-3 (EPA)	-	0.1±0.1	14.1±0.2	-
22:5n-3	-	-	2.7±0.1	-
22:6n-3(DHA)	-	-	16.1±0.1	-
Total n-3 PUFA[4]	0.7±0.1	0.5±0.1	34.7±0.2	-
Total PUFA[5]	15.9±0.6	7.6±0.1	37.3±0.0	55.4±0.8

Values are mean ± SD

1.5.2 Previous work with fish

1.5.2.1 Overview

Although dipterans larvae are not particularly part of the natural feed intake of aquatic species, other invertebrates are, suggesting that farmed fish can benefit from MM as a feed ingredient. Comprehensive reviews have already gathered the outcomes of the previous research studies assessing the suitability of BSF or housefly MM as substitutes to FM in farmed fish species (Makkar *et al.*, 2014; Riddick, 2014; Henry *et al.*, 2015).

Early studies were mostly carried out using whole, chopped or frozen maggots often dispensed to fish with their frass, as supplementary feeds or in combination with other feeds or feedstuffs in low-income developing countries (LIDC) in Asia and Africa where access to good quality feed and feedstuffs is challenging (1.5.2.2 below). Research is now globally focusing replacing FM with MM in nutritionally balanced compound diets that meet fish species specific requirements (Figure 1.4). Principally because of the high interspecies variability of the results of the studies, findings were presented below on a fish group basis according to the species assessed (see 1.5.2.3 to 1.5.2.6). Thus, unless indicated otherwise, most recent studies looked at crude or defatted MM from housefly or BSF larvae, fed on animal manures, as FM substitutes in compound diets for juvenile fish. Other factors such as the larval rearing substrate, the stage of development of the fly larvae at harvest (i.e. larvae, prepupae, pupae), the MM processing methods which might influence its nutritional composition (reviewed by Henry *et al.*, 2015), the diet formulations (i.e. other ingredients involved) and the MM dietary inclusion levels and the fish nutritional requirements (related to the species and its life stage) often make difficult the comparison and generalisation of the outcomes of the various case studies.

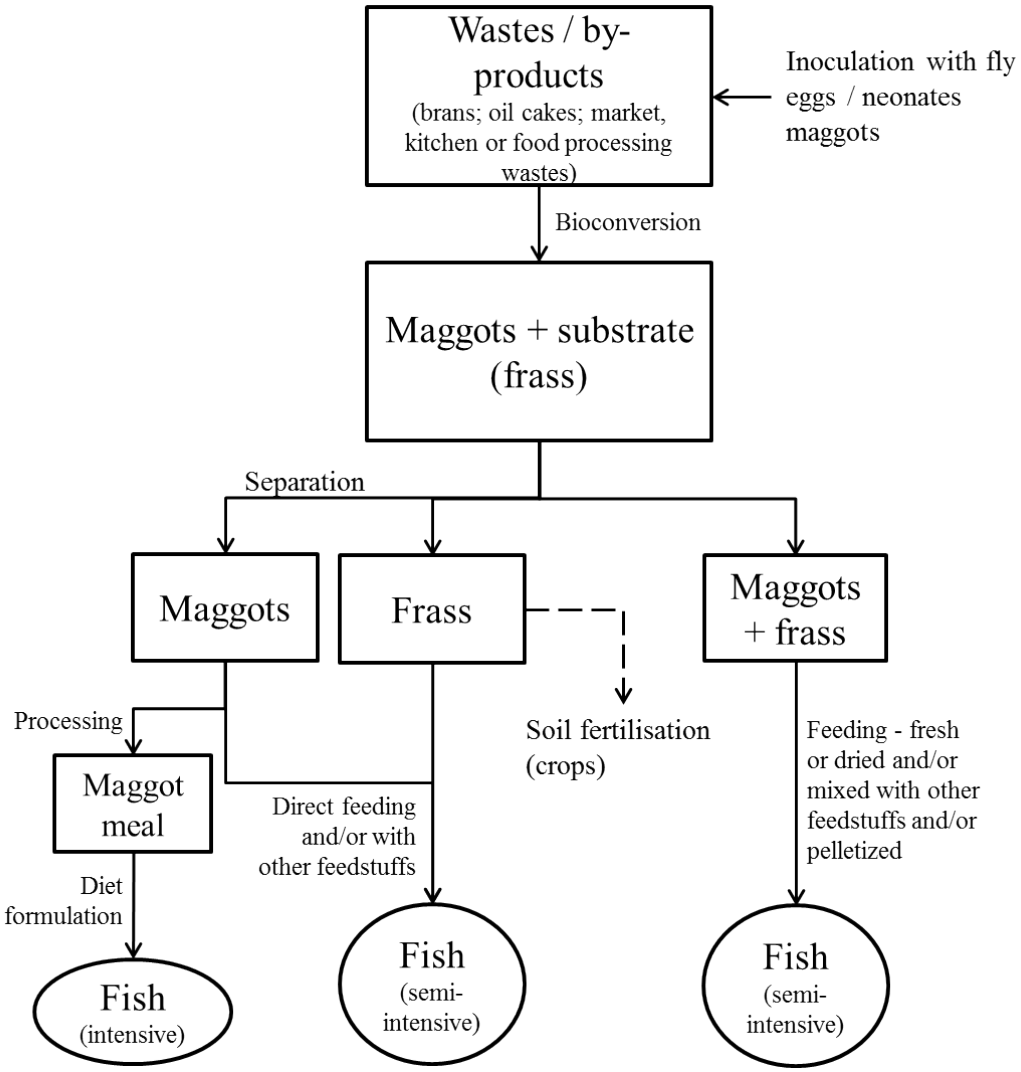

Figure 1.4 Use of maggots and frass as a source of nutrient for fish in intensive or semi-intensive aquaculture (adapted from Spinelli (1980)

1.5.2.2 Use in semi-intensive farming systems

Although the use of complete well-balanced diets will certainly increase given the development of the aquaculture sector (see 1.1 above), semi-intensive aquaculture systems will surely continue to benefit populations from LIDC; therefore, the potential of non-processed maggots should not be neglected. Brans (wheat, rice, etc.) and low FM or low protein feeds are commonly used in semi-intensive farming (Tacon, 1996; Tacon and De Silva, 1997). Several studies showed that chopped, frozen or live maggots (housefly or BSF) offered to Blue tilapia, Nile tilapia, Channel catfish, or African catfish in supplement of a nutrient-poor or cheap supplementary feed allowed significantly better growth performance than the fish fed the supplementary feed (SF) only (Bondari and Sheppard, 1981; Ebenso and Udo, 2003; Madu and Ufodike, 2003; Oyelese, 2007; Kareem and Ogunremi, 2012). However, Blue tilapia and Channel catfish growth were seriously depressed when fed solely whole or chopped BSF compared to a commercial diet (Bondari and Sheppard, 1987). To a larger extent, given the difficulties that may be associated with the separation of the maggots from their growing medium or substrate (see 1.5.3 below), and providing that the substrate is nutritionally suitable (feed-grade) and does not present risks for the fish, both maggots and frass could be dispensed to fish as a sole SF or blended with other feedstuffs after drying and roughly pelletized as indicated in Figure 1.4 (Spinelli, 1980).

1.5.2.3 Catfish

Many studies have looked at feeding catfish juveniles (1-10 g, initial weight) for 6 to 10 weeks with MM-based diets with generally positive outcomes. Dietary inclusions of housefly MM between 75 and 250 g/kg did not affect the fish performance of the African catfish (*Clarias gariepinus*) and the hybrid catfish (*Heterobranchus longifilis* x *C. gariepinus*) compared to FM-based diets (Sogbesan *et al*., 2006; Aniebo *et al*., 2009; Michael and Sogbesan, 2015); similar results were reported for BSF MM dietary inclusion up to 300 g/kg for the Channel catfish, *Ictalurus punctatus* (Newton *et al*., 2005b). On the contrary, darkbarbel catfish (*Pelteobagrus vachelli*) fed a diet containing 390 g/kg housefly MM showed significant reduction of the feed and protein efficiencies and the growth compared to fish fed a FM-based diet, probably related to a lower antioxidant activity (Dong *et al.*, 2013). Fasakin *et al.* (2003) reported that at least 320 g/kg defatted housefly meal could be included in a compound diet for *C. gariepinus*

(25% FM replacement) without impacting the fish performance in comparison with a FM-based diet whereas 335-350 g/kg crude MM inclusions were detrimental to the fish growth. In FM-free diets (100 % substitution with MM), only Aniebo *et al*. (2009) reported fish performance similar to FM-based diets for *C. gariepinus* and the differences with other studies results (Idowu *et al.*, 2003; Sogbesan *et al.*, 2006) might be attributed to the better quality of the MM which was produced from maggots reared on a mixture of cow blood and wheat bran. In fact, it is highly probable that the flies attracted by this substrate were from various species, including blowflies (Calliphoridae) and housefly, leading to MM of better quality thanks to balanced AA composition and a different FA composition related to the chosen substrate (Henry *et al.*, 2015).

1.5.2.4 Tilapia

Assessment of housefly MM has been limited to Nile tilapia fingerlings, *Oreochromis niloticus* (2 to 15 g, initial weight) fed experimental diets for 8 to 10 weeks. BSF larvae were only used whole or chopped to feed the blue tilapia, *Oreochromis aurea* (Bondari and Sheppard, 1987, 1981). Results indicated that dietary inclusion of MM comprised between 150 and 680 g/kg, thus, replacing between 20 and 100 % FM respectively, did not affect tilapia performance compared to FM-based diets and did not have adverse effects on the haematology and homeostasis of the fish (Ajani *et al*., 2004; Ogunji *et al*., 2008a, 2008b, 2007; Omoyinmi and Olaoye, 2012). In another study, Ogunji *et al*. (2008c) used a low-protein MM (28.6 % crude protein, on a dry matter (DM) basis, compared to 37.5 % DM in previous studies) and reported that fish growth was significantly depressed with diets containing 150 and 300 g/kg MM; however, the differences between the dietary protein/dietary lipid ratios among experimental diets might have biased the results (Henry *et al.*, 2015).

1.5.2.5 Carps

Carps have also been subjects of feeding experiment with housefly MM. In particular, the growth of 12.2 g juvenile gibel carp (*Carassius auratus gibelio*) fed for 6 weeks with a diet containing 390 g/kg MM (maggots reared on a mixture of wheat and rice brans with soya bean dregs) was not affected and antioxidant activity was even enhanced compared to fish fed the FM control diet (Dong *et al.*, 2013). Similarly, 72 g black carp (*Mylopharyngodon piceus*) showed an enhanced antioxidant capacity when

fed a basal diet supplemented with 25 g/kg MM; results also concluded in an improved growth, non-specific immunity of and disease resistance of the fish supplemented with MM (Ming *et al.*, 2013). Moreover, MM may enhance nutrient digestibility in common carps (*Cyprinus carpio*) of 100 g compared to Nile tilapia of similar size; the latter were found to be poorly efficient in utilising nutrients provided by housefly MM; however the spawning activity of the tilapia during the study could have biased the results (Ogunji *et al.*, 2009).

1.5.2.6 Carnivorous species

More sporadic work was done on carnivorous species, although several species are known to naturally ingest insects, particularly in their juvenile stages (Scott and Crossman, 1973; Bell *et al.*, 1994; Amundsen *et al.*, 2001). In the Rainbow trout (*Oncorhynchus mykiss*), a dietary inclusion of 92 g/kg housefly MM was found detrimental to the fish growth whereas at 150 g/kg BSF MM inclusion seemed to perform better since the fish performance were similar to those of the fish fed FM-based feeds (St-Hilaire *et al*., 2007b). However, above this level BSF meal was systematically associated with growth and performance reductions (St-Hilaire *et al*., 2007b; Sealey *et al*., 2011). In addition, it was not advised to lower dietary fish oil levels while substituting FM with lipid-rich BSF MM as it resulted in lower trout fillets quality with significantly reduced n-3 LcPUFA contents (St-Hilaire *et al*., 2007b). On the other hand, when MM quality was improved through defatting or by improving its FA composition (feeding maggots on EFA-rich substrates to increase in particular the n-3 LcPUFA content), dietary inclusions comprised between 180 and 400 g/kg did not affect the fish performance and fillet quality (Sealey *et al.*, 2011; Gasco *et al.*, 2015). In Atlantic salmon (*Salmo salar*) post-smolts however, the defatting method used on BSF MM used at 50 and 250 g/kg inclusions have affected the fish performance compared to the control diet whereas diets containing up to 250 g/kg crude BSF MM performed equally to the FM-based control diet (Lock *et al.*, 2015). Although the nutrients digestibility of both crude and defatted BSF meals were reported excellent in Atlantic salmon post-smolt, a low protein digestibility of defatted BSF MM was reported for young turbot (*Psetta maxima*). Growth depression was positively related to the MM inclusion in turbot diets and above 330 g/kg dietary inclusion, FCR increased significantly indicating a low palatability of the diets potentially caused by the high chitin content of the insect meal (Kroeckel *et al.*, 2012).

1.5.3 Bottlenecks and limitations

Methods used for the maintenance of fly colonies (dipteran) in captivity within laboratory or small-scale farming facilities mainly for research purposes or in mass-rearing systems for the production of natural enemies, pollinators, or baits for recreational fishing are already commercial realities; in the EU they have been developed under strict legal frameworks, including strict measures to prevent accidental escapes (Leppla and Ashley, 1978; Hardouin and Mahoux, 2003; DEFRA, 2005).

As stated in 1.5.1.3 above, up-scaled systems to produce fly larvae intended for the animal feed industry are developing. Nevertheless, the task is challenging it requires a strong knowledge of the species biology and its requirements and an adaptation to site-specific conditions (controlled environment chambers, management of the resources available and public acceptance for instance). Scale and technology level of large-scale systems depend on several factors such as some site-specific aspects, the investment capacity, the production objectives and the finality of the products. Housefly and BSF large-scale farming are relatively new and technical issues are still being progressively identified and solved to develop innovative and efficient mass-rearing systems in their respective contexts (Sheppard *et al*., 2002; Zhang *et al*., 2010; Diener *et al*., 2011b; Čičková *et al*., 2012c; van Huis *et al*., 2013; Caruso *et al*., 2014; Devic and Maquart, 2015; van Zanten *et al*., 2015). The major challenges related to the industrialisation of the farming processes include the economic viability and the cost-competitiveness which should meet or exceed systems producing conventional sources of protein for aquaculture. This can be achieved only through optimised production and processing processes using economically competitive and sustainable resources (Rumpold and Schlüter, 2013; van Huis *et al*., 2013; Pastor *et al*., 2015). As previously stated (see 1.5.1 above), the choice of the substrate on which the maggots will be farmed is important as it influences the nutritional composition (in particular the FA profile) of the larvae, potentially improving their quality as a feedstuff for fish significantly; however, the choice of the substrate should certainly account for other criteria such as the sustainability (circular economy, see 1.4.3 above), the consistency of supply and the cost (Rumpold and Schlüter, 2013; van Huis *et al.*, 2013). In addition, safety aspects concerning the substrates should be considered as the presence of heavy metals or pathogens could impair the insect survival, growth, fertility or affect the quality of the

final product by bioaccumulation (Borowska *et al*., 2004; Tylko *et al*., 2005; Diener, 2010; Borowska and Pyza, 2011; Diener *et al*., 2011a), thereby becoming a risk for the fish through ingestion (Banjo *et al.*, 2005). On the other hand, good practices including the quality control and the traceability of both inputs (substrates) and outputs (insect-based products) are substantial advantages of farming that ensure the product safety.

To date, together with the regulatory framework and the nutritional aspects of the MM already discussed (see 1.4.4 and 1.5.2 above), the volumes of MM that can be produced remain one of the main limitations to its use in aquaculture. Nevertheless, this is related to the current production capacity of the emerging insect farming industry which is expected to improve and upscale gradually its technology, once the market is ready.

The economic aspect of the MM production is also an important point to consider as it is one of the main drivers of using alternative feed ingredients (Rust *et al.*, 2011). Market price is defined by the profit margin and the production costs, and to answer the vast market demand of the aquafeed industry, insect-based products have to be supplied at a competitive price compared to conventional sources of protein; nevertheless, being of animal origin it can be expected that market price would be higher than plant proteins thanks to a better digestibility (Table 1.3). Production costs can be high in industrialised country if the system is not fully automatized and requires expensive labour. Similarly, the use and maintenance of environment control facilities (light, temperature, humidity) are expensive but critical to maintain fly colonies under temperate climates (Pastor *et al.*, 2015). In Europe, due to the legislation, MM is currently sold at high prices to the pet food industry which is a sector less price-sensitive for raw materials as pet owners are more willing to pay a high price for a quality product. Compared to industrialised countries, small and medium-scale production systems in LIDC are less demanding economically and might be profitable providing some improvements of the current methods used to improve the productivity and the costs (Caruso *et al.*, 2014). MM and maggots are considered as cheap alternative feedstuffs in LIDC (Gabriel *et al.*, 2007) and several studies have positively concluded on the cost-effectiveness of MM as a feedstuff for catfish or tilapia in replacement of expensive, inconsistent and often poor quality FM (Ajani *et al*., 2004; Sogbesan *et al*., 2006; Ezewudo *et al*., 2015). According to Drew and Pieterse (2015), who are developing a large-scale MM production system in South Africa (AgriProtein),

once the legislation will change in favour of the use of insect-based products in animal feeds, the remaining challenge will be to aligned the market price of bulk insect protein to the price of other conventional feed ingredients; in this context, they assumed that MM produced for less than $ 1,000 per tonne, would result in a profitable business and an immediate incentive to use MM in animal and fish feeds.

Table 1.3 Market prices of various protein sources.

Feed Ingredient	Market price (USD/tonne meal)	Description and port of origin	Date	Protein content (%, as fed)	Protein price (USD/tonne protein)	Reference
Peru Fish meal	1,586	C&F Bremen	June 2016	65	2,440	World Bank (2016)
Soy protein concentrate	940	CIF, n/a	June 2016	65	1,446	Barentz Animal Nutrition
Corn gluten meal	700 - 710	C&F Asia	June 2016	60	1,167 - 1,183	Bacon (2016)
Soybean meal	467	CIF Rotterdam	June 2016	48	973	World Bank (2016)
Meat and Bone meal (porcine)	490 - 510	C&F Asia	June 2016	50	980 – 1,020	Bacon (2016)
Poultry by-product meal	580 - 600	C&F Asia	June 2016	60	967 – 1000	Bacon (2016)
Maggot meals	-		-	37.5 – 55.8	-	Feedipedia (2015)

1.6 Research hypothesis and objectives

The pressing need for the identification of sustainable alternative sources of protein supporting the development of the aquaculture sector globally has led to consider insects, in particular, the housefly and BSF larvae, as promising candidates. In both industrialised and low-income developing countries, the development of a new industry producing MM, frass and other by-products when resources and technology allow it (oil, chitin extract), has been initiated around the concept of circular economy which supposes the valorisation of low or no-value resources (wastes) through a sustainable process from which each output (product) is also a resource that can be integrated into other processes. Previous research indicated that MM and by-products can be used in existing aquaculture systems as a source of nutrients. From the foregoing discussion it is clear that the major problems are first related to the current volumes of MM available that are not yet sufficient to cover the ever growing demand of the aquafeed industry; secondly, although insect-based materials are suitable for fish, the high variability of the results from the previous studies does not allow a generalisation.

Therefore, contextualised and commercially relevant research should investigate where and how insect-based products could be integrated into aquaculture. In particular, MM and frass, produced in farming systems established under site-specific conditions (environmental and socio-economic context), should be assessed as sources of nutrients for farmed fish. It is expected that the strategic use of consistent high-quality MM and frass can meet the specific requirements of various fish species cultured in different aquaculture systems, thereby contributing to food security. Indeed, critical parts of intensive aquaculture processes (juvenile stages) could benefit from MM as a suitable FM substitute whereas frass could be valorised in low-value farming process (semi-intensive) and/or in crop culture as a bio-fertiliser. Volumes required in each case should also be considered in the assessment.

As part of the European Union Seventh Framework Programme (EU FP7) project untitled PROteINSECT, the main objective of this study was to assess housefly and BSF MM and BSF frass as sources of nutrients for two commercially important farmed species (imposed by the consortium): Atlantic salmon (carnivorous, intensive farming) and Nile tilapia (omnivorous/herbivorous, intensive and semi-intensive farming) in their respective contexts. Specifically, the objectives were:

- To assess the suitability of crude and defatted housefly MM as a FM substitute for Atlantic salmon parr (freshwater stage) by examining the effects of increasing dietary inclusion on the fish performance, digestibility and whole body composition;

- To investigate and compare the cost-efficiency of crude and defatted BSF MM and crude housefly MM as FM substitutes in Nile tilapia sex-reversal process;

- To assess the suitability of crude BSF MM as a FM substitute in advanced nursing of Nile tilapia diet by examining the effects of increasing dietary inclusion on the fish performance and whole body composition;

- To compare the performance of the BSF frass used either as a supplemental feed in semi-intensive Nile tilapia culture or as a soil conditioner (bio-fertiliser) for spring onion;

- To model the main flows of input and outputs of insect production systems in relation to the MM requirements of a specific fish farm in pre-define contexts and site-specific conditions.

1.7 Thesis structure

This thesis is structured into eight chapters as shown in Figure 1.5 below.

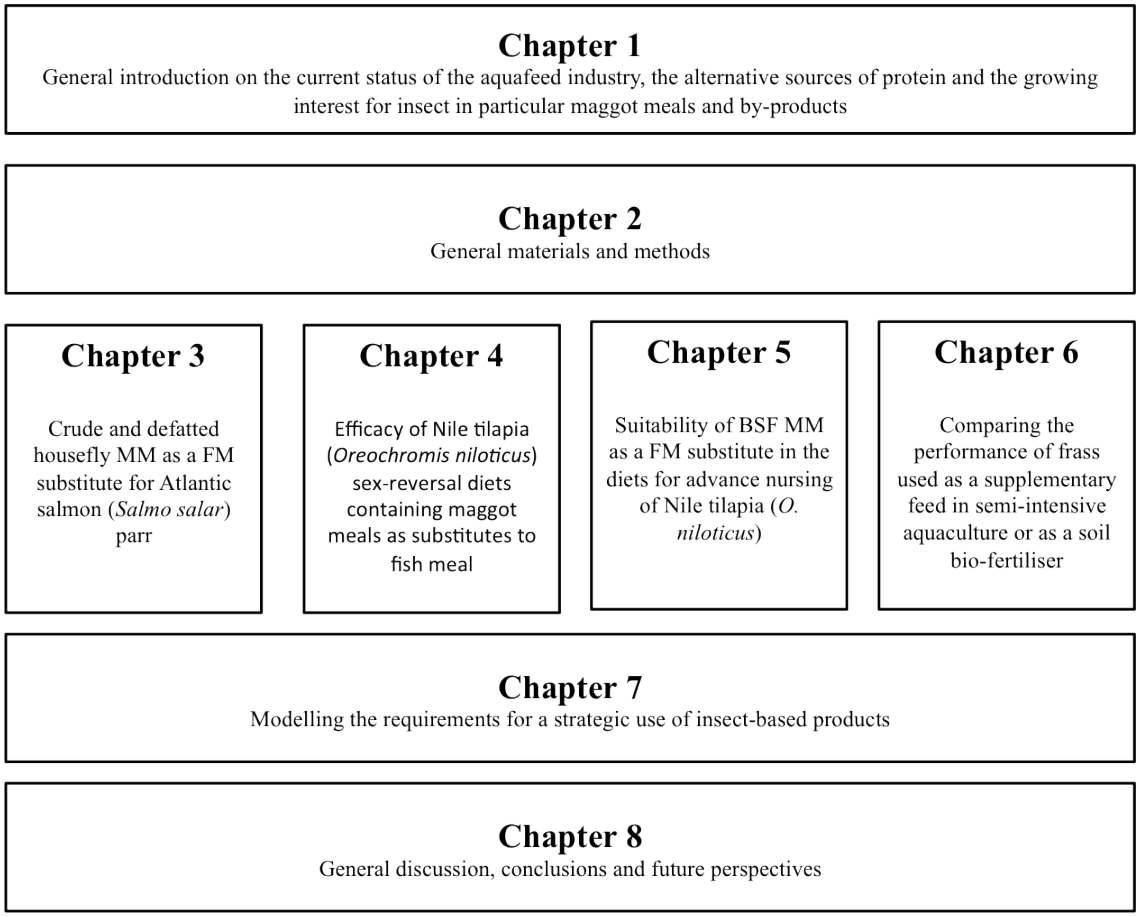

Figure 1.5 Thesis structure

Chapter 2. General materials and methods

2.1 Experimental diets and feed ingredients

2.1.1 Maggot meals and frass

MM and frass used as feed ingredients / soil conditioner in the following experiments were sourced from pilot production systems of common housefly larvae (*M. domestica*) cultured on poultry manure in the United Kindom (Grant Bait Ltd.; Yorkshire, UK; Chapters 3 and 4) or black soldier fly larvae (*H. illucens*) fed on processed food wastes in Malaysia (Entofood Sdn Bhd; Kuala Lumpur, Malaysia; Chapter 4) or on a mixture of brewery wastes and processing wastes from a fish feed factory in Ghana (PROteINSECT production pilot, Ashaiman, Ghana; Chapter 5). Frass derived either from processed food wastes or from brewery spent grains digested by BSF larvae in Malaysia (Entofood Sdn Bhd; Kuala Lumpur, Malaysia Chapter 6). Insect products were selected and used according to their local or regional availability; MM were consistently used to substitute FM in simple or complete diets for fish whereas frass was used as a supplementary feed and bio-fertiliser.

2.1.2 Experimental diets

Housefly larvae meal was used as a FM substitute in complete diets for salmon parr, formulated and manufactured at the University of Stirling in the UK (Chapter 3). Sex-reversal tilapia fry were fed simple diets prepared on-farm, in Thailand, by gradually replacing FM with either housefly larvae meal (shipped from the UK for convenience, but available in China for instance) or BSF larvae meals (Chapter 4). Advanced nursing of tilapia in Ghana were fed diets formulated and manufactured by Raanan Fish Feed West Africa (Prampram, Ghana), thereby replacing gradually the FM inclusion of the commercial feed formulation with locally produced BSF larvae meal (Chapter 5). Finally, the two types of BSF frass were dispensed as supplementary feed (single feed ingredients) to juvenile tilapia kept in semi-intensive conditions in green water ponds in Thailand (Chapter 6).

Diets formulation, manufacture and storage were detailed in each relevant chapter. Complete diets were formulated to satisfy the nutritional requirements of the species according to their life stage (NRC, 2011).

2.2 Experimental designs and set up

2.2.1 On-farm experiments

All the experiments were conducted on-farm using the facilities and benefited from the technical assistance of commercial running systems in the UK, Ghana and Thailand. Commercial husbandry practices were reproduced at experimental scale in order to demonstrate the commercial relevance of the results.

Constraints associated with research procedures in commercial conditions were numerous. In the first place, precautions were taken for the experiments not to interfere physically with commercial farming units (separate ponds dedicated to the experiments, distance between commercial and experimental cages, etc.). In addition, because the farm staff was involved in the experiment, it was important that research-related activities did not impair the farming activities and productivity. Thus, while ensuring a scientific approach and validity of the findings, experiments and related activities (feeding, samplings, water quality monitoring, etc.), which were time-consuming given the size of the trials (large numbers of fish, dimensions of the structures, etc.), were carried out simply and efficiently. Moreover, each trial was individually adapted to the space, material and fish available (experimental units, ponds and dimensions, feed, etc.).

2.2.2 Experimental design

In each relevant chapter, the number of treatments, replications and fish chosen for the experiment were described in details. Although all the trials were conducted in conditions similar to commercial practices, amounts of insect meal were often limiting and influenced greatly the size and the design of the experiment. A minimum of three replicates per treatment was used in each experiment; fish were maintained in experimental units: tanks, cages or hapas of a smaller size compared to commercial units and stocked at commercial densities. Appropriate feeding was consistently ensured by skilled technicians and feed was distributed using methods representative of commercial practices in the relevant systems (feeding rate, distribution method, etc.).

Atlantic salmon (*Salmo salar*) and Nile Tilapia (*Oreochromis niloticus*) were the two species considered for these trials due to their economic significance in temperate and

tropical zones, respectively (see Chapter 1). The duration of the trials varied from 3 weeks to 3 months depending on the species studied and the farming process or system considered. All the investigations were initiated with juveniles (fry or fingerlings) and experimental periods allowed a significant increase in body weight of at least 300% as recommended in the NRC (2011) guidelines.

Throughout most trials, fish and treatments were randomly allocated to the experimental units (tanks or hapas). In the trial conducted in Ghana (Chapter 5), fish were randomly allocated to the experimental cages, but a simple segregation by treatment was applied in order to simplify the trial management and to avoid any confusion errors from the farm staff when feeding or sampling (i.e. dispensing the wrong feed to the wrong cage), which could have led to the invalidation of the results. Therefore, the experimental site was chosen very carefully according to the farmer's knowledge and experience of the area; treatments and replicates were strictly maintained under similar and optimal conditions to ensure that the design would not affect the results of the study (Schank and Koehnle, 2009). Indeed, the dimensions, water dynamic and bathymetry of the Volta Lake (Ghana), where the trial was conducted, characterised a very well mixed site with little opportunity for environmental variation between cages which were separated by a maximum distance of 3 meters.

In all experiments, water quality was monitored using appropriate and available equipment (thermometer, DO meter and spectrophotometer) and experimental units were maintained clean at all time as appropriate measures were applied to prevent excessive fouling (cleaning, nets changes, etc.).

2.2.3 Experimental sampling

Fish were systematically weighed at the start of the experimental feeding periods (i.e. after the acclimation periods) and on termination of the experiments; frequency of intermediate samplings was detailed in relevant chapters. Due to the number of fish in all the trials, fish were bulk weighed; in most cases, three sub-samples, representative of the total population of each experimental unit, were randomly selected, weighed and fish were counted.

Fish were euthanised by anaesthetic overdose (using metacaine sulfonate MS-222), for the collection of the samples required for the biochemical analysis and on termination of the experiments. Fish samples were systematically pooled (on the basis of the initial population or the experimental unit), homogenised and stored at -20°C until further analysis.

2.2.4 Fish performance and feed utilisation indicators

Fish growth performance and feed utilisation efficiency were evaluated according to the following indices:

Weight Gain (WG)

$$WG\ (g) = Final\ Weight\ (Wf, in\ g) - Initial\ Weight\ (Wi, in\ g)$$

Specific Growth Rate (SGR)

$$SGR\ (\%\ \text{body weight/day}) = [\frac{\ln Wf - lnWi}{Number\ of\ days}] * 100$$

Feed Conversion Ratio (FCR)

$$FCR = \frac{Feed\ distributed\ (g, on\ a\ DM\ basis)}{WG\ (g)}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{WG\ (g)}{Amount\ of\ protein\ fed\ (g, on\ a\ DM\ basis)}$$

Daily feeding rate

$$Daily\ feeding\ rate\ \ (\%\ \text{biomass/day}) = \left[\frac{Feed\ distributed\ (g, on\ a\ DM\ basis)}{Number\ of\ feeding\ days}\right] * \left[\frac{100}{Biomass\ fed\ (g)}\right]]$$

Survival rate (%) was determined by difference between the number of fish initially stocked and the final count or estimation of fish remaining on termination of the experiment.

2.3 Biochemical analyses

Proximate composition of the feedstuffs, experimental diets, fish whole body and faeces were analysed at the University of Stirling using methods based on the Association of Official Analytical Chemists (AOAC, 1990) or standard methods as detailed below. Dry matter determination was carried out on wet samples. Protein, ash and gross energy of the whole fish body and faeces samples were evaluated from finely ground and homogenised dried samples whereas diets and feedstuffs were systematically analysed 'as is'. Two methods were used for the lipid determination as described below. Each sample was analysed in duplicates.

2.3.1 Dry matter

Approximately 1.0 g feedstuffs or diet; 15.0 g homogenised fish body samples (wet weight) or 5.0 g soil samples (Chapter 6) were placed in a drying oven (Gallenkamp Oven 300) at 110°C until constant weight was achieved (AOAC, 1990).

Faeces samples were freeze-dried using a CHRIST Alpha 1-4 LSC freeze dryer (Osterode am Hartz, Germany) at -50°C under vacuum for 48 h.

2.3.2 Crude protein

The protein content of the samples was determined from the nitrogen (N) content of each sample which assumes that protein contains 16% nitrogen, using the automated Tecator Kjeltec TM 2300 analyser (Foss, Warrington, UK) according to the standard method (Persson, 2008) and the manufacturer's instructions. Briefly, about 250 mg of each sample was placed in a Kjeldahl digestion tube with 2 mercury Kjeltabs and 5 ml conc. sulphuric acid and boiled at 420°C for 1 hour. After cooling to room temperature, distillation was carried out using the Tecator Kjeltec TM 2300.

2.3.3 Crude Lipid

2.3.3.1 Soxhlet method

Lipid content of the feedstuffs and the diets was determined by Soxhlet extraction with petroleum ether (Christie, 2003) following an acid hydrolysis with HCL. Acid hydrolysis was performed on 1.0 g finely ground samples using a fully automated

hydrolysis apparatus (Tecator Hydrotec^{TM} 8000, Foss Analytical, Hillerød, Denmark), according to the manufacturer's instructions. Hydrolyzed samples were then dried at 60°C for 16-18 hrs and transferred to the Soxhlet apparatus (Tecator Soxtec system 2050 Auto Extraction apparatus, Foss Analytical, Hillerød, Denmark).

2.3.3.2 Folch method

Folch non-destructive method was applied to samples (feedstuffs, diets, fish whole body samples and freeze-dried faeces) to extract crude lipid used for subsequent fatty acid analyses. 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, UK) according to Folch *et al*. (1957) and determined gravimetrically after an overnight desiccation under vacuum.

2.3.4 Fatty acid composition

Fatty Acid Methyl Esters (FAME) were determined after extraction of total lipid from samples (feedstuff, diets, whole fish body and faeces) as described in 2.3.3.2 above. FAME were prepared from total lipid re-dissolved in chloroform/methanol (2:1,v/v) at a concentration of 10 mg/ml by acid-catalysed transesterification at 50^{o}C for 16 h (Christie, 1993). Extraction and purification of FAME were performed as described by Tocher and Harvie (1988) and separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30mx0.32mm i.d. x 0.25µm ZB-Wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionisation detection. Hydrogen was used as carrier gas with initial oven thermal gradient of 50^{o}C to 150^{o}C at 40^{o}C.min^{-1} to a final temperature of 230^{o}C at 2^{o}C.min^{-1}. Individual FAME were identified by comparison to known standards (Supelco™ 37-FAME mix; Sigma-Aldrich Ltd., Poole, UK) and published data (Tocher and 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

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

A similar method (Loss on Ignition) was used to determine soil samples organic matter (OM) content (Chapter 6). Approximately 5.0 g samples placed in a pre-weighed porcelain crucibles place were loaded in a muffle furnace (Gallenkamp Gallenkamp Muffle Furnace) at 430°C for 16 hours.

2.3.6 Crude fibre

Crude fibre was determined according to AOAC, AACC and AOAS standards using FibreCap 2021/2023 system (Foss application note ASN3801) by removing off all the digestible materials from 1.0 g sample placed in Foss FiberCap devices (Foss Analytical, Hillerød, Denmark) through successive defatting in petroleum ether, boiling in acid and alkali solutions, drying and incineration in a muffle furnace (Gallenkamp Gallenkamp Muffle Furnace) at 600°C for at least 4 hours.

2.3.7 Nitrogen Free extract (NFE)

NFE was determined by subtracting the sum of the protein, lipid, ash and fibre to the dry matter content.

2.3.8 Gross energy

Gross energy of the feedstuffs and the diets was measured by bomb calorimetry (Parr 6200 bomb calorimeter, calibrated with benzoic acid) according to the manufacturer's instructions. Briefly, 1.0 g sample was combusted in a container filled with oxygen; the heat released and the temperature variation was used by the instrument to calculate automatically the energy content of the sample (in KJ/g).

2.3.9 Amino acid composition

The amino acid compositions of feedstuffs and the diets were determined by ALS Food & Pharmaceutical (Cambridgeshire, UK) and Eurofins Food and Feed Testing (Moss,

Norway) using High-Performance Liquid Chromatography (HPLC) method according to their respective commercial procedures.

2.4 Digestibility analysis

For the digestibility analysis (Chapter 3), Yttrium oxide (Y_2O_3), an inert marker was added to the diets at a 0.1% inclusion level. Yttrium in diets and faeces (collected by stripping on termination of the experiment) was measured using the acid digestion technique by Inductively Coupled Plasma Mass-Spectrometry (ICP-MS). Briefly, 0.1 g samples were digested in 5 ml of concentrated nitric acid in a CEM Mars Xpress microwave digester for 20 minutes at 190°C. Each sample 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 analysed in a Thermo Scientific Series 2 ICP-MS (Cheshire, UK).

The Apparent Digestibility Coefficients (ADC) for the nutrients and gross energy of the diets were calculated as follows (Maynard *et al*., 1969):

ADC of dry matter of diet = $1–(Y_{diet}/Y_{faeces})$

ADC of nutrients and energy of diets = $1–(Y_{diet}/Y_{faeces})*(N_{faeces}/N_{diet})$

where Y_{diet} = concentration of yttrium oxide in the diet; Y_{faeces} = concentration of yttrium oxide in the faeces; N_{faeces} = concentration of nutrient (or gross energy) in the faeces; N_{diet} = concentration of nutrient (or gross energy) in the diet.

2.5 Statistical analyses

All statistical calculations were carried out using IBM SPSS Statistics (version 21). A significance level of 5% ($P<0.05$) was chosen for all the analyses. Data were presented as the arithmetic mean together with the standard error of the mean (mean ± SE) or as arithmetic mean together with the standard deviation (mean ± SD) when stated.

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.

When significant differences in final body weights (covariate) were identified (Chapter 3), body compositions were statistically compared between treatments using an analysis of covariance, controlling for the effect of the covariate (Shearer, 1994).

Correlations between the dietary inclusions of crude MM and the performance or nutritional results were analysed using Pearson’s coefficient or Spearman’s coefficient when preliminary assumptions were violated.

A significance of $P<0.05$ was considered for all analyses performed.

Chapter 3. Assessing housefly larvae meal (*Musca domestica*) as a substitute for fish meal in the diet of Atlantic salmon (*Salmo salar*) parr

3.1 Introduction

Current research on animal nutrition is actively looking at alternative feedstuffs to reduce the dependence on the traditionally used marine ingredients (FM and FO) which provide quality nutrients to support efficient growth and performance of farmed fish. Aquaculture is one of the fastest growing animal food-producing sectors but it is also one of the main global consumers of FM and FO for the culture of carnivorous species such as Atlantic salmon, *Salmo salar* (Tacon and Metian, 2008; FAO, 2014). The exploration and identification of several alternative protein sources in recent times have significantly contributed to the decreased use of FM in aquafeeds (Olsen and Hasan, 2012). Plant products, such as soybean meal, offer sustainable alternatives to conventional sources of proteins and are now widely used within the aquafeed industry (Gatlin *et al.*, 2007). However, the inclusion of such products in feeds, especially for salmonids, are limited due to their nutritional characteristics that can negatively impact fish performance, health and welfare (Francis *et al*., 2001; Pratoomyot *et al*., 2011).

Insects have been identified as promising candidates for fish and livestock in the global assessment of potential feedstuffs (Makkar *et al.*, 2014; Sánchez-Muros *et al.*, 2014). Indeed, the nutritional profile of insects, in particular dipteran larvae (maggots), are similar to FM except for their fatty acid (FA) composition, that is often low in the omega-3 (n-3) long-chain polyunsaturated fatty acids (PUFA), eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids, typically associated with marine ingredients (Barroso *et al.*, 2014). Several studies have been conducted to test the use of insect-derived protein in experimental feeds on different species of cultured finfish (reviewed by Henry *et al.*, 2015), although limited work has been performed on salmonids. Species such as rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon post-smolts have shown positive responses to diets containing partial inclusion of black soldier fly (*Hermetia illucens*) larvae meal (Sealey *et al*., 2011; Lock *et al*., 2015) or mealworm (*Tenebrio molitor*) meal (Belforti *et al.*, 2015). Moreover, in the early freshwater stage of Atlantic salmon parr, 85% of the natural diet is composed of invertebrates, mainly aquatic and terrestrial insects (Scott and Crossman, 1973; Amundsen *et al*., 2001). Therefore, juvenile salmon represent an ideal candidate with which to test FM replacement with insect meal.

The common housefly (*Musca domestica*) is a ubiquitous species that is adapted to a wide range of environment conditions. In the framework of the EC FP7 project PROteINSECT, housefly mass-rearing systems have been developed in several locations, including the United Kingdom (Charlton *et al.*, 2015). Local production (UK / Europe) would be a substantial advantage if maggot meal (MM) is identified as a suitable feed ingredient for farmed Atlantic salmon as the aquafeed industry could benefit from reduced transport costs and environmental impacts compared to imported materials such as soybean meal for instance. In addition, as the Atlantic salmon is a high-value species with specific nutritional requirements, the use of refined materials of improved quality, such as defatted MM is considered (Fasakin *et al.*, 2003).

Thus, the aim of the current study was to investigate the effects of FM substitution with crude and defatted common housefly (*Musca domestica*) larvae meals in the diet of Atlantic salmon parr (*S. salar*), with a particular focus on fish performance, digestibility and nutritional composition.

3.2 Materials and methods

3.2.1 Experimental diets

Six experimental diets were formulated so that the main protein source, FM, was gradually replaced with insect meal. Housefly larvae (*M. domestica*) were produced by Grant Bait Ltd. (Yorkshire, UK) using poultry manure as a feeding substrate according to Charlton *et al.* (2015). Dried larvae (3 hours at 65°C) were ground into a crude MM, with a sub-sample defatted (DMM). Defatting was performed by Nuscience (Drongen, Belgium) using a commercial solvent extraction method with hexane (Merck, Germany); no hexane residues were identified in the DMM. Other ingredients used in the diet preparation were supplied by BioMar Ltd. (Grangemouth, UK). In order to assist in diet formulation, the three main protein sources (FM, MM and DMM) were analysed at the University of Stirling for proximate composition (Table 3.1).

A practical control diet (FM100) was formulated with 400 g/kg FM inclusion and four of the experimental diets substituted 25, 50, 75 and 100% of the FM (w/w) with MM (MM25, MM50, MM75 and MM100, respectively), whereas in the final diet 50% of the FM was replaced with DMM (DMM50). Diets were formulated to be isonitrogenous and isoenergetic with 530 g/kg crude protein; 160 g/kg crude lipid and 21 MJ/kg gross

energy (Table 3.2). Since MM and DMM showed compositions different than FM, the other ingredient levels were adjusted to maintain protein, lipid and other essential nutrient levels similar to the FM100 control diet. In addition, the diets included yttrium oxide (0.3 g/kg) as an inert marker for digestibility determination. Feeds (1.3 mm pellets) were produced at the University of Stirling using a compression pellet mill (California Pellet Mill; San Francisco, USA), stored at room temperature (feed storage room) and used within 3 months from manufacture. All diets were formulated to satisfy the nutritional requirements of juvenile salmonid fish (NRC, 2011). Proximate, amino acid and fatty acid compositions of the control and experimental diets were analysed as described below (Table 3.2).

Table 3.1 Analysed composition of the different test ingredients: fishmeal (FM), common housefly larvae (*M. domestica*) crude maggot meal (MM) and defatted maggot meal (DMM)

	Ingredients		
	FM	**MM**	**DMM**
Proximate Composition (g/kg)			
Dry matter	907.8	956.1	975.7
Crude protein	710.8	457.4	562.5
Crude lipid	70.9	242.5	120.5
Ash	118.3	98.5	79.2
Crude fibre	2.2	74.7	91.8
Nitrogen Free Extract (NFE)	5.6	83.0	121.7
Gross Energy (MJ/kg)	19.3	23.7	21.4
Essential Amino Acid Composition (g/100g meal)			
Histidine	1.38	1.26	1.61
Arginine	4.75	2.18	2.99
Threonine	2.88	1.95	2.45
Valine	3.11	2.18	2.67
Methionine	1.60	1.01	1.28
Lysine	5.19	3.39	4.32
Iso-Leucine	2.59	1.59	1.96
Leucine	4.62	2.65	3.36
Phenylalanine	2.37	2.53	3.38
Fatty Acid (g/100g meal)			
14:0	0.30	0.37	0.22
16:0	1.22	4.57	2.66
18:0	0.19	0.44	0.24
Total Saturated[1]	**1.75**	**5.49**	**3.19**
16:1n-7	0.35	2.36	1.40
18:1n-9	0.95	5.07	2.95
22:1n-11	0.60	0.00	0.00
Total monounsaturated[2]	**2.87**	**8.44**	**5.00**
18:2n-6	0.11	3.57	1.87
20:4n-6	0.04	0.00	0.01
Total n-6 PUFA[3]	**0.20**	**3.59**	**1.88**
18:3n-3	0.07	0.56	0.31
18:4n-3	0.11	0.07	0.03
20:5n-3 (EPA)	0.55	0.02	0.01
22:5n-3	0.06	0.00	0.00
22:6n-3(DHA)	0.89	0.01	0.00
Total n-3 PUFA[4]	**1.71**	**0.65**	**0.35**
Total PUFA[5]	**1.99**	**4.27**	**2.27**
Total FA content	**6.61**	**18.20**	**10.48**

Values are presented 'as is', based on duplicate analyses
[1]Includes 15:0; 20:0; 22:0 and 24:0; [2]Includes 16:1n-9; 17:1; 18:1n-7; 20:1n-9; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9; [3]Includes 18:3n-6; 20:2n-6; 20:3n-6; 22:4n-6 and 22:5n-6; [4]Includes 20:3n-3; 20:4n-3 and 21:5n-3; [5]Includes 16:2; 16:3 and 16:4

3.2.2 Experimental design and set-up

The trial was conducted at the University of Stirling's Niall Bromage Freshwater Research Unit, Stirling, Scotland (56.02°N, 4.00°W) between June and August 2015, in accordance with commercial husbandry practices. Three thousand six hundred (3,600) Atlantic salmon parr, sourced from Howietoun Fishery (Stirling, UK), with an initial weight of 5.19 ± 0.09 g (mean ± SD) were equally distributed (200 fish per tank) among eighteen circular glass-reinforced plastic tanks (1 m^3 water volume) and supplied with freshwater of ambient temperature (13.8 ± 0.8°C, range 11.0-14.0°C) by flow-through at a rate of 1 L/min. Fish were acclimated for a period of 7 days prior to the commencement of the 8-week experimental feeding period, fed a commercial diet for Atlantic salmon (EWOS 1P diet), and maintained under constant photoperiod (24L:0D) throughout the duration of the experiment. Dietary treatments were randomly assigned to triplicate tanks with fish fed daily using automatic belt feeders at a constant rate of 2.5% of biomass (adjusted weekly by estimation of the biomass from batch test weights) over 24 hours. Mortalities were recorded daily. The experiment was subjected to ethical review by the University of Stirling's Ethics Committee and carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

One day prior to the start of the experimental feeding period and repeatedly every 2 weeks until the termination of the trial, accurate batch test weights using 3 sub-samples per tank (representing 25% of the tank population) were conducted to monitor fish growth. Whole fish samples were collected at the start (n=3 fish/tank) and end (n=5 fish/tank) of the experiment following an overdose of metacaine sulfonate (MS-222) anaesthetic. While initial fish were pooled as three separate samples (n=18 fish/pool), final fish samples were pooled on a tank basis; samples were then homogenised and stored at -20°C until analysis. On termination of the trial, fish from each tank were euthanised (MS-222 overdose) and faeces were collected by stripping individual fish, between 2 and 4 hours after last feed ingestion (Austreng, 1978; Refstie *et al.*, 1998). Faeces were pooled on a tank basis, freeze-dried and used for digestibility analysis.

3.2.3 Biochemical analyses

Feed ingredients, diets and whole fish samples were analysed using standard methods described in Chapter 2 to determine dry matter (DM), crude protein, crude lipid, ash,

crude fibre, gross energy and FA composition. Amino acid compositions of the feed ingredients and diets were determined by HPLC (subcontracted by ALS Food & Pharmaceutical in UK and Eurofins Food and Feed Testing in Norway).

The moisture content of faeces samples was determined by freeze-drying (48 hours) before samples were homogenised and analysed as described above.

Yttrium oxide in diets and faeces was measured using the acid digestion technique by Inductively Coupled Plasma Mass-Spectrometry (ICP-MS).

3.2.4 Calculations

Fish performance and feed utilisation were assessed by determination of the Weight gain (g), Specific Growth Rate (SGR, %body weight/day), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Survival (%) as described in Chapter 2.

The Apparent Digestibility Coefficients (ADC) for the nutrients and gross energy of the diets were calculated as follows (Maynard *et al.*, 1969):

ADC of dry matter of diet = $1–(Y_{diet}/Y_{faeces})$

ADC of nutrients and energy of diets = $1–(Y_{diet}/Y_{faeces})*(N_{faeces}/N_{diet})$

where Y_{diet} = concentration of yttrium oxide in the diet; Y_{faeces} = concentration of yttrium oxide in the faeces; N_{faeces} = concentration of nutrient (or gross energy) in the faeces; N_{diet} = concentration of nutrient (or gross energy) in the diet.

Table 3.2 Ingredients, proximate composition (g/kg), gross energy (MJ/kg), essential amino acid and fatty acid compositions (g/100g diet) of the six experimental diets

	Experimental diets					
	FM100	**MM25**	**MM50**	**MM75**	**MM100**	**DMM50**
Component (g/kg)						
Fishmeal	400.0	300.0	200.0	100.0	0.0	200.0
Crude MM	0.0	100.0	200.0	300.0	400.0	0.0
Defatted MM	0.0	0.0	0.0	0.0	0.0	200.0
Fish Oil	50.0	50.0	50.0	50.0	46.5	50.0
Rapeseed oil	60.0	44.0	25.0	8.0	0.0	50.0
Soy protein concentrate	150.0	170.0	190.0	210.0	230.0	177.5
Wheat gluten	150.0	170.0	190.0	210.0	231.5	177.5
Wheat grain	138.0	120.0	109.0	94.0	72.5	95.0
Vitamin & mineral premix[1]	4.3	4.3	4.3	4.3	4.3	4.3
Di-Calcium Phosphate	35.0	32.0	25.0	20.0	12.5	34.0
Histidine	10.0	7.0	4.0	1.0	0.0	9.0
Antioxidant	0.4	0.4	0.4	0.4	0.4	0.4
Choline chloride	2.0	2.0	2.0	2.0	2.0	2.0
Yttrium oxide	0.3	0.3	0.3	0.3	0.3	0.3
Analysed Composition (g/kg)						
Dry matter	947.3	970.7	970.6	941.2	942.9	940.9
Crude protein	535.5	526.4	530.9	524.6	525.7	520.7
Crude lipid	154.3	168.5	160.5	158.7	162.1	160.8
Ash	99.0	100.0	99.5	82.5	76.9	93.6
Crude fibre	6.6	13.8	21.3	25.3	31.6	24.5
NFE	152.0	162.0	158.4	150.1	137.0	141.2
Gross Energy (MJ/kg)	20.7	21.3	21.5	21.5	21.9	21.4
Essential amino acid composition (g/100g diet)						
Histidine	1.97	1.8	1.67	1.46	1.29	2.04
Arginine	2.74	2.66	2.6	2.51	2.41	2.54
Threonine	1.94	1.91	1.84	1.89	1.86	1.94
Valine	2.41	2.35	2.34	2.37	2.4	2.38
Methionine	1.02	0.89	1.02	0.93	0.89	1.06
Lysine	3.02	2.86	2.74	2.61	2.51	2.81
Iso-Leucine	2.12	2.07	2.06	2.07	2.08	2.03

	FM100	MM25	MM50	MM75	MM100	DMM50
Leucine	3.8	3.66	3.59	3.72	3.68	3.68
Phenylalanine	2.23	2.37	2.49	2.74	2.92	2.64
Fatty Acid composition (g/100g diet)						
14:0	0.39	0.37	0.36	0.39	0.37	0.31
16:0	1.30	1.64	1.77	2.32	2.46	1.48
18:0	0.22	0.23	0.22	0.25	0.26	0.20
20:0	0.04	0.03	0.02	0.02	0.02	0.03
Total Saturated[2]	***2.00***	***2.33***	***2.43***	***3.04***	***3.17***	***2.07***
16:1n-7	0.45	0.61	0.74	1.05	1.15	0.57
18:1n-9	3.67	3.49	2.69	2.60	2.56	3.29
18:1n-7	0.30	0.27	0.21	0.18	0.15	0.24
20:1n-9	0.61	0.70	0.52	0.53	0.43	0.49
22:1n-11	0.84	0.89	0.76	0.74	0.61	0.70
Total monounsaturated[3]	***6.08***	***6.26***	***5.30***	***5.53***	***5.35***	***5.61***
18:2n-6	1.45	1.60	1.51	1.78	1.95	1.51
20:2n-6	0.02	0.01	0.01	0.01	0.00	0.01
20:4n-6	0.02	0.02	0.02	0.02	0.01	0.02
Total n-6 PUFA[4]	***1.51***	***1.64***	***1.55***	***1.81***	***1.97***	***1.54***
18:3n-3	0.43	0.40	0.31	0.29	0.29	0.36
18:4n-3	0.19	0.17	0.15	0.16	0.14	0.13
20:4n-3	0.04	0.03	0.03	0.02	0.02	0.02
20:5n-3 (EPA)	0.55	0.45	0.36	0.34	0.28	0.34
22:5n-3	0.05	0.04	0.04	0.03	0.03	0.03
22:6n-3(DHA)	0.67	0.53	0.41	0.37	0.29	0.39
Total n-3 PUFA[5]	***1.94***	***1.64***	***1.31***	***1.22***	***1.07***	***1.30***
Total PUFA[6]	***3.53***	***3.35***	***2.92***	***3.09***	***3.09***	***2.90***
Total FA content	***11.6***	***11.9***	***10.6***	***11.7***	***11.6***	***10.6***
n-3/n-6	1.3	1.0	0.8	0.7	0.5	0.8

Values are presented 'as is', based on duplicate analyses.
Abbreviations: FM100 – control diet; MM25 – diet where 25 % FM was replaced with housefly maggot meal (MM); MM50 – diet where 50 % FM was replaced with MM; MM75 – diet where 25 % FM was replaced with MM; MM100 – diet where 100 % FM was replaced with MM; DMM50 – diet where 50 % FM was replaced with defatted MM
[1]Vitamin and mineral premix with limestone carrier added according to the commercial standards of BioMar Ltd. ; [2]Includes 15:0; 22:0 and 24:0 ; [3]Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; [4]Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6 ; [5]Includes 20:3n-3 and 21:5n-3 ; [6]Includes 16:2; 16:3 and 16:4

3.2.5 Statistical analyses

Statistical analyses were carried out using IBM SPSS Statistics software (version 21). Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's HSD test for unplanned multiple comparisons or using one-way ANOVA on ranks (Kruskal-Wallis test) when preliminary assumptions were violated. Due to the significant differences in final body weights (covariate), body compositions were statistically compared between treatments using an analysis of covariance, controlling for the effect of the covariate (Shearer, 1994). Correlations between the dietary inclusions of crude MM and the performance or nutritional results were analysed using Pearson's coefficient or Spearman's coefficient when preliminary assumptions were violated. A significance of $P<0.05$ was considered for all analyses performed.

3.3 Results

3.3.1 Growth performance and feed utilisation

During the 8-week experimental period, fish grew from an initial weight of 5.19 ± 0.09 g to final weights ranging from 18.15 ± 1.39 g (MM100) to 23.74 ± 1.77 g (MM25) (Table 3.3). Growth performance (final weight, weight gain and SGR) of fish fed MM25, MM50 and DMM50 did not differ significantly ($P>0.05$) from the FM100 control. However, diets with a higher inclusion of MM (MM75 and MM100) appeared to suppress growth. Fish fed MM75 and MM100 showed significantly lower weight gain and SGR compared to FM100 ($P<0.05$), although MM75 was not significantly different than MM50 and DMM50. The complete replacement of FM with MM (MM100) also influenced the feed utilisation resulting in the highest FCR (1.04), which was significantly different from FM100 (0.86), and the lowest PER (0.0173). The highest PER values were recorded in fish fed MM25 (0.0210) and DMM50 (0.0214), although these were not significantly different from the FM100, MM50 and MM75 fed groups (0.0208; 0.0197 and 0.0190, respectively). In addition, strong negative correlations were observed between the dietary inclusion of MM and weight gain (r -0.842; $P<0.05$), SGR (r -0.836; $P<0.05$) and PER (r -0.712; $P<0.05$), whereas FCR and MM inclusion were positively correlated (r 0.711; $P<0.05$). No difference in survival was found among treatments.

Table 3.3 Performance of Atlantic salmon parr fed experimental diets for 8 weeks (n=3). The significance of the correlation (P<0.05) between the MM dietary inclusion and the fish performance parameter is indicated in the final column as negative (Neg), positive (Pos) or no correlation (-)

	Dietary treatments						Pooled SEM	P-value	Correlation
	FM100	**MM25**	**MM50**	**MM75**	**MM100**	**DMM50**			
Initial weight (g)	5.34	5.17	5.16	5.09	5.26	5.14	0.037	0.217	-
Final weight (g)	22.85^{a}	23.74^{a}	21.25^{abc}	18.96^{bc}	18.15^{c}	21.83^{ab}	0.892	0.000	Neg
Weight gain (g)	17.51^{a}	18.58^{a}	16.08^{ab}	13.86^{bc}	12.89^{c}	16.69^{ab}	0.888	0.000	Neg
SGR[1] (%bw/day)	2.55^{a}	2.67^{a}	2.48^{ab}	2.30^{bc}	2.16^{c}	2.53^{a}	0.076	0.000	Neg
FCR[2]	0.86^{b}	0.88^{ab}	0.93^{ab}	0.94^{ab}	1.04^{a}	0.87^{ab}	0.028	0.046	Pos
PER[3]	0.02^{ab}	0.02^{a}	0.02^{ab}	0.02^{ab}	0.02^{b}	0.02^{a}	0.001	0.024	Neg
Survival rate (%)	100.0	99.8	100.0	99.8	100.0	100.0	0.066	0.514	-

Means with different superscripts within each row are significantly (P<0.05) different
Abbreviations: FM100 – control diet; MM25 – diet where 25 % FM was replaced with housefly maggot meal (MM); MM50 – diet where 50 % FM was replaced with MM; MM75 – diet where 25 % FM was replaced with MM; MM100 – diet where 100 % FM was replaced with MM; DMM50 – diet where 50 % FM was replaced with defatted MM
[1]Specific Growth Rate (percent of the body weight per day)
[2]Feed Conversion Ratio
[3]Protein Efficiency Ratio

3.3.2 Whole fish body composition

No significant differences ($P>0.05$) were observed between the different treatments for dry matter, protein and ash contents of whole fish (Table 3.4). The lipid levels of fish fed the MM25 and MM100 diets were significantly higher than FM100-fed fish ($P<0.05$). Although no significant differences were reported for the dry matter among treatments, crude lipid and moisture were inversely correlated (r -0.820; $P<0.05$). Strong linear relationships were also detected between the dietary inclusion of MM and the fish whole body FA profile, with increasing MM inclusion resulting in increased levels of total saturated FA (r 0.946; $P<0.05$) as well as total n-6 PUFA (r 0.924; $P<0.05$) but decreased levels of n-3 FA (r -0.840; $P<0.05$). However, changes in FA composition were also induced by decreasing rapeseed oil dietary inclusions, which were negatively correlated to the MM dietary inclusions (r -0.985; $P<0.05$); thus the fish whole body FA levels were found inversely correlated to the rapeseed oil inclusions ($P<0.05$). Except for 18:4n-3, other n-3 PUFA were significantly affected by the dietary treatments, in particular, total n-3 PUFA contents for MM50, MM75, MM100 and DMM50 (0.95 ± 0.10; 0.97 ± 0.01; 0.91 ± 0.05 and 1.04 ± 0.02 g/100g, respectively) were significantly lower ($P<0.05$) than FM100 and MM25 (1.46 ± 0.15 and 1.20 ± 0.02 g/100g, respectively). The n-3/n-6 ratio illustrated well the impact of the different treatments on these two PUFA with values decreasing with increasing MM dietary inclusions (r -0.961; $P<0.05$) and decreasing rapeseed oil inclusions (r 0.957; $P<0.05$).

Table 3.4 Whole body compositions (g/kg, on a wet basis) and fatty acid content (g/100g of whole fish body) of Atlantic salmon parr prior to the start (initial; mean ± SD) and at the end of the experimental period (n=3)

	Initial	Dietary treatments						Pooled SEM	P-value
		FM100	MM25	MM50	MM75	MM100	DMM50		
Analysed Composition (g/kg)									
Dry matter	270.3±0.6	277.8	288.8	283	282.7	282.9	284.6	1.447	0.073
Crude protein	150.0±3.7	156	155.9	155.7	154.7	153.5	155.2	0.388	0.482
Crude lipid	71.8±3.0	89.6^{b}	97.7^{a}	93.4^{ab}	94.4^{ab}	96.9^{a}	95.0^{ab}	1.176	0.031
Ash	23.6±1.1	20.9	21.2	20.5	20.6	19.1	20.5	0.295	0.300
Fatty Acid (g/100g fish body)									
14:00	0.33±0.03	0.27	0.28	0.28	0.28	0.28	0.27	0.002	0.716
16:00	0.97±0.09	0.93^{d}	1.12^{c}	1.23^{b}	1.31^{b}	1.45^{a}	1.10^{c}	0.074	0.000
18:00	0.17±0.02	0.22^{c}	0.24^{bc}	0.26^{ab}	0.26^{ab}	0.28^{a}	0.24^{bc}	0.009	0.001
20:00	0.01±0.00	0.02^{a}	0.02^{a}	0.01^{b}	0.01^{c}	0.01^{c}	0.02^{a}	0.001	0.000
***Tot.Saturated*[1]**	***1.51±0.15***	***1.46^{d}***	***1.70^{c}***	***1.82^{bc}***	***1.88^{b}***	***2.06^{a}***	***1.66^{c}***	0.084	0.000
16:1n-7	0.35±0.04	0.30^{e}	0.41^{d}	0.49^{c}	0.59^{b}	0.68^{a}	0.40^{d}	0.057	0.005
18:1n-9	0.88±0.12	2.24^{ab}	2.30^{a}	2.05^{bc}	1.88^{c}	1.89^{c}	2.38^{a}	0.088	0.000
18:1n-7	0.16±0.02	0.22^{ab}	0.23^{a}	0.21^{b}	0.21^{b}	0.20^{b}	0.22^{ab}	0.004	0.002
20:1n-9	0.56±0.05	0.49^{a}	0.52a	0.49^{ab}	0.47^{ab}	0.42^{b}	0.51^{a}	0.014	0.005
22:1n-11	0.66±0.06	0.45^{b}	0.49^{a}	0.46^{ab}	0.43^{b}	0.39^{b}	0.45^{c}	0.013	0.000
***Tot.monounsat.*[2]**	***2.84±0.30***	***3.83^{ab}***	***4.13^{a}***	***3.91^{ab}***	***3.83^{b}***	***3.86^{b}***	***4.15^{a}***	0.061	0.014

	Initial	Dietary treatments						Pooled SEM	P-value
		FM100	MM25	MM50	MM75	MM100	DMM50		
18:2n-6	0.30±0.03	0.68^{c}	0.78^{b}	0.76^{b}	0.82^{ab}	0.88^{a}	0.78^{b}	0.027	0.000
20:2n-6	0.03±0.00	0.06	0.06	0.06	0.06	0.06	0.06	0.001	0.060
20:4n-6	0.03±0.00	0.02^{c}	0.03^{ab}	0.02^{ab}	0.03^{b}	0.04^{a}	0.03^{ab}	0.003	0.000
Tot. n-6 PUFA[3]	***0.38±0.04***	***0.82^{d}***	***0.94^{bc}***	***0.93^{c}***	***1.01^{b}***	***1.12^{a}***	***0.95^{bc}***	0.041	0.000
18:3n-3	0.07±0.01	0.17^{a}	0.17^{a}	0.14^{b}	0.12^{bc}	0.11^{c}	0.16^{a}	0.010	0.009
18:4n-3	0.14±0.01	0.11	0.11	0.1	0.11	0.11	0.11	0.002	0.169
20:4n-3	0.06±0.01	0.04^{ab}	0.05^{a}	0.04^{bcd}	0.04^{cd}	0.03^{d}	0.04^{abc}	0.002	0.014
20:5n-3 (EPA)	0.23±0.03	0.14^{ab}	0.15^{a}	0.11^{c}	0.11^{c}	0.10^{c}	0.12^{bc}	0.008	0.013
22:5n-3	0.09±0.01	0.06^{a}	0.06^{a}	0.05^{b}	0.05^{b}	0.05^{b}	0.05^{b}	0.003	0.017
22:6n-3(DHA)	0.83±0.07	0.64^{a}	0.62^{a}	0.49^{b}	0.52^{b}	0.48^{b}	0.53^{b}	0.028	0.080
Tot. n-3 PUFA[4]	***1.46±0.15***	***1.20^{a}***	***1.18^{a}***	***0.95^{b}***	***0.97^{b}***	***0.91^{b}***	***1.04^{b}***	0.050	0.011
Tot. PUFA[5]	***1.93±0.19***	***2.06^{ab}***	***2.17^{a}***	***1.91^{b}***	***2.03^{ab}***	***2.07^{ab}***	***2.03^{ab}***	0.033	0.030
Tot. FA content	***6.28±0.37***	***7.35^{b}***	***8.00^{a}***	***7.64^{ab}***	***7.74^{ab}***	***8.00^{a}***	***7.84^{ab}***	0.100	0.019
n-3/n-6	3.82	1.46^{a}	1.25^{b}	1.02^{c}	0.95^{cd}	0.81^{d}	1.09^{ab}	0.094	0.000

Means with different superscripts within each row are significantly (P<0.05) different and comparisons were made between dietary treatments and excluded the initial values.
Abbreviations: FM100 – control diet; MM25 – diet where 25 % FM was replaced with housefly maggot meal (MM); MM50 – diet where 50 % FM was replaced with MM; MM75 – diet where 25 % FM was replaced with MM; MM100 – diet where 100 % FM was replaced with MM; DMM50 – diet where 50 % FM was replaced with defatted MM
[1]Includes 15:0; 22:0 and 24:0 ; [2]Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; [3] Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6 ; [4]Includes 20:3n-3 and 21:5n-3 ; [5]Includes 16:2; 16:3 and 16:4

3.3.3 Digestibility

The apparent digestibility coefficients (ADC) of nutrients and energy in salmon fed the experimental diets are presented in Table 3.5. Protein ADC for MM25, MM50 and MM100 (0.885; 0.880 and 0.885, respectively) were significantly higher ($P<0.05$) than FM100 (0.86). Crude lipid ADC of MM75 and MM100 (0.876) were significantly lower than FM100 (0.949) ($P<0.05$) and slightly, but not significantly ($P>0.05$), reduced compared to MM25, MM50 and DMM50. ADC of nutrients and energy obtained for MM75 were generally lower than MM50 and MM100. Nutrient ADC for DMM50 were not significantly different ($P>0.05$) from other treatments, excepting for energy which was significantly higher than MM75. Differences in FA digestibility were only observed for the saturated FA as well as for 18:1n-7 and 20:1n-9, where the highest FA ADC were reported for FM100 and MM25 for the palmitic acid (16:0). However, significant differences observed for 18:1n-7 and 20:1n-9 did not affect the digestibility of the total monounsaturated FA.

Table 3.5 Apparent Digestibility Coefficients (ADC) of nutrients, gross energy and fatty acids of the six experimental diets (n=3)

	Experimental diets						Pooled	P
	FM100	**MM25**	**MM50**	**MM75**	**MM100**	**DMM50**	**SEM**	**value**
ADC								
Dry matter	0.671^{a}	0.659^{a}	0.658^{a}	0.619^{b}	0.647^{ab}	0.642^{ab}	0.007	0.007
Crude protein	0.862^{c}	0.885^{a}	0.880^{ab}	0.865^{bc}	0.885^{a}	0.870^{abc}	0.004	0.034
Crude lipid	0.949^{a}	0.914^{ab}	0.904^{ab}	0876^{b}	0.876^{b}	0.915^{ab}	0.011	0.026
Gross Energy	0.779^{a}	0.773^{a}	0.769^{a}	0.732^{b}	0.752^{ab}	0.763^{a}	0.007	0.006
Fatty Acid ADC								
14:0	0.964^{a}	0.931^{ab}	0.914^{ab}	0.884^{b}	0.877^{b}	0.917^{ab}	0.013	0.013
16:0	0.932^{a}	0.905^{a}	0.889^{ab}	0.852^{b}	0.851^{b}	0.881^{ab}	0.013	0.016
18:0	0.908^{a}	0.864^{ab}	0.810^{bc}	0.783^{c}	0.771^{c}	0.815^{bc}	0.021	0.017
20:0	0.935^{a}	0.897^{ab}	0.857^{bc}	0.818^{c}	0.811^{c}	0.849^{bc}	0.019	0.000
***Total Saturated*[1]**	***0.936^{a}***	***0.905^{ab}***	***0.885^{abc}***	***0.850^{cb}***	***0.847^{b}***	***0.879^{bc}***	0.014	0.002
16:1n-7	0.975	0.954	0.946	0.942	0.939	0.950	0.005	0.330
18:1n-9	0.980	0.954	0.936	0.924	0.920	0.949	0.009	0.090
18:1n-7	0.972^{a}	0.938^{ab}	0.912^{abc}	0.879^{bc}	0.855^{c}	0.929^{abc}	0.017	0.005
20:1n-9	0.971^{a}	0.936^{ab}	0.909^{ab}	0.901^{ab}	0.889^{b}	0.917^{ab}	0.012	0.044
22:1n-11	0.973	0.949	0.936	0.921	0.919	0.937	0.008	0.117
***Tot.monounsat.*[2]**	***0.977***	***0.950***	***0.934***	***0.923***	***0.920***	***0.943***	0.008	0.050
18:2n-6	0.964	0.964	0.962	0.960	0.964	0.963	0.001	0.832
20:2n-6	0.885	0.776	0.716	0.660	-	0.737	0.034	0.161
20:4n-6	0.948	0.957	0.933	0.932	0.911	0.943	0.007	0.129
***Total n-6 PUFA*[3]**	***0.962***	***0.960***	***0.957***	***0.956***	***0.959***	***0.960***	0.001	0.867
18:3n-3	0.981	0.975	0.972	0.969	0.972	0.975	0.002	0.290
18:4n-3	0.985	0.968	0.966	0.967	0.968	0.972	0.003	0.232
20:4n-3	0.976	0.938	0.936	0.934	0.932	0.945	0.007	0.192
20:5n-3(EPA)	0.986	0.984	0.983	0.983	0.985	0.984	0.000	0.745
22:5n-3	0.972	0.943	0.947	0.939	0.938	0.952	0.005	0.229
22:6n-3(DHA)	0.962	0.956	0.951	0.948	0.949	0.953	0.002	0.864
***Total n-3 PUFA*[4]**	***0.976***	***0.968***	***0.966***	***0.965***	***0.967***	***0.969***	0.002	0.598
***Total PUFA*[5]**	***0.970***	***0.965***	***0.961***	***0.960***	***0.962***	***0.964***	0.001	0.731
Total FA content	***0.968^{a}***	***0.945^{ab}***	***0.930^{ab}***	***0.914^{b}***	***0.911^{b}***	***0.936^{ab}***	0.009	0.013

Means with different superscripts within each row are significantly (P<0.05) different.
Abbreviations: FM100 – control diet; MM25 – diet where 25 % FM was replaced with housefly maggot meal (MM); MM50 – diet where 50 % FM was replaced with MM; MM75 – diet where 25 % FM was replaced with MM; MM100 – diet where 100 % FM was replaced with MM; DMM50 – diet where 50 % FM was replaced with defatted MM
[1]Includes 15:0; 22:0 and 24:0; [2]Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9; [3]Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6; [4]Includes 20:3n-3 and 21:5n-3; [5]Includes 16:2; 16:3 and 16:4

3.4 Discussion

The current study aimed to evaluate MM as a FM replacement in juvenile salmon feeds. Insect meals have increasingly been studied as an alternative protein source to FM in fish feeds (reviewed by Henry *et al.*, 2015), with Diptera species displaying the most similar nutritional profile to FM, and therefore the most likely to suit as a substitute in fish feeds, among the various insect species examined (Barroso *et al.*, 2014). The current study used a housefly larvae meal which was lower in protein and higher in lipid content compared to the FM, similar to the findings previously reported by Barroso *et al.* (2014). The amino acid profile of both MM and FM also displayed high similarities, although the essential amino acid level was generally higher in FM. Moreover, the defatting process of the MM led to the concentration of the proteins and amino acids, resulting in DMM exhibiting a similar nutritional profile to FM, particularly with respect to the amino acid profile. The FA composition of the FM, MM and DMM however, showed some differences in accordance with that previously reported by Barroso *et al.* (2014). For instance, compared to the FM, n-6 levels were greater in both insect meals (MM and DMM), whereas total n-3 PUFA was lower as well as being devoid of EPA and DHA.

Accordingly, the nutritional composition of the experimental diets mirrored those of the main ingredients. For instance, dietary crude fibre and ash were directly related to the MM and DMM inclusions since these two ingredients contained more fibre and slightly less ash than FM. Moreover, by substituting FM, the n-3 and n-6 dietary levels decreased and increased respectively, despite the dietary lipid source (FO) being maintained at a constant level among treatments thereby keeping these essential FA present in the diets. FA compositions of DMM50 and FM100 diets were similar to each other except for the total n-3 PUFA content which was lower in DMM50. Consequently, replacing greater than 25% FM with either MM or DMM in feeds resulted in the n-3/n-6 ratio falling below 1.0, which is usually recommended for salmonids to support optimal growth and health of the fish (NRC, 2011). Nonetheless, this statement may be mitigated for freshwater Atlantic salmon parr. In fact, Bell *et al*. (1994) suggested that feeding salmon parr with a diet that mimics their natural food FA composition, namely invertebrates that are relatively low in EPA and DHA (Scott and Crossman, 1973; Bell *et al*., 1994; Amundsen *et al*., 2001), would benefit farmed

smolts by facilitating their transition to seawater. Therefore, unlike the previous study on seawater-adapted post-smolts (Lock *et al.*, 2015), the low levels of dietary n-3 PUFA detected were of low concern and less effort was required to improve the balance n-3/n-6 ratio. Moreover, the amino acid composition of the experimental diets indicated that, although histidine, lysine and arginine levels decreased slightly with increasing dietary levels of MM, essential amino acids were sufficient to meet the requirements for Atlantic salmon parr (NRC, 2011).

As in the study by St-Hilaire *et al*. (2007b), diets were formulated to contain equals amounts of protein, lipid and energy. However, retrospective analyses showed some minor differences between diets that could have affected the comparative fish growth and nutrient utilisation. As MM gradually replaced FM, soy protein concentrate and wheat gluten inclusions were increased to compensate for the protein deficiency. Similarly, rapeseed oil was reduced to correct the dietary lipid levels. It is, therefore, reasonable to assume that these ingredients may have contributed, to some extent, to the overall results of this study. Nevertheless, as 40% of the diet was comprised of FM, MM or DMM, these key ingredients were the main factors influencing the results.

Impaired fish growth and feed utilisation following dietary inclusion of MM were also found in other studies (St-Hilaire *et al*., 2007b; Kroeckel *et al*., 2012), and only fish survival was not affected by the dietary changes. Diets containing up to 200 g/kg MM or DMM led to a similar performance observed in the FM-based diet, whereas a total substitution of FM resulted in a significantly lower growth and a higher FCR compared to FM100. MM75 results, on the other hand, were intermediate between MM50 and MM100. This is consistent with Lock *et al.* (2015) who found that black soldier fly (BSF) larvae meal could replace up to 50% FM in post-smolt diets without affecting fish growth. Nevertheless, the same authors also reported a decreased FCR with increasing dietary inclusions of BSF meal. Similarly, in a study on rainbow trout (*O. mykiss*), Belforti *et al.* (2015) found an improvement in FCR for the insect meal-based diets compared to the FM control, suggesting that the lipid content or the FA composition of the insect meal (mealworm, *T. molitor*) probably reduced the fish voluntary feed intake. In the current study, although the feeding response of each treatment was considered good (visual assessment), uneaten feed was not collected and FCR were calculated according to commercial practices by considering the feed

distributed equalled to the feed consumed. Therefore, although FCR values were very good across treatments, even for MM100 with the highest FCR (1.04), values may have been slightly overestimated. Likewise, PER values may have been slightly underestimated and the tendency towards lower PER with increased dietary levels of MM suggested that dietary protein was less efficiently used by the fish as the MM inclusion increased.

Overall performance of the fish fed the defatted MM-based diet (DMM50) was comparable with the control (FM100), MM25 and MM50 fed fish. Interestingly, and although not significantly different, DMM50 performed slightly better than MM50 with higher weight gain, SGR, PER and a lower FCR. In contrast, Lock *et al.* (2015) reported that dietary inclusions of defatted BSF meal significantly affected the growth of post-smolts; however, it is thought that the high-temperature drying method used after the defatting process resulted in lipid oxidation that subsequently led to the reduction in the quality of the meal (Henry *et al*., 2015; Lock *et al*., 2015).

Dietary treatments had no significant influence on the dry matter, crude protein and ash content of whole body fish. Moreover, in accordance with previous studies, the FA composition of the fish reflected that of the diet (Turchini *et al*., 2009; Sealey *et al*., 2011; Belforti *et al*., 2015). Nonetheless, the lipid deposition was found to be significantly higher in fish fed MM25 and MM100 compared to FM100. Whole body lipid storage in fish is generally determined by the available the levels of dietary lipid and dietary energy or by an imbalance in protein-to-energy ratio (Shearer, 1994) which were slightly higher in MM25 (168.2 g/kg) and MM100 (21.9 MJ/kg) respectively, compared to FM100 (154.3 g/kg and 20.7 MJ/kg).

Faecal collection by stripping has been widely discussed in the literature and is often considered as a method that results in underestimates of digestibility because the material collected (digesta) might not be completely digested (Glencross *et al.*, 2007). ADC values determined for MM75, in particular, dry matter, protein and gross energy ADC, were not consistent with the pattern suggested from other treatments and were probably biased and substantially underestimated. This could result from a contamination of the faeces samples during stripping with undigested material or endogenous material (Glencross *et al.*, 2007). Disregarding MM75, the protein digestibility of the MM-based diets was significantly better than FM100. On the other

hand, lipid and gross energy digestibility were inversely correlated to the MM dietary levels. These results may also explain the tendency for greater lipid storage in the fish fed the MM-based diets. Indeed, well-digested dietary proteins of MM-based diets may have compensated for the poorly digested dietary lipid and energy and were, therefore, less efficiently used for growth (lower PER). Dietary inclusions of both BSF and mealworm meals impaired the protein digestibility of turbot (*Psetta maxima*) and rainbow trout, respectively (Kroeckel *et al.*, 2012; Belforti *et al.*, 2015), whereas in post-smolt salmon comparable amino acid digestibility coefficients for BSF and FM-based diets were reported (Lock *et al.*, 2015). Differences in dietary FA digestibilities were principally found for saturated FA as well as for 18:1n-7 and 20:1n-9, contrasting the findings of Lock *et al.* (2015) who found no difference in digestibility of any FA. Ingredient composition of the experimental diets has surely contributed to these results. Indeed, soy protein concentrate and wheat gluten protein are highly digestible for Atlantic salmon with respective ADC equal to 0.90 and 0.99 (NRC, 2011), thus increasing dietary levels concomitant with that of the MM may have improved the overall protein ADC of the diets. Lipid digestibility, on the other hand, was mostly related to MM dietary inclusion as when the latter increased, the levels of rapeseed oil decreased. In addition, it has been mentioned that chitin, the main constituent of insect cuticle, could interfere with the lipid digestibility in carnivorous species such as Atlantic salmon and turbot by inhibiting nutrient absorption in the gastro-intestinal tract (Olsen *et al*., 2006; Kroeckel *et al*., 2012).

DMM50 nutrients digestibility was similar to MM50 and FM100, except for the stearic (18:0) and arachidic (20:0) acids. In addition, the crude lipid digestibility of DMM50 (0.92) was slightly better, although not significant, than MM50 (0.90) suggesting that defatted meal may slightly improve the digestibility of the dietary lipid.

The present study is among the first, to our knowledge, to examine crude and defatted housefly larvae meal (*M. domestica*) as a substitute to FM in Atlantic salmon diets. Previous studies on African catfish (*Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*), have demonstrated that up to 30% of the FM inclusion could be replaced with crude housefly larvae meal (Fasakin *et al*., 2003; Ogunji *et al*., 2008c, 2009; Aniebo *et al*., 2009). The use of defatted housefly larvae meal was also encouraged by Fasakin *et al*. (2003) who found that the African catfish

fed FM-free DMM-based diets performed better (growth and nutrient utilisation) than the fish fed FM-free MM-based diets and equal to the fish fed the FM control diet. Thus, in addition to the results from the current study it may be of interest to look at higher substitution rates with DMM for salmon parr. Fish feeding trials using BSF and housefly larvae meal, widely reviewed by Henry *et al*. (2015) and Makkar *et al*. (2014), highlighted the variability of the results from one fish species to another. The choice of insect species used to substitute FM can be dictated by various factors such as the availability of the material, but the nutritional requirements of the fish being farmed as well as the chemical composition of the insect meal are also essential criteria that must be considered. It should be noted that although the FA composition of the MM, devoid of essential n-3 PUFA such as EPA and DHA, might have been limiting for carnivorous fish species given their requirements in terms of essential FA, this can be remedied slightly by modifying the insect rearing substrate (St-Hilaire *et al*., 2007a). Furthermore, differences between the current study and that of Lock *et al.* (2015) may be related to the stage of development of the fish used in the study but also to the insect meal itself.

The findings of the present study suggested that common housefly larvae meal is a very good source of protein and a suitable replacement for FM in the diets of salmon parr during their freshwater stage in a commercial hatchery setting. Moreover, dietary inclusions of up to 200 g/kg are recommended to ensure performance and body composition similar to FM-based diets. The results also highlighted the high potential of refined meal (defatted) which could probably replace more than 50% FM in salmon parr diets (dietary inclusions ≥ 200 g/kg). As Atlantic salmon is an anadromous fish species whose nutritional requirements change throughout life, further studies are needed to evaluate the suitability of MM and DMM in other life stages.

Chapter 4. Efficacy of Nile tilapia (*Oreochromis niloticus*) sex-reversal diets containing maggot meals as substitutes to fish meal

4.1 Introduction

Tilapia monosex culture (all-male) is often preferred in grow-out systems as it limits recruitment and continuous breeding of this highly prolific species, leading to more uniform marketable fish crops produced at a faster rate due to the significant difference in growth between males and females (Mair and Little, 1991; Little and Hulata, 2000; Phelps and Popma, 2000). Masculinisation methods are various but the oral administration of 17α-methyltestosterone (MT), a synthetic male hormone, is mostly used because it is the most effective and economically feasible existing method (Guerrero and Guerrero, 1988; Phelps, 2006; El-Greisy and El-Gamal, 2012). In Thailand, a cost-effective hatchery technology that includes broodstock management, seed collection and incubation, larval rearing and fry masculinisation using MT-treated fish meal has been developed over several years of research; it is now a commercial process in use worldwide to produce millions of sex-reversed tilapia fry (Little, 1989; Bhujel, 1997; Turner, 2015).

Good farming practices have ensured over the years the success of commercial hatcheries to produce monosex tilapia fry. Quality of the seed, environment (water temperature, level of natural food) and husbandry are important factors to consider; however quality and feed management remain key parameters for success (Popma and Green, 1990; Phelps, 2006; Little and Hulata, 2000). Daily intake of well-prepared MT-feed (including a uniform distribution of the hormone, applied at a dose of 60 mg per kg of feed) should start prior to the start of the gonad differentiation, hence right after the yolk-sack absorption, of the swim-up fry (D'Cotta *et al.*, 2001). Therefore, high quality, palatability and floatability are key drivers that led to the selection of pure FM as the hormone carrier. High quality commercial feeds for fish juveniles are also suitable when quality FM is not available, however, compounded diets must contain at least 40% crude protein (usually provided by high levels of FM) and fish oil to enhance palatability (Phelps, 2006; NRC, 2011). Global rise of the commodities prices, FM in particular, and concerns about sustainability led to the exploration and the identification of alternatives feedstuffs that contribute to the decreasing use of FM in aquafeeds (Olsen and Hasan, 2012).

Insect meals have been identified as promising candidates for fish and livestock in the global assessment of potential feedstuffs (Makkar *et al.*, 2014; Sánchez-Muros *et al.*,

2014). Except for their fatty acid composition, that is often low in essential omega-3 fatty acids, MM, in particular, seem to have a strong potential for fish thanks to their nutritional profile similar to FM (Barroso *et al.*, 2014). Lipid-rich MM could also be defatted to improve their nutritional quality and answer better to the fish nutritional requirements (Fasakin *et al.*, 2003), which depends on several factors such as the species, the rearing substrate or the processing method applied (Henry *et al.*, 2015). Although the insect farming industry is developing and progressively moving towards industrialisation, current production capacity is far from being able to meet demand for the whole aquaculture industry to replace, even partially, FM. A strategic use of this ingredient should, therefore, be considered and as such, the specific requirements of sex-reversal process in terms of feed quality and amounts required suggest the potential for MM to be used.

The aim of the current study, conducted in a commercial hatchery in Thailand, was to investigate the efficacy of diets for sex-reversal of Nile tilapia fry containing various levels of crude BSF (*H. illucens*) MM, defatted BSF MM or crude housefly (*M. domestica*) MM. Quality, floatability and palatability of the MT-treated feeds are key drivers ensuring successful sex-reversal process and insect meal-based diets were expected to perform as well as the control diet composed of FM only. Indicators of a successful sex-reversal process were considered as (ranked by order of importance) (i) the male percentage, (ii) the survival rate, (iii) the size homogeneity of the fish produced (evenness) and (iv) the fish performance (growth and feed utilisation); the profit index (PI) and economic conversion ratio (ECR) were also calculated to assess the economic efficiency of the diets.

4.2 Materials and methods

4.2.1 Experimental diets

The fish meal (FM, tuna by-products) used in this experiment was supplied locally from T.C. Union Agrotech Co. Ltd. (Bangkok, Thailand). The crude housefly larvae (*M. domestica*) meal (HM) was supplied by Grant Bait Ltd. (Yorkshire, UK) and produced according to Charlton *et al.* (2015) using poultry manure; BSF larvae (*H. illucens*) meals, crude (BM) and defatted (DM), were produced (using a large-scale BSF pilot farming system) by Entofood Sdn Bhd (Kuala Lumpur, Malaysia) from processed food

wastes. Chemical compositions of the feed ingredients were analysed at the University of Stirling (Stirling, UK) as dry matter; crude protein; crude lipid; ash; crude fibre and energy as described in Chapter 2 (Table 4.1).

Hormone-treated diets were prepared on-farm according to the recommended procedures (Guerrero, 1975) as a control diet composed of pure FM (F100) along with twelve (12) experimental diets where 25; 50; 75 and 100 % of the FM was replaced (w/w) with HM, BM or DM (Table 4.1). Briefly, a stock solution of 17α-methyltestosterone (MT) dissolved in 95% pure ethanol was also prepared at a concentration of 250 mg/L and used within a day. Following sieving through a 0.6 mm mesh screen, the ingredients were thoroughly mixed together in relevant proportions (Table 4.1) before adding 240 ml/kg of MT stock solution in a large stand mixer (Tong Hor, Lex product) ensuring a homogenous distribution of the MT at a dose of 60 mg/kg diet. Air-dried treated feeds (spread into a thin layer on a mesh rack placed in the shade at ambient for 6 hours) were packed in airtight plastic bags, refrigerated (4°C) and used within 30 days (Figure 4.1).

Table 4.1 Proximate composition (g/kg) and gross energy (MJ/kg) of the fish meal, black soldier fly meal (BSF), defatted BSF meal and housefly meal used in the composition (g/kg) of the 13 experimental diets

	Fish meal (FM)	**BSF meal (BM)**	**Defatted BSF meal (DM)**	**Housefly meal (HM)**
Proximate composition (g/kg)				
Dry matter	911.0	929.8	915.4	956.1
Crude protein	558.5	334.1	473.4	457.4
Crude lipid	119.2	415.6	174.8	283.1
Ash	214.3	84.9	129.8	98.5
Crude fibre	5.3	56.9	68.9	74.7
NFE	13.7	38.3	68.4	42.4
Gross Energy (MJ/kg)	18.3	25.9	20.2	23.7
Diets formulations (g/kg)				
F100	1000	-	-	-
B25	750	250	-	-
B50	500	500	-	-
B75	250	750	-	-
B100	-	1000	-	-
D25	750	-	250	-
D50	500	-	500	-
D75	250	-	750	-
D100	-	-	1000	-
H25	750	-	-	250
H50	500	-	-	500
H75	250	-	-	750
H100	-	-	-	1000

Values are presented 'as is', based on duplicate analyses. Abbreviations: F100 – control diet made of 100 % MT-impregnated fish meal (FM); B25, B50, B75 and B100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by Black Soldier Fly larvae (BSF) meal, D25, D50, D75 and D100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by defatted BSF meal, H25, H50, H75 and H100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by housefly larvae meal.

4.2.2 Experimental design and set up

The experiment was conducted on-farm, within the facilities of Nam Sai Farms Co. Ltd. (Prachinburi, Thailand) in November 2015. A 2,000 m^2 earthen pond of 1.2 m depth, located at Nam Aig site (13°58'35.29"N; 101°14'50.74"E), was drained, limed (1,875 kg/ha as $Ca(OH)^2$) and sun-dried for a week. The pond was then filled with predator-free water, screened through a fine mesh, from an on-farm reservoir and 20 kg NPK inorganic fertiliser (15-15-15; Saksiam Inter Supply Co. Ltd., Bangkok, Thailand), dissolved in a small volume of pond water, was then broadcasted into the pond, one week prior to the start and then repeatedly once a week to promote natural productivity during the course of the experiment. Treatments were replicated 5 times and randomly allocated to 65 hapas (1.0 m^3) set up in the pond (Figure 4.1). Stage V swim up fry (10.5±0.1 mg; mean ± SD, n=3), derived from a single batch of GIFT tilapia strain eggs hatched in Nam Sai Farms in a recirculated hatchery system, were stocked at a density of 5,600 fry per hapa according to standard practices (Little, 1989; Bhujel, 2014, 2013). The sex-reversal process lasted 21 days, during which fry were fed with the control and experimental MT-treated diets at 14, 30, 50 and 85 g/day/hapa (total 980 g of feed/hapa in total) for the period of days 1-5, days 6-10, days 11-15 and days 16-21, respectively; daily feeding rations were divided into five equal portions and fed, by hand, five times a day to each hapa (Bhujel, 2014). Aeration of the water was provided by a blower and 14 homemade diffusers (tubular bags made of textile material) place around the pond. Air was turned on twice a day from 21:00 hrs to 08:00 hrs and from 13:00 hrs to 15:30 hrs and hapa nets changed once a week to prevent fouling and low dissolved oxygen (DO). Water temperature was measured and recorded every 2 hours with RFID (Radio Frequency Identification) temperature sensors (LOG-IC® data loggers, American Thermal Instruments) placed at 10 and 50 cm under the water surface in the pond. DO was measured on alternate days at 08.00 hrs and 15.00 hrs using a YSI 550A digital probe and water samples were collected twice a week to measure pH, ammonia, nitrite and alkalinity levels using a Hanna HI83200 spectrophotometer.

Growth was monitored by test weighing the fish at the beginning, halfway through (day 10) and at the end of the experimental period. The sampling procedure consisted of removing three separate sub-samples using a scoop net of fish concentrated in a corner of the hapa before counting them and recording bulk weights (Tanita K-200 digital

scale, precision: 0-1000gx1g). At the end of the 21-day treatment period, fish were graded into 3 sizes (small, medium and large) using hand graders with meshes of 7.5 mm and 9.0 mm, respectively, to assess the uniformity of the harvested fish. Total biomass of each size class was recorded and a sub-sample of fish for each group size (scoop-net) was counted and weighed. Survival rate (%) was determined by difference between the initial and the final fish stock in each hapa. Simpson's dominance index or evenness index (D) was calculated to assess the uniformity of the fry size distribution within each hapa at the end of the 21-day sex-reversal period (Heip *et al.*, 1998):

$$D = \sum_{i} (Ni/N)^2$$

where Ni/N is the relative abundance of fish in each size class. Greater D values (tending towards 1) indicating greater the homogeneity of the treatment populations.

Biomass and number of fish at harvest were also determined from the sampling and feed conversion ratio (FCR) was calculated as described in Chapter 2.

4.2.3 Sex determination

Evaluation of the treatment efficacy was achieved seven weeks after the end of the 21-day treatment period. 220 fish from each replicate were retained after the treatment period and, because the size can be influenced by the sex (Guerrero, 1975), fish sub-samples were collected randomly using a scoop net and then transferred to 65 new hapas-in-pond of 5.0 m^2 each (Figure 4.1). During this period, fish were fed *ad libitium*, 4 times per day with a 32 % crude protein farm-made nursery feed consisting of a mixture of FM, soybean meal, corn meal, cassava meal, rice bran and meat and bone meal. A representative sample of 100 fish per replicate was sacrificed by an overdose of metacaine sulfonate (MS-222) anaesthetic and gonadal examination was performed according to Guerrero and Shelton (1974). Slides of squashed gonads were examined using a Seek SK-500E microscope (magnification 4x) to identify and record the frequency of testicular and ovarian tissue.

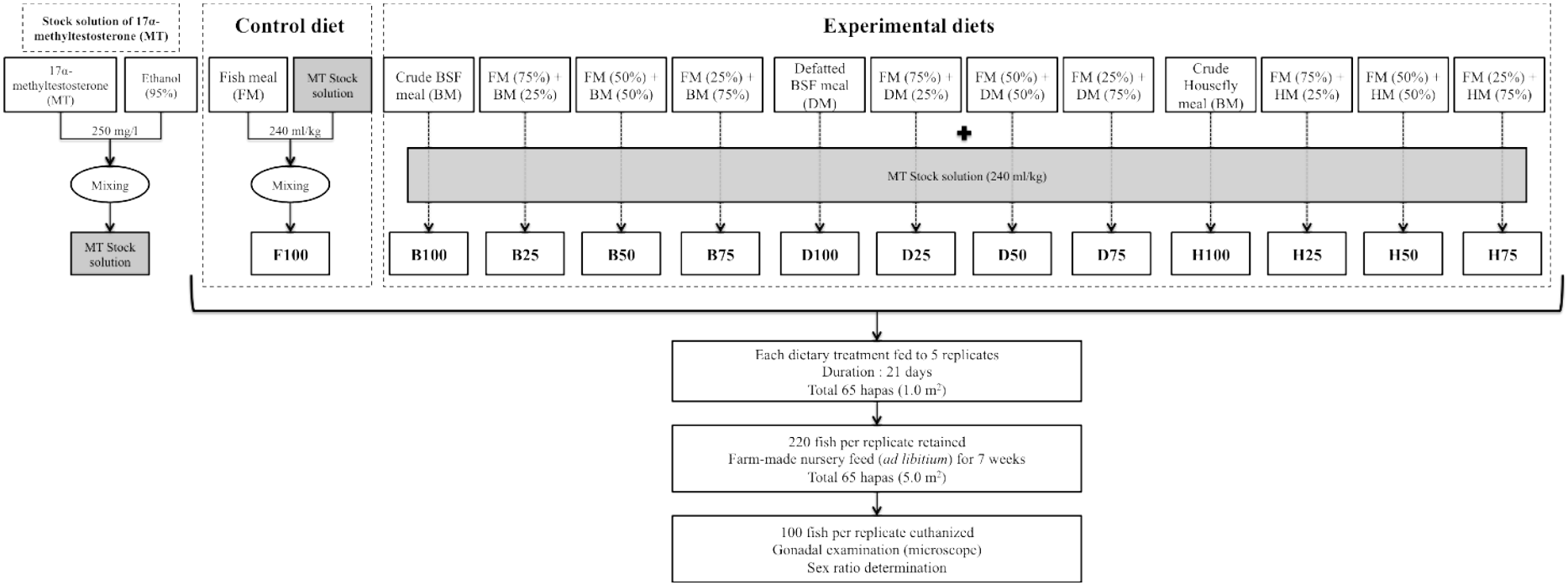

Figure 4.1 Diagram representing the investigation plan for the study including a detailed protocol for the control and experimental diets preparation and the main steps of the experimentation

4.2.4 Economic analyses

A simple economic analysis was performed to assess the cost-effectiveness of the diets. The evaluation used the US Dollar (USD) as currency and was conducted for a production of 1 m^2 (5,600 fry initially stocked and 980 g of feed used per m^2) based on the experimental performance results for each treatment. Price of the FM (1.0 USD/kg) was based on the Thai market price (conversion rate: 1.0 USD = 35.6 Thai Baht, November 2015); however, the lack of commercially traded insect meals led to sensitivity analyses being used for each type of insect meal independently, considering prices varying between 30% more or less the price of the FM (i.e. from 1.3 to 0.7 USD/kg). The price for each diet (USD/kg) was calculated according to the assumption made on the price of the meals and their relevant inclusions in the diets and further used to calculate the cost of the feed required per production unit (USD/m^2). Value of the production accounted for the size of the fry harvested at the end of the 21-day sex-reversal period; farm-gate prices applied were 14.0 USD/1000 fry for the large fish (>300 mg) and 12.0 USD/1000 fry for the medium and small fish (<300 mg) (Turner, 2015). Assumptions also included that all other variable and fixed costs remained constant independently of the diet.

The economic evaluations were based on the Profit Index (PI) and the Economic Conversion Ratio (ECR) (Goddard, 1996; Martínez-Llorens *et al*., 2012) calculated as:

- PI (USD/m^2) = Value of the production (USD/m^2) / Feed cost (USD/m^2)
- ECR (USD/kg of fish)= FCR * Feed price (USD/kg)

4.2.5 Statistical analyses

Statistical analyses were carried out using IBM SPSS Statistics software (version 21). Data were treated using one-way analysis of variance (ANOVA) or Kruskal-Wallis non-parametric test when preliminary assumptions were violated. Tukey's HSD test was applied for unplanned multiple comparisons. Differences among means with $P<0.05$ were accepted as representing statistically significant differences. Results are presented as mean ± standard error (SE) unless otherwise stated.

Quadratic regression analyses were applied, where PI and ECR were a function of the insect meal inclusion level using the expression $Y=c+bX+aX^2$. Optimum insect meal inclusion levels that maximised the PI and minimised the ECR were obtained by deriving these equations and equalising to zero (Shearer, 2000; Martínez-Llorens *et al.*, 2012).

4.3 Results

4.3.1 Sex-reversal success and performance

Water temperature and dissolved oxygen varied slightly during the course of the experiment and the diurnal periods with values ranging from 28.5 to 34.4°C and 2.4 to 6.8 g/L, respectively. Water pH (7.7±0.5; mean ± SD) remained stable during the 21-day experimental period, whereas nitrite, ammonia and alkalinity levels have decreased during the first 10 days and then stabilised around 0.6±0.1 mg/L, 0.9±0.5 mg/L and 66.1±7.2 mg/L, respectively. Nevertheless, all the values were within tolerance limits for tilapia (Beveridge and McAndrew, 2000; El-Sayed, 2006).

A high proportion of males (99.8 to 100 %) was achieved across treatments and although only a few females were identified in F100, B75 and H50, the success of the sex-reversal process was not significantly affected ($P>0.05$) by the dietary treatments (Table 4.2). Similarly, survival rates ranging between 68.3 % and 84.0 %, were good and did not differ significantly between dietary treatments ($P>0.05$). The grading process at the end of the 21-day sex-reversal period led to large, medium and small fry with mean individual weights of 458.2±2.6 mg, 263.1±2.9 mg and 147.8±2.1 mg, respectively. Groups of fry fed diets containing 25 to 75 % DM or HM and 25% BM showed Simpson dominance indexes significantly higher (0.6) than fry fed F100 (0.5) ($P<0.05$), thereby indicating that these treatments led to significantly more homogenous size populations than the control. Relative abundance of each size class varied across treatments ($P<0.05$) (Figure 4.1). Groups of fry fed diets containing up to 50 % BM and up to 75 % DM or HM were mainly composed of medium size fish (67.8 to 76.6 % of the total biomass), hence, their higher evenness indexes (except for B50) compared to F100. All the diets containing 25 to 75 % MM led to significantly less fish of small size (8.0 to 21.0 %) than the control (36.4 %) ($P<0.05$), whereas B25-50-75 and H50-75

treatments led to significantly more large fish (16.7 to 23.5%) than F100 (6.7 %) ($P<0.05$).

Diets containing 100 % MM (B100, D100 and H100) led to population distributions comparable to F100 ($P>0.05$) with 2.2 to 6.7 % large fry, 48.0 to 63.5 % medium fry and 34.3 to 46.0 % small fry. None of the treatments led to performance results significantly lower than the control, F100 (Table 4.3). However, excepting for D75, biomass of the fish fed diets containing 25 to 75 % MM were significantly higher than F100 ($P<0.05$). Although feed efficiency was good across treatments, FCR were significantly improved ($P<0.05$) when MM replaced 25 to 75 % FM (0.8 to 0.9) compared to F100 (1.1). Accordingly to the survival rates, the number of fish at harvest (end of the 21-day sex-reversal period) in each treatment did not differ significantly from the control ($P>0.05$). Performance (biomass and FCR) of the fish fed 100 % MM diets were comparable to F100 ($P>0.05$).

Table 4.2 Indicators assessing the success of the 21-day sex-reversal process for each dietary treatment compared to the control diet (F100)

Dietary treatment	Males (%)	Survival rate (%)	Evenness
F100	99.8±0.2	74.5±2.5	0.5±0.0
B25	100.0±0.0	68.3±5.6	0.6±0.0*
B50	100.0±0.0	73.5±6.8	0.5±0.0
B75	99.8±0.2	79.6±5.6	0.5±0.0
B100	100.0±0.0	84.0±4.4	0.5±0.0
D25	100.0±0.0	78.6±3.1	0.6±0.0*
D50	100.0±0.0	80.4±2.6	0.6±0.0*
D75	100.0±0.0	82.9±5.0	0.6±0.0*
D100	100.0±0.0	75.7±4.8	0.5±0.0
H25	100.0±0.0	81.8±2.5	0.6±0.0*
H50	99.8±0.2	79.0±6.7	0.6±0.0*
H75	100.0±0.0	72.9±5.4	0.6±0.0*
H100	100.0±0.0	73.7±3.0	0.5±0.0

Abbreviations: F100 – control diet made of 100 % MT-impregnated fish meal (FM); B25, B50, B75 and B100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by Black Soldier Fly larvae (BSF) meal, D25, D50, D75 and D100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by defatted BSF meal, H25, H50, H75 and H100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by housefly larvae meal
*Mean ± SE (n=5) significantly different from the control, F100

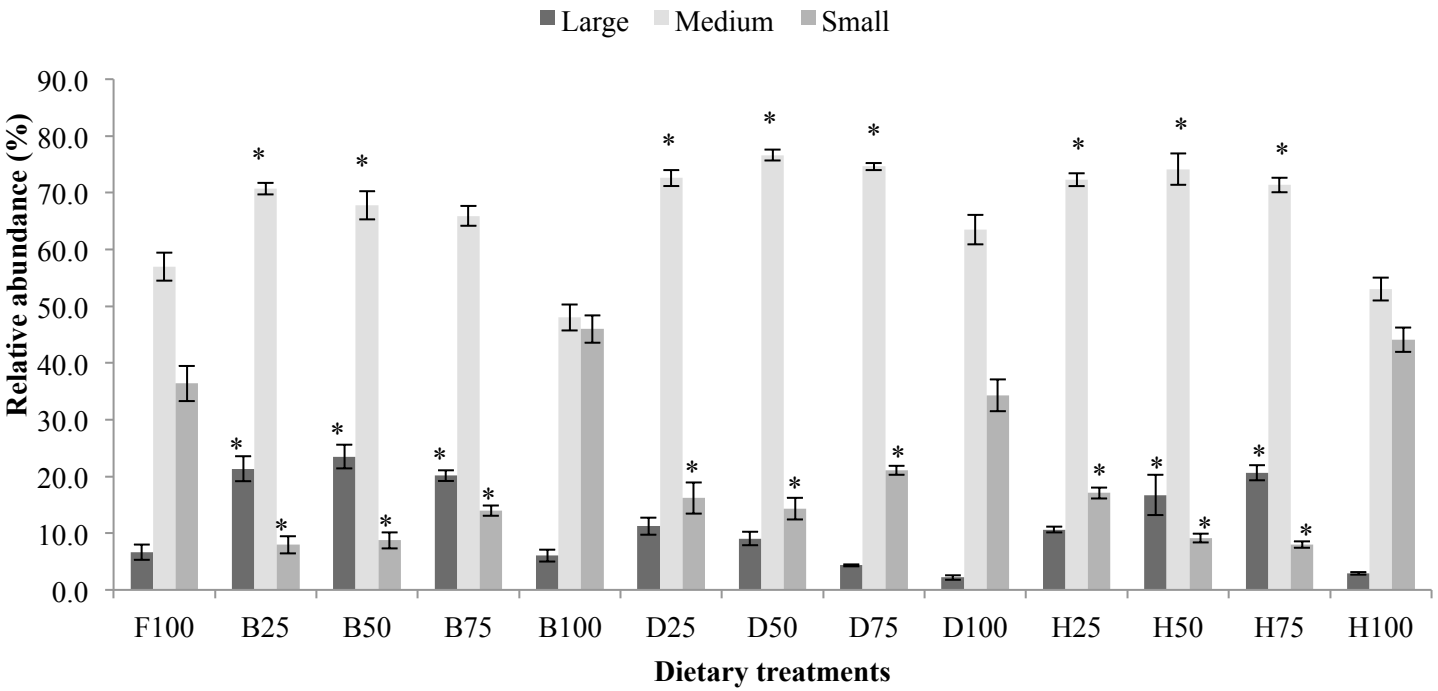

Figure 4.2 Relative abundance (% of total biomass harvested) of large (458.2±2.6 mg), medium (263.1±2.9 mg) and small (147.8±2.1 mg) fry at the end of the 21-day experimental period. For comparable columns (class size), mean ± SE (n=5) bearing an asterisk (*) were significantly different from the control, F100 ($P<0.05$)

4.3.2 Economic analyses

The price of F100 diet was fixed (1.0 USD/kg); therefore, assuming a production unit of 1 m^2 that required 980 g of MT-treated feed for the 21-day sex-reversal period (ensuring a daily dose of hormone intake to the fish stocked), the cost of feed amounted to 0.98 USD/m^2. The price of the other diets and, consequently, the cost of the feed required per m^2 varied depending on the price of the MM (0.7-1.3 USD/kg) and the dietary inclusion levels (Table 4.3). Costs of the MM-based diets were reduced with MM inclusion increasing; in addition, when MM prices were below 1.0 USD/kg, the MM-diets cost less than F100. Value of the production (USD/m^2), based on the relative abundance of fish in each class size and their assumed farm-gate prices, indicated that none of the dietary treatment led to values significantly different from the control diet F100 ($P>0.05$). PI_{F100} and ECR_{F100} were respectively equal to 51.7 USD/m^2 and 1.1 USD/kg of fish. Equations (1); (2) and (3) for estimating, respectively, the optimum BM, DM and HM levels that maximise PI considering the variation of the MM price were developed in the first model.

(1) $^{PI}BM_{level} = - 43.356 + 30282 * BM_{price} + 41.095 * BM_{price}^2$

(2) $^{PI}DM_{level} = 414.140- 545.542 * DM_{price} + 183.377* DM_{price}^2$

(3) $^{PI}HM_{level} = 420.701 + 560.451 * HM_{price} + 179.991 * HM_{price}^2$

The model 1 indicated two opposite trends depending on the MM considered; the optimum inclusion level of BM that maximised PI ($^{PI}BM_{level}$) increased with the price of the BM whilst optimum $^{PI}DM_{level}$ and $^{PI}HM_{level}$ decreased with increasing DM and HM prices (Figure 4.3A). However, at these optimum inclusions levels, PI_{BM}, PI_{DM} and PI_{HM} decreased as the price of the MM increased (Figure 4.3B).When BM was considered, it appeared that the maximum PI_{BM} obtained was always lower than PI_{F100} suggesting that BM-based diets were less profitable than F100. In addition, below 0.8 USD/kg, the model 1 recommended to not include BM in the diet in order to maximise the profit. Conversely, PI_{DM} and PI_{HM} at optimum DM and HM inclusion levels were greater or equal to PI_{F100} when MM prices were comprised between 0.8 and 1.3 USD/kg with values ranging respectively from 76.4 to 51.6 USD/m^2 and 72.1 to 52.7 USD/m^2. Model 1 suggested that below 0.8 USD/kg, a total substitution of FM with either DM or HM would maximise the PI (76.4 and 72.1 USD/m^2, respectively).

Table 4.3 Figures used for the economic analyses based on the experimental performance of the fry during the 21-day sex-reversal period (biomass and number of fish at harvest and Feed Conversion Ratio, FCR) under each treatment. Price of the diets (USD/kg); cost of the feed required per unit of production (USD/m^2) and value of the fish produced (USD/m^2) for each dietary treatment were also calculated

Dietary treatments	Biomass produced (g)	Number of fish at harvest	FCR	Diet price[1] (USD/kg)	Feed cost[2] (USD/m^2)	Production value[3] (USD/m^2)
F100	857.0±42.3	4173±141	1.1±0.1	1.00	1.00	50.1±0.8
B25	1150.3±88.7*	3827±311	0.8±0.1*	0.93 - 1.08	0.91 - 1.05	47.5±3.8
B50	1203.8±101.6*	4114±380	0.8±0.1*	0.85 - 1.15	0.83 - 1.13	51.2±4.6
B75	1197.3±98.3*	4456±314	0.8±0.1*	0.78 - 1.23	0.76 - 1.20	53.6±3.1
B100	831.8±39.5	4707±245	1.2±0.1	0.70 - 1.30	0.69 - 1.27	57.0±3.8
D25	1200.8±18.6*	4399±173	0.8±0.0*	0.93 - 1.08	0.91 - 1.05	55.4±2.5
D50	1159.8±35.4*	4500±144	0.8±0.0*	0.85 - 1.15	0.83 - 1.13	54.8±1.8
D75	1109.5±35.9	4643±280	0.9±0.0*	0.78 - 1.23	0.76 - 1.20	53.6±3.3
D100	941.0±35.0	4240±270	1.0±0.0	0.70 - 1.30	0.69 - 1.27	52.3±3.1
H25	1151.6±12.4*	4580±142	0.8±0.0*	0.93 - 1.08	0.91 - 1.05	53.6±3.9
H50	1241.3±76.5*	4425±377	0.8±0.1*	0.85 - 1.15	0.83 - 1.13	57.3±1.4
H75	1175.2±62.7*	4080±302	0.8±0.0*	0.78 - 1.23	0.76 - 1.20	36.5±2.7
H100	847.0±14.8	4127±170	1.2±0.0	0.70 - 1.30	0.69 - 1.27	52.1±2.8

Abbreviations: F100 – control diet made of 100 % MT-impregnated fish meal (FM); B25, B50, B75 and B100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by Black Soldier Fly larvae (BSF) meal, D25, D50, D75 and D100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by defatted BSF meal, H25, H50, H75 and H100: diets where, respectively, 25; 50; 75 and 100 % FM was replaced by housefly larvae meal

*Mean ± SE (n=5) significantly different from the control, F100. 1Price of the diet calculated with the FM price (1.0 USD/kg) and considering the variation of price from 0.7 to 1.3 USD/kg for each MM; 2Cost of the feed (USD/m2) calculated from the price of the diet considering 980g of feed required for 1 m2 production unit; 3Value of the fish produced (mean ± SE) per unit of production, assuming farm-gate prices of 14.0 USD/1000 fry for large fry and 12.0 USD/1000 fry for medium and small fry

Similarly, equation (4), (5) and (6) were developed for estimating, respectively, the optimum BM, DM and HM levels that minimise the ECR considering the variation of the MM prices (model 2).

(4) $^{ECR}BM_{level} = 108.333 – 81.164 * BM_{price} + 21.400 * BM_{price}^2$

(5) $^{ECR}DM_{level} = 113.266 – 77.569 * DM_{price} + 16.725 * DM_{price}^2$

(6) $^{ECR}HM_{level} = 102.161 – 72.822 * HM_{price} + 18.990 * HM_{price}^2$

As the price of the MM increased, optimum MM dietary inclusion levels that minimise ECR ($^{ECR}MM_{level}$) decreased; the trends were comparable for the 3 types of MM considered (Figure 4.4A). Inclusions of BM and HM that minimise ECR were similar with values comprised between 38.9-62.1% and 39.5-60.6 %, respectively when MM cost 0.8-1.3 USD/kg whereas $^{ECR}DM_{level}$ were slightly higher with values ranging from 67.2 to 40.7 % as DM price increased (0.8 to 1.3 USD/kg). Although ECR_{MM} increased with the price of the MM, up to 1.3 USD/kg, at the optimum MM levels, ECR_{MM} were lower than ECR_{F100} with values comprised between 0.6-0.7 USD/kg at 0.8USD/kg and 0.8-0.9 USD/kg at 1.3 USD/kg (Figure 4.4B).

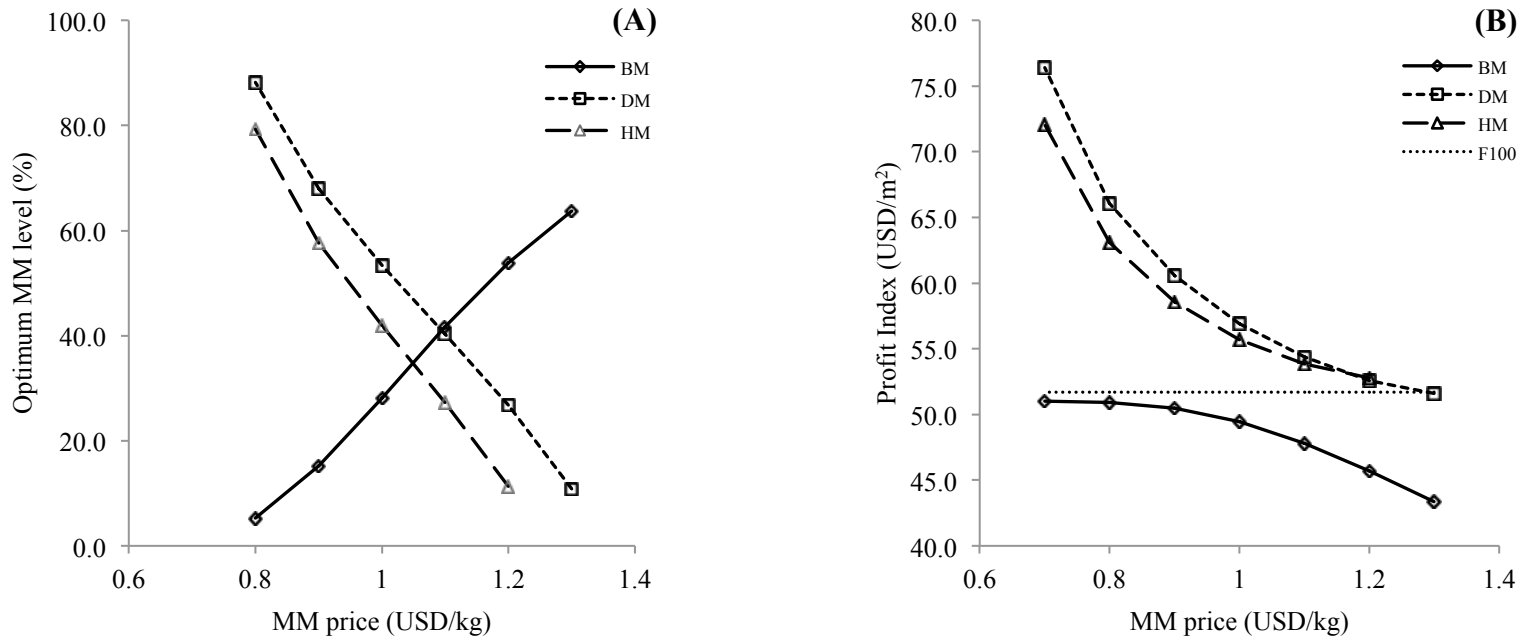

Figure 4.3 Simulation of (A) optimum inclusion levels of maggot meal (MM) maximising the profit index (PI) and (B) PI for these optimal MM levels respectively, according to the MM price variation (USD/kg). MM considered were crude BSF meal (BM), defatted BSF meal (DM) and crude housefly meal (HM). F100 reference on graph (B) being the control diet made of 100 % MT-impregnated FM

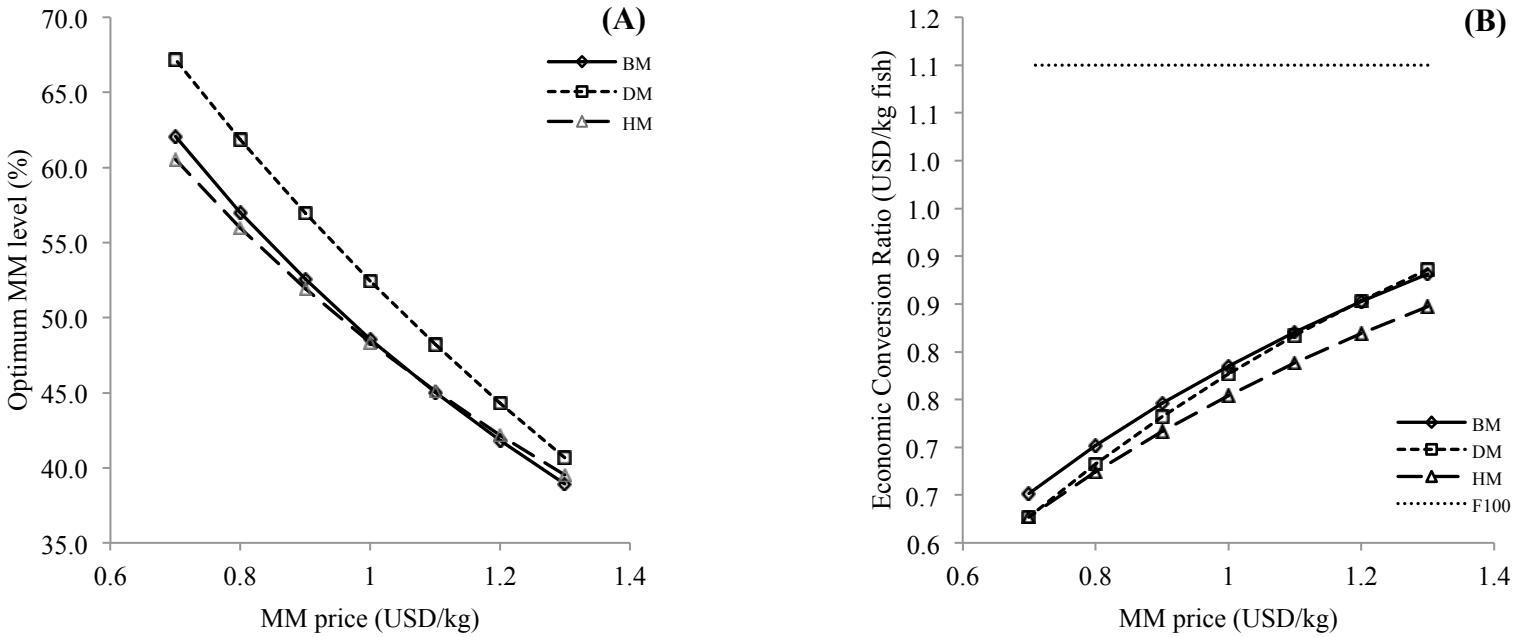

Figure 4.4 Simulation of (A) optimum maggot meal (MM) inclusion levels minimising the economic conversion ratio (ECR) and (B) ECR for these optimal MM levels respectively, according to the MM price variation (USD/kg). MM considered were crude BSF meal (BM), defatted BSF meal (DM) and crude housefly meal (HM). F100 reference on graph (B) being the control diet made of 100 % MT-impregnated FM

4.4 Discussion

Successful sex-reversion of tilapia fry relies on good farming and feeding practices. Quality, floatability and palatability of the MT-treated feed are key drivers ensuring intake of daily dose of hormone and although pure FM treated with MT is preferred to produce monosex Nile tilapia in Thailand (Bhujel, 1997), commercial feeds formulated for juveniles fish (crude protein >40%) are also widely used (Phelps, 2006). The type of feed chosen depends on its availability, cost and consistency. In the current study, although the quality of the FM used was not the highest compared to anchovy, herring or menhaden FM, it was considered to be relatively good value for money in Thai market (NRC, 2011; Bhujel, 2013). However, just like everywhere else, FM is becoming a scarce resource, resulting in increasing prices that undoubtedly stimulate adulteration, affecting dramatically the efficiency of the treatment and the hatchery reputation which is critical in such a competitive market (W. Turner, pers. communication 2016); thus, the importance to identify cost-efficient alternatives.

High lipid content of the BM led to lumpy diets, in particular for B100 and B75, that sank quickly whereas other MM-based diets floatability was comparable to F100; floatability of the diets containing BM was improved with increasing levels of FM. Nevertheless, the results indicated that, although few females were identified in groups of fry fed the control diet (F100), B75 and H50, the sex-reversal process was very efficient across treatments with high proportions of male reported (99.8 to 100 %). This suggested that all the fish received sufficient hormone dose during the 21-day process and that the quality of BM (sinking feed) did not compromise the feeding. Perhaps, the latter was highly palatable, encouraging the fry to feed before it sank. Although limited work was conducted to compare the effects of different feed mixtures on the efficiency of tilapia sex-reversal process (Abucay and Mair, 1997; Bhujel, 2013), the present results were consistent with previous research which showed that oral administration of androgen was efficient (high percentage of males reported) providing the use of high-quality and well-prepared feed, including single feed ingredients (FM in most cases), commercial diets or simple farm-made mixes (Mair and Little, 1991; Phelps, 2006). Sex-reversal is considered ineffective when less than 96 % males are produced (Mair and Little, 1991). According to Vera Cruz and Mair (1994), to avoid unwanted and significant impacts on grow-out fish crop in ponds, sex-reversed populations counting

more than 98 % male are recommended. Indeed, even a small proportion of females can lead to substantial recruitment and to heterogeneous growth resulting in non-uniformly sized marketable fish. Commercial hatcheries, such as Nam Sai Farms, that sell mainly fry for pond culture on a competitive market, aim at producing more than 99 % males and below 99.5 %, seed is often considered as a low quality (Bhujel, 2014; Turner, 2015). Purchase of fry is a major cost for farmers and investment in monosex seed, which is more costly than mixed-sex fry, contributes to better performance during grow-out, thus the importance of a high quality and in particular, a high percentage of males (Little, 1989).

Survival is also an important parameter to consider, firstly for economic reasons (number of fish stocked / bought vs. number of fish harvested) but also because during the high-density sex-reversal process, it is a factor that influences indirectly the success of the process. High survival is particularly required to maintain the high density of fry which first, creates a crowding effect ensuring an active feeding response (Phelps and Popma, 2000); secondly, high density may reduce the hierarchical interactions between the fish, thereby resulting in a more uniform population (size) and therefore a more uniform hormone intake by all the fry (Little (1991) in Vera Cruz and Mair (1994). In the present study, survival rates were good and not significantly different across treatments (77.3±4.6 % in average); rates were also comparable to those reported by Vera Cruz and Mair (1994) when fry was stocked at 6,000 fish/m^3 (76.1 %). This result suggested that a high density of fry was similarly maintained across treatments; however, the grading results indicated significant differences in the distribution of the fry sizes in the sex-reversed populations depending on the dietary treatment. In fact, fry fed diets containing 25 % BM or 25 to 75 % DM or HM led to more uniform populations (Simpson dominance index = 0.6) than the control (0.5). Greater evenness of these groups was related to the significantly greater abundance of medium size fry (70.7-76.6 % of the total biomass) and lower abundance of small size fry (8.0-21.0 %) compared to the control (F100). Non-significance of B50 evenness index, a dietary treatment that also led to significantly more medium and less small size fry than F100, was attributed to a lower difference between the relative abundances of large and medium fry (23.5 and 67.8 %, respectively) compared to B25, D25-50-75 and H25-50-75. Dietary treatments made of MM only (B100, D100 and H100) led to sex-reversed population distributed similarly to F100 with mostly medium and small fish. Contrary

to Vera Cruz and Mair (1994), the significantly greater abundance of large fish in the groups of fry fed B25-50-75 and H50-75 compared to F100, did not lead to the establishment of size hierarchies as this would have resulted in higher mortalities associated with the aggressiveness and cannibalistic behaviour from the larger fish (Dambo and Rana, 1992). In addition, the fact that the treatment was carried out in green water, reduced greatly the risks of cannibalism which are worst in clearwater systems (D. Little, pers communication 2016). Furthermore, although the fry populations were not even in size, the success of the sex-reversal process was not compromised, indicating that all the fry had similar access to the MT-treated feed, regardless the treatment.

Among the figures used for the economic analyses, biomass and number of fish at harvest mirrored the size distributions and survival rates in each treatment, respectively. Indeed, accordingly to the survival rates, number of fish at harvest was not significantly different across treatments. Diets that contained both FM and MM inclusions resulted in greater biomass than single feed ingredient diets and significantly greater than F100, excepting for D75. This result was logically explained by the significantly lower abundance of small size fish in the groups of fry fed mixed-ingredient diets compared to the fry fed F100. Non-significantly different biomass of D75, a treatment which also lead to significantly less small size fish than F100, was attributable to its lower abundance of large size fish (4.3 %) compared to the other mixed ingredients treatments (9.0 to 23.5%). It has been mentioned that mean individual weights of fry at the end of the sex-reversal process, which usually ranges between 100 and 500 mg, was influenced by the water temperature and the feed quality (Popma and Green, 1990; Popma and Lovshin, 1995); given the design here, the difference in growth was more likely explained by the differences in the nutritional composition of the diets. Similarly, FCR, which was also calculated from the weight gained during the 21-day process, was strongly influenced by the biomass at harvest since the quantity of feed used in the experiment did not vary among treatments. With values significantly lower than F100, FCR calculated for the diets containing 25 to 75 % MM indicated a better efficiency of the mixed ingredients treatments. Finally, it is common knowledge that the combination of feed ingredients in fish diets leads to better performance than any single source due to an improved nutrient balance provided by the contribution of both ingredients (NRC,

2011; Parker, 2011). Hence, it was not surprising to observed improved fish performance while feeding with diets mixing insect meal and FM.

Tilapia fry of approximately 250 mg (1.0 inch) are the standard and most desired marketed size because of a lower farm-gate price and the ease of 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 stunt the fish growth, thereby leading to evenly small/medium size fish that can be, if necessary, further grown to marketable size during additional nursing phases using a cheaper feed (Little and Hulata, 2000). For the reasons stated above (dominance, aggressiveness, cannibalism) individual large fry are a disadvantage at harvest (Bhujel, 2014; Turner, 2016), apart from requiring more MT-treated feed (large fish consume more feed), which is an expensive input given the price of the hormone (2.8 USD/g) and the high quality of the feed.

Economic evaluation is essential to assess the cost-effectiveness of an alternative feedstuff and to determine the optimum substitution level that improves the profitability. Other studies looking at alternatives to FM for Nile tilapia focused mainly on fingerlings stages and in most cases, the alternative ingredients considered were locally available, less expensive sources of protein (El-Sayed, 1999; Ogello *et al.*, 2014). Thus, when economic evaluations were performed, the costs of the alternative ingredient-based feeds were lower than control diets (FM-based diets in general); this compensated for the lower fish performance measured, resulting in better economic performance (El-Sayed, 1999). Although replacing FM with MM in the diet for sex-reversal tilapia did not compromise the success of the process and even improved the fish performance in some cases, farmers would be more willing to use it if there was an economic incentive. MM current market price might be very high due to the limited production (volumes) and to the main buyer being the pet food industry, who is willing to pay high prices for high-quality products. Therefore, it is not considered as representative of the situation where it would be allowed and integrated into fish and livestock feeds; indeed, in this case, MM price would have to be competitive with the price of FM and its current alternatives (Drew and Pieterse, 2015). Thus, in the present study, economic analyses considered MM prices ranging from 1.3 to 0.7 USD/kg, which corresponded to 30 % more or less the market price of the FM used in the

experiment (i.e. 1.0 USD/kg); moreover, it can be expected that the price of the defatted MM might be superior to crude meals given the additional processing steps related to the fat removal.

Obviously, at a price of 1.0 USD/kg or less, total or partial MM inclusion resulted in diets prices equal or lower to the price of F100; reciprocally when MM cost more than 1.0 USD/kg, diets including MM were more expensive than F100. However, the diet price was not the only factor influencing the results of the economic analyses which accounted also for the performance results of each dietary treatment. In particular, the number of fry produced per class size was used to calculate the value of the production for each treatment, which was not significantly different than F100 despite the differences in the size distributions, and the FCR was turned into an economic index (ECR) by integrating the price of the diets. Since the previously discussed results clearly showed that MM inclusions did not impair the success of the sex-reversal process, the present economic models were used to assess the optimum inclusions of MM that either maximised the PI (model 1) or minimised the ECR (model 2) considering the variation of the MM prices. Similarly to that previously reported by Martínez-Llorens *et al.* (2012), optimum MM inclusion levels differ depending on the economic index considered and varied substantially with the price of the MM. Indeed, optimal inclusion levels were more sensible to the price changes when the maximisation of the PI was sought (values varying between 0 and 100 % inclusion depending on the MM) than for the minimisation of the ECR (values ranging between 38.9 and 67.2% inclusion depending on the MM).

According to the first model, at prices ranging from 0.7 to 1.3 USD/kg, the maximum PI obtained for the optimal dietary inclusion of BM were always below PI_{F100} suggesting that BM-based diets were less profitable than the control diet, F100. Surprisingly, the model also indicated that, as the price of BM increased, greater inclusions would maximise the PI although the latter decreased with increasing BM price; the positive relationship between the value of the production and the BM inclusion level explained this result. The two other MM presented trends opposite to BM; with negative relationships between the optimum inclusion levels and the prices of the MM. According to this model, at prices below 0.8 USD/kg, a total substitution of FM using DM and HM would result in maximum PI (76.4 and 72.1 USD/m^2,

respectively when MM cost 0.7 USD/kg), whereas above 1.2 USD/kg only an inclusion of HM would still be slightly more profitable than F100 (52.7 USD/m^2, when MM cost 1.3 USD/kg). The results indicated also that DM was more competitive than HM since, at the same price, optimum DM dietary inclusions were higher than HM and led to greater PI except at prices higher than 1.2 USD/kg. Thus, if hatcheries are looking at maximising their profits, the use of BM would not be recommended even at prices lower than the FM; on the contrary, up to 1.1 USD/kg inclusion of DM or HM would certainly benefit farmers. Previous studies looking at insect meals (termite and housefly larvae) as FM substitutes in the diets of catfish have also reported higher PI with insect-based diets than with FM-based control diets (Sogbesan and Ugwumba, 2008b; Michael and Sogbesan, 2015); however, this was certainly related to the lower price of the insect meals compared to FM. In the present study, at prices up to 20% more than the price of the FM, dietary inclusions of DM or HM were still considered more profitable than the 100 % FM-based diet because mixed-ingredient diets were found to result in higher performance than single ingredients.

When the ECR was considered (model 2), optimum inclusion levels of MM showed similar decreasing trends as the price of the MM increased and corresponding ECR values increased with the price of the MM. In this case, the model suggested that at the same price, optimum dietary inclusions of BM that minimise ECR were comparable to HM, however, ECR values obtained for HM were always slightly better (lower) than for BM. On the other hand, optimum dietary inclusions of DM that minimise ECR were greater than for the two other MM, and corresponding ECR value were intermediate between ECR_{BM} and ECR_{HM}. In all cases, ECR values obtained for the MM were substantially lower than ECR_{F100}. Thus, according to this model, at prices between 0.7 and 1.3 USD/kg, dietary inclusions levels of approximately 39 to 62 % BM or HM and 40 to 67 % DM would always lead to better ECR than F100. Moreover, consistent with the model 1, using DM, if available, would allow the substitution of greater levels of FM compared to BM or HM, producing improved economic returns (better PI and ECR than F100).

The higher efficiency of the defatted MM over the two other crude meals (BM and HM) was already suggested by (Fasakin *et al.*, 2003) and in Chapter 3. In the present study, in addition to the economic results favouring DM over HM and BM, other performance

parameters also suggested the higher potential of this MM with levels of 100 % males and high survival (75.7 to 82.9%) obtained in all DM-based treatments. Furthermore, fry produced with DM-based diets were very homogenous in size (significantly more medium size fry and less small size fry for D25-50-75 compared to F100) and D100 led to a size distribution very similar to F100 (with relative abundances of large, medium and small being 2.2; 63.5 and 34.3 % respectively for D100 and 6.7; 57.0 and 36.4 % respectively for F100). On the contrary, although using BM-based diets was not detrimental to the efficiency of the sex-reversal process, the economic analysis highlighted some limitations, in particular, to improve the profit of commercial hatcheries (model 1). At last, HM-based diets also led to efficient sex-reversal process but were slightly less attractive economically than DM-based diets. HM has already shown great potential as a feed ingredient for tilapia or catfish (Ajani *et al*., 2004; Sogbesan *et al*., 2006; Ogunji *et al*., 2008a, 2008b, 2008c; Aniebo *et al*., 2009; Omoyinmi and Olaoye, 2012) and ubiquity of the common housefly could be an advantage for sourcing HM. Equations (1) to (6) can be good tools for hatcheries to determine the optimal MM inclusion levels that would either maximise the PI or minimise the ECR according to the price of the meal considered.

Although the hatchery technology used at Nam Sai Farms, and in several commercial hatcheries globally, is economically viable, the use of FM is questionable in terms of sustainability and the evolution of the prices might sooner or later affect the profitability of the system. Research for alternatives to FM for sex-reversed tilapia fry in Thailand have already identified the potential of insects with the particular case of silkworm pupae; however the availability of the product was limited and did not match the demand (Bhujel, 2013). In the recent years, the development of the insect industry toward the industrialisation of the farming process (various commercial pilots and projects emerging worldwide) restored the possibilities of use for aquaculture as the quantity, quality and consistency would not be limiting factors anymore. Economies of scale should allow the industrialisation of insect farming and development of markets with very competitively priced products, especially if the price of FM continues to climb. Hatcheries could, therefore, buy insect meal from a local producer or invest in the development of their own production system (on-farm) to reduce their costs.

Chapter 5. Partial replacement of fish meal with Black Soldier Fly (*Hermetia illucens*) larvae meal in commercial diets for advanced nursing of Nile tilapia (*Oreochromis niloticus*)

5.1 Introduction

Farmed fish contribute to food security and represent a rich source of dietary animal protein, micronutrients and FA in LIDC; however, per capita consumption levels of fish can vary greatly even within the same country and this is often linked to the availability (Beveridge *et al*., 2013). In Ghana, for instance, most aquaculture production (around 80 %) consisted of Nile tilapia (*Oreochromis niloticus*) (FAO, 2005-2016), but local fish farmers struggle to compete with cheaper imports from China; they are also constrained by both availability, quality and cost of pelleted fish feeds and feed ingredients (Hecht, 2007; Rurangwa *et al*., 2015). Conventional feed ingredients such as FM and FO and their alternatives (oilseed cakes, soybean meal, poultry by-products etc.) are available in LIDCs such as Ghana but consist either of poor quality local products or high-cost imported ingredients (Gabriel *et al*., 2007; Obirikorang *et al*., 2015). Moreover, the intensification of the farming methods, relying on complete fish feeds, result in an increasing demand for feed and feedstuffs (Tacon and Metian, 2008).

The importance of quality feeds and feed ingredients, even for omnivorous species such as tilapia, makes perfect sense at critical stages (juveniles or broodstock) when fish are maintained under intensive clear-water farming conditions and depend entirely on nutritionally complete diets (Tacon, 1988). Global research for the identification of cost-effective substitutes to conventional feedstuffs continues (El-Sayed and Tacon, 1997; El-Sayed, 2004; Hasan *et al*., 2007; Karalazos, 2007; Ayoola, 2010; Obirikorang *et al*., 2015) and interest is rising towards unconventional feedstuffs such as insects (van Huis *et al*., 2013). Fly larvae or maggots (Insecta, Diptera) have been identified as a high protein and valuable feed ingredient for livestock in general (Veldkamp *et al*., 2012; van Huis *et al*., 2013; Makkar *et al*., 2014) and fish specifically, given their natural feeding habits (Bailey and Harrison, 1948; Randall, 1967; Odesanya *et al*., 2011; Barroso *et al*., 2014; Henry *et al*., 2015). The nutritional profile of dipteran larvae is highly similar to FM except for the FA composition, that is often low in the omega-3 (n-3) long-chain polyunsaturated fatty acids (PUFA), eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids which are typically associated with marine ingredients (Barroso *et al*., 2014).

The Black Soldier Fly (BSF, *Hermetia illucens*) is a non-pest species commonly found in tropical or sub-tropical areas, that can be mass-produced using various sources of

organic material as a food substrate (Maurice, 1960; Leclercq, 1997). Manures, market wastes and brewery wastes, for instance, are available resources in urban and peri-urban areas and their disposal via insect conversion to animal feed has been identified as a potential strategy (Žáková and Borkovcová, 2013; Nguyen *et al*., 2015). This could be an important part of efforts to enhance food security whilst contributing to the development of a circular economy (Veldkamp *et al*., 2012; Marchant, 2014).

Previous research on tilapia juveniles has shown that both meal from housefly larvae (*M. domestica*) and blowfly larvae (*Chrysomya megacephala*) can replace up to 100% of the FM in practical diets for tilapia fingerlings without affecting fish performance compared to FM-based control diets (Ogunji *et al*., 2008a, 2008b, 2008c; Sing *et al*., 2014). On the other hand, fresh BSF larvae (*H. illucens*) fed whole or chopped to blue tilapia (*Oreochromis aureus*) significantly reduced the fish growth (Bondari and Sheppard, 1987). BSF larvae meal has been used as a substitute to FM in several fish species diets except tilapia (Makkar *et al*., 2014; Henry *et al*., 2015).

This study investigated the effects on the performance, feed utilisation efficiency and body composition of Nile tilapia (*O. niloticus*) fingerlings fed commercially formulated diets containing BSF MM as a substitution for fish meal and fish oil.

5.2 Materials and Methods

5.2.1 Experimental diets

BSF larvae (*H. illucens*) meal (BM) was produced within a pilot system located in Greater Accra (Ghana) described by Charlton *et al.,* (2015). Larvae were fed on a substrate mix composed of 35% spent grain (brewery solid waste) or wheat bran (depending on availability), 22% processing wastes from a local fish feed factory, 12% yeast slurry (brewery waste water) and 31% water (bringing the moisture content to approximately 60%) and were harvested after 13 days of development (prior to the prepupae stage). Oven-dried larvae (60-80ºC, 2 hours) were subsequently ground into a fine and homogeneous meal (particle size between 600-800 μm) using an artisanal flour mill machine. Nutritional composition of the MM was analysed (Table 5.1) in order to assist in diet formulation.

Table 5.1 Proximate composition (g/kg), gross energy (MJ/kg), essential amino acid composition (g/100 g of BM) and fatty acid composition (g/100 g of BM) of the BSF larvae meal (BM)

	Black soldier fly larvae meal
Proximate composition (g/kg)	
Dry matter	950.3
Crude protein	416.4
Crude lipid	232.4
Crude fibre	76.6
Ash	116.5
Gross Energy (MJ kg^{-1})	21.7
Essential amino acids (g/100 g BM)	
Histidine	1.18
Arginine	2.00
Threonine	1.72
Valine	2.63
Methionine	0.75
Lysine	2.70
Iso-Leucine	1.84
Leucine	2.90
Phenylalanine	1.75
Fatty acid composition (g/100 g BM)	
14:0	1.02
16:0	3.33
18:0	0.47
20:0	0.01
Total saturated[1]	***4.92***
16:1n-7	0.60
18:1n-9	2.67
18:1n-7	0.55
22:1n-9	0.03
Total monounsaturated[2]	***4.08***
18:2n-6	1.86
18:3n-6	0.03
20:4n-6	0.02
Total n-6	***1.92***
18:3n-3	0.17
18:4n-3	0.19
20:5n-3 (EPA)	0.09
22:6n-3 (DHA)	0.01
Total n-3	***0.46***
Total Polyunsaturated[3]	***2.38***
Total fatty acids	***11.39***

Values are presented 'as is', based on duplicate analyses
[1]Includes 15:0 and 22:0 ; [2]Includes 16:1n-9; 17:1 and 20:1n-11 ; 3Includes 16:2; 16:3 and 16:4

Diets were formulated and prepared by Raanan Fish Feed West Africa (Prampram Fishfeed Factory, Ghana). Raanan PG40 commercial diet, formulated with a 100 g/kg FM inclusion, was used as control for the experiment (FM100). In the three test diets, 25, 50 and 75 % of the FM inclusion in FM100 were replaced (w/w) with BM (BM25, BM50 and BM75, respectively). Test diets were formulated to be isonitrogenous and isoenergetic with 380 g/kg crude protein, 90 g/kg total lipid and 19 MJ/kg gross energy, by adjusting other ingredient dietary levels (Table 5.2); in particular, FO was not included the three BM-based diets due to the high lipid content of the crude BM (244.5 g/kg). Commercially packaged diets were kept on-farm under cool and shaded conditions (25°C, 50-60% relative humidity) and used within two months following manufacture. Proximate, amino acid and fatty acid compositions of the control and experimental diets were analysed as described below (Table 5.2).

Table 5.2 Ingredient composition (g/kg), proximate composition (g/kg) and gross energy (MJ/kg), essential amino acid and fatty acid compositions (g/100 g of diet) of the commercial control diet (FM100) and the three test diets (BM25; BM50 and BM75)

	Dietary treatments			
	FM100	**BM25**	**BM50**	**BM75**
Ingredient composition (g/kg)				
Fish meal	100.0	80.0	50.0	30.0
Soybean meal	200.0	180.0	160.0	130.0
BSF meal	-	30.0	50.0	80.0
Poultry byproduct meal	50.0	80.0	100.0	130.0
Fish oil	20.0	-	-	-
Corn meal	304.0	304.0	304.0	304.0
Wheat bran	130.0	130.0	140.0	130.0
Poultry blood meal	100.0	100.0	100.0	100.0
Feather meal	90.0	90.0	90.0	90.0
Vitamin premix	3.0	3.0	3.0	3.0
Anti-mold	1.5	1.5	1.5	1.5
Klinofeed ®	1.0	1.0	1.0	1.0
Methionine	0.5	0.5	0.5	0.5
Proximate composition (g/kg)				
Dry matter	949.4	957.5	952.5	958.3
Crude protein	372.8	378.4	371.7	376.7
Crude lipid	94.8	78.3	77.6	93.4
Crude fibre	30.5	33.1	35.0	34.4
Ash	62.9	67.6	68.1	66.8
NFE	388.4	400.1	400.1	387.0
Gross Energy (MJ/kg)	19.6	19.2	19.4	19.7
Essential amino acid composition (g/100 g diet)				
Histidine	2.25	2.37	2.34	2.41
Arginine	1.36	1.37	1.31	1.33
Threonine	3.41	3.43	3.44	3.44
Valine	1.93	1.96	1.96	1.90
Methionine	1.12	1.17	1.14	1.15

	FM100	BM25	BM50	BM75
Lysine	2.18	2.18	2.15	2.18
Iso-Leucine	2.17	2.18	2.24	2.22
Leucine	0.66	0.58	0.51	0.59
Phenylalanine	1.62	1.57	1.63	1.66
Fatty acid composition (g/100 g diet)				
14:0	0.10	0.10	0.13	0.16
16:0	1.44	0.97	1.03	1.07
18:0	0.36	0.32	0.35	0.36
20:0	0.03	0.02	0.02	0.02
***Total saturated*[1]**	***1.97***	***1.44***	***1.56***	***1.65***
16:1n-7	0.14	0.12	0.13	0.14
18:1n-9	2.41	1.69	1.71	1.69
18:1n-7	0.15	0.13	0.13	0.14
22:1n-11	0.07	0.04	0.03	0.03
***Total monounsaturated*[2]**	***2.92***	***2.09***	***2.09***	***2.09***
18:2n-6	1.51	1.29	1.33	1.25
20:2n-6	0.03	0.02	0.02	0.01
20:4n-6	0.02	0.02	0.01	0.01
***Total n-6*[3]**	***1.58***	***1.34***	***1.37***	***1.29***
18:3n-3	0.17	0.13	0.12	0.11
18:4n-3	0.02	0.02	0.01	0.01
20:5n-3 (EPA)	0.08	0.05	0.04	0.04
22:6n-3 (DHA)	0.18	0.12	0.08	0.07
***Total n-3*[4]**	***0.51***	***0.35***	***0.27***	***0.25***
***Total Polyunsaturated*[5]**	***2.11***	***1.71***	***1.66***	***1.55***
Total fatty acids	***7.00***	***5.25***	***5.31***	***5.29***
n-3/n-6	0.32	0.26	0.20	0.19

Values are presented 'as is', based on duplicate analyses. Abbreviations: FM100 – Control diet (Raanan PG40 commercial diet); BM25 – diet where 25 % fish meal (FM) was replaced by Black Soldier Fly larvae meal (BM); BM50 – diet where 50 % FM was replaced by BM; BM75 – diet where 75 % FM was replaced by BM.
[1]Includes 15:0; 22:0 and 24:0 ; [2]Includes 16:1n-9; 20:1n-11; 20:1n-7; 22:1n-9 and 24:1n-9 ; [3]Includes 18:3n-6; 20:3n-6 and 22:4n-6; [4]Includes 20:3n-3; 20:4n-3 and 22:5n-3 ; [5]Includes 16:2 and 16:3

5.2.2 Experimental design and set up

In order to demonstrate the relevance of the results, the experiment was conducted on-farm (commercial tilapia producer, Volta Lake, Ghana) under conditions similar to commercial husbandry practices. All-male, hormonally sex-reversed Nile tilapia fingerlings (*O. niloticus*) were obtained from a local commercial hatchery following advanced nursing procedure as described for hapas- in- ponds (Little *et al.*, 2003). Prior to the start of the experiment, twenty-five thousand (25,000) fish were transferred into a single floating cage (3x3 m) suspended in Volta Lake where they were fed six times a day with a standard diet (480 g/kg crude protein and 50 g/kg total lipid) for 3 weeks as an acclimation period to the lake conditions. Twelve floating cages (1.0 m^3 each), set up in the outermost part of the grow-out and nursery site of the farm (500 m from the shore, water column of 30-35 m depth), were stocked at random with one thousand five hundred (1,500) acclimated fingerlings (5.7±0.5 g; mean ± SE) each. The experiment was conducted between the months of September and October 2014, for 32 days which was equivalent to the commercial advanced nursing period and allowed a body increase of at least 300% recommended for juvenile fish studies (NRC, 2011). Control and test diets were distributed daily by hand to triplicates cages; fish were fed to visual satiety, over 6 feeding sessions per day (at regular intervals of 2 hours) and amount of feed distributed was determined by difference with pre-weighed feed containers prepared daily. Water temperature (°C), pH and dissolved oxygen (DO; mg/L) were recorded daily at 07:00 hrs and 16:00 hrs using OxyGuard® Handy digital probes (Polaris and pH) immersed at 50 cm under the water surface within cages.

At the start and on termination of the experiment, all the fish in each cage were counted and bulk weighed (Tanita KD 200 digital scale, precision: 0-1000gx1g). Growth was monitored through intermediate samplings carried out every 10 days, by counting fish and recording bulk weights of 3 separate sub-samples from each cage (representing approximately 20% of the population), using a scoop net of fish concentrated in the corner of the cage. Fish were starved for 24 hours prior to the samplings in order to limit stress and mortalities related to handling. Whole fish samples were collected at the start (n=20 fish from the initial population) and on termination (n=5 fish per cage) of the experiment, following an overdose of metacaine sulfonate (MS-222) anaesthetic. While initial fish were pooled as four separate samples (n=5 fish/pool), final whole fish

samples were pooled on a cage basis; pooled samples were subsequently homogenised and stored under freezing conditions (-20ºC) until further analyses.

5.2.3 Biochemical analyses

BSF meal, experimental diets and whole fish samples were analysed using standard methods described in Chapter 2 to determine dry matter, crude protein, crude lipid, ash, crude fibre, gross energy and FA composition. Amino acid compositions of the feed ingredients and diets were determined by HPLC (subcontracted by ALS Food & Pharmaceutical in UK and Eurofins Food and Feed Testing in Norway).

5.2.4 Fish performance and feed utilisation

Fish performance and feed utilisation were assessed by determination of the weight gain (g), Specific Growth Rate (SGR, %body weight/day), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), daily feeding rate (% biomass/day) and survival (%) as described in Chapter 2.

5.2.5 Statistical analyses

Statistical analyses were carried out using IBM SPSS Statistics software (version 21). Data were subjected to one-way ANOVA followed by Tukey's HSD test for unplanned multiple comparisons. Correlations between the dietary inclusions of MM and the performance or nutritional results were analysed using Pearson's coefficient. A significance of $P<0.05$ was considered for all analyses performed. Values are presented as mean ± SE unless otherwise stated.

5.3 Results

5.3.1 Growth performance and feed utilisation

Water temperature and dissolved oxygen varied slightly during the course of the experiment and the diurnal periods with values ranging from 26.8 to 30.5°C and 5.1 to 8.1 g/L, respectively.

Growth and feed utilisation of the fish fed the control and experimental diets were not affected by treatments (Table 5.3). During the 32-day experimental period, fish grew from an average initial weight of 5.7±0.1 g to 16.6±0.1 g. Live weight gain and SGR of

the fish fed the control and the MM-based diets were not significantly different across treatments ($P>0.05$).

Overall feeding response was good with total amounts of feed distributed (26.0±0.2 kg per cage) and feeding rates (4.2±0.1 % biomass/day) not significantly different between treatments ($P>0.05$). This indicated similar feed intakes for the fish fed the control and experimental diets. Feed utilisation efficiency (FCR and PER) was not compromised by the dietary treatments ($P>0.05$). However, BM25 treatment indicated a significantly lower survival (81.7±1.1 %) compared to others ($P<0.05$) and BM75 survival rate (90.1±0.3 %) was found significantly higher than FM100 (86.1±0.2 %).

5.3.2 Whole fish body composition

Analysed fish body compositions compared between treatments indicated no significant differences ($P>0.05$) for dry matter, crude protein, crude lipid, ash and crude fibre (Table 5.4). However, whole body FA composition varied significantly between dietary treatments. Strong linear relationships were found between the dietary inclusion of BM and selected FA; in particular, BM dietary inclusion was positively correlated to the total saturated FA (r 0.672; $P<0.05$) and a negatively to the n-3 PUFA (r -0.725; $P<0.05$).

Table 5.3 Growth performance and feed utilisation indices determined for nursing tilapia fingerlings fed control and experimental diets for 32 days

	Dietary treatments			
	FM100	**BM25**	**BM50**	**BM75**
Initial live weight (g)	5.5±0.1	5.1±0.1	6.1±0.4	6.1±0.2
Final live weight (g)	16.0±0.4	16.9±1.0	17.0±0.6	16.5±0.5
Live weight gain (g)	10.4±0.5	11.8±1.1	10.9±0.9	10.4±0.4
SGR (% bw/day)	3.3±0.1	3.7±0.2	3.2±0.3	3.1±0.0
Total feed distributed (kg)	25.9±0.5	25.7±0.2	26.2±0.1	26.4±0.3
FCR	2.2±0.1	2.1±0.2	2.0±0.1	2.1±0.0
PER	1.2±0.0	1.2±0.1	1.3±0.1	1.2±0.0
Feeding rate (% biomass/day)	4.4±0.0	4.3±0.2	4.0±0.1	4.1±0.1
Survival rate (%)	86.1±0.2^{b}	81.7±1.1^{c}	89.5±1.3 ^{ab}	90.1±0.3^{a}

Means ± SE (n=3) bearing different superscripts within each row are significantly different ($P<0.05$)
Abbreviations: FM100 – Control diet (Raanan PG40 commercial diet); BM25 – diet where 25 % fish meal (FM) was replaced by Black Soldier Fly larvae meal (BM); BM50 – diet where 50 % FM was replaced by BM; BM75 – diet where 75 % FM was replaced by BM.

Table 5.4 Proximate composition (g/kg of fish, wet weight basis) and fatty acid composition (g/100 g of fish) of Nile tilapia fingerlings whole body at the start (Initial; mean ± SD; n=4) and on termination of the 32-day experimental period (n=3)

	Initial	Dietary treatments				Pooled SEM	P-value
		FM100	BM25	BM50	BM75		
Proximate composition (g/kg)							
Dry matter	238.1±3.4	286.0	278.5	282.0	285.2	1.701	0.071
Crude protein	148.8±1.5	153.6	152.7	152.9	154.3	0.369	0.653
Crude lipid	37.0±1.4	107.8	96.1	99.9	102.2	2.452	0.095
Ash	48.8±0.9	33.1	34.5	33.9	35.7	1.099	0.276
Crude fibre	0.7±0.2	0.8	0.8	0.8	0.8	0.016	0.975
Fatty acid composition (g/100 g fish)							
14:0	0.05±0.00	0.16^{c}	0.17^{c}	0.23^{b}	0.29^{a}	0.031	0.000
16:0	0.05±0.01	1.59^{ab}	1.46^{b}	1.67^{ab}	1.80^{a}	0.072	0.053
18:0	0.18±0.07	0.48	0.47	0.50	0.52	0.012	0.359
20:0	0.01±0.00	0.02	0.02	0.02	0.02	0.000	0.435
Total saturated[1]	***0.76±0.03***	***2.27^{ab}***	***2.13^{b}***	***2.44^{ab}***	***2.66^{a}***	***0.113***	***0.035***
16:1n-7	0.09±0.00	0.27^{b}	0.26^{b}	0.29^{ab}	0.33^{a}	0.016	0.014
18:1n-9	0.70±0.03	2.54	2.21	2.47	2.57	0.082	0.165
18:1n-7	0.08±0.00	0.20^{ab}	0.20^{b}	0.23^{ab}	0.25^{a}	0.013	0.035
22:1n-11	0.01±0.00	0.04^{a}	0.02^{b}	0.02^{b}	0.02^{b}	0.004	0.000
Total monounsat.[2]	***0.97±0.05***	***3.30***	***2.89***	***3.26***	***3.43***	***0.114***	***0.127***
18:2n-6	0.25±0.02	0.82	0.76	0.82	0.91	0.030	0.125
20:2n-6	0.02±0.00	0.06	0.06	0.06	0.07	0.002	0.208
20:4n-6	0.04±0.00	0.06^{b}	0.06^{b}	0.07^{ab}	0.08^{a}	0.004	0.033
Total n-6[3]	***0.37±0.03***	***1.09***	***1.02***	***1.11***	***1.23***	***0.044***	***0.110***
18:3n-3	0.02±0.00	0.07	0.06	0.06	0.07	0.003	0.071
20:4n-3	0.00±0.00	0.01^{a}	0.01^{b}	0.01^{b}	0.01^{b}	0.001	0.000
20:5n-3 (EPA)	0.01±0.00	0.01^{a}	0.01^{b}	0.01^{b}	0.01^{b}	0.001	0.002
22:5n-3	0.02±0.00	0.05^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.004	0.001
22:6n-3 (DHA)	0.09±0.00	0.19^{a}	0.14^{b}	0.12^{b}	0.12^{b}	0.015	0.002
Total n-3[4]	***0.14±0.01***	***0.36^{a}***	***0.27^{b}***	***0.24^{b}***	***0.26^{b}***	***0.026***	***0.002***
Total polyunsat.[5]	***0.53±0.03***	***1.48***	***1.32***	***1.38***	***1.52***	***0.046***	***0.218***
Total fatty acids	***2.26±0.11***	***7.04***	***6.35***	***7.07***	***7.60***	***0.258***	***0.111***

Means with different superscripts within each row are significantly ($P<0.05$) different and comparisons were made were made between dietary treatments and excluded the initial values.

Abbreviations: FM100 – Control diet (Raanan PG40 commercial diet); BM25 – diet where 25 % fish meal (FM) was replaced by Black Soldier Fly larvae meal (BM); BM50 – diet where 50 % FM was replaced by BM; BM75 – diet where 75 % FM was replaced by BM.

[1]Includes 15:0; 22:0 and 24:0 ; [2]Includes 16:1n-9; 17:1; 20:1n-11; 20:1n-9; 20:1n-7; 22:1n-9 and 24:1n-9 ; [3]Includes 18:3n-6; 20:3n-6; 22:4n-6 and 22:5n-6 ; [4]Includes 18:4n-3and 20:3n-3 ; [5]Includes 16:2; 16:3 and 16:4

5.4 Discussion

Compared to high-quality FM such as anchovies, herring or menhaden (645-720 g/kg crude protein and 76-96 g/kg crude fat; 'as is' basis (NRC, 2011), the BSF meal produced in Ghana for the purpose of the experiment, had a lower protein and a higher lipid content. A similar observation was made by Barroso *et al*. (2014) for a BSF meal which presented even lower levels of crude protein, lipid and ash (362 g/kg; 180 g/kg and 93 g/kg, respectively) compared to the present meal. Insect life stage, feeding substrate and processing methods influence their nutritional composition (Aniebo and Owen, 2010; van Huis *et al*., 2013) which explains the differences reported here. Also similar to that previously found by Barroso *et al.* (2014), the amino acid profile of BM was comparable to conventional FM including 9 out of 10 of the essential amino acids (BSF meal is known to be low in tryptophan (Newton *et al*., 1977; Henry *et al*., 2015), which is a considerable advantage over other common FM substitutes (soybean meal, for example) that can be deficient in some EAA (El-Sayed and Tacon, 1997). Although individual AA levels of the BSF meal from Ghana were found lower than in conventional FM (NRC, 2011), it can be considered as a good source of protein. The MM used in the current experiment was also a rich source of FA, in particular saturated and monounsaturated and it was slightly richer in EPA and DHA compared to MM used in other studies (St-Hilaire *et al*., 2007b; Kroeckel *et al*., 2012; Barroso *et al*., 2014) owing to the substrate mix on which the larvae fed (St-Hilaire *et al*., 2007a). Indeed, the enrichment of the BM with essential PUFA is an advantage here as FO was not included in any of the BM-based diets.

Nutritional composition of the control and experimental diets met the requirements for tilapia fingerlings (Jauncey, 1998; El-Sayed, 2006; NRC, 2011). The substitution of FM with BM required the adjustment of other feed ingredients dietary levels, mainly FO, soybean meal and poultry by-product meal, thereby ensuring isonitrogenous and isoenergetic diets; however, these adjustments affected other nutrients dietary levels such as the crude fibre and ash. Indeed, the high fibre content of the BM-based diets was directly related to the increasing inclusions of BM which had substantially more fibre (76.6 g/kg) than conventional FM (6.0-10.0 g/kg, as is, NRC (2011). However, the ash content of the BM (116.5 g/kg) was comparable or lower than conventional FM (102.0-215.0 g/kg, as is, NRC (2011), thus, higher ash levels in the BM-based diets

compared to FM100 could be attributable to other feed ingredients. Moreover, in the BM-based diets, FO inclusion was reduced to zero due to the high lipid content of BM. This resulted in BM25 and BM50 dietary lipid contents being about 18% lower than FM100 and BM75. Low-fat diets are preferred for warmwater omnivorous fish such as tilapia (El-Sayed, 2006) and the recommended dietary lipid content for tilapia fingerlings varies between 80 and 120 g/kg (Jauncey, 1998). The replacement of greater levels of FM with BM in such a formulation would have certainly led to lipid levels exceeding those recommended for tilapia. Nevertheless, a possible solution could be to use defatted MM instead of crude, which would enable higher inclusion levels as suggested by Fasakin *et al*. (2003) and in Chapter 3 and 4. The FA composition of the BM-based diets was also affected by the substitution of FM and FO, nonetheless, essential FA requirements for optimal growth of tilapia fingerlings (C_{18} PUFA such as 18:2n-6 and 18:3n-3) were satisfied (NRC, 2011).

Considering the design of the experiment, a particular attention was given to the location and setting of the experimental cages ensuring no interaction with the commercial practices and good water flow and oxygenation throughout each replicate. Therefore, the position of the experimental set up was determined in accordance with the farmer's knowledge and experience of the area. Moreover, given the surface occupied by the Volta Lake (8,500 km2), the water dynamics and the bathymetry at the farm location, the experimental structures were optimally positioned to benefit from optimal environmental conditions (van Zwieten *et al*., 2011). In addition, the water quality parameters monitored during the 32-day experiment were similar across cages and remained within optimal recommendations for tilapia farming (Beveridge and McAndrew, 2000; El-Sayed, 2006).

Fish performance were acceptable for tilapia farmed in cages (El-Sayed, 2013) and not significantly different among treatments, indicating that the dietary treatments did not compromise the fish growth. In accordance with these results, up to 100 % FM was replaced with housefly (*M. domestica*) or blowfly (*C. megacephala*) larvae meals in practical diets for tilapia fingerlings without compromising the fish growth compared to a FM-based control diet (Ogunji *et al*., 2008a, 2008b; Sing *et al*., 2014). Also, similar to that previously reported in other studies (Fasakin *et al*., 2003; Ogunji *et al*., 2008c; Karapanagiotidis *et al*., 2014; Sing *et al*., 2014), overall survival was good during the

32-day experimental period. The significant differences reported among the treatments survival rates were more likely explained by the stress related to the frequent sampling and handling (every 10 days) that would have more deeply affected the smaller fish than the larger one (MacNiven and Little, 2001; Bolivar *et al*., 2004). Indeed, despite the initial weights not being significantly different among treatments (ANOVA and Tukey's HSD test failed to detect differences with a P-value=0.05, close to the significance level though), the fish that were stocked at a slightly smaller size (namely BM25 and FM100) had significantly lower survival rates than the larger fish (BM50 and BM75). Moreover, compared to the other treatments, the significantly lower survival of BM25-fed fish can explain the slightly (but not significant) greater weight gain and SGR reported (11.8±1.9 g/fish and 3.7±0.4 % biomass/day, respectively), probably the competition for the resources was reduced with the number of fish.

The feeding method applied in the experiment (manual distribution), which is common practice in countries where labour costs are low, limits feed wastage and prevents starvation as it is based on the fish feeding response (El-Sayed, 2013). Multiple feeding can also improve growth and feed efficiency in species such as tilapia with relatively small stomachs and a continuous foraging behaviour (Shiau, 2002; NRC, 2011). Feed utilisation efficiency, measured through feeding rates, FCR and PER, was comparable between treatments. Feed intake was not affected by the BM dietary inclusions and the retroactively calculated feeding rates indicated that the fish were appropriately fed. Indeed, at 28°C, it is recommended to feed 5 to 20 g tilapia fingerlings at 6-4 % biomass/day (Shiau, 2002; Ng and Romano, 2013). Palatability of feeds containing insect meal seems to be related to various factors such as the fish species and its feeding response but also the insect meal characteristics (species, farming and processing methods) (Henry *et al*., 2015). For instance, a diet containing defatted BSF meal seemed to be poorly palatable for juvenile turbot, *Psetta maxima* (Kroeckel *et al*., 2012), whereas inclusion of blowfly meal in feed for juvenile red tilapia did not affect the feed intake (Sing *et al*., 2014). In Chapter 4, fish performance results suggested that Nile tilapia fry feeding response towards crude and defatted BSF meals (produced in different conditions) was good, indicating a high palatability of the material used as a single feed ingredient or mixed with FM. Also consistent with other studies using BM-based diets for tilapia fingerlings (Ogunji *et al*., 2008a, 2008c; Sing *et al*., 2014), PER values were comparable between dietary treatments. The latter indicated that dietary

proteins were similarly and efficiently used by the fish fed the different diets (Steffens, 1989; De Silva and Anderson, 1994). Thus, the replacement of up to 75% FM with BSF meal in a commercially formulated diet for nursing tilapia did not affect the dietary protein quality. This result was expected as the BM used in the present study was provided with a set of essential amino acids similar to FM.

The proximate composition of the whole fish body was also not affected by the dietary treatments. However, the FA profile mirrored that of the diets and strong correlations between selected FA and the dietary inclusions levels of BM indicated that the latter influenced the FA composition of the whole fish body. The total substitution of the FO in the 3 experimental diets explained the n-6 and n-3 PUFA levels (respectively increasing and decreasing with increasing MM inclusions). Sánchez-Muros *et al.* (2015) made similar observations while replacing 50 % FM and 100 % FO with a *Tenebrio molitor* larvae meal in a diet for Nile tilapia fingerlings. At the juvenile stages, farmers prioritise, in general, optimal growth and survival of the fish, using cost-effective and sustainable feeds and ingredients, and therefore, the FA composition of the fish whole body is less concerning at this stage than for a market-size fish (Turchini *et al*., 2009). To restore the n-3 PUFA levels, which have beneficial effects on human health (Ruxton *et al*., 2004), finishing diets containing essential PUFA could be used during the last weeks of farming (fattening stage), thereby improving the nutritional quality of the marketable fish (Karapanagiotidis *et al*., 2007).

Commercial aquafeed manufacturers continue to produce feeds for tilapia including 20 to 250 g/kg FM in their formulations because of its high nutritional quality and the high feeding response that it elicits (FAO, 2012); but this also suggests that the market price may still too low to avoid it completely and inclusion is retained to improve the feed quality (palatability) in a highly competitive feed market. In the present study, the absence of differences between the fish growth, feed utilisation and body composition was also probably related to the low inclusions of BM in the test diets (between 30 and 80 g/kg). However, in comparison with other studies (Ogunji *et al*., 2008a, 2008b, 2008c; Sing *et al*., 2014) where the FM dietary inclusions considered were greater, leading to higher inclusions of MM when used as FM substitute, and although farm-made feed are still widely used in LIDC, the present study and results seem more economically relevant due to the application to a commercial feed formulation. As

previously stated, with the intensification of the fish farming systems, commercial compound diets are increasingly used. Although no economic evaluation was conducted, given the results of the study, it can be expected that, up to 80 g/kg, a dietary inclusion of BM could benefit both feed manufactured and fish farmer. Indeed, providing that MM market price is competitive, feed production costs would be alleviated by the reduction of FM, FO and soybean meal dietary levels (expensive feedstuffs). The strategic use of quality ingredients such as MM for juvenile tilapia, containing essential nutrients comparable to FM (Barroso *et al*., 2014), could support the sustainable intensification of aquaculture and contribute more broadly to food security.

Chapter 6. Evaluation of frass performance when used as supplementary feed in semi-intensive tilapia farming or as a soil conditioner (bio-fertiliser)

6.1 Introduction

Frass, secondary product of fly larvae production systems, consists of undigested substrate residues thoroughly mixed with insect excreta (Alvarez, 2012; Čičková *et al*., 2012a). Compared to the initial substrates offered to the larvae to ensure their growth and development, frass are homogenous, odour-free, friable and stable materials with a moisture content usually reduced by half (Čičková *et al*., 2012c; Zhu *et al*., 2012; Wang *et al*., 2013). Dry matter and volume reductions of between 50 and 80% were reported for animal manures, municipal organic wastes, faecal sludge or agricultural by-products treated with maggots, thereby leading to a material containing soluble and available nutrients, such as nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) (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). Noteworthy, the maggot bioconversion process is dependent on the environmental parameters (temperature, relative humidity), the type and composition (chemical and physical) of the substrate and the larval density or feeding rate applied.

Frass is not a negligible by-product since, according to the literature (Calvert, 1979; Čičková *et al*., 2012b; Wang *et al*., 2013; Caruso *et al*., 2014), amounts produced represent 80 to 95 % of the total outputs of a bioconversion process by weight (i.e. larval biomass + frass; wet weight); therefore it seems important to find a suitable application for this by-product (van Zanten *et al*., 2015). Li *et al*. (2011c) indicated that maggot digested dairy manures can be further hydrolyzed into fermentable sugar, suitable for the food industry (ethanol fermentation). Čičková *et al*. (2012c) suggested that frass derived from swine manure could be further processed (dried, ground and packaged) in order to stabilised the product, thereby facilitating handling and storage, whereas Zhu *et al*. (2012), proposed to use fly larvae bioconversion process as a cost-efficient way to shorten the composting period usually necessary for swine manure through a 2-stage composting process. According to Newton *et al*. (2005b), frass derived from animal manures could be used for vermicomposting, thereby converting frass into earthworm castings, a valuable product in horticulture. In most studies, frass composition was compared to organic fertilisers owing to their optimal levels of N, P and K (Choi *et al.*, 2009; Zhu *et al.*, 2012; Wang *et al.*, 2013; Lalander *et al.*, 2014); however, limited and unclear results are available from the use of frass as soil

conditioners or organic fertilisers (NC State University, 2006; Choi *et al.*, 2009). In fact, maggot frass could be a competitive product on the overall composts/bio-fertilisers market due to its availability (large amounts) and thanks to a composition comparable to compost or vermicompost, which are high-value products but available in limited quantities (NC State University, 2006). Hypothetically, when feed-grade materials (food industry by-products; oil cakes; food, kitchen or market wastes, etc.) are used as rearing substrates for maggots, resulting frass might still be suitable feedstuff for omnivorous fish species.

Freshwater fish in the tropics, mostly herbivorous and omnivorous species, are still predominantly farmed under semi-intensive conditions (Bostock *et al.*, 2010). Semi-intensive farming usually occurs in ponds with fish, stocked at low density, relying primarily on natural food productivity enhanced through fertilisation (using manures or chemical fertilisers) and/or supplementary feeding to supply nutrient deficiencies and improve carrying capacity (De Silva, 1993; Tacon and De Silva, 1997). Good management practices suggested the application of supplementary feeding once a critical standing crop (CSC) is reached (Hepher, 1978), in order to simulate fish growth which would decline if relying solely on natural food (Figure 6.1). Supplemental feeds (SF) are various, from single feed ingredients to more complex mixtures and can be dispensed in a powder form, dough or pellets that are either broadcasted manually or placed in feed dispensers of various types (trays, perforated bags, etc.). The choice for suitable SF depends on various factors such as cost, availability, processing and handling requirements prior to feeding (minimal) and nutritional value; nonetheless, it is in general selected according to availability which, is in turn, related to surrounding agricultural activities (by-products) (De Silva, 1993; Jauncey, 1998).

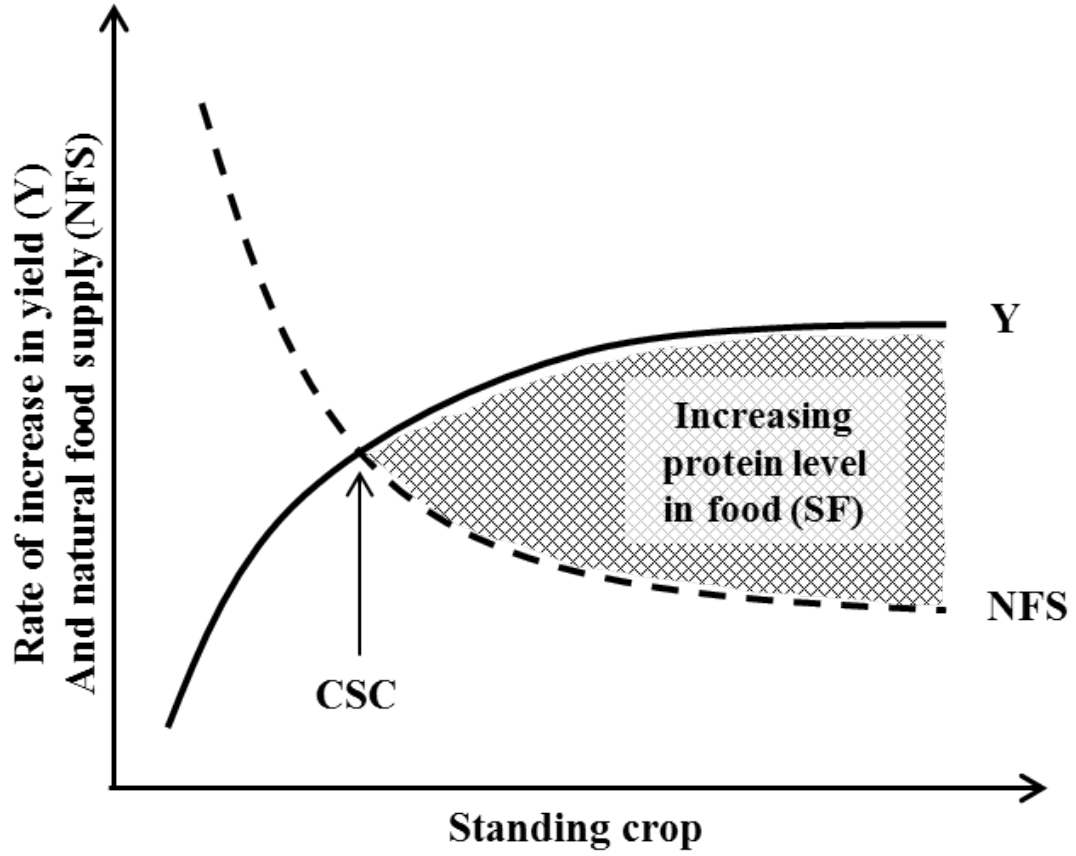

Figure 6.1 Schematic representation of changes in natural food supply (NFS) and fish yields (Y) in ponds, in relation to standing crop of the farmed species and the ensuing protein needs of the supplemental feeding (SF) once critical standing crop level (CSC) is reached (modified after De Silva (1993)

Global intensification of aquaculture systems is also reflected in semi-intensive practices by the change from subsistence to income-generating enterprises of even small-scale farmers' activities, increasing the competition for primary resources such as land, water and nutrients sources (De Silva, 1993). In this context, supplementary feeding is important because it permits to almost double the yields and production rates compared to a pond fertilisation only; therefore, cost-efficiency is a key factor.

The aim of this study is to compare the performance of two types of feed-grade derived insect frass used as supplementary feed for Nile tilapia (*O. niloticus*) farmed under semi-intensive conditions or used as a soil organic fertiliser for spring onion, an important cash-crop relevant for relevant farmers in Asia and West-Africa.

6.2 Materials and methods

6.2.1 Frass

The frass used in the following experiments were sourced from a large-scale pilot farming system of BSF located in Malaysia (Entofood Sdn Bhd; Kuala Lumpur, Malaysia). Two types of frass were compared, one was derived from processed food wastes (FW) and the other from brewery spent grains (BW) fed to BSF larvae. Following the bioconversion process and the separation of the larvae from the residues, both frass types were dried for 2 to 3 hours at 70°C.

Both frass and rice bran were analysed for proximate, fatty acid and mineral compositions at the University of Stirling (Stirling, UK). Dry matter, crude protein (total N), crude lipid, ash, crude fibre, gross energy and fatty acids were analysed as described in Chapter 2. Amino acid (AA) compositions of the frass and rice bran were determined by HPLC (subcontracted by Eurofins Food and Feed Testing in Norway). Mineral analysis was carried out by ICP-MS analysis, similarly to Yttrium analysis (see paragraph 2.4 above). Chemical compositions of the frass and rice bran are detailed in the Table 6.1 below**.**

Table 6.1 Proximate, essentials and total amino acid, fatty acid and macro-mineral compositions of the rice bran and the two types of Black Soldier Fly frass derived from brewery wastes (BW) and food wastes (FW)

	Rice Bran	**BW Frass**	**FW Frass**
Proximate composition (g/kg)			
Dry Matter	865.4	875.5	923.3
Crude protein	122.2	207.2	184.6
Crude lipid	125.4	22.9	22.6
Ash	59.3	59.2	204.7
Fibre	34.6	234.0	228.5
NFE	523.9	352.2	283.0
Gross energy (MJ/kg)	18.7	24.1	23.2
Amino Acid composition (g/100 g meal)			
Histidine	0.32	0.18	0.21
Arginine	0.91	0.41	0.51
Threonine	0.45	0.39	0.44
Valine	0.66	0.53	0.62
Methionine	0.27	0.11	0.17
Lysine	0.56	0.42	0.51
Isoleucine	0.43	0.42	0.45
Leucine	0.85	0.71	0.77
Phenylalanine	0.54	0.46	0.47
Total amino acid	***10.99***	***8.63***	***10.60***
Fatty acid composition (g/100g meal)			
Total saturated	2.10	0.38	0.40
Total monounsaturated	3.35	0.27	0.32
Total n-6 PUFA	2.68	0.62	0.53
Total n-3 PUFA	0.10	0.08	0.03
Total PUFA	2.78	0.70	0.56
Total FA content	8.23	1.35	1.28
Macro-mineral composition (g/kg)			
Calcium (Ca)	8.2	17.6	361.2
Magnesium (Mg)	72.1	23.9	14.5
Sodium (Na)	214.1	253.5	484.3
Potassium (K)	144.3	31.4	192.6
Phosphorus (P)	151.8	102.6	148.7

Values are presented 'as is', based on duplicate analyses.

6.2.2 Supplemental feeding trial

6.2.2.1 Experimental design and set up

The experiment was conducted on-farm at Nam Sai Farms Co. Ltd. (Prachinburi, Thailand) between December 2015 and March 2016 (14 weeks). A 2,260 m^2 earthen pond (1.2 m depth), located on Nam Sai main site (13°59'19.95"N; 101°12'58.50"E) was primarily drained, limed (1,875 kg/ha as $Ca(OH)^2$) and dried for a week. The pond was then filled with fish-free water, screened through a fine mesh, from an on-farm reservoir and conditioned with 30 kg 15-15-15 NPK inorganic fertiliser (Saksiam Inter Supply Co. Ltd., Bangkok, Thailand) and 150 kg Marl ($CaCO_3$), 10 days before stocking the fish, in order to promote natural productivity. Fertilisation and liming operations were then repeated every 10 days with 40 kg of 15-15-15 NPK and 150 kg of Marl during the first 4 weeks of experimentation or 40 kg of 15-15-15 NPK and 150 kg of Dolomite ($CaMg(CO_3)^2$) to provide magnesium (Mg) from week 5 and until the end of the experiment. A paddle wheel system (2.24 kW), located on one side of the pond, was activated daily from 09:00 PM to 08:00 AM and 01:00 to 03:30 PM to improve water circulation, oxygenation and productivity of the pond.

Four dietary treatments consisting of supplementary feeding with single feed ingredients, namely rice bran (RB), BSF frass derived from brewery wastes (BW) or BSF frass derived from processed food wastes (FW), or no supplementary feeding (Control) were randomly allocated to 16 hapas nets (40 m^2), as 4 replicates per treatment. Each experimental unit (hapa) was stocked with 97 monosex Nile tilapia (17.6±0.8 g initially, mean ± SD) and the pond (outside hapas) was stocked with 1,362 mixed-sex Nile tilapia (individual weight of 30g, approximately). Hapa nets were changed every 2 weeks. After a week of acclimation (week 1) during which no feed was distributed in order to allow the fish get used to the natural feed supply, supplementary feeding was started (week 2) by dispensing daily RB, BW or FW using feeding trays (30*30*5 cm) immersed at 30 cm depth in the designated hapas. During the first feeding week (week 2), hapas were supplemented with 50 g (3% of the biomass) SF in order to accustom the fish. From week 3 and until termination of the experiment (end of week 14), fish were fed to apparent satiation, over a one-hour feeding session (9:30 to 10:30 AM). Briefly, each day, fish were first served the same amount of feed offered and consumed the day before, after 30 minutes trays were checked and more feed was

distributed if necessary (quantity added was left at the at the feeder's discretion). Appropriate feeding was ensured by a skilled farm technician in response to the fish feeding response. At the end of the day, all the trays were lifted up to prevent fouling. The amount of feed distributed daily was determined by the difference between the weight of daily prepared feed containers before and after the feeding session.

Growth and survival of the fish under different treatments were evaluated at the start (stocking day), at mid-term (week 7) and on termination (end of week 14) of the experiment. Samplings involved of weighing all the fish of each hapa in batches of 10 fish/batch or less depending on the number of fish remaining in the last batch (UWE DW-3000E digital scale, precision: 0-3000gx1). Fish stocked outside the hapas were counted and bulk weighed at the start of the experiment (initial biomass = 40.6 kg); on termination of the experiment, the pond was drained and captured fish were weighed in batches of 10 as described previously.

Weight gain (g); SGR (% body weight/day); FCR; feeding rate (% body weight/day) and survival rate (%) were determined as described in Chapter 2.

Water temperature was measured and recorded every 2 hours with RFID (Radio Frequency Identification) temperature sensors (LOG-IC® data loggers, American Thermal Instruments) placed at 10 and 50 cm under the water surface in the pond. Dissolved oxygen (DO) was measured twice a week at 07:00 AM and 03:00 PM using a YSI 550A digital DO meter. Due to a delay in the procurement of the reagents for pH, ammonia, nitrite and alkalinity on one hand (Hanna HI83200 spectrophotometer) and filtering equipment for Total Suspended Solids (TSS) on the other hand, measurements were started on week 8 until termination of the experiment. Water samples were collected in 4 locations in the pond (within hapas) and analysed weekly for pH, ammonia, nitrite and alkalinity; TSS was determined following Stirling *et al*. (1985)'s method, thereby collecting every two weeks 5 water samples of 0.5 L each from different 5 locations in the pond (in and outside hapas); samples were immediately filtered through dried, pre-weighed 47-mm Whatman GF/C papers, using a filter funnel placed on a 1.0 L Buchner flask connected to a venture suction pump fitted to the water tap; paper filters were subsequently dried in an artisanal oven (temperature $\leq 40°C$) in aluminium foil for 5 - 6 hours until constant weight. The difference in weight of the filters divided by the volume of water filtered corresponded to the TSS.

6.2.2.2 Justification of the design

The experiment was designed to keep variability, cost and management to the minimum; hence the choice of placing all the treatment replicates within a single pond. A minimum of twelve ponds (4 treatments in triplicates) would have been necessary otherwise. Fish farmed under semi-intensive conditions are generally stocked at low density (0.5 to 3 fish/m^2 (Yi *et al*., 2008) directly in the pond, but in order to keep different treatment replicates independent fish had to be stocked in enclosed structures. Large hapas (40 m^2) permitted to apply dietary treatments to a significant number of fish (97 fish per hapa), stocked at 2.4 fish/m^2. In addition, in order to balance the overall pond density, maintain the water quality even and contribute to fouling control on the hapa nets, larger fish were stocked in the pond, outside the hapas (1 fish/m^2).

A preliminary test indicated that both frass and rice bran sink quickly when broadcasted (0. 1-0.2 m/min) leading to the use of feeding trays to dispense the feed daily. Single ingredient SF were preferred to simple feed formulation, again, to minimise management but also to compare, as a preliminary experiment, the fish performance without interaction with other ingredients.

6.2.3 Agronomy trial

A pot trial was conducted within the Controlled Environment Facility (CEF) of the Biological and Environmental Sciences Department, University of Stirling (Stirling, UK) between the months of March and May 2016. Unamended brown earths soil was collected from the University of Stirling grounds in a place with no fertiliser history. Dried soil (72 hours, ambient temperature in CEF) was sieved (0.8 mm) in order to remove stones and unwanted materials. BSF frass derived from brewery wastes (BW) or BSF frass derived from processed food wastes (FW) were respectively rehydrated at 1:1 and 1:0.8 (frass:water) rehydration ratios, in order to adjust the moisture content to approximately 50 % (comparable with compost) and applied during soil preparation at 5.0; 10.0 and 15.0 tonnes/ha (BW5, BW10 and BW15; FW5, FW10 and FW15, respectively). A positive control with commercial 15:3:15 NPK fertiliser (Dobbies Garden Centres Ltd, Lasswade, UK) applied at 0.3 tonne/ha (NPK) and a negative control with no amendment were also prepared (NF). For each of the 5 treatments, 4 replicate pots (10 cm diameter; 350 cm^3 volume) were placed in individual watering

saucers and were filled with the respective soil mixture, seeded with 7 to 10 spring onion seeds (*Allium cepa*, variety White Lisbon) and watered. Pots were subsequently arranged in a completely randomised design in a climate-controlled cabinet (Microclima Jumo Imago F3000, Snijders Scientific) at 24.0°C and 70.0 % relative humidity with a light-dark regime of 16:8 hrs (irradiance 400 µmol/m^2/sec).

The experiment was conducted for 8 weeks according to the seed distributor's instructions (Thompson & Morgan, Ipswich, UK). Plants were watered from below every 3 to 4 days and weeds were removed when necessary. On termination of the experiment, plants were harvested by removing delicately the soil from the root system. Soil samples were stored under refrigerated conditions (-4°C) until further analysis. Shoots (including the onion bulbs) and roots were separated and pooled on a pot basis; individual shoot length (cm) was recorded immediately after harvest. Pooled shoot and root dried weights (g) were determined after drying in an oven (Gallenkamp Oven 300) for 48 hours at 70°C in order to calculate the root to shoot ratios (root:shoot), a good indicator commonly used to assess the plant health and culture conditions (Bernier *et al.*, 1995; Andrews *et al.*, 1999). Expected yield (tonnes/ha) was determined on a dry matter (DM) basis and accounted for the whole plant (shoots+roots) since whole spring onion plants are usually sold in West-African markets to allow fast rehydration and increase shelf life (C. Adeku, pers communication, April-May 2016).

Soil analyses were conducted at the end of the experiment. Moisture content and soil organic matter (OM) were determined from 5.0 g soil samples using the standard methods described in 2.3.1 and 2.3.5 above, respectively. Electrical conductivity and soils pH were measured on 5.0 g samples in solution in 30 ml deionised water using Hanna HI 98311 and HI 2550 digital probes, respectively.

6.2.4 Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics software (version 21). Data were subjected to one-way ANOVA followed by Tukey's HSD test for unplanned multiple comparisons or using one-way ANOVA on ranks (Kruskal-Wallis test) when preliminary assumptions were violated. Correlations (agronomy trial) between the frass application rate and the performance of the plants were analysed using Pearson's

coefficient. Data are presented as mean ± SE, unless otherwise stated and a significance of $P<0.05$ was considered for all analyses performed.

In the supplemental feeding trial with tilapia, the aerator (paddle wheel) located at one extremity of the pond had a significant but not homogenous effect on the growth of the fish stocked in the 4 closest hapas (one replicate of each treatment). No significant correlation was found between the growth of the fish and the distance from the aerator, therefore some unexplained random effects might also explain the differences. A statistician was consulted and advised to exclude these 4 hapas (outliers) from the statistical analyses comparing the treatment effects and the discussion. The completely randomised design of the experiment ensured that the remaining treatments kept on a balanced design allowing an ANOVA followed by post-hoc test to be applied as described above.

6.3 Results

6.3.1 Supplemental feeding trial

Water temperature and dissolved oxygen (mean ± SD) varied slightly during the course of the experiment and with the diurnal periods with average values ranging between 28.0±2.1°C and 2.6±0.6 g/L in the morning (07:00 AM) and 30.6±2.2°C and 7.6±0.1 g/L in the afternoon (03:00 PM). Water pH, ammonia, nitrite and TSS levels remained stable during the last 7 weeks of the experiment with values ranging from 8.3-8.5; 1.9-3.9 mg/L; 0.6-0.8 mg/L and 5.2-10.3 mg/L, respectively. Alkalinity indicated decreasing values between week 7 and 14 (from 211.3 to 97.5 mg $CaCO_3$/L) despite the regular application of marl and dolomite.

Because no feed was dispensed during the first week of the 98-day experimental period, fish were fed during 91 days in total. Fish performance indicated significant differences among dietary treatments (Table 6.2). Growth performance (final weight, weight gain and SGR) and feed utilisation indicators (FCR and feeding rate) of fish fed with RB were significantly higher than for BW and FW treatments ($P<0.05$). FCR and feeding rates were not calculated for the Control treatment as no SF was dispensed. The growth performance of the fish fed the frass (BW and FW) were not significantly different ($P>0.05$) from those of the fish relying on the natural food supply (Control). A significantly greater amount of RB (2.5 more, on a dry matter basis) was dispensed to

the fish compared to the frass ($P<0.05$). Overall survival was good and not affected by the dietary treatments ($P>0.05$).

A total of 890 fish were captured from the pond (fish outside the hapas) on termination of the experiment, with a final individual weight of 240.8±24.1 g (mean ± SD). Weight gain of these fish was, therefore, higher than for the fish hold in hapas (210.8 g compared to 145.8-94.2 g in hapas). On the contrary, survival (65.3 %). was lower than for the fish in hapas.

Total biomass in the pond on termination of the experiment was 406.7 kg (including also the replicates that were excluded from the statistical comparisons and the fish outside the hapas). The adjusted yield was equivalent to 2,033.5 kg/ha.

Table 6.2 Performance of Nile tilapia fed rice bran, brewery wastes (BW) frass or food wastes (FW) frass and those kept in hapas (Control) relying on natural food

	Dietary treatments			
	Rice bran	**BW frass**	**FW frass**	**Control**
Initial weight (g)	17.8±0.4	17.6±0.5	17.4±0.5	17.7±0.5
Final weight (g)	163.6±0.8[a]	124.0±2.8[b]	117.7±3.6[b]	117.5±4.3[b]
Weight gain (g)	145.8±1.1[a]	106.5±3.2[b]	94.2±3.5[b]	99.9±3.9[b]
SGR (% body weight/day)	2.4±0.0[a]	2.1±0.1[b]	2.0±0.0[b]	2.1±0.0[b]
Total feed distributed (kg DM/hapa)	25.3±0.1[a]	9.6±0.4[b]	10.3±0.1[b]	-
FCR	2.1±0.1[a]	1.1±0.1[b]	1.3±0.0[b]	-
Feeding rate (% body weigh/day)	2.0±0.1[a]	1.0±0.1[b]	1.2±0.0[b]	-
Survival (%)	86.6±2.4	84.9±3.5	90.4±1.8	87.4±2.1

Means± SE (n=3) bearing different superscripts within each row are significantly different ($P<0.05$)
[1]Fish outside the hapas, initial biomass = 40.6 kg for a total of 1,362 fish

6.3.2 Agronomy trial

Both frass showed great water retention and absorption capacity when water was added to the dried materials during rehydration process. In addition, the application of 5, 10 or 15 tonnes/ha frass (50% moisture) to the brown earths soil resulted in a growth medium more aerated than the unamended soil; on the contrary, NPK application resulted in a more compact growth medium compared to the NF control.

Significant differences were identified in the growth performance of the spring onion plants under the different fertilisation treatments, measured 8 weeks after sowing (Figures 6.2 and 6.3). Shoot length measurements indicated that NF treatment resulted in significantly smaller plants (13.5±0.3 cm) than all other treatments ($P<0.05$) whereas FW15 fertilisation led to shoots significantly longer (27.9±1.0 cm) than NPK (22.8±0.6 cm), BW5 (20.5±0.5 cm), BW10 (22.0±1.5 cm), BW15 (19.6±1.4 cm) and NF treatments ($P<0.05$). Dried shoot and root biomasses were used to determine the root to shoot ratios. NF treatment led to the greatest root:shoot ratio (0.95) which was also significantly different from BW5 (0.54), BW15 (0.51) and all the FW-based treatments 0.58; 0.55 and 0.53 for FW applied at 5, 10 and 15 tonnes/ha, respectively) ($P<0.05$). The root:shoot ratios calculated for NPK (0.67) and BW10 (0.62) were not significantly different from all the other treatments ($P>0.05$). The numbers of plant per pot (3.7±0.1 plants/pot) were not significantly different among treatments ($P>0.05$); thus the expected yields per hectare were extrapolated from the experimental results and indicated that FW10 and FW15 treatments resulted in significantly higher yields (0.34±0.01 and 0.35±0.05 tonne/ha, respectively) than NF and all the BW-based treatments ($P<0.05$). NPK fertilisation led to a yield substantially higher (0.27±0.02 tonne/ha) than NF and BW5 ($P<0.05$), but comparable to the other treatments (Figure 6.3).

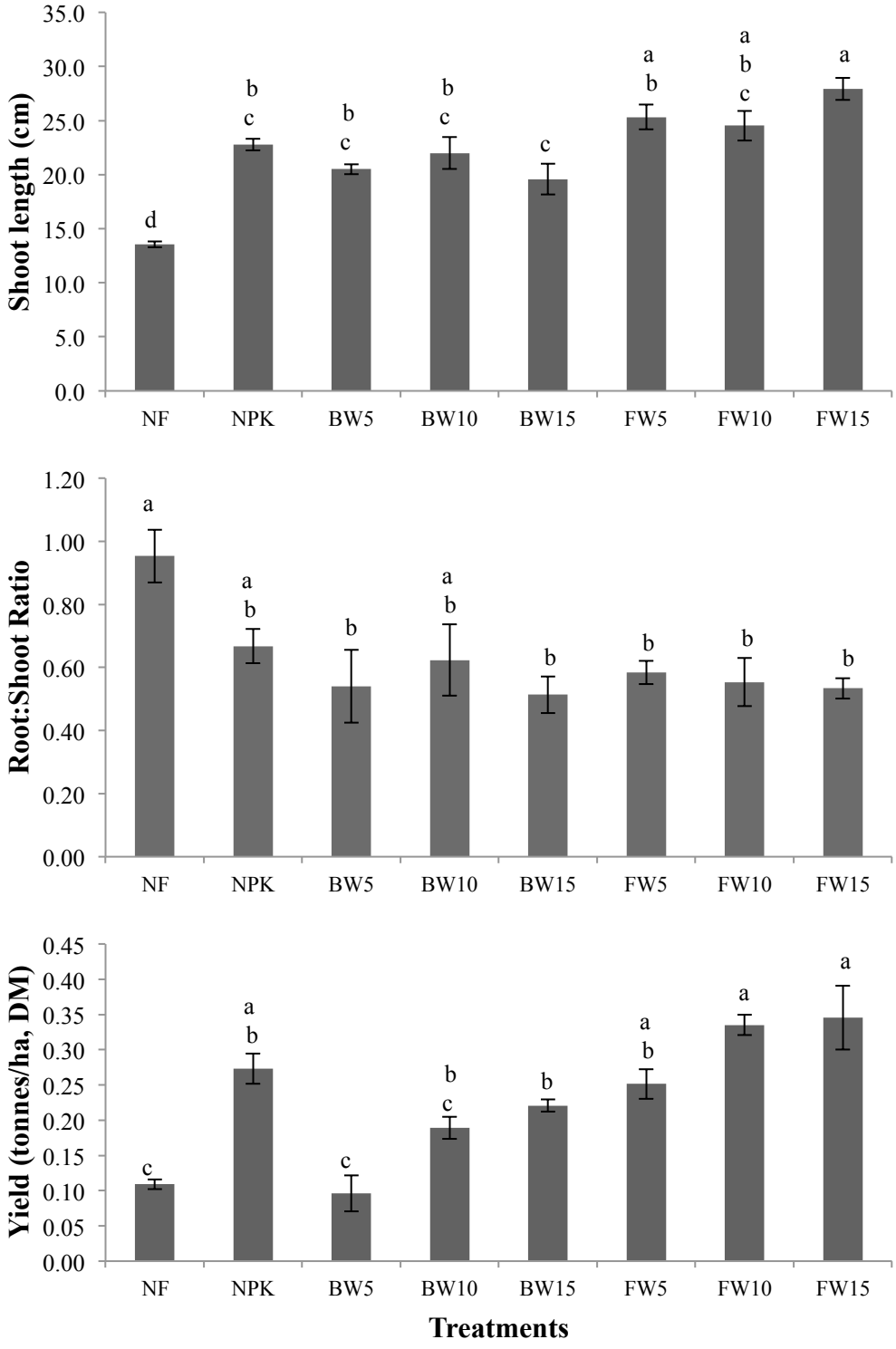

Figure 6.2 Effect of soil fertilisation treatment (NF: no fertiliser; NPK: 0.3 tonne/ha commercial NPK fertiliser; BW: brewery waste frass applied at 5; 10 or 15 tonnes/ha and F: food wastes frass applied at 5; 10 or 15 tonnes/ha) on the spring onion shoot length (cm); root:shoot ratio and expected yield (tonnes/ha, on a dry matter basis)

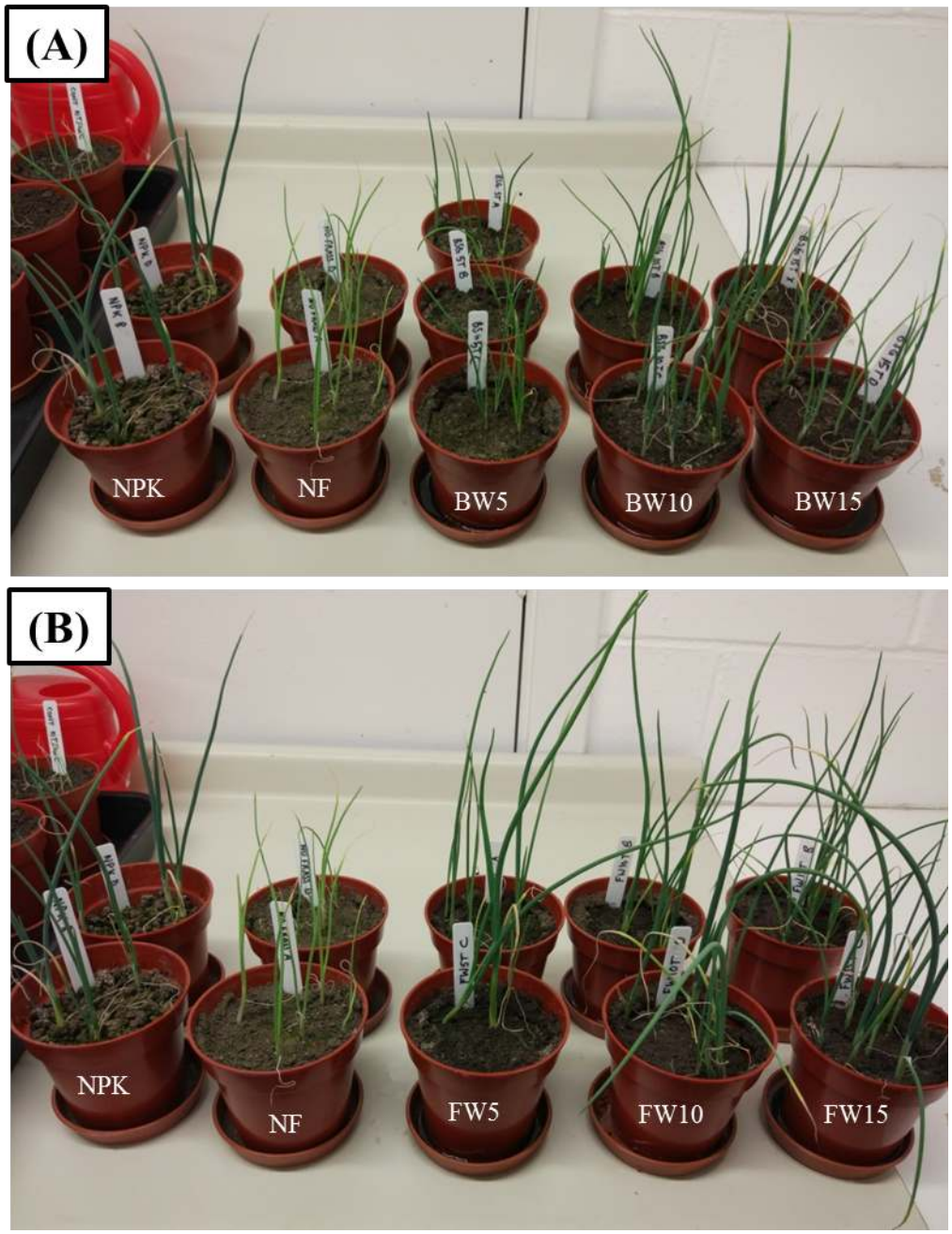

Figure 6.3 Comparison between spring onions (*Allium Cepa*, White Lisbon) plant growth, 8 weeks after sowing in unamended (NF) soil or soil fertilised with NPK (0.3 tonne/ha), brewery waste frass (BW) applied at 5, 10 or 15 tonnes/ha (A) or food wastes frass applied at 5, 10 or 15 tonnes/ha (B)

Significant positive correlations ($P<0.05$) were identified between the FW and BW application rates and the shoot length (r 0.817 and 0.591, respectively) and the expected yield (r 0.812 and 0.861, respectively) whereas significant negative relationships ($P<0.05$) were found between the FW and BW application rates and the root:shoot ratios (r -0.710 and -0.573, respectively).

Soil analyses also indicated significant differences among treatments (Figure 6.4). Among all the treatments, NF (6.7±0.1 %) and NPK (6.6±0.1 %) had the significantly lowest soil organic matter (OM) content ($P<0.05$). No significant difference ($P>0.05$) was identified between BW5 and FW5 (7.4±0.1 and 7.2±0.1 %, respectively); BW10 and FW10 (8.4±0.1 and 8.3±0.1 %, respectively) and BW15 and FW15 (9.0±0.2 and 9.1±0.1 %, respectively); however, strong positive correlations were found between BW and FW application rates and the OM content of the soil (r 0.978 and r 0.974, respectively) indicating that increasing levels of frass increased substantially the soil OM ($P<0.05$). The pH of the soil fertilised with 0.3 tonne/ha NPK was significantly more acidic (5.6±0.0) than the other fertilisation treatments (pH values ranging between 6.4±0.1 and 6.6±0.1) ($P<0.05$). Electrical conductivity varied widely with the treatments; low electrical conductivity was found in NF soils (53.3±7.3 µS) and in the soils fertilised with 5, 10 and 15 tonnes/ha BW (142.0±25.4; 191.3±19.3 and 168.3±34.5 µS, respectively). NF electrical conductivity was significantly lower than NPK (702.8±97.2 µS); FW5 (538.5±96.92 µS); FW10 (958.8±164.22 µS) and FW15 (1393.3±192.22 µS) ($P<0.05$). FW5 and FW10 electrical conductivities were not significantly different from NPK ($P>0.05$). In addition, a strong positive relationship was found between the soil electrical conductivity and the FW application rate (r 0.904; $P<0.05$) indicating that increasing FW frass level in soil improved the soil conductivity.

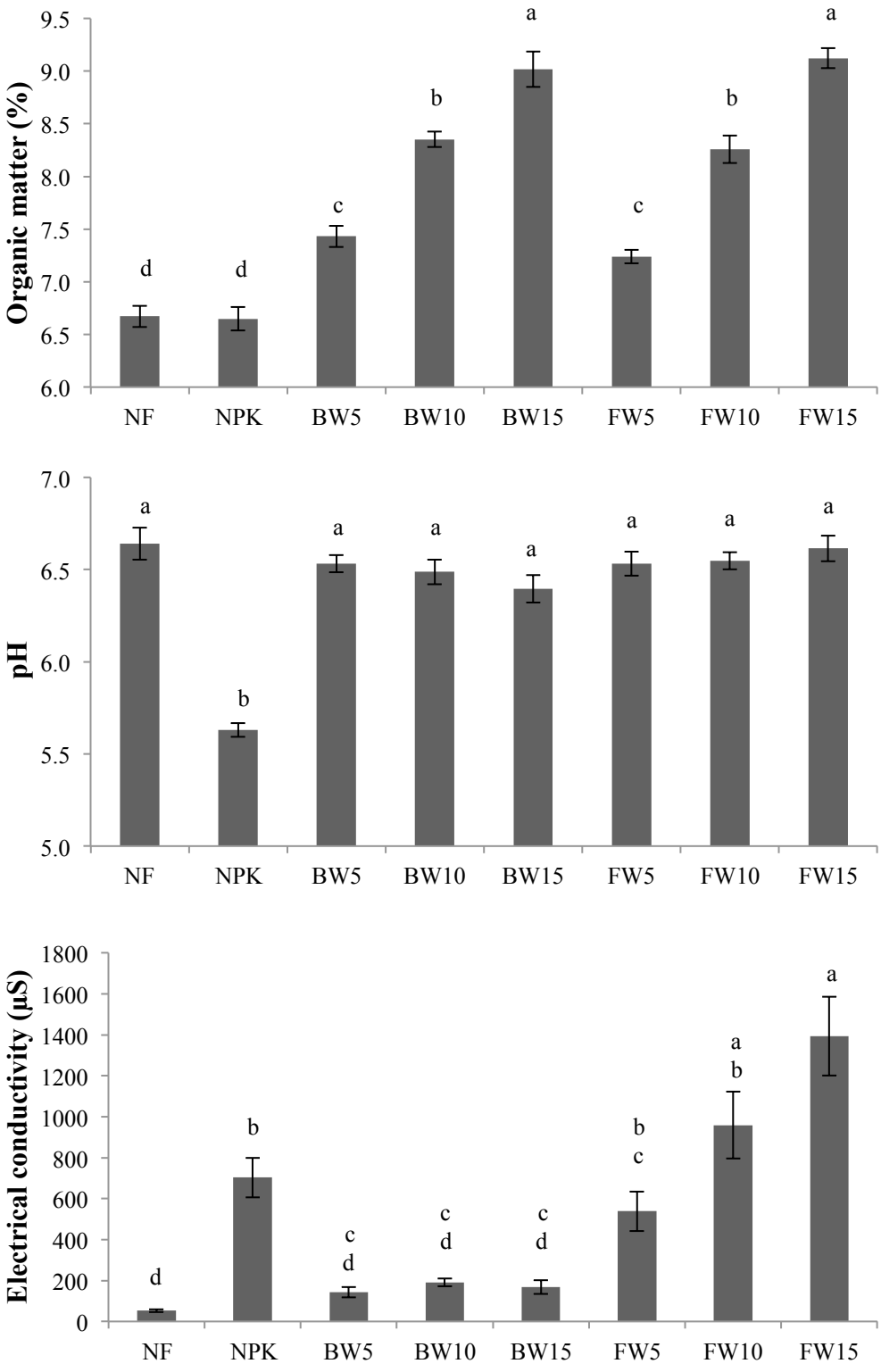

Figure 6.4 Effect of soil fertilisation treatment (NF: no fertiliser; NPK: 0.3 tonne/ha commercial NPK fertiliser; BW: brewery waste frass applied at 5; 10 or 15 tonnes/ha and F: food wastes frass applied at 5; 10 or 15 tonnes/ha) on the soil organic matter, pH and electrical conductivity after the harvest of the spring onions, 8 weeks after sowing

6.4 Discussion

The circular economy strategy considers that all outputs of a production system are valuable resources and must become inputs of other processes. In the case of insect farming, frass, which might be a lower-value product compared to the insect biomass or meal, represent however the main output in term of weight and have been suggested as good soil conditioner, source of fermentable sugar or suitable material for further vermicomposting (Newton *et al*., 2005b; Choi *et al*., 2009; Li *et al*., 2011c; Čičková *et al*., 2012c; Zhu *et al*., 2012). In the current study, frass derived from feed-grade materials (brewery and processed food wastes) showed compositions which led first, to the idea of using them as SF for tilapia farmed under semi-intensive conditions. Indeed, both types of frass were substantially higher in protein (total N) and gross energy than the RB. Nevertheless, the protein content of the frass might have been overestimated by the Kjeldahl method which assumes that the total nitrogen measured in a sample is related to the AA that compose the proteins. Indeed, when Kjeldahl protein contents (total N multiplied by a conversion factor; i.e. 6.25) were compared to the total AA contents of the frass (often considered as a better measure of the protein), substantial differences were reported (82.4 and 54.1 % differences for BW and FW, respectively), thereby indicating large proportions of non-protein-nitrogen in both frass. For the RB, a difference of only 10.6 % between total N and total AA was measured. The comparison of the total AA contents of the three SF indicated, therefore, similar protein levels for RB and FW (11.0 and 10.6 g/100 g meal, respectively) and slightly lower content for BW (8.6 g/100 g meal). The high crude fibre contents of both frass were concerning because fish do not possess the appropriate enzyme set to digest cellulose-rich materials and high dietary fibre might reduce the gut transit time and therefore the digestibility (Jauncey, 1998). However, low lipid and thus, FA contents, of the frass could be an advantage for the frass compared to the RB, firstly during storage under ambient tropical conditions as it reduces the risks of rancidity but also because low-fat diets are usually preferred for warmwater omnivorous fish such as tilapia (El-Sayed, 2006). Nevertheless, FA serve also as energy sources for fish (NRC, 2011), thus low content could also impact negatively the growth. Minerals are essential elements of fish nutrition contributing to the maintenance of normal metabolic and physiological functions (Jauncey, 1998), contents varied widely between the three SF used in the feeding trial. According to Dato-Cajegas and Yakupitiyage (1996), except phosphorus,

which improve significantly the growth and the feed utilisation of tilapia in semi-intensive systems, the supplementation of other minerals is not essential because the requirements are usually satisfied by the levels of minerals available in the pond water and the natural food.

The fundamental principle of using SF at low stocking density in fertilised ponds is to complement the high protein levels of the natural food, which availability decreases as the fish grow, with an additional substrate for the energy metabolism as it becomes limiting using exogenous feeds (Jauncey, 1998). Thus, the nutritional composition and the nutrient availability for the fish of the SF is crucial to ensure effectiveness of the method.

Evaluation of the water quality during the course of the experiment led to values within tolerance limits for tilapia (Beveridge and McAndrew, 2000; El-Sayed, 2006). Fertiliser and lime were added according to commercial practices in order to maintain optimum water quality and natural productivity for the fish growth. Liming was initially applied to increase the response to fertilisation and to neutralise water acidity, which leads generally to increasing alkalinity and hardness (Boyd, 1982). However, according to Wurts and Masser (2013), once the pH and alkalinity stabilise above 8.0 and 50.0 mg/L, respectively, limestone does not dissolve properly and regular application might be ineffective and unnecessary, which explains the results observed in this study. In addition, as previously reported by Diana *et al*. (1994), the declining levels of alkalinity were also probably due to the carbon use by photosynthesis or other natural processes in the pond. Although the natural food level could not be measured in the present study, it was assumed to remain constant thanks to the regular fertilisation and liming and the relatively stable water quality.

The good growth (2.1 - 2.3 % body weight/day) of the fish that were not fed (inside and outside the hapas) indicated that natural food was abundant and sufficient to support the fish dietary requirements at the density stocked. These growth rates were, comparable to, or higher than the value reported for semi-intensive conditions in other studies (Green, 1992; Thakur *et al.*, 2004). The slightly better growth (weight gain and SGR) of the fish outside the hapas compared to the unfed fish kept in-hapas (Control treatment) was probably related to several factors such as the lower density of fish and a better

access to the natural food, in particular periphyton developing at the bottom of the pond (Jauncey, 1998).

The growth performance of the fish under the different dietary treatments indicated that the fish fed RB grew significantly better than the fish fed the frass (BW and FW). In addition, the growth performance of the frass-fed fish and unfed fish (in-hapas) were not significantly different. These results suggested that the production rates were not improved by the supplementation with frass in comparison to the fish relying only on the natural foods and, therefore, that the BSF frass were not suitable and cost-effective single-ingredient SF for tilapia. Moreover, comparable performance of the fish fed BW, FW and the Control treatment suggested that these fish benefitted only from the natural foods available in the pond. Nevertheless, at the end of each day, when the feeding trays were lifted out of the water to dry overnight, there was systematically some feed remaining from the morning feeding session but the amounts were residual. Although further investigations are required to explain this result, it supposes two hypotheses:

(i) The fish have not ingested the frass, which would have then filtered through the mesh of the feeding tray, due to a poor palatability. Indeed, farmed tilapia in semi-intensive ponds usually prefer SF to natural foods (Schroeder, 1983), but the low palatability of the frass could explain the non-interest from the fish. Although these frass derived from feed-grade materials, the bioconversion by the larvae followed by the drying process could have affected the palatability of the materials.

(ii) The fish have ingested the frass but did not assimilate the nutrients properly. Poor nutritional quality of the frass related to low lipid contents, high levels of non-protein N or high fibre contents which could have led to a poor digestibility (Jauncey, 1998; Liti *et al.*, 2006), could explain the poor nutrient utilisation and assimilation.

FCR values obtained for BW and FW were significantly better than for RB because the amount of frass distributed to the fish were significantly lower than the RB, thus the FCR was not considered as a good feed utilisation indicator in the present study since the fish under BW and FW treatments did not rely on the frass as a source of nutrients supporting their growth, as discussed above.

The experimental design could have also influenced the results of the study; indeed, comparisons with previous studies suggested that various factors might have contributed to the frass inefficiency as SF. For instance, although farmers often start supplementary feeding 2 weeks after stocking the fish in pond (El-Sayed, 2008, 2007), previous research showed that supplemental feeding is more cost-effective if started at fattening stages, once the CSC is reached, namely 75-80 days post-stocking or when fish reach 100-150 g (Diana, 1996; Brown *et al.*, 2000). Thus, other experiments were usually conducted for 21 to 23 weeks to allow sufficient time to reach CSC before dispensing SF (Green, 1992; Diana *et al.*, 1994; Green *et al.*, 2002; Thakur *et al.*, 2004). Proper timing when adding SF have also proved to avoid wastage of resources (El-Sayed, 2008). In the current study, the experiment was terminated 14 weeks after stocking due to a time constraint, which seemed to correspond to the CSC threshold as, at this time, the fish average individual weights varied between 164 g (RB) and 124-118 g (BW, FW and Control) and the production yield was equivalent to 2,000 kg/ha. Therefore, during the first 14 weeks of the experiment, the fish could have neglected the poorly palatable frass and rely solely on the abundant and not limiting natural food without compromising growth (hypothesis (i) above), whereas the fish fed the RB would have been encouraged to feed on the SF thanks to its high palatability. An extended experimental period could have probably led to different results because, as the fish grow, they would have found themselves in an environment with limiting natural resources and may have been forced to feed on the frass to support their requirements. However, in the case where frass nutrients were not available and digestible (hypothesis (ii)), an extended experimental period would have probably depressed the fish growth and resulted in poor survival but clearer experimental outcome.

The method used to dispense the SF to the fish in the present study is debatable and could have also affected the results. In fact, as hypothesised earlier, frass particles and nutrients could have leached throughout the mesh of the feeding trays before the fish fed after some time in the water, and the use of feeding trays might not have been the best method to encourage a good feeding response. In semi-intensive farming systems, SF that are in a powdery form such as RB, are usually broadcasted manually once or twice a day but the use of feeding trays or perforated bag suspended above the water surface is also common practice (De Silva, 1993; Tacon and De Silva, 1997). However,

according to De Silva (1993), there is little knowledge on the efficacy of these forms of feeding and it is always difficult to determine the amount of feed wasted or really ingested by the fish. Farm-made feeds consisting of simple ingredient mixtures, dispensed as pellets or dough, are also commonly used in semi-intensive aquaculture systems and might reduce nutrients leaching and feed wastage (New *et al*., 1994). Compounding the frass with other ingredients as pellet or dough could have eventually contributed to better outcomes as suggested by Thompson *et al*. (2016) who found that BSF frass from distiller's dried grains with solubles (DDGS) can replace FM in diets for tilapia juveniles when combined with highly digestible animal (poultry by-product meal) and plant proteins (soybean meal).

Frass was often compared to compost or vermicompost due to its chemical composition and in an earlier study, Edwards *et al*. (1983) found that compost could be used as a supplementary feed for Nile tilapia and that uneaten compost would contribute to the pond fertilisation. Unlike compost, frass used in the current study had been dried to improve shelf-life and storage, thereby removing microorganism activity and probably reducing significantly the nutritional quality for fish. Nevertheless, it could be interesting to look at the possibility of using these frass to fertilise the pond (organic fertiliser such as compost and animal manures are often used in semi-intensive farming) rather than using them as direct SF.

Indeed, in line with previous research claims about the potential of insect frass as bio-fertiliser (Newton *et al*., 2005b; Lalander *et al*., 2013, 2014; Wang *et al*., 2013), the results of the agronomy trial presented here indicated clearly the positive effects of the frass on the soil fertility and on the plants growth. Consistently with Singer *et al*. (1998)'s observations related to the use of organic fertiliser, the soil structure was improved (more aerated) by the addition of frass compared to the unamended soil (NF) and the soil fertilised with NPK. Improvement of the soil structure is a characteristic of organic soil conditioners that usually encourages the development of the root system and therefore the growth of the plants (Singer *et al.*, 1998).

Root to shoot ratio, which is influenced by a range of environmental factors (weather, aeration, nutrient supply, etc.), is a good indicator of the quality of the culture conditions and of the plant health (Bernier *et al.*, 1995; Andrews *et al.*, 1999). High values (highest value being 1.0) indicate equivalent root and shoot biomasses (dry

weights) which are often related to a soil nutrient deficiency (Wilson, 1988); low values indicate that the plants have developed a greater shoot biomass compared to the root system. In the present study, root:shoot ratios of the spring onions cultured in the soil fertilised with BW or FW were significantly lower than the plants cultured on unamended soil (NF treatment) and NPK treatment showed a ratio intermediate between NF and the frass treatments; thus, growing conditions were improved by application of fertiliser, particularly the organic frass. Because the plants were cultured under strictly controlled environment, the improvement of the culture conditions was mostly related to a better soil fertility (Harris, 1992). Plants rely, *inter alia*, on soil nutrients to ensure their growth and development; ammonium, nitrate, potassium and phosphorous are available nutrients to plant (Horneck *et al*., 2011) and N-based nutrients, in particular, stimulate top growth at the expense of the roots (Harris, 1992; Andrews *et al*., 1999). Soil nutrients levels and availability of the previously cited nutrients couldn't be determined in this study, but the results suggested that frass improved the growing conditions by increasing the nutrients available to the plants in the soil. In addition, the results indicated that increasing levels of frass (up to 15 tonnes/ha) increased significantly the nutrients availability in soil. Because shoot length and yields were positively correlated to the frass application rate, it is assumed that frass supplied, in particular, nitrogen-based nutrients in soil. Abdissa *et al*. (2011) also reported that N improved significantly growth, quality and yield of *A.cepa.* According to Lalander *et al*. (2014), it can also be assumed that most of the non-protein N measured in the frass was mainly ammonium nitrogen (NH_4-N).

Soil OM (representing the soil carbon content) and electrical conductivity (representing the soil soluble salts content) are known to be positively correlated; thus, high OM content in soil induces a high cation exchange capacity and subsequently a high electrical conductivity, thereby suggesting excellent growing conditions (Horneck *et al*., 2011; Valente *et al*., 2012). Frass, like other organic residues or composts, increased significantly the OM content and electrical conductivity of the soil, leading to higher soil fertility compared to unamended soil, thereby contributing to the plant growth (Horneck *et al*., 2011). This result corroborates with the previous assumption as a soils with high OM content contain also more total N and intuitively more plant-available N (Horneck *et al*., 2011). Management of soils OM is crucial to ensure long-term soil productivity and, therefore, sustainable agriculture practices (Chander *et al*., 1997).

Increasing the soil OM by using organic rather than inorganic fertilisers have several benefits, including economic benefits, as it improves the soil water-holding capacity (reducing irrigation requirements), microbiological activity (nutrient turnover) and stability (reducing nutrient leaching). Another advantage highlighted by the present study was the non-influence of the frass application rate on the soil pH. Indeed, unlike the NPK, which led to a moderately acidic soil (pH 5.6), the application of frass did not significantly alter the soil pH in comparison to the unamended treatment (NF). Moreover, contrary to NPK, the soil pH of both NF and frass treatments were within the values recommended for most crops, namely between 6.0 to 8.2 (Horneck *et al*., 2011).

Excepting for BW5 treatment which led to a yield comparable to the unamended (NF) treatment, significantly lower than the yield obtained under NPK fertilisation, in most cases, growth performance of the spring onion cultured on FW or BW amended soils were not significantly different from those of the plants growing on NPK fertilised soil, although growing conditions (OM content and pH mainly) were improved by the use of the organic residues compared to the inorganic fertiliser. This indicates a minimum of 10.0 tonnes/ha BW or 5.0 tonnes/ha FW is required to expect yields comparable to those obtained with conventional inorganic fertiliser. The results (correlations) have also highlighted that increasing levels of frass, from 5 to 15 tonnes/ha, resulted in increasing OM and electrical conductivity and therefore, better yields. Moreover, the yields achieved with soil amended at 10 and 15 tonnes/ha FW were significantly greater than the yields obtained with BW suggesting that applied at 10 tonnes/ha, FW frass performed better than BW frass as bio-fertilisers, probably related to the significantly lower electrical conductivity (soluble salts) reported in the soil amended with BW compared to FW10 and FW15. This could encourage farmers to choose to apply higher rates of frass, in particular FW frass, if available at a price competitive with other conventional fertilisers. According to the present study, up to 15 tonnes/ha, the application of frass as soil organic fertiliser is suitable for spring onion and may benefit the farmer by improving the soil fertility, nutrient stability (less fertiliser would be needed for the next crop) and productivity. Nevertheless, if frass becomes marketable as fertiliser, nutrients analysis will be essential in order to define recommended and maximum application rates. This is important to limit the risks of pollution related to nutrient leaching in water (D'Haene *et al.*, 2014), but also because, at high

concentrations, nutrients such as N-based elements may become toxic and suppress plants growth (Gerendás *et al.*, 1997; Jaynes *et al.*, 2004).

Previous studies with frass were mostly limited to nutrient composition analyses and very few agronomy trials using frass as bio-fertiliser were reported in the literature. Similarly to the present study, in Korea, Choi *et al*. (2009) reported that BSF frass, also derived from food wastes and used as an organic fertiliser for cabbages led to growth performance and yields similar to those obtained with a commercial fertiliser; however the study did not detail the application rates used and the type of fertiliser used as control (assumed to be inorganic). Another study showed that BSF frass derived from swine manure did not perform as well as a commercial potting soil mix for basil (*Ocimum basilicum*) when applied at increasing rates comprised between 5 and 50 % mixture with sand or clay soil (nutrient poor growth medium) or for sudan grass (*Sorgham sundanense*) when applied at increasing rates comprised between5 and 20 % mixture with sand or clay soil (Newton *et al*., 2005b). Although data were not compared statistically, results of the latter study suggested that growth of basil was suppressed with BSF frass mixed with sand soil whereas above 10 % frass in clay soil plants growth was blocked (hypothetically the nutrients levels provided by the frass became toxic); similarly above 5% frass in soil, growth of sudan grass was suppressed compared to the control (commercial potting soil mix). Comparison with the present study outcomes is difficult due to the different application methods (rates); however it is likely that despite the nutrient reduction achieved through the bioconversion process with fly larvae (NC State University, 2006; Myers *et al*., 2008), frass derived from swine manure may contain higher nutrient levels than FW and BW, and was probably toxic for plants even at low application rates in soil. Thus, it is important to proceed to nutrient composition analysis prior to the application as a soil fertiliser, as suggested earlier.

In most countries, inorganic and organic fertilisers are subjected to regulations; thus, comprehensive assessment using standard procedures for quality and safety testing will be surely required for frass to be marketed as bio-fertilisers. In Europe, the current circular economy strategy might be a significant programme promoting frass as bio-fertilisers because it aims at revising, *inter alia*, the current legislations on wastes and

fertilisers, thereby allowing a better waste management and waste recycling into valuable resources to which access to market would be facilitated (EC, 2016a, 2016b).

In conclusion, although the outcomes of the feeding trial did not satisfy the first hypothesis, further investigations might be considered in order to determine with more certitudes if frass can be valuable source of nutrients for tilapia farmed under semi-intensive conditions. On the other hand, the results of the agronomy trial confirmed that both FW and BW frass derived from BSF bioconversion processes, based on feed-grade substrates, are relatively good organic fertiliser improving soil fertility and stability and leading to yields comparable to those achieved with inorganic products. This is encouraging as it could contribute to reduce the use of inorganic fertiliser in crop culture and to encourage better soil and crop management practices providing that frass market price is competitive with conventional fertiliser. These results suggested that, similarly to manures or composts, frass could probably be used as organic fertilisers in ponds (extensive and semi-intensive farming) to support the development of sustainable aquaculture. Thus, frass are definitely valuable products and should not be considered as wastes but rather as resources that can provide nutrients for other food-producing systems.

Chapter 7. Modelling the requirements for the strategic use of insect-based products

7.1 Introduction

Global concerns are rising toward the amount of waste generated by human activities nowadays, in particular organic wastes. Indeed, with a global population expected to reach almost 10 billion individuals by 2050, it is anticipated that, together with rapid urbanisation and rising incomes, food demand (in particular for Animal Source Food, ASF) will continue to rise (United Nations Department of Economic and Social Affairs, 2015). Paradoxically, 30 to 40 % of the food produced globally is currently wasted or lost (FAO, 2011). In addition, the livestock industry which produces ASF, is expected to double the production by 2050, generating therefore twice more animal waste (manures) which can be a great source of pollution and cause significant damages to the environment if suitable treatment or disposal strategies are not implemented (FAO, 2006). In fact, as wastes of any sort increasingly represent costs for those who generate them; their re-use or recycling can be a win-win solution. Wastes streams can become resources and provide additional incomes. As previously suggested, maggots efficiently convert organic resources into a nutrient-rich insect biomass that can be used as feed ingredient for farmed fish once it is processed into a meal (Chapter 3, 4 and 5) and a valuable by-product (frass) recoverable in crop culture as bio-fertiliser (Chapter 6). In order to limit the impacts on the environment and the costs (transport), a sustainable aquaculture industry should consider using locally produced materials (MM) and frass could also be integrated into local agriculture / horticulture depending on the demand (cash-crops, gardening, etc.). However, application of this strategy, based on the circular economy principles, may differ depending on the geographic and economic contexts considered.

In this concluding study using both primary and secondary data, two different contexts were considered as examples: (1) the United Kingdom (UK), where Atlantic salmon (*S. salar*) farming is predominant in tonnage and value (Ellis *et al.*, 2015) and (2) Ghana, where the aquaculture sector is dominated by Nile tilapia (*O. niloticus*) culture (FAO, 2005-2016). Housefly MM and BSF MM production systems developed, respectively, in the UK and Ghana within the project PROteINSECT were used to support the models. This analysis aims to model first the volumes of MM required in each relevant aquaculture system and to determine and discuss the implications in terms of production

with a focus on the fly species, the amounts of substrates required and the potential applications for the frass in each context.

7.2 Materials and methods

7.2.1 General

Two simple models based on the annual production of commercial fish farms in two locations (UK and Ghana) were developed to determine the amount of MM (tonnes/year) that would be required according to the recommended FM substitution (w/w basis) defined in the previous experiments of the present study (Chapter 3 to 5) and the literature. In addition, primary data from the maggot farming systems developed in UK and Ghana, within the project PROteINSECT, to produce housefly or BSF MM, respectively, were applied to the models to determine the annual quantity (tonnes/year) of fresh maggots to produce and consequently the prerequisite amount of substrate (tonne/year) to grow the maggots. Finally, the amounts of frass resulting from the bioconversion process were estimated also from primary knowledge. Substrate opportunities, processing methods of the MM and frass produced and sale opportunities for frass were further discussed.

All the calculations were carried out using Microsoft Excel 2010.

7.2.2 United Kingdom

This model (1) is based on secondary data from the largest Atlantic salmon company in the UK (Marine Harvest Scotland) producing almost one-third of the total volume of salmon in the UK (179,022 tonnes of salmon produced in 2014 in Scotland); the leading supplier of salmonid feeds in Scotland (Ewos) and the literature (Bergheim *et al*., 2009; Crampton *et al*., 2010; Taylor *et al*., 2011; Marine Harvest, 2015; Munro and Wallace, 2014; Tacon and Metian, 2015). Data related to the MM production were sourced from the housefly production system developed in the UK (Grant Bait Ltd.; Yorkshire, UK) through the project PROteINSECT (2015) and the results from the study on Atlantic salmon parr (Chapter 3) and Lock *et al.* (2015) were applied to model the fish performance.

Marine Harvest Scotland is a well-established company which produced 48,900 tonnes Atlantic salmon in 2014 Scotland. Mostly imported (75.8 %; Munro and Wallace,

(2014), ova are hatched in-house and fish are then farmed up to market size (between 4.0 and 6.0 kg, individual weight) at several sites. The model built in the present study simplified the reality by simplifying the production stages, thereby considering parr from 5 to 30 g; smolts from 30 to 100g (in Scotland, smolts are generally transferred to the sea between 70 and 120 g; (Munro and Wallace, 2014) and grouping the post-smolt stage to the rest of the production under 'grow-out' stage in seawater (from 100 g to 5.0 kg, average market size).

The current model considered the replacement of 50 % FM in diets for parr (according to Chapter 3), smolt and grow-out (assumed from Lock *et al.*, 2015) with defatted housefly MM. Lock *et al.* (2015) showed that a 100 g/kg dietary inclusion of crude BSF MM (50 % FM substitution) in post-smolt diets led to fish performance similar to FM-based control diet; therefore, the model here assumed that defatted housefly MM, which had a nutritional profile comparable to crude BSF (except for the lipid content, significantly lower in defatted housefly MM) would reasonably perform like BSF MM for smolt and grow-out as modelled here.

MM was produced from housefly larvae fed on poultry manure (60 % DM) generated by the broiler farming industry as described by Charlton *et al.* (2015); specifically, from 1.0 tonne of manure (fresh), 0.02 tonnes of defatted MM (97.5 % DM) were produced and 1.0 tonne of fresh maggots resulted in 0.2 tonne MM after processing (drying, grinding, defatting). Finally, substrate weight was reduced by 70.0 % through the bioconversion process, resulting after drying, in a frass assumed to be suitable as a high-quality soil conditioner (Chapter 6; Miller *et al.*, 1974; Teotia and Miller, 1974; Morgan and Eby, 1975; Čičková *et al.*, 2012c). The model considered that frass was subsequently dried (2-3 hours at 70°C, as described in Chapter 6) following bioconversion process in order to reduce the moisture to approximately 10 % which facilitates storage and improves shelf life of the product. The model assumed also that the housefly MM was defatted as described in Chapter 3, using a solvent method with hexane as commonly applied in the industry (Russin *et al.*, 2011).

All the data required to build the model (1) are summarised in Table 7.1.

Table 7.1 Primary and secondary data (yearly production figures) used to build the model based on Scottish salmon industry and housefly pilot-scale production system in the UK

	Ova	**Fry**	**Parr**	**Smolt[4]**	**Grow-out[5]**
Total production (t/year)	-	67.6	401.8	1,326.1	48,900.0
Individual average weight (g)	-	5.0	30.0	100.0	5000.0
Number of individuals	$19.3x10^6$	$13.5x10^6$	$13.4x10^6$	$13.3x10^6$	$9.8x10^6$
Survival (%)	70.0	99.0	99.0	-	-
Feed Conversion Rate (FCR)	-	-	0.9	1.0	1.1
Total feed required (t/year)[1]	-	-	361.7	1,326.1	53,790.0
FM inclusion in feed (g/kg)[2]	-	-	400.0	200.0	200.0
Maggot meal[3]	-	-	Defatted housefly	Defatted housefly	Defatted housefly
FM substitution with MM[2] (%)	-	-	50.0	50.0	50.0

[1]Calculated as Feed required (tonne/year) = FCR * total production (tonne/year); [2]Adapted from Crampton *et al.* (2010) and Lock *et al.* (2015); [3]Adapted from Chapter 3 and Lock *et al.* (2015); [4]Smolts are transferred to the sea between 70 and 120 g; [5]Includes post-smolts; [4]

7.2.3 Ghana

This second model (2) is based on secondary data from the literature (Little *et al*., 1997; New and Wijkström, 2002; Bhujel, 2014). Primary and secondary data from the main aquafeed manufacturer in Ghana (Raanan Fish Feeds West Africa, Ghana) and primary data collected from a pilot-scale production system set up in Accra periphery (Ghana) to produce BSF MM. Moreover, secondary data from hatchery, nursery, and grow-out tilapia operations, at various locations were used to model the production figures of the largest commercial tilapia farm in Ghana (Volta region) which harvest more than 6,000 tonnes fish annually.

The modelled tilapia farm includes a hatchery site where broodstock is maintained all-year long and all-male fingerlings are produced through hormonal sex-reversal process in hapas as described in Chapter 4. Following a first nursing phase (I) also achieved in hapas, set up in fertilised ponds, using a farm-made feed made of rice bran and FM (2:1 ratio), advanced nursing (as described in Chapter 5) and grow-out operations were considered in cages-in-lake using commercial feeds (Raanan feeds).

This model considered the replacement of 100 % FM with defatted BSF MM in MT-treated feed for sex-reversal Nile tilapia fry in earthen ponds according to Chapter 4's recommendations (better economic performance than crude MM). Substitution of 75 % FM using crude BSF MM was then applied to nursing (I and advanced) fry and fingerlings according Chapter 5's results and reasonably, although not supported by experimental results, the model assumed a dietary inclusion of 37.5 g/kg crude BSF MM (representing a dietary substitution of 75 % FM, similarly to nursing phases) in compound diets fed to food-fish fish up to commercial size (approximately 400 g). This study did not consider the use of MM in the diets for broodfish.

MM was produced from BSF larvae fed on a substrate mix composed of brewery wastes and processing wastes from a local fish feed factory as described in Chapter 5; specifically, from 1.0 tonne of substrate mix (40 % DM), 0.012 tonnes of MM (95 % DM) were produced and 1.0 tonne of fresh maggots resulted in 0.3 tonne MM after processing (drying, grinding and defatting of necessary). Finally, substrate weight was reduced by 66.0 % through the bioconversion process, resulting after drying process, in a frass assumed to be comparable to BW frass described in Chapter 6. The model

considered that frass was dried (sun drying) following bioconversion process in order to facilitate storage and improve shelf life reducing moisture to approximately 10 %. Defatted MM was assumed to be processed using the solvent method with hexane described in Chapter 3 for housefly MM and defatted material was applied only to sex-reversal fry feeding since small amounts of meal are required for the process. Defatting larger amounts was not applicable in the conditions of production in Ghana (medium-scale artisanal process).

All the data required to build the model (2) are summarised in Table 7.2.

Table 7.2 Primary and secondary data (yearly production figures) used to build the model based on tilapia commercial farm and BSF pilot-scale production system in Ghana

	Broodstock[1]	**Sex-reversal fry**	**Nursing I**	**Advanced nursing**	**Grow-out**
Total production (t/year)	6.0	9.0	112.5	405.0	6,480.0
Individual average weight (g)[2]	200.0	0.3	5.0	20.0	400.0
Number of individuals	30,000	$30.0x10^6$	$22.5x10^6$	$20.3x10^6$	$16.2x10^6$
Survival (%)	-	75.0	90.0	85.0	-
Feed Conversion Rate (FCR)[3]	-	1.0	1.2	2.0	1.5
Total feed required (t/year)[4]	-	9.0	135.0	810.0	9,720.0
FM inclusion in feed (g/kg)[3,5]	-	1000	333.3	100.0	50.0
Maggot meal[5]	-	Defatted BSF	Crude BSF	Crude BSF	Crude BSF
FM substitution with MM (%)[5]	-	100.0	75.0	75.0	75.0

[1]Adapted from Little *et al.* (1997); [2]Adapted from Bhujel (2014); [3]Adapted from New and Wijkström (2002);
[4]Calculated as Feed required (tonne/year) = FCR * total production (tonne/year); [5]Adpated from Chapters 4 and 5

7.3 Results and discussion

7.3.1 General

In this chapter, the use of MM for aquaculture was studied using a site-specific approach because the socioeconomic and environmental conditions related to a particular geographic area influence substantially the aquaculture sector (species, farming methods, development, etc.) but also the potential for the insect farming (scale, substrates, requirements, processing, etc.). Therefore, following the logic and the results of the previous studies, defatted housefly MM produced in the UK from poultry manure was integrated into a model (1) where it was used to substitute FM in Atlantic salmon feeds and BSF MM produced in Ghana from a mixture of agro-industrial wastes was integrated into a model (2) where it was used to substitute FM in Nile tilapia feeds. Results and recommendations from the previous studies on the optimum MM dietary levels to apply at the different stages (i.e. fry, fingerlings or grow-out) were considered for both models respectively and reasonably extrapolated for the stages for which no experimental results were available.

For instance, model (1) was based on the use of housefly MM for the Atlantic salmon as primary data were available for parr (Chapter 3) and for the insect production system (PROteINSECT, 2015). For the other production stages (smolts and grow-out), the results of Lock *et al.* (2015)'s study on post-smolt were considered although BSF MM was used as a FM substitute rather than housefly MM. Because housefly MM and BSF MM from both studies had different nutritional profiles, it was decided to model the FM substitution with defatted rather than crude MM in order to minimise the differences. In addition, it was assumed that most of the differences between BSF and housefly MM lied mainly in their respective FA compositions which were strongly related to the nutritional composition of the substrates used to grow the maggots (St-Hilaire *et al*., 2007a); thus, defatting process lessened the differences as it reduced the FA levels by half, approximately. Although Lock *et al.* (2015) did not recommend the use of defatted meal because of the poor quality of the material used in their study, the results of Chapter 3 showed, on the contrary, that defatted meal might actually be more suitable than crude MM given that a solvent method with hexane, preserving the quality of the meal processed and widely used for other feed-grade material such as soy products (Russin *et al.*, 2011), is applied. Furthermore, the insect fat extracted during the

defatting process (not accounted in the models) can also be refined into a very stable oil and sold to the animal feed industry or further processed into biodiesel (Li *et al*., 2011b; Zheng *et al*., 2012; Mariod, 2013; Byrne, 2016), thereby generating additional income.

Despite the study modelled the largest operating fish farms in both contexts, the comparison between the volumes of MM required for salmon and for tilapia is difficult because of the differences in the production methods, the scales and the fish requirements. Indeed, with the present models, annual salmon tonnage harvested (48,900 tonnes/year) was 7.5 times greater than tilapia (6,480 tonnes/year). Considering this, it was estimated that 55,478 tonnes of feed would be required annually to support the production described for salmons against 10,674 tonnes/year for tilapia (Table 7.2). In both cases, the volumes of feed required for fry and juveniles were significantly lower than for the grow-out operations because feeding rates are usually based on the fish body weight; however, FM inclusions in feeds decreased as the fish grow bigger. As a result, the quantities of MM required to substitute between 50 and 100 % FM in salmon and tilapia diets were significantly lower for the juvenile stages than for the for grow-out.

7.3.2 MM requirements

According to the assumption made on the FM dietary inclusions in Atlantic salmon diets and the levels of substitution with defatted housefly MM suggested by the previous studies (50 % replacement), the total amount of MM required to cover the annual requirements equalled to 5,583 tonnes (Table 7.3). Specifically, 72; 133 and 5,379 tonnes of MM/year were required to substitute 50 % FM in parr, smolt and grow out salmon diets, respectively. In the case of tilapia (model (2)), it was estimated that 9.0 tonnes of defatted BSF MM would be required to ensure the total substitution of the FM used for the production of 30 million monosex fry annually (Table 7.4). Reasonably, a substitution of 75 % FM in farm-made and commercially formulated diets for nursing and fry and fingerlings and for grow-out tilapia with crude BSF MM was assumed; thus, the model (2) estimated an additional 459 tonnes of crude BSF MM required to support the production system, making a total of 468 tonnes of MM annually.

The salmon system required nearly 12 times more MM than the tilapia system, which is understandable given that 5 times more feed was necessary for salmons with greater dietary inclusions of FM to substitute with MM. However, even if the model (1) was adjusted to a foodfish production of 6,500 tonnes salmon/year, which can be the case of smaller operators in Scotland (Munro and Wallace, 2014), it would still require 1.6 times more MM than the tilapia system. Thus, salmon farming is more demanding than tilapia in terms of insect protein to supply. In addition, the estimated figures in both models indicated clearly that fry and juveniles required less MM than grow-out accounting for 96.3 % and 77.9 % of the total MM tonnages estimated for salmon and tilapia, respectively. Consequently, similar observations were made on the volumes of larvae to produce, substrates required to grow the larvae and the resulting volumes of frass (Tables 7.3 and 7.4). Model (1) estimated that a total of 27,920 tonnes of housefly larvae (fresh biomass) would need to be farmed and processed annually to cover the volumes of MM required whereas according to model (2), 1,560 tonnes of BSF larvae would need to be produced.

The production of hundreds or thousands tonnes of MM per annum still seems to be an ambitious objective for the emerging insect industry. Very little information about the current production capacity of the insect farming industry developing globally is available, but it can be expected that if the market demand for insect meal increases, providing that the legislation changes and becomes more flexible towards the use of insect in animal feeds using a broader range of substrates, production levels will not be an issue for commercial companies who will certainly develop novel technologies to tackle the problem. Very few systems described in the literature were, however, capable of producing large volumes of maggots as most work was conducted on pilot system or in laboratories. Burtle *et al*. (2012) have designed a system in the USA which could, in theory, produce 3,750 tonnes BSF MM per year using 360 tonnes of daily food leftovers or swine manure. The development and multiplication of this kind of system could contribute significantly to the MM supply globally. In China, a housefly larvae bioreactor has also shown promising results with a total production of 760-960 tonnes of fresh larvae per year (corresponding to 570-720 tonnes MM/year) using swine manure (Wang *et al.*, 2013). Pilot systems developed in the UK and Ghana within the project PROteINSECT produced respectively, 364-520 kg of housefly MM and 416-780 kg of BSF MM annually, which is far from the requirements modelled in this study.

Private company Enviroflight, located in the USA, stated on its website (www.enviroflight.net) that they can produce about 300 tonnes of BSF MM per year from 6.0 tonnes of organic material per day.

The automation of the most handling tasks and a good control of the production parameters (temperature, humidity, light), in brief, the development of a specific novel technology, adapted to site-specific conditions, would probably be the most favourable options to consider to increase the production levels. In addition, models (1) and (2) estimated only the requirements for one large-scale farm in each location; thus it must be expected that the volumes of MM and substrate would be significantly greater if applied to the whole salmon or tilapia industries. Thus, until the insect farming industry increases its production capacity, a strategic use of MM, in particular, must be considered. From the previous results, it is clear that MM could be used in priority for young fish (fry, fingerlings, parr and smolts) given the lower amounts of MM required and the specific requirements at these stages (nutritional requirements, quality, palatability, etc.). However, the incentive to use MM in fish diets will rely on its market price, which will be determined mainly by the production costs, which are site-specific dependant. It is critical that insect based-products market prices are competitive with conventional feed ingredients and remain low with production costs preferably not exceeding 1,000 USD/tonne for the MM, according to Drew and Pieterse (2015). In the case of the systems developed in the UK and in Ghana within the project, production costs were estimated to 13.1 USD/kg of housefly MM and 4.3 USD/kg of BSF MM, respectively, which can be considered as excessive and not competitive compared to market price of conventional feed ingredients (Table 1.3). Labour, costs related to the processing (drying, grinding) and substrate accounted in both cases for the most of the estimated costs; in the UK context the energy related costs ranked fourth (PROteINSECT, 2016b). Nevertheless, these systems were far from being industrial and required substantial technical improvements such as automation, control of the environmental parameters, etc.. On the contrary, in China, the full-scale housefly larvae bioreactor described above showed profitable performance by selling both MM (1,430 USD/tonne in 2010) and composted frass (6.5 USD/m^3) to local feed manufacturers and fertiliser dealers (Wang *et al.*, 2013). Economies of scale and technological improvements will certainly contribute to reduce the production costs resulting in the production of competitive insect-based products.

Table 7.3 Adjusted dietary inclusion levels (%) of FM and MM, quantities of MM, fresh maggot and substrate (poultry manure) required (tonne/year) and amount of frass produced (tonne/year) resulting from the substitution of FM in Atlantic salmon parr, smolt and grow-out diets.

	Parr	**Smolt**	**Grow-out**	**Total**
Total feed required (t/year)	361.7	1,326.1	53,790.0	55,477.7
Maggot meal		Defatted housefly		-
FM substitution with MM (%)	50.0	50.0	50.0	-
Corrected FM inclusion in MM-based feed (g/kg)	200	100	100	-
MM dietary inclusion (g/kg)	200	100	100	-
Quantity of MM required (t/year)	72.3	132.6	5,379.0	5,583.9
Quantity of fresh maggot required (t/year)[1]	361.7	663.0	26,895.0	27,919.7
Quantity substrate required (t/year)[2]	4,339.8	7,956.4	322,740.0	335,036.2
Frass produced (t/year)[3]	1,302.0	2,386.9	96,822.0	100,510.9

[1]Assuming 1.0 tonne fresh housefly larvae = 0.2 tonne housefly MM; [2]Assuming 1.0 tonne poultry manure (60% DM) = 0.02 tonne MM;
[3]Assuming a substrate weight reduction of 70.0 % into frass

Table 7.4 Adjusted dietary inclusion levels (%) of FM and MM, quantities of MM, fresh maggot and substrate (mix) required (tonne/year) and amount of frass produced (tonne/year) resulting from the substitution of FM in Nile tilapia sex-reversal fry, advanced nursing and grow-out diets.

	Sex-reversal fry	**Nursing I**	**Advanced nursing**	**Grow-out**	**Total**
Total feed required (t/year)	9.0	135.0	810.0	9 720.0	10,674.0
Maggot meal	Defatted BSF	Crude BSF	Crude BSF	Crude BSF	-
FM substitution with MM (%)	100.0	75.0	75.0	75.0	-
Corrected FM inclusion in MM-based feed (g/kg)	0.0	83.3	25.0	12.5	-
MM dietary inclusion (g/kg)	1000.0	250.0	75.0	37.5	-
Quantity of MM required (t/year)	9.0	33.7	60.75	364.5	468.0
Quantity of fresh maggot required (t/year)[1]	30.0	112.4	202.5	1,215.0	1,559.9
Quantity substrate required (t/year)[2]	750.0	2,809.7	5,062.5	30,375.0	38,997.2
Frass produced (t/year)[3]	255.0	955.3	1,721.3	10,327.5	13,259.0

[1]Assuming 1.0 tonne fresh maggot = 0.3 tonne MM; [2]Assuming 1.0 tonne fresh substrate (40% DM) = 0.012 tonne MM; [3]Assuming a substrate weight reduction of 66.0% into frass

Both housefly and BSF species can be farmed in the UK and in Ghana, or more widely under temperate or tropical conditions. The farming methods are not widely different between both species; under temperate climates, the maintenance of the optimum parameters (temperature, light, humidity) for the fly survival and productivity are essential and required controlled environment facilities and substantial levels of energy compared to tropical conditions. The choice of a species over another, under site-specific conditions, might be therefore driven by factors other than the climate, such as (i) the final use of products (MM mainly) or (ii) the substrate availability. Because the frass resulting from the bioconversion process with one species or the other is assumed, according to the results of Chapter 6 and other authors, to be a suitable soil conditioner it is not likely to influence the choice of the insect species.

(i) The final use of the MM (i.e. used as feed ingredient for salmon or tilapia) is not influencing much the choice of the insect species to grow as both MM are good protein sources with nutritional profiles highly similar to FM (Barroso *et al.*, 2014 and previous studies), thus likely to be suitable for any fish species. Despite the fact that the number of studies comparing housefly and BSF MM for a particular fish species and in similar conditions (i.e. same FM substitution levels) is reduced to that of St-Hilaire *et al*. (2007b) on Rainbow trout (*O. mykiss*) and that of Chapter 4 on sex-reversal Nile tilapia, it can be assumed that both MM led to comparable fish performance. Additional processing (i.ie defatting) or feed formulation adjustment (balancing nutrients levels using an ingredient blend) can also contribute significantly to use efficiently the MM selected (Chapter 4).

(ii) Rearing substrates that are essential to grow the maggots can influence the choice of the fly species due to the availability related to site-specific conditions. Firstly, as highlighted in numerous studies, housefly larvae perform more efficiently on animal manures whereas BSF larvae are more resilient to a wider range of organic substrates (Čičková *et al*., 2015). As already stated, the use of waste streams or materials with no or low economic value, not yet harnessed in other value chains (no/low competition), is the most favourable option to consider to farm maggots cost-efficiently and sustainably (PROteINSECT, 2016a). However, mass

flows that are considered as wastes in a particular context can be considered resources in another. That is, for instance, the case of animal manures which are, on one hand, costly to treat or dispose in industrialised countries because of the their abundance (intensive livestock industry) and the limited usage options associated with a high risk for the environment (DEFRA, 2013) but are, on the other hand, valuable resources in LIDC, where the livestock industry is limited and dispersed (making it difficult to collect) resulting in low volumes and poor availability but high demand of these materials commonly used as bio-fertiliser (for cash-crops or aquaculture ponds). Therefore, it seems more relevant to farm housefly larvae in industrialised contexts, such as in the UK, using manures and BSF larvae in LIDC, where the type of substrates available might be broader and availability less consistent, requiring, therefore, a greater adaptability. That said, because in Europe the precautionary approach in application does not authorise the use of animal manures or kitchen and table wastes in the diet of farmed animals intended for animal feed (EC regulation 1069/2009) further research is still required to assess the risks of biological or chemical contamination when insects farmed on manures are fed to fish and livestock (EFSA Scientific Committee, 2015).

7.3.3 Substrate requirements and implications

To farm the housefly larvae in the UK and the BSF larvae in Ghana to meet the requirements as feed ingredients to local aquaculture, it was estimated that 335,036 tonnes of poultry manure and 38,997 tonnes of substrate mix (brewery wastes and feed factory wastes), respectively, were a prerequisite (Tables 7.3 and 7.4).

In 2015, in the UK, 929.9 million broilers were farmed with approximately 84 % of the production being in England and Wales (DEFRA, 2016a). Assuming that 1,000 broilers produce 0.42 tonne of manure per week and that they are farmed for 42 days (6 weeks), a total of 2.343 million tonnes manure was produced in 2015 (Chambers *et al*., 2001). This represents about 7 times the amount (335,000 tonnes/year) required in the model (1). Thus, poultry litter is generated in large quantity in the UK and requires to be properly managed in order to avoid public health and environmental risks (Burton and Turner, 2003). Currently poultry litter is mainly spread on lands as a fertiliser (700,000

tonnes/year), but that represented only 2 % of the farms in the UK in 2015 which preferred cattle or pig slurries (consistent and easy to manage and spread) to poultry wastes (DEFRA, 2016b), or it is used to produce energy in power stations (670,000 tonnes/year according to Slade *et al*. (2010). Lower volumes are also recycled in anaerobic digestion plants (approximately 30,000 tonnes/year) or in mushroom culture (approximately 10,000 tonnes/year) (P. Metcalfe, pers. communication 2016). Since 2014, an European Commission regulation (EU 592/2014) authorised farmers to use poultry manures as fuel in on-farm combustion plants, however this is still considered as negligible given the newness of the amendment (estimated < 10,000 tonnes/year). According to this, it is estimated that about 930,000 tonnes of poultry litter (approximately 40 % of the annual production) are stored on-farm annually (Figure 7.1) and despite the recommendation from the Food Standards Agency to store manure for no more than 8 weeks to reduce the risk of spreading resistant bacteria, it is often stacked for an average period of 7 months (DEFRA, 2010a, 2016b).

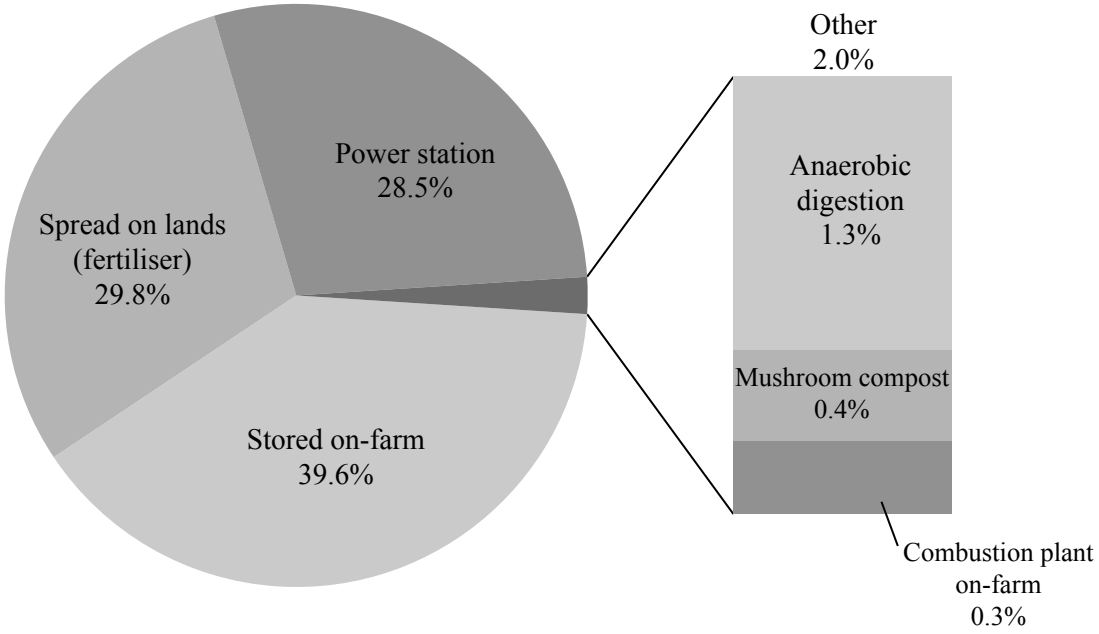

Figure 7.1 Uses of poultry litter produced within the UK in 2015

Manures stored on-farm may still be considered as potential sources of hazards (Nicholson *et al*., 2011) and represent the portion really available for the insect industry. On the farms where manure is stacked because it is produced during the period when manure cannot be spread (i.e. from October to April, according to DEFRA, 2009) or in excess with respect to the surface available for the application as a fertiliser (defined by the Department for Environment, Food and Rural Affairs and the European regulations), there are environmental and economic benefit to transfer / sell these wastes to another user such as insect farms which are able to recycle nutrients. Indeed, effective storage methods that reduce nitrogen losses from solid manure involve substantial investments (capital cost ~ 11,000 USD) and average annual cost are estimated at approximately 1,000 USD/farm (N-TOOLBOX, 2011; converted from British pounds as 1.0 USD = 0.70 GBP). Given the average nutrient composition of broiler litter (33; 22 and 34 kg of nitrogen phosphate and potash per tonne of manure, respectively) and the value of these nutrients (0.52; 0.42 and 0.30 USD/kg, respectively) manure surplus could be sold at 36.6 USD per tonne and generate additional incomes for the farm (DEFRA, 2006, 2010b). If one of the largest poultry farms in the UK is considered, producing 5.6 million broilers annually and 8,500 tonnes of manure subsequently; with 115 ha of arable land where 920 tonnes of broiler litter are spread annually (at a rate of 8.0 tonnes/ha/year to supply 250 kg/ha of total nitrogen as recommended by DEFRA (2003) and a biomass heating plant where 2,700 tonnes of manure are burnt as fuel annually (Clements, 2016; Uphouse Farm Ltd.) that means 4,880 tonnes of litter required storage annually, equivalent to 178,600 USD additional income if sold at 36.6 USD/tonne. However, to maintain production costs as low as possible, manure intended for insect production would have to be sold for less, especially considering that transportation by Heavy Goods Vehicle (HGV) might also be involved (1.14 USD/tonne/km in the UK (P. Metcalfe, pers. communication 2016). In 2007, Thetford power station (Norfolk, UK) was supplied with 420,000 tonnes of poultry litter per year from about 100 farms at a price of 14.3 USD/tonne, including transport (Metcalfe, 2007), suggesting that insect farms could consider paying less than 36.6 USD/tonne. Any co-location with the livestock farming industry would greatly limit the costs and proximity to concentration activity would certainly facilitate the tradability and value of the frass (discussed below). A similar approach could be applied to swine manure, also suitable for housefly larvae farming (Pastor, 2011; Čičková *et al*., 2012c; Zhu *et al*., 2015). When scientific evidences is available to

satisfy the EFSA standards, the environmental and economic benefits related to the conversion of manure into MM and frass will be additional incentives for policy makers to change the current regulation, thereby leading to the development of the insect farming industry in accordance with a circular economy strategy.

Leading actors of the insect sector have recently organised as a non-profit organisation called IPIFF (International Platform of Insects for Food and Feed) which aims at promoting insects as a sustainable solution to the global growing protein deficit. In Europe, IPIFF has made the case for the EU Regulation 999/2001 to change in order to facilitate the use of PAP of insects in aquaculture, providing the use of vegetal origin substrate only; they also indicate that insect companies are planning to invest more than €100 million once PAP of insects will be allowed for aquaculture (IPIFF, 2014). BSF larvae can be reared on vegetal origin substrates, which can come from the food wastes, for example. In the UK, despite the efforts made to continuously reduce wastage, it is estimated that 15 million tonnes of food waste are produced per annum including 7.0 million tonnes/year from households, 3.9 million tonnes/year from the food manufacturing industry (not including 4.2 million tonnes of food and animal by-products that are already diverted to animal feed manufacture or rendering derived), 0.9 million tonnes/year from the hospitality and food services and 0.2 million tonnes/year from the retail & wholesale sector (WRAP, 2013). Among those, only the food wastes from the retail & wholesale are currently authorised for the production of insects (EC regulation 1069/2009) and although not yet authorised, it would also be interesting to use the wastes from the hospitality and food services sectors and from households (i.e. table wastes). However, several problems related to the consistency and the collection remain; in fact, most of the food wastes generated by the household in the UK (92.0 %) are still not properly separated from the general waste (DEFRA, 2015) which make is difficult to access.

In Ghana, many wastes are often turned into resources: demand and price of animal manures, used as bio-fertiliser, are high (76 USD/tonne; considering 1.0 USD = 3.94 GHS) as availability is low and scattered (estimated to 64,000 tonnes/year), agro-wastes from large plantations (banana, pineapple, etc.) are often recycled in-house through composting; however, market wastes (fruits and vegetables) and organic wastes originated from the breweries, the abattoirs (18,000 tonnes/year of rumen content

estimated), etc. are free for whom is interested, in particular farmers who collect it to feed their livestock (C. Adeku, Osei-Boaten, A. Pile, N. Danion and M. Kape, pers. communications, 2013-2014). In addition, local conditions make waste selection, collection and transportation (25.3 USD/tonne delivered within 50 km from the collection point) difficult and expensive; thus, similarly to the situation in the UK and also applicable to other geographic contexts, proximity to substrate sources must be seriously considered to reduce the costs. Considering these constraints (cost, quantity, consistency of the substrates), it seemed that small or medium-scale production systems would be more suitable for the local conditions and therefore it is unlikely that amounts of MM produced would be sufficient to cover the demand of a large fish farm. However, small insect production units could focus on quality rather than quantity and MM produced could target the critical stages of intensive and semi-intensive aquaculture system such as fry and juveniles where specific requirements could be met with MM (Chapter 4 and 5). Thus, the final use and quality of the MM to produce, in this case, will drive the choice of the substrate since it influences the nutritional composition, in particular the FA composition, of the larvae. In LIDC it is more likely that processing methods such as defatting would be limited to very small amounts of MM owing to the quantity of solvent and equipment required but also the safety measures related to the handling and the process which might require large investments that cannot be considered at small or medium production scale. Cheaper and more applicable alternatives defatting methods, such as the use of a mechanical press, could be considered in these conditions, however no information is available on the efficiency of this method with insect material. Moreover, it is assumed that the mostly herbivorous and omnivorous fish species (tilapia, catfish, etc.) farmed in the tropics would cope well with crude MM. Moreover, as showed in Chapter 5, although MM couldn't be defatted, the enrichment of its nutritional quality with essential FA (n-3 PUFA) benefitted the feed manufacturer who alleviated his formulation by 75 % FM and 100 % FO without compromising the performance of tilapia fingerlings. In the latter study, enrichment was possible thanks to the addition of n-3 PUFA-rich processing wastes in the BSF larvae rearing substrate mix sourced from the local fish feed plant and clearly this was questionable in terms of sustainability and relevance (using fish feed processing wastes to produce a fish feed ingredient) but time constraints motivated this choice. Nevertheless, as previously demonstrated by St-Hilaire *et al*. (2007a) and Caruso *et al*.

(2014), fish offal sourced on local markets can be used instead. This enriched MM demonstrated the successful strategic use for tilapia fingerlings (Chapter 5).

The bioconversion processes with insects contribute in all cases to waste remediation by reducing volumes, dry matter, nutrients and pathogen organisms such as *E. coli*, *Salmonella* spp. of the considered substrates, lowering thereby public health and environmental risks associated with excess production, poor disposal and management and the related costs (Erickson *et al*., 2004; Newton *et al*., 2005a; Liu *et al*., 2008; Diener *et al*., 2011a; Wang *et al*., 2013; Lalander *et al*., 2014). So, cost and environmental benefit are incentives that encourage the development of the sector.

The development of small or medium-scale insect farms in LIDC could contribute to diversify rural incomes and may improve livelihoods. This approach opens opportunity for further research looking at the social aspects of the insect farming development. Nevertheless, such rural development raise an important question related to the technology transfer: how would the technological package required will be made available to farmers? Local examples such as existing operational sites (for example cricket farming or sericulture in South East Asia or maggot farming in Africa) or pilots developed in research projects would certainly contribute as knowledge platforms.

7.3.4 Frass

Finally, models (1) and (2) estimated that the bioconversion process of the respective substrates into MM would also result in 100,511 tonnes and 13,259 tonnes of frass, respectively (Tables 7.3 and 7.4). The volumes of frass generated are therefore substantial and consistent with previous studies (Calvert, 1979; Čičková *et al*., 2012b; Wang *et al*., 2013; Caruso *et al*., 2014), frass represented 94.4 and 96.5 % of the total weight of the products (i.e. MM + frass) generated by the bioconversion processes in models (1) and (2), respectively. Thus, it is critical to find appropriate applications and markets for the these by-products, to ensure the profitability and maximise the operational and environmental performance of insect farms (PROteINSECT, 2016a). According to the previous results (Chapter 6), frass are efficient fertilisers, but the tradability of these materials depends also on site-specific markets. Nevertheless, it is assumed that in every context considered, frass can become a competitive product on the organic fertilisers market. In the UK and other industrialised countries, frass would

probably need to be officially assessed (nutrient composition) in order to be listed as authorised organic fertilisers and to allow the development of recommendations. In Europe, as a Circular Economy Strategy is being developed (EC, 2016a), innovative bio-fertilisers produced according to the revised regulation (EC, 2015, 2016b) will be most favoured to contribute to the expected 30 % reduction of the inorganic fertiliser usage. Therefore, there is an opportunity here, for insect frass, to obtain the authorisation to access the market (CE-marked), thereby benefiting the economy (insect and crop industry) but also the environment. End-users could range from large-scale agriculture / crops sector to niche markets such as organic agriculture, small-scale or backyard horticulture and vegetable gardening. In Ghana, and more broadly in LIDC where inorganic fertilisers are intensively used for crops due to the limited availability and the cost of organic fertiliser such as manures (C. Adeku, pers. communication 2014), buying incentive towards frass may develop rapidly among small-holder farmers that cultivate crops near insect farming sites because the proximity is also important with respect to organic fertiliser sourcing (Owusu-Bennoah and Visker, 1994). Another solution for the frass, in particular in temperate areas, is the use as fuel in biomass heating systems as already applied for chicken manure. For instance, in the UK, as the use of fertiliser on arable land is limited and seems already saturated as farms are stocking excess manure, other solutions should be considered. Although this would induce additional investments, it would certainly be paid back within few years as the energy produced could be re-invested in a system warming up the facilities during cooler seasons (using a water boiling system) and to produce electricity, thereby reducing considerably the energy-related costs. According to processes previously set up in the UK (BHSL, 2016; Broiler Guide, 2011), about 1.3 million USD should be considered to set up a 500 kWth biomass burner which has a burning capacity of 10 tonnes biomass per day to provide 300 kWth of thermal and 40 kW of electricity. This would result in a cost saving of approximately 95,000 USD/year for gas (heating) and 31,500 USD/year for electricity; in addition, the ash produced can also be sold as a soil conditioner.

7.3.5 Conclusion

To summarise, although MM is a suitable feed ingredient that can be used in aquafeeds in particular as a source of protein, simple models developed in this study have

highlighted some remaining challenges related to the production capacity of the emerging insect farming industry and to site-specific related conditions. In the models presented here, figures were estimated only for one large-scale farm in each location; therefore, volumes of MM, substrate and frass to consider would be significantly greater if a wider approach was considered. It would be recommended to consider BSF farming in LIDC because the species is more resilient to adapt to a wide range of substrate sources, whereas, in industrialised contexts housefly or BSF would be both suitable. In both cases, the production of MM and frass, ultimately used in food production systems (aquaculture and crop culture), lead to waste remediation opportunities, economic and environmental benefits which contribute, thereby, to the development of sustainable circular economies. Moreover, as highlighted in this study, a strategic use of MM is recommended by targeting in priority fry and juvenile stages rather than grow-out, given that MM is a consistent high-quality raw material that can meet specific requirements at these critical stages but also to show proof of commercial concept.

Chapter 8. General discussion, conclusions and future perspectives

8.1 General

In the present study, different insect-based products, namely maggot meals (MM) and frass, produced in pilot-scale operation systems, were assessed as strategic feed ingredients for two major farmed fish species, Atlantic salmon (*Salmo salar*) and Nile tilapia (*Oreochromis niloticus*). Specifically, crude and defatted housefly larvae meals were first used to replace FM in diets for Atlantic salmon parr (Chapter 3). Then, cost-efficiency of crude and defatted Black Soldier Fly (BSF) larvae meals and crude housefly larvae meal were compared when replacing FM in simple diets for sex-reversal tilapia (Chapter 4). In Chapter 5, locally produced BSF meal was assessed as a FM and FO substitute in commercial diets for advanced nursing of tilapia. Efficacy of BSF frass derived from two different waste sources (food and brewery wastes) were also compared when used as supplementary feeds for tilapia in semi-intensively managed ponds or as soil bio-fertiliser for spring onions (Chapter 6). Finally, results of the previous studies were integrated in two contextualised models to estimate the volume of MM required for the aquaculture systems considered (Atlantic salmon in the UK and Nile tilapia in Ghana); this was further used to determine and discuss the implications for the production of the insect-derived products, with a focus on the volumes of MM and substrates required and the potential applications for the frass in each context (Chapter 7).

8.2 Quality of insect-based products

The aquafeed industry is relatively small compared to the livestock feed industry as compound feeds manufactured for the aquaculture sector represent only 4.0 % of the one billion tonne global annual production of animal feed (IFIF, 2014). However, aquaculture represents the largest consumer of marine ingredients with 60.8% of the global FM production and 73.8% of the global FO production in 2008 going to aquafeeds (Tacon *et al.*, 2011). Reliance on marine resources as important sources of essential amino acids and fatty acids has become a major issue over the last decades due to the volatility of the commodity markets related to the collapse of natural stocks (Naylor *et al*., 2009; FAO, 2014). Growth rate and intensification of the aquaculture sector, including a rapid increase of the proportion of fed fish and crustacean species, created a pressing need for alternative feed ingredients (Tacon and Metian, 2015).

In the global assessment of alternative feedstuffs, insects have been identified as candidates with a great potential. In particular, research recently acknowledged the similarities between the nutritional composition of fly larvae (Diptera Order; Insecta) and FM, and therefore the potential of maggot meals as feed ingredients for aquaculture species (Barroso *et al.*, 2014). In the present study, different insect-based products, namely BSF and housefly MM, have been assessed as potential feed ingredients for two major farmed species: Atlantic salmon (*S. salar*) and Nile tilapia (*O. niloticus*). These products, originating from pilot-scale farming systems located in the UK, Ghana and Malaysia, showed very interesting nutritional profiles.

In the first place, as previously reported by Henry *et al.* (2015), the MM used in the different studies had different nutritional compositions mainly due to the rearing substrates on which the larvae have fed and the processing method (Table 8.1). Specifically, MM lipid contents and FA compositions were strongly influenced by the rearing substrates; this was either advantageous (enrichment with essential FA, Chapter 5) or disadvantageous (FA composition that does not meet fish dietary requirements, Chapter 3 or high lipids levels complicating handling or affecting floatability, Chapter 4) depending on the context. However, the studies also showed that these issues can be overcome by using suitable processing methods to remove excessive fat; indeed, contrary to Lock *et al.* (2015) who reported the poor quality of a defatted BSF MM dried at high temperature, in Chapter 3, a solvent extraction method (hexane) followed by drying at low temperature led here to a MM of high quality (housefly). Nevertheless, in line with the findings of Barroso *et al.* (2014), MM from both fly species (housefly and BSF) used in the present study, were excellent sources of protein with comprehensive essential amino acid profiles comparable to FM (Chapter 3, 4 and 5).

Table 8.1 Substrates and nutritional compositions of the MM used in the study compared to fish meal (tuna, Thailand).

Meal	**Crude housefly**	**Defatted housefly**	**Crude BSF (Malaysia)**	**Defatted BSF (Malaysia)**	**Crude BSF (Ghana)**	**Fish meal (Tuna, Thailand)**
Substrate	Poultry manure	Poultry manure	Processed food wastes	Processed food wastes	Brewery + feed processing wastes	-
Proximate Composition (g/kg)						
Dry matter	956.1	975.7	929.8	915.4	950.3	911.0
Crude protein	457.4	562.5	334.1	473.4	416.4	558.5
Crude lipid	242.5	120.5	415.6	174.8	232.4	119.2
Ash	98.5	79.2	84.9	129.8	76.6	214.3
Crude fibre	74.7	91.8	56.9	68.9	116.5	5.3
NFE	83.0	121.7	38.3	68.4	108.4	13.7
Gross Energy (MJ/kg)	23.7	21.4	25.9	20.2	21.7	18.3
Essential Amino Acid Composition (g/100g meal)						
Histidine	1.26	1.61	1.21	2.10	1.18	1.59
Arginine	2.18	2.99	2.29	3.27	2.00	3.31
Threonine	1.95	2.45	1.48	2.39	1.72	2.42
Valine	2.18	2.67	2.43	3.85	2.63	2.76
Methionine	1.01	1.28	0.72	1.18	0.75	1.38
Lysine	3.39	4.32	2.21	3.53	2.70	3.93
Iso-Leucine	1.59	1.96	1.83	2.86	1.84	2.26
Leucine	2.65	3.36	2.79	4.11	2.90	3.86
Phenylalanine	2.53	3.38	1.67	2.51	1.75	2.24
Fatty Acid (g/100g meal)						
14:00	0.37	0.22	2.05	0.85	1.02	0.41
16:00	4.57	2.66	5.85	2.49	3.33	1.79
18:00	0.44	0.24	0.80	0.33	0.47	0.51
***Total Saturated*[1]**	***5.49***	***3.19***	***16.94***	***7.11***	***4.92***	2.86
16:1n-7	2.36	1.40	0.79	0.31	0.60	0.49
18:1n-9	5.07	2.95	5.50	2.36	2.67	1.11
22:1n-11	0.00	0.00	0.00	0.00	0.03	0.12
***Total monounsat.*[2]**	***8.44***	***5.00***	***6.73***	***2.75***	***4.08***	2.29
18:2n-6	3.57	1.87	2.51	1.19	1.86	0.23
20:4n-6	0.00	0.01	0.00	0.03	0.02	0.11
***Total n-6 PUFA*[3]**	***3.59***	***1.88***	***2.58***	***1.22***	***1.92***	0.47
18:3n-3	0.56	0.31	0.18	0.08	0.17	0.08
18:4n-3	0.07	0.03	0.09	0.02	0.19	0.07
20:5n-3 (EPA)	0.02	0.01	0.13	0.06	0.09	0.44
22:5n-3	0.00	0.00	0.00	0.00	0.00	0.07
22:6n-3 (DHA)	0.01	0.00	0.02	0.00	0.01	0.96
***Total n-3 PUFA*[4]**	***0.65***	***0.35***	***0.42***	***0.16***	***0.46***	1.67
***Total PUFA*[5]**	***4.27***	***2.27***	***3.02***	***1.39***	***2.38***	2.23
Total FA content	***18.20***	***10.48***	***26.70***	***11.25***	***11.39***	7.39

Values are presented 'as is'

Frass, the second insect-based product considered in this study, consisting of undigested substrate residues thoroughly mixed with maggots' excreta, also showed interesting nutrient profile (Chapter 6). First, the nutritional approach suggested that these BSF farming residues, derived from feed-grade materials (brewery spent grain or processed food wastes), could be suitable feedstuffs for low-trophic level fish such as tilapia cultured in semi-intensive conditions. Indeed, compared to rice bran, the common supplementary feed for tilapia, frass had roughly similar protein contents and gross energy levels but high fibre levels as well. However, further analyses indicated that both frass were actually low in protein-nitrogen (total AA), suggesting that the nutrient compositions of both type of frass were more similar to those of organic soil conditioners (compost or vermicompost). This corroborated studies from other authors (Choi *et al.*, 2009; Zhu *et al.*, 2012; Wang *et al.*, 2013; Lalander *et al.*, 2014), highlighting the high quality of frass as bio-fertilisers.

Although some challenges still remain in terms of production (Chapter 7), the emerging insect farming industry is, however, able to supply consistently and almost globally high-quality products (MM and frass) to food producing systems such as aquaculture and agriculture, thereby contributing significantly to food security.

8.3 A strategic use of maggot meals

Numerous studies have demonstrated the suitability of insect meals, in particular MM, as feed ingredients for fish (see paragraph 1.5.2). Nevertheless, the interspecies (fish and insects species studied) variability of the results makes a generalisation difficult. In the present study, MM were assessed as FM substitutes in the diets for Atlantic salmon parr, Nile tilapia fry and fingerlings because they are considered as critical stages in intensive farming processes. At these stages, the nutritional requirements are usually high and specific; thus, it was assumed that MM could contribute to reduce the reliance on FM because of their consistency and high-quality. Moreover, all the experiments described in this study were conducted on-farm in order to demonstrate the commercial relevance of the results.

8.3.1 Atlantic salmon

Although research has contributed to the decrease of FM in salmon diets over the last few years by identifying alternative sources of proteins, FM dietary inclusion remains

high in salmon feeds (200 to 500 g/kg (Tacon and Metian, 2008), especially in feeds intended for juveniles. After marine shrimp and fish, farmed Atlantic salmon is among the top consumers of FM, as the ingredient is considered ideal owing to its nutritional composition, meeting the fish dietary requirements (Tacon and Metian, 2008; NRC, 2011). In the present study (Chapter 3), the replacement of up to 50 % FM with crude or defatted housefly MM in a diet formulated for Atlantic salmon parr (containing initially 400 g/kg FM) led to growth performance (final weight, weight gain and SGR) and feed utilisation (FCR, PER, digestibility) similar to those observed with the FM-control diet. This is consistent with Lock *et al.* (2015) who found that BSF larvae meal could replace up to 50 % FM in post-smolt diets without affecting fish growth. However, lipid storage was greater in fish fed MM-based diets compared to the control and whole body FA composition mirrored that of the diets with higher saturated and n-6 PUFA levels and lower n-3 PUFA levels with increased levels of MM in the diet, similar to that previously reported by other authors (Turchini *et al.*, 2009; Sealey *et al.*, 2011; Belforti *et al.*, 2015). Despite the main dietary lipid source (fish oil) being maintained at a constant level among treatments, thereby keeping essential FA present in the diets, apparent digestibility of the lipids and saturated FA decreased with increasing MM inclusions; this suggested that the MM lipid and FA composition might affect the quality of salmon parr diets. On the other hand, the trend of the results reported with the diet containing 200 g/kg defatted housefly MM, demonstrated slightly better fish performance and lipid digestibility than the diet containing 200 g/kg crude MM. The use of defatted MM as feed ingredient for fish was already encouraged by Fasakin *et al.* (2003) but the present results were contrary to those of Lock *et al.* (2015) who used a defatted BSF MM whose quality might have deteriorated during the drying process. This point to a requirement for refinement in meal processing technologies for any industrial scale-up.

This is the first time MM is used in Atlantic salmon parr diets and the results obtained are really encouraging. In addition, protein digestibility of the MM-based diets was significantly improved compared to the control, confirming the high quality of the MM proteins. Although in this study the diets were formulated to represent modern formulations for Atlantic salmon parr, it is expected that further improvements according to the aquafeed industry standards (least-cost formulation and nutrients balance) could lead to better performance.

According to these results and assuming that housefly MM could substitute 50 % FM in farmed Atlantic salmon diets throughout the whole life cycle, it was estimated that for a farm producing nearly 49,000 tonnes salmon per annum, about 5,600 tonnes of MM would be required (Chapter 7). The model also predicted that 335,000 tonnes of poultry manure (60 % DM) would be required to grow the maggots (28,000 tonnes/year). Although some concerns about the production capacity of the emerging insect farming industry remain, it is likely that if MM is authorised as feed ingredient in aquaculture, this issue will rapidly be overcome. Meanwhile, because the largest amounts of MM would be required for the grow-out stages (estimated to more than 95 % of the total amount of MM required for the whole process), it would be recommended to start using this feed ingredient strategically. Thus, freshwater stages (fry and smolt), which are more likely to perform well with insect meals considering their natural feeding behaviour (Scott and Crossman, 1973; Amundsen *et al*., 2001), should be considered first; then, post-smolt, which showed good response as well, according to Lock *et al.* (2015), could also be included. The lack of data for the post-smolt stage (number of fish produced annually) did not allow the estimation of MM required for this stage. In addition, this would be a start to show proof of commercial concepts.

Furthermore, according to secondary data (national surveys), it seems that the resources (substrates) needed to farm the insects are available in large quantities and consistently in the UK, and probalbly more broadly in other industrialised countries. In fact, with the development of a Circular Economy Strategy in Europe (EC, 2016a), it is expected that enterprises developing solutions to convert wastes into valuable products, such as the maggot farming industry which contributes to waste remediation and food security, will be encouraged. The use of waste sources to farm maggots is encouraged; poultry manure for instance can be used to farm housefly larvae as demonstrated in a pilot system developed in the UK. Large volumes produced each year are mainly spread on arable lands (30%), turned into energy in power stations (30%) or stored on-farm (40%). The recycling of stored manures, which can represent a source of risks for the environment (pollution) or for the public health (Nicholson *et al*., 2011), through bioconversion processing involving fly larvae can contribute significantly to the development of waste remediation strategies. Related economic and environmental benefits would certainly be significant incentive encouraging both farmers and policy makers to engage in this novel industry, thereby contributing to the development of

sustainable circular economies. Moreover, implication for the Scottish salmon industry might be significant as it would reduce its dependency on imported soy and fish meal while integrating local livestock industry (i.e. poultry farming) into a more wider economy.

8.3.2 Nile tilapia

Low-trophic omnivorous and herbivorous fish species such as the Nile tilapia are more flexible in terms of feed ingredients and require less FM than carnivorous fish species (Tacon and Metian, 2015). Nevertheless, FM is often included in tilapia diets, particularly for fry and fingerlings because it is considered as an excellent source of essential nutrients and improve the feeding response, which an important criteria in the competitive feed industry (Jauncey, 1998). This also suggest that FM is still too cheap despite its increasing price? Because tilapia has a low market value, the feed related costs in farming systems are restricted. Moreover, considering that most tilapia are produced in LIDC, where high-quality FM is expensive and poorly available or inconsistently, locally produced alternative sources of protein such as MM, could contribute to support the development of sustainable and economically viable tilapia aquaculture.

In the present study, BSF and housefly MM were used to substitute FM in the diets of sex-reversal fry and nursing Nile tilapia in commercial set up in Thailand and Ghana, respectively. In the first experiment (Chapter 4), the results indicated that, at the end of a 21-day period, successful sex-reversal was achieved (99.8 to 100 % males) with fry fed MT-treated diets containing 25 to 100 % crude or defatted BSF MM or crude housefly MM. High survival was reported for all the treatments indicating no influence of the MM dietary inclusions; survival rates were also comparable to those reported by Vera Cruz and Mair (1994). This means that, similarly to FM which commonly used as hormone carrier in commercial hatcheries, MM are of high quality and highly palatable feed ingredients for fish. However, evenness of the fish harvested and fish performance results suggested that mixed ingredient diets performed better than single ingredients (FM or MM alone); the better nutrient balance resulting from the ingredients blend explained this result (NRC, 2011; Parker, 2011). A simple economic study was also conducted in order to compare the cost-effectiveness of the different MM, assuming that MM market price ranged between 30 % more or less the current price of the FM

(1.0 USD, Thai market price, November 2015). Results suggested that, although in the experiment it performed well as a hormone carrier and similarly to FM, crude BSF meal was economically less attractive than crude housefly and defatted BSF MM and, thus not recommended for sex-reversal fry. Moreover, in line with Fasakin *et al.* (2003) and Chapter 3's results, the economic analysis highlighted the great potential of defatted MM as it led to the better economic performance than the two types of crude MM. Therefore, this study also recommended the use of defatted MM. Depending on the market price, crude housefly MM or defatted BSF MM can be used as a single ingredients (substituting totally FM) or key ingredients in a formulated diet for Nile tilapia sex-reversal fry; similarly, silkworm pupae meal was suggested as potential alternative to FM for sex-reversal tilapia fry in Thailand, but the limited availability of the product could not match the demand and the research were stopped (Bhujel, 2013).

The experiment presented in Chapter 5 was conducted in Ghana, over 32 days, to evaluate the suitability of crude BSF MM, as partial substitute to FM (25 to 75 % substitution) in commercially formulated diets for advanced nursing of Nile tilapia, containing initially 100 g/kg FM. Due to the high lipid content of the MM, sufficient to cover tilapia juvenile dietary lipid requirement for (NRC, 2011), FO was not included in the test diets. Similarly to that reported in other studies where FM was replaced by housefly or blowfly MM in practical diets for Nile tilapia fingerlings (Ogunji *et al*., 2008a, 2008b, 2008c; Sing *et al*., 2014), growth performance (final weight; weight gain and SGR); feed utilisation efficiency indices (FCR and PER) and feed intake of the fish cultured in cage-in-lake were not significantly different between treatments. Despite the differences in survival between the treatments, which were more likely related to the stocking size of the fish (smaller fish were probably less resistant to frequent handling) than being a treatment effect, overall survival was good. Fish whole body composition (dry matter, crude protein, lipid, ash and fibre) was also not affected by the treatments, except for the FA compositions, which mirrored that of the diets, especially by increasing the n-6 PUFA and decreasing the n-3 PUFA levels consequently to the FO substitution. This was also reported in Chapter 3 and by Sánchez-Muros *et al*. (2015) while replacing 50 % FM and 100 % FO with a *Tenebrio molitor* larvae meal in a diet for Nile tilapia fingerlings. Judging by the results of Ogunji *et al*. (2008a, 2008b, 2008c) and Sing *et al*. (2014), MM could probably be included in tilapia fingerlings diets at rates greater than 75.0 g/kg (present study), thereby substituting other sources of

protein concurrently to FM. Unlike these studies, higher inclusions could not be tested due to limited amounts of MM available; thus further investigations would be required to test greater inclusion levels in least-cost diets.

According to the results of the two latter discussed experiments (Chapter 4 and 5) and assuming that the substitution of 75 % FM is applicable to first nursing and grow-out stages, it was estimated that, about 500 tonnes of BSF MM per annum were necessary to manufacture the 10,700 tonnes of feeds required by a tilapia farm producing nearly 6,500 tonnes fish annually (Chapter 7). The model also predicted approximately 40,000 tonnes of substrate (40 % DM) would be required to grow the maggots (1,600 tonnes/year). Contrary to the expectations of development in industrialised countries, it is suggested that small or medium-scale insect farming systems would be more suitable in LIDC given to the economic and local constraints and the limited availability of substrates/wastes to grow the maggots (PROteINSECT, 2016a). Indeed, what is usually considered as wastes in industrialised area is often a valuable resource in developing countries, restricting substantially the list potential substrates available to farm maggots cost-efficiently. In addition, local conditions make waste selection, collection and transportation difficult and expensive. Therefore, it is unlikely that amounts of MM produced would be sufficient to cover the demand of large fish farm; for example, the production capacity of the BSF pilot developed in Ghana was estimated at 416-780 kg MM/year. Nevertheless, by selecting the substrates cautiously (St-Hilaire *et al*., 2007a; Caruso *et al*., 2014), small-holder farmers could focus on quality rather than quantity and the small amounts of high-quality MM produced could be used as key ingredients targeting strategically the critical stages of intensive and semi-intensive aquaculture systems, such as fry and juveniles where, like for salmons, the requirements for high-quality feeds and feed ingredients are significantly higher but the volumes required are substantially lower than for food-fish. As this perspective is an opportunity to diversify local rural incomes and improve livelihood, technology transfer plans would have to be implemented, using, for example, traditional operation already existing (i.e. cricket farming or sericulture in South East Asia) or the pilots developed in related projects (PROteINSECT in West Africa).

8.4 Recommended applications for frass

First assessment of BSF frass derived from two feed-grade materials, namely brewery spent grains (BW) and processed food wastes (FW), suggested a potential application in aquaculture feed (Chapter 6). Therefore, frass were used as supplementary feeds to 17.0 g tilapia in green water pond (semi-intensive-like conditions) for 3 months and fish performance were evaluated in comparison to unfed fish (relying on the natural productivity) or to those fed rice bran. This was the first time insect frass were fed directly to fish as single feed ingredients but the results did not satisfy the hypothesis. Indeed, frass-fed fish performed similarly to the fish relying on the pond natural food, whereas, rice bran fed fish performed significantly better than those fed the frass or relying on the natural food. These results indicated that frass might not be a direct source of nutrients for fish owing to high fibre and non-protein nitrogen levels making its value as an organic fertiliser and soil conditioner greater than as a feedstuff.

Even if frass could be used as a feed ingredient or a supplementary feed for fish, the amounts required would be negligible compared to quantities of frass that result from a maggot farming system (Chapter 7). Operational results and environmental performance of maggot farms rely on the tradability of these co-products (PROteINSECT, 2016a); thus, like MM, a strategic use should be considered.

In the second experiment presented in Chapter 6, the same BSF frass used in the first experiment with tilapia were also assessed as soil organic conditioners. The results of this pot trial, conducted under climate controlled conditions, indicated that frass are effective organic fertilisers; similar observations were reported by Choi *et al*. (2009) with cabbages. However, the study suggested that efficacy and minimum application rates might depend on the type of frass (i.e. type of substrate initially used to grow the maggots) which might influence the frass nutrients composition. Yields comparable to those obtained with a soil fertilised with NPK inorganic fertiliser, were achieved with a minimum of 5.0 tonnes/ha FW frass or 10.0 tonnes/ha BW, but contrary to the NPK, the use of frass did not alter the soil pH and improved the soil organic matter (OM) and electrical conductivity. In addition, the results highlighted that increasing levels of frass resulted in increasing soil OM and electrical conductivity and therefore, increasing yields and that FW frass performed better than BW frass as bio-fertilisers. Thus, soil

fertility, stability and productivity (crop response) were positively related to the organic fertilisers' application rates.

This is could encourage farmers to apply greater amounts of frass to achieve better productivity; nevertheless, if frass becomes marketable as fertiliser, it will become crutial to define recommended and maximum application rates in order to limit the risks of pollution related to nutrient leaching in water (D'Haene *et al.*, 2014), but also because, at high concentrations, nutrients such as N-based elements may become toxic and suppress plants growth (Gerendás *et al.*, 1997; Jaynes *et al.*, 2004). In most countries, inorganic and organic fertilisers are subjected to regulations; thus comprehensive assessment using standard procedures for quality and safety testing will be surely required for frass to be marketed as bio-fertilisers. In Europe, the current Circular Economy Strategy might be a significant programme promoting frass as bio-fertiliser as it aims at revising, *inter alia,* the current legislations on wastes and fertilisers, thereby allowing better waste management through recycling into valuable resources to which access to market would be facilitated (EC, 2016a). In Chapter 7, it has been showed that in countries like Ghana, large plantations usually recycle their own wastes to produce compost (A. Pile; N. Danion, pers. communications 2013- 2014) whereas small-holder farmers rely mostly on inorganic fertilisers and applied organic soil conditioner if the resources are locally available because of the prohibitive transportation costs (Owusu-Bennoah and Visker, 1994; IFDC, 2012). Considering the evolution of the commodities prices (manures, inorganic fertilisers, etc.), it is likely that frass market prices will be competitive with those of the local, commonly used, organic fertilisers, thereby suggesting that this secondary insect-based product could also be marketable in LIDC.

8.5 Future perspectives

This study highlighted the high potential of housefly and BSF MM as quality feed ingredients for farmed fish, specifically during the first life stages where low amounts of MM can meet the specific requirements of fry and fingerlings. Accounting for site-specific socioeconomic and environmental conditions, the production of consistent high-quality MM and frass, based on a circular economy strategy that allows the recycling of wastes sources into valuable products, can support the development of sustainable aquaculture globally and, through making the by-product frass available as

high-quality organic fertiliser for soil, improve sustainability and profitability of surrounding crop culture.

That said, further investigations are required to better assess the potential of defatted MM in particular. The outcomes of this study suggested that greater inclusion rates and better performance could be achieved using defatted MM rather than crude MM. Research could investigate further the cost-benefits of using this highly processed material over crude MM for different fish species in commercial settings.

For omnivorous and herbivorous species, such as tilapia, further research could consider higher dietary inclusions of MM than those applied here. The use of MM in marine ingredients-free diets, as a feedstuff that can provide essential nutrients such as EAA and EFA, could greatly benefit the aquafeed industry.

Insect frass are undeniably suitable soil fertilisers; nevertheless more research is needed to assess this valuable by-product. Further investigations could look more deeply at the nutrient composition of frass derived from various organic materials in order to define sustainable and optimum use in soil. Furthermore, there is still an opportunity for frass to contribute to fish farming if used as an organic fertiliser in ponds and this is yet to be tested.

Finally, although the Atlantic salmon and Nile tilapia are two economically relevant species in aquaculture, future research should extend towards other species such as shrimps and prawns, intensively farmed in various countries. Also, ornamental fish market may represent an interesting niche for high-value applications of insect based products.

References

Abdissa, Y., Tekalign, T., Pant, L.M., 2011. Growth, bulb yield and quality of onion (*Allium cepa* L.) as influenced by nitrogen and phosphorus fertilization on vertisol I. growth attributes, biomass production and bulb yield. African J. Agric. Res. 6, 3252–3258. doi:10.5897/AJAR10.1024

Abowei, J.F.N., Ekubo, A.T., 2011. A Review of Conventional and Unconventional Feeds in Fish Nutrition. Br. J. Pharmacol. Toxicol. 2, 179–191.

Abucay, J.S., Mair, G.C., 1997. Hormonal sex reversal of tilapias: implications of hormone treatment application in closed water systems. Aquac. Res. 28, 841–845. doi:10.1046/j.1365-2109.1997.00878.x

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 Aquac. Mag. 35, 52–54.

Alvarez, L., 2012. The Role of Black Soldier Fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) in Sustainable Waste Management in Northern Climates. University of Windsor.

Amundsen, P.-A., Gabler, H.-M., Riise, L.S., 2001. Intraspecific food resource partitioning in Atlantic salmon (*Salmo salar*) parr in a subarctic river. Aquat. Living Ressources 14, 257–265.

Andrews, M., Sprent, J.I., Raven, J.A., Eady, P.E., 1999. Relationships between shoot to root ratio, growth and leaf soluble protein concentration of *Pisum sativum*, *Phaseolus vulgaris* and *Triticum aestivum* under different nutrient deficiencies. Plant, Cell Environ. 22, 949–958. doi:10.1046/j.1365-3040.1999.00452.x

Aniebo, A.O., Erondu, E.S., Owen, O.J., 2009. Replacement of fish meal with maggot meal in African catfish (*Clarias gariepinus*) diets. Rev. UDO Agrícola 9, 666–671.

Aniebo, A.O., Owen, O.J., 2010. Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal (HFLM). Pakistan J. Nutr. 9, 485–487. doi:10.3923/pjn.2010.485.487

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

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13, 265–272. doi:10.1016/0044-8486(78)90008-X

Awoniyi, T.A.M., 2007. Health, Nutritional and Consumers' Acceptability Assurance of Maggotmeal Inclusion in Livestock Diet: A Review. Int. J. Trop. Med. 2, 52–56.

Ayoola, A.A., 2010. Replacement of Fishmeal with Alternative Protein Sources in Aquaculture Diets. North Carolina State University.

Bailey, R.M., Harrison, H.M.J., 1948. Food Habits of the Southern Channel Catfish

(*Ictalurus Lacustris Punctatus*) in the Des Moines River, Iowa. Trans. Am. Fish. Soc. 75, 110–138. doi:10.1577/1548-8659(1945)75[110:FHOTSC]2.0.CO;2

Banjo, A.D., Lawal, O.A., Adeduji, O.O., 2005. Bacteria and fungi isolated from housefly (*Musca domestica* L.) larvae. African J. Biotechnol. 4, 780–784.

Barroso, F.G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. Aquaculture 422-423, 193–201. doi:10.1016/j.aquaculture.2013.12.024

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., Zoccarato, I., Gasco, L., 2015. *Tenebrio molitor* meal in rainbow trout (*Oncorhynchus mykiss*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. Ital. J. Anim. Sci. 14. doi:10.4081/ijas.2015.4170

Bell, J.G., Ghioni, C., Sargent, J.R., 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. Aquaculture 128, 301–313. doi:10.1016/0044-8486(94)90319-0

Bergheim, A., Drengstig, A., Ulgenes, Y., Fivelstad, S., 2009. Production of Atlantic salmon smolts in Europe-Current characteristics and future trends. Aquac. Eng. 41, 46–52. doi:10.1016/j.aquaeng.2009.04.004

Bernier, P.Y., Lamhamedi, M.S., Simpson, D.G., 1995. Shoot:Root ratio is of limited use in evaluating the quality of container conifer stock. Tree Plant. Notes 46, 102–106.

Beveridge, M.C.M., McAndrew, B.J., 2000. Tilapias: Biology and Exploitation. Kluwer Academic Publishers.

Beveridge, M.C.M., Thilsted, S.H., Phillips, M.J., Metian, M., Troell, M., Hall, S.J., 2013. Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. J. Fish Biol. 83, 1067–1084. doi:10.1111/jfb.12187

BHSL, 2016. Uphouse Farm, Norfolk. Transforming Poultry Production [WWW Document]. URL <http://www.bhsl.com/portfolio-item/uphouse-farm-norfolk/>

Bhujel, R.C., 1997. Fry quality is the main factor behind the success of AIT's joint venture tilapia hatchery. AASP Newsl. 3.

Bhujel, R.C., 2013. Feed management practices for Nile tilapia in Thailand, in: On-Farm Feeding and Feed Management in Aquaculture. pp. 159–189.

Bhujel, R.C., 2014. A manual for tilapia business management. CABI Publisher.

Biancarosa, I., Liland, N.S., Biemans, D., Bruckner, C., Amlund, H., Torstensen, B., Lock, E., 2015. Changes in nutrient and heavy metal composition of the Black Soldier Fly larvae (*Hermetia illucens*) fed on marine microalguae, in: Aquaculture Europe 2015. Rotterdam, Netherlands.

Bolivar, R.B., Jimenez, E.B.T., Sugue, J.R.A., Brown, C.L., 2004. Effect of stocking

sizes on the yield and survival of Nile tilapia (*Oreochromis niloticus* L.) on-grown in ponds, in: 6th International Symposium on Tilapia in Aquaculture Philippine International Convention Center Roxas Boulevard, Manila, Philippines September 12-16, 2004. pp. 574–583.

Bondari, K., Sheppard, D.C., 1981. Soldier fly larvae as feed in commercial fish production. Aquaculture 24, 103–109. doi:10.1016/0044-8486(81)90047-8

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). Aquac. Fish. Manag. 18, 209–220. doi:10.1111/j.1365-2109.1987.tb00141.x

Borowska, J., Sulima, B., Niklinska, 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 Environ. Bull. 13, 1402–1409.

Borowska, J., Pyza, E., 2011. Effects of heavy metals on insect immunocompetent cells. J. Insect Physiol. 57, 760–770. doi:10.1016/j.jinsphys.2011.02.012

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., Corner, R., 2010. Aquaculture: global status and trends. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 2897–2912. doi:10.1098/rstb.2010.0170

Boyd, C.E., 1982. Water quality management for pond fish culture. ElsevierScience Publishers B. V.

Bradley, S.W., Sheppard, D.C., 1984. Housefly oviposition inhibition by larvae of *Hermetia illucens*, the Black Soldier Fly. J. Chem. Ecol. 10, 853–859.

Broiler Guide, 2011. Poultry Farmers Investing in Biomass [WWW Document]. URL <https://www.boilerguide.co.uk/poultry-farmers-investing-in-biomass>

Brown, C.L., Bolivar, R.B., Jimenez, E.T., Szyper, J., 2000. Timing of the onset of supplemental feeding of Nile tilapia (*Oreochromis niloticus*) in ponds, in: Fitzsimmons, K., Filho, J.C. (Eds.), Tilapia Culture in the 21st Century. Proc. 5th Int. Symp. Tilapia Aquacult., American Tilapia Association and ICLARM. Rio de Janeiro, Brazil, pp. 237–240.

Bukkens, S.G.F., 1997. The nutritional value of edible insects. Ecol. Food Nutr. 36, 287–319. doi:10.1080/03670244.1997.9991521

Burtle, G., Newton, G.L., Sheppard, D.C., 2012. Mass Production of Black Soldier Fly Prepupae for Aquaculture Diets, A Manuscript for Aquaculture International. University of Georgia, Tifton Campus, Tifton, GA.

Burton, C.H., Turner, C., 2003. Manure management. treatment strategies for sustainable agriculture, 2nd ed. Silsoe Research Institute, Bedford, UK.

Byrne, J., 2016. Coppens starts using insect fats in feed. Feednavigator.com.

Calvert, C.C., 1979. Use of Animal Excreta for Microbial and Insect Protein Synthesis.

J. Anim. Sci. 48, 178–192.

Caruso, D., Devic, E., Subamia, I.W., Talamond, P., Baras, E., 2014. Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) *Hermetia illucens*, Stratiomyidae. PT Penerbit IPB Press.

Chambers, B., Nicholson, N., Smith, K., Pain, B., Cumby, T., Scotford, I., 2001. Managing Livestock Manures. Booklet 1: Making better use of livestock manures on arable land.

Chander, K., Goyal, S., Mundra, M.C., Kapoor, K.K., 1997. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biol. Fertil. Soils 24, 306–310. doi:10.1007/s003740050248

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R., Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. J. Insects as Food Feed 1, 7–16. doi:10.3920/JIFF2014.0020

Choi, Y., Choi, J., Kim, J., Kim, M., Kim, W., Park, K., Bae, S., 2009. Potential Usage of Food Waste as a Natural Fertilizer after Digestion by *Hermetia illucens* (Diptera : Stratiomyidae). Int. J. Indust. Entomol. 19, 171–174.

Christie, W.W., 1993. Preparation of Ester Derivatives of Fatty Acids for Chromatographic Analysis, in: Christie, W.W. (Ed.), Advances in Lipid Methodology - Two. The Oily Press, Dundee, pp. 69–111.

Christie, W.W., 2003. Lipid Analysis - Isolation, Separation, Identification and Structural Analysis of Lipids, 3rd ed. The Oily Press.

Čičková, H., Kozánek, M., Moravek, I., Takáč, P., 2012a. A Behavioral Method for Separation of House Fly (Diptera : Muscidae). J. Econ. Entomol. 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. Bull. Entomol. Res. 103, 119–125. doi:10.1017/S000748531200065X

Čičková, H., Pastor, B., Kozánek, M., Martínez-Sánchez, A., Rojo, S., Takáč, P., 2012c. Biodegradation of Pig Manure by the Housefly, *Musca domestica*: A Viable Ecological Strategy for Pig Manure Management. PLoS One 7. doi:10.1371/journal.pone.0032798

Čičková, H., Newton, L.G., Lacy, C.R., Kozánek, M., 2015. The use of fly larvae for organic waste treatment. Waste Manag. 35, 68–80. doi:10.1016/j.wasman.2014.09.026

Clements, M., 2016. Burning poultry litter creates clean energy for producer. [WWW Document]. URL <http://www.wattagnet.com/articles/25689-burning-poultry-litter-creates-clean-energy-for-producer>

Crampton, V.O., Nanton, D.A., Ruohonen, K., Skjervold, P.O., El-Mowafi, A., 2010. Demonstration of salmon farming as a net producer of fish protein and oil. Aquac. Nutr. 16, 437–446. doi:10.1111/j.1365-2095.2010.00780.x

D'Haene, K., Salomez, J., De Neve, S., De Waele, J., Hofman, G., 2014. Environmental performance of nitrogen fertiliser limits imposed by the EU Nitrates Directive. Agric. Ecosyst. Environ. 192, 67–79. doi:10.1016/j.agee.2014.03.049

D'Cotta, H., Fostier, A., Guiguen, Y., Govoroun, M., Baroiller, J.F., 2001. Aromatase plays a key role during normal and temperature-induced sex differentiation of tilapia *Oreochromis niloticus*. Mol. Reprod. Dev. 59, 265–276. doi:10.1002/mrd.1031

Dalla Valle, A.Z., Iriti, M., Faoro, F., Berti, C., Ciappellano, S., 2008. In vivo prion protein intestinal uptake in fish. Apmis 116, 173–180. doi:10.1111/j.1600-0463.2008.00863.x

Dambo, W.B., Rana, K.J., 1992. Effect of stocking density on growth and survival of *Oreochromis niloticus* (L.) fry in the hatchery. Aquac. Fish. Manag. 23, 71–80.

Das, M., Ganguly, A., Haldar, P., 2010. Nutrient Analysis of Grasshopper Manure for Soil Fertility Enhancement. Am. J. Agric. Environ. Sci. 7, 671–675.

Dato-Cajegas, C.R.S., Yakupitiyage, A., 1996. The need for dietary mineral supplementation for Nile tilapia, *Oreochromis niloticus*, cultured in a semi-intensive system. Aquaculture 144, 227–237. doi:10.1016/S0044-8486(96)01292-6

De Silva, S.S., 1993. Supplementary feeding in semi-intensive aquaculture systems, in: New, M.B., Tacon, A.G.J., Csavas, I. (Eds.), Farm-Made Aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992, Bangkok, Thailand. FAO-RAPA/AADCP, Bangkok, Thailand, 434p. pp. 24–60.

De Silva, S.S., Anderson, T.A., 1994. Fish nutrition in aquaculture, 1st ed. Chapman & Hall, London, UK.

Defoliart, G.R., 1995. Edible insects as minilivestock. Biodivers. Conserv. 4, 306–321. doi:10.1007/BF00055976

DEFRA, 2003. Manure Management Plan.

DEFRA, 2005. Process Guidance Note 6/5 (05). Secretary of State's Guidance for Maggot Breeding Processes. Crown, London, UK.

DEFRA, 2006. Saving money by reducing waste. Waste minimisation manual: a practical guide for farmers and growers. Crown, London, UK.

DEFRA, 2009. Guidance for Farmers in Nitrate Vulnerable Zones. Storage of organic manure. Crown, London, UK.

DEFRA, 2010a. Pigs and Poultry Farm Survey 2009 - England.

DEFRA, 2010b. Fertiliser Manual (RB209), 8th ed. TSO.

DEFRA, 2013. Nutrients, fertilisers and manures [WWW Document]. URL <https://www.gov.uk/guidance/managing-nutrients-and-fertilisers>

DEFRA, 2015. Digest of Waste and Resource Statistics – Department for Environment

Food & Rural Affairs 2015 Edition.

DEFRA, 2016a. UK Poultry Slaughterings, Weights and Poultry Meat Production - Monthly Dataset.

DEFRA, 2016b. The British Survey of Fertiliser Practice. Fertiliser Use on Farm Crops for Crop Year 2015. Crown.

Devic, E., Maquart, P.-O., 2015. *Dirhinus giffardii* (Hymenoptera: Chalcididae), parasitoid affecting Black Soldier Fly production systems in West Africa. Entomologia 3, 25–27. doi:10.4081/entomologia.2015.284

Diana, J.S., Lin, C.K., Jaiyen, K., 1994. Supplemental Feeding of Tilapia in Fertilized Ponds. J. World Aquac. Soc. 25, 497–506. doi:10.1111/j.1749-7345.1994.tb00818.x

Diana, S., 1996. Timing of Supplemental Feeding for Tilapia Production. J. World Aquac. Soc. 27, 410–419. doi:10.1111/j.1749-7345.1996.tb00625.x

Diener, S., 2010. Valorisation of Organic Solid Waste using the Black Soldier Fly, *Hermetia illucens*, in Low and Middle-Income Countries 5–73. doi:10.3929/ethz-a-6559779

Diener, S., Studt Solano, N.M., Roa Gutiérrez, F., Zurbrügg, C., Tockner, K., 2011a. Biological Treatment of Municipal Organic Waste using Black Soldier Fly Larvae. Waste and Biomass Valorization 2, 357–363. doi:10.1007/s12649-011-9079-1

Diener, S., Zurbrügg, C., Gutiérrez, F.R., Nguyen, D.H., Morel, A., Koottatep, T., Tockner, K., 2011b. Black soldier fly larvae for organic waste treatment - prospects and constraints, in: Proceedings of the WasteSafe 2011 – 2nd International Conference on Solid Waste Management in the Developing Countries.

Dong, G.F., Yang, Y.O., Song, X.M., Yu, L., Zhao, T.T., Huang, G.L., Hu, Z.J., Zhang, J.L., 2013. Comparative effects of dietary supplementation with maggot meal and soybean meal in gibel carp (*Carassius auratus gibelio*) and darkbarbel catfish (*Pelteobagrus vachelli*): Growth performance and antioxidant responses. Aquac. Nutr. 19, 543–554. doi:10.1111/anu.12006

Drew, D.J.W., Pieterse, E., 2015. Markets, money and maggots. J. Insects as Food Feed 1, 227–231. doi:10.3920/JIFF2014.0018

Ebenso, I.E., Udo, M.T., 2003. Effect of live maggot on growth of the Nile perch, *Oreochromis niloticus* (Cichlidae) in South Eastern Nigeria. Glob. J. Agric. Sci. 2, 72–73.

EC (European Parliament Council of the European Union), 2001. Regulation (EC) 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Off. J. Eur. Union L 147, 1–40.

EC (European Parliament Council of the European Union), 2009. Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not

intended for human consumption and repealing. Regulation (EC) No 1774/2002 (Animal by-products Regulation). Off. J. Eur. Union L 300, 1-27.

EC (European Commission), 2013. Commission Regulation (EU) No 56/2013 of 16 January 2013 amending amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. Off. J. Eur. Union L 21, 3–16.

EC, 2014. Commission regulation (EU) No 592/2014 of 3 June 2014 amending Regulation (EU) No 142/2011 as regards the use of animal by-products and derived products as a fuel in combustion plants. Off. J. Eur. Union L 165, 33–40.

EC (European Commission), 2015. Revision of the Fertilisers Regulation (EC) No 2003/2003.

EC (European Commission), 2016a. Circular Economy Strategy. Closing the loop - An EU action plan for the Circular Economy [WWW Document]. URL <http://ec.europa.eu/environment/circular-economy/index_en.htm>

EC, 2016b. Proposal for a regulation of the european parliament and of the council laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009.

Edwards, P., Polprasert, C., Pacharaprakiti, C., Rajput, V.S., Suthirawut, S., 1983. Compost as fish feed, a practical application of detritivory for the cultiation of tilapia. Aquaculture 32, 409–413.

EFSA Scientific Committee, 2015. Risk profile related to production and consumption of insects as food and feed EFSA Scientific Committee. doi:10.2903/j.efsa.2015.4257

El Boushy, A.R., 1991. House-fly pupae as poultry manure converters for animal feed: A review. Bioresour. Technol. 38, 45–49. doi:10.1016/0960-8524(91)90220-E

El-Greisy, Z.A., El-Gamal, A.E., 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. Egypt. J. Aquat. Res. 38, 59–66. doi:10.1016/j.ejar.2012.08.005

Ellis, T., Gardiner, R., Gubbins, M., Reese, A., Smith, D., 2015. Aquaculture statistics for the UK , with a focus on England and Wales 2012.

El-Sayed, A.-F.M., Tacon, A.G.J., 1997. Fishmeal replacers for tilapia: A review, in: Tacon, A.G.J., Basurco, B. (Eds.), Feeding Tomorrow's Fish. Cahiers Options Méditerranéennes No 22. pp. 205–224.

El-Sayed, A.-F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture 179, 149–168. doi:10.1016/S0044-8486(99)00159-3

El-Sayed, A.-F.M., 2004. Protein nutrition of farmed tilapia: searching for unconventional sources, in: New Dimensions in Farmed Tilapia: Proceedings of the Sixth International Symposium on Tilapia Aquaculture. pp. 364–378.

doi:10.1080/10454438.2011.627780

El-Sayed, A.-F.M., 2006. Tilapia Culture. CABI Publishing, Wallingford, UK.

El-Sayed, A.-F.M., 2007. Analysis of feeds and fertilizers for sustainable aquaculture development in Egypt, in: Hasan, M.R. (Ed.), Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. FAO Fisheries Technical Paper No.497. FAO, Rome, pp. 401–422.

El-Sayed, A.-F.M., 2008. Tilapia Feed and Feeding, in: Proceedings of the 8th International Symposium on Tilapia Aquaculture. Cairo, Egypt, pp. 717–723.

El-Sayed, A.-F.M., 2013. Tilapia feed management practices in sub-Saharan Africa, in: Hasan, M.R., New, M.B. (Eds.), On-Farm Feeding and Feed Management in Aquaculture. FAO Fisheries and Aquaculture Technical Paper No.583. FAO, Rome, pp. 377–405.

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. J. Food Prot. 67, 685–690.

EU (European Commission), 2011. Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. Off. J. Eur. Union L 54, 1-254.

Ezewudo, B.I., Monebi, C.O., Ugwumba, A.A.A., 2015. Production and utilization of *Musca domestica* maggots in the diet of *Oreochromis niloticus* (Linnaeus, 1758) fingerlings. African J. Agric. Res. 10, 2363–2371. doi:10.5897/AJAR2014.9274

FAO, 2005-2016. National Aquaculture Sector Overview. Ghana. National Aquaculture Sector Overview Fact Sheets. Text by Awity, L. [WWW Document]. URL <http://www.fao.org/fishery/countrysector/naso_ghana/en>

FAO, 2006. Livestock's long shadow - environmental issues and options. Rome.

FAO, 2011. Global Food Losses And Food Waste - Extent, causes and prevention. Rome.

FAO, 2012. The State of World Fisheries and Aquaculture 2012. Rome.

FAO, 2014. The State of World Fisheries and Aquaculture 2014. Rome.

Fasakin, E.A., Balogun, A.M., Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquac. Res. 34, 733–738. doi:10.1046/j.1365-2109.2003.00876.x

Feedipedia, 2015. Animal Feed Resources Information System – INRA, CIRAD, AFZ and FAO [WWW Document]. URL <http://www.feedipedia.org/>

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for isolation and

purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish, Aquaculture. doi:10.1016/S0044-8486(01)00526-9

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. Environ. Int. 91, 201–214. doi:10.1016/j.envint.2016.02.022

Gabriel, U.U., Akinrotimi, O.A., Bekibele, D.O., Onunkwo, D.N., Anyanwu, P.E., 2007. Locally produced fish feed : potentials for aquaculture development in subsaharan Africa. African J. Agric. Res. 2, 287–295.

Gasco, L., Stas, M., Schiavone, A., Rotolo, L., Marco, M. De, Dabbou, S., Renna, M., Malfatto, V., Lussiana, C., Katz, H., Zoccarato, I., Gai, F., 2015. Use of Black Soldier Fly (*Hermetia illucens*) meal in Rainbow trout (*Oncorhynchus mykiss*) feeds, in: Aquaculture Europe 2015. Rotterdam, Netherlands.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., 2007. Expanding the utilization of sustainable plant products in aquafeeds : a review. Aquac. Res. 38, 551–579. doi:10.1111/j.1365-2109.2007.01704.x

Gerendás, J., Zhu, Z., Bendixen, R., Ratcliffe, R.G., Sattelmacher, B., 1997. Physiological and Biochemical Processes Related to Ammonium Toxicity in Higher Plants. Z. Pflanzenernähr. Bodenkd. 160, 239–251.

Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. Aquac. Nutr. 13, 17–34. doi:10.1111/j.1365-2095.2007.00450.x

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

Goddard, S., 1996. Feed Management in Intensive Aquaculture, Chapman an. ed.

Green, B.W., 1992. Substitution of organic manure for pelleted feed in tilapia production. Aquaculture 101, 213–222. doi:10.1016/0044-8486(92)90025-G

Green, B.W., El Nagdy, Z., Hebicha, H., 2002. Evaluation of Nile tilapia pond management strategies in Egypt. Aquac. Res. 33, 1037–1048. doi:10.1046/j.1365-2109.2002.00767.x

Guerrero, R.D., Shelton, W.L., 1974. An Aceto-Carmine Squash Method for Sexing Juvenile Fishes. Progress. Fish-Culturist 36, 56–56. doi:10.1577/1548-8659(1974)36[56:AASMFS]2.0.CO;2

Guerrero, R.D., 1975. Use of Androgens for the Production of All-Male *Tilapia aurea* (Steindachner). Trans. Am. Fish. Soc. 104, 342–348. doi:10.1577/1548-8659(1975)104<342:UOAFTP>2.0.CO;2

Guerrero, R.D., Guerrero, L.A., 1988. Feasibility of commercial production of sex-reversed Nile tilapia fingerlings in the Philippines, in: Pullin, R.S. V., Bhukaswan, T., Tonguthai, K., Maclean, J.L. (Eds.), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623pp. Departement of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Halloran, A., Münke, C., 2014. Discussion paper: Regulatory frameworks influencing insects as food and feed.

Hardouin, J., Mahoux, G., 2003. Zootechnie d'insectes - Elevage et utilisation au bénéfice de l'homme et de certains animaux. BEDIM.

Hardy, R.W., Tacon, A.G.J., 2002. Fishmeal: historical uses, production trends and future outlook for supplies, in: Stickney, R.R., MacVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, New York, pp. 311–325.

Hardy, R.W., 2006. Worldwide Fish Meal Production Outlook and the Use of Alternative Protein Meals for Aquaculture, in: Cruz Suárez, L.E., Ricque Marie, D., Tapia Salazar, M., Nieto López, M.G., Villarreal Cavazos, D.A., Puello Cruz, A.C., García Ortega., A. (Eds.), Avances En Nutrición Acuícola VIII. VIII Simposium Internacional de Nutrición Acuícola. 15 - 17 Noviembre. Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México. pp. 410–419.

Harris, R.W., 1992. Root-Shoot Ratios. J. Arboric. 18, 39–42.

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 No. 497. FAO, Rome.

Hecht, T., 2007. Review of feeds and fertilizers for sustainable aquaculture development in sub-Saharan Africa, in: Hasan, M.R., Hecht, T., De Silva, S.S., Tacon, A.G.J. (Eds.), Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. FAO Fisheries Technical Paper. No. 497. FAO, Rome, pp. 77–109.

Heip, C.H.R., Herman, P.M.J., Soetaert, K., 1998. Indices of diversity and evenness. Oceanis 24, 61–87.

Hem, S., Rini, M., Hadadi, A., Larue, M., Pouyaud, L., 2007. Valorization of Palm Kernel Meal via Bioconversion: Indonesia's initiative to address aquafeeds shortage, in: International Conference on Oil Palm and Environment (ICOPE). 15-16 November 2007, Bali, Indonesia.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203, 1–22. doi:10.1016/j.anifeedsci.2015.03.001

Hepher, B., 1978. Ecological aspects of warm-water fishpond management, in: Gerking, S.D. (Ed.), Ecology of Fish Production. John Wiley and Sons, New York, USA, pp. 447–468.

Horneck, D.A., Sullivan, D.M., Owen, J.S., Hart, J.M., 2011. Soil Test Interpretation

Guide. EC 1478. Corvallis, OR Oregon State Univ. Ext. Serv. 1–12. doi:10.1017/CBO9781107415324.004

Idowu, A.B., Amusan, A.A.S., Oyediran, A.G., 2003. The response of *Clarias gariepinus* fingerlings (Burchell 1822) to the diet containing Housefly maggot (*Musca domestica*) (L). Niger. J. Anim. Prod. 30, 139–144. doi:10.4314/njap.v30i1.3325

IFDC, 2012. Ghana Fertilizer Assessment. Alabama, USA.

IFFO, 2013. IFFO, The Marine Ingredients Organization [WWW Document]. URL <http://www.iffo.net/>

IFIF, 2014. Global feed production. IFIF estimates/National and Regional Associations [WWW Document]. URL <http://www.ifif.org/>

Index Mundi, 2016. Commodity Prices [WWW Document]. URL <http://www.indexmundi.com/>

Ingrosso, L., Novoa, B., Valle, A.Z.D., Cardone, F., Aranguren, R., Sbriccoli, M., Bevivino, S., Iriti, M., Liu, Q., Vetrugno, V., Lu, M., Faoro, F., Ciappellano, S., Figueras, A., Pocchiari, M., 2006. Scrapie infectivity is quickly cleared in tissues of orally-infected farmed fish. BMC Vet. Res. 2, 21. doi:10.1186/1746-6148-2-21

IPIFF, 2014. The contribution of the EU insect sector to alternative protein sources for animals. Brussels.

Jackson, A.J., 2012. Fishmeal and fish oil and its role in sustainable aquaculture. Int. Aquafeed 18–21.

Jauncey, K., 1998. Tilapia Feeds and Feeding. Pisces Press.

Jaynes, D.B., Colvin, T.S., Karlen, D.L., Cambardella, C.A., Meek, D.W., 2004. Surface Water Quality: Nitrate Loss in Subsurface Drainage as Affected by Nitrogen Fertilizer Rate. J. Environ. Qual. 33, 645–655. doi:10.2134/jeq2001.3041305x

Karalazos, V., 2007. Sustainable alternatives to fish meal: Effects on growth, tissue fatty acid composition and lipid metabolism. University of Stirling.

Karapanagiotidis, I.T., Bell, M. V., Little, D.C., Yakupitiyage, A., 2007. Replacement of Dietary Fish Oils by Alpha-Linolenic Acid-Rich Oils Lowers Omega 3 Content in Tilapia Flesh. Lipids 42, 547–559. doi:10.1007/s11745-007-3057-1

Karapanagiotidis, I.T., Daskalopoulou, E., Vogiatzis, I., Rumbos, C., Mente, E., Athanassiou, C.G., 2014. Substitution of Fishmeal by Fly *Hermetia illucens* Prepupae Meal in the Diet of Gilthead Seabream (*Sparus aurata*), in: HydroMedit 2014, 1st International Congress of Applied Ichthyology & Aquatic Environment, November 13-15, Volos, Greece. pp. 110–114.

Kareem, A.O., Ogunremi, J.B., 2012. Growth Performance of *Clarias gariepinus* fed compounded rations and maggots. J. Environ. Issues Agric. Dev. Ctries. 4, 1–5.

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 364-365, 345–352. doi:10.1016/j.aquaculture.2012.08.041

Krome, C., 2014. Studies on the suitability of Jatropha curcas kernel meal as an alternative protein source in diets of carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). University of Stirling.

Lalander, C., Diener, S., Magri, M.E., Zurbrügg, C., Lindström, A., Vinnerås, B., 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) - From a hygiene aspect. Sci. Total Environ. 458-460, 312–318. doi:10.1016/j.scitotenv.2013.04.033

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. Agron. Sustain. Dev. 261–271. doi:10.1007/s13593-014-0235-4

Larde, G., 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. Biol. Wastes 33, 307–310. doi:10.1016/0269-7483(90)90134-E

Leclercq, M., 1997. A propos de *Hermetia illucens* (Linnaeus, 1758) (“soldier fly”) (Diptera Stratiomyidae: Hermetiinae). Bull. Annls Soc. r. belge Ent. 133, 275–282.

Leppla, N.C., Ashley, T.R., 1978. Facilities for insect research and production. USDA, Washington DC.

Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., Zhou, S., 2011a. From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. Fuel 90, 1545–1548. doi:10.1016/j.fuel.2010.11.016

Li, Q., Zheng, L., Hou, Y., Yang, S., Yu, Z., 2011b. Insect Fat, a Promising Resource for Biodiesel. J. Pet. Environ. Biotechnol. S2, 2–4. doi:10.4172/2157-7463.S2-001

Li, Q., Zheng, L., Qiu, N., Cai, H., Tomberlin, J.K., Yu, Z., 2011c. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Manag. 31, 1316–1320. doi:10.1016/j.wasman.2011.01.005

Liti, D.M., Mugo, R.M., Munguti, J.M., Waidbacher, H., 2006. Growth and economic performance of Nile tilapia (*Oreochromis niloticus* L.) fed on three brans (maize, wheat and rice) in fertilized ponds. Aquac. Nutr. 12, 239–245. doi:10.1111/j.1365-2095.2006.00397.x

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., Turner, W.A., Bhujel, R.C., 1997. Commercialization of a hatchery process to produce MT-treated Nile tilapia in Thailand, in: Alston, D.E., Green, B.M., Clifford, H.C. (Eds.), The Fourth Symposium on Aquaculture in Central America: Focusing on Shrimp and Tilapia, 22-24 April 1997, Tegucigalpa, Honduras,

Asociacion Nacional de Acucultores de Honduras and the Latin American Chapters of WAS. pp. 108–118.

Little, D.C., Hulata, G., 2000. Strategies for tilapia seed production, in: Beveridge, M.C.M., McAndrew, B.J. Tilapias: Biology and Exploitation. Kluwer Academic Publishers, pp. 267–326.

Little, D.C., Bhujel, R.C., Pham, T.A., 2003. Advanced nursing of mixed-sex and mono-sex tilapia (*Oreochromis niloticus*) fry, and its impact on subsequent growth in fertilized ponds. Aquaculture 221, 265–276. doi:10.1016/S0044-8486(03)00008-5

Liu, Q., Tomberlin, J.K., Brady, J.A., Sanford, M.R., Yu, Z., 2008. Black Soldier Fly (Diptera: Stratiomyidae) Larvae Reduce Escherichia coli in Dairy Manure. Environ. Entomol. 37, 1525–1530. doi:0046-225X/08/1525-1530$04.00/0

Lock, E.-J., Arsiwalla, T., Waagbø, R., 2015. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquac. Nutr. 1–31. doi:10.1111/anu.12343

Lomas, N., 2012. Comparison and Selection of Saprophagous Diptera Species for Poultry Manure Conversion. The University of Guelph.

MacNiven, A.M., Little, D.C., 2001. Development and evaluation of a stress challenge testing methodology for assessment of Nile tilapia (*Oreochromis niloticus*, Linn.) fry quality. Aquac. Res. 32, 671–679. doi:10.1046/j.1365-2109.2001.00574.x

Madu, C.T., Ufodike, E.B.C., 2003. Growth and Survival of Catfish (*Clarias anguillaris*) Juveniles Fed Live Tilapia and Maggot as Unconventional Diets. J. Aquat. Sci. 18, 47–52.

Mair, G.C., Little, D.C., 1991. Population Control in Farmed Tilapias. NAGA, ICLARM Quarterly. 8–13.

Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33. doi:10.1016/j.anifeedsci.2014.07.008

Marchant, B., 2014. Closing the food waste loop: a new angle for insect-based feeds. Int. Aquafeed 28–30.

Marine Harvest, 2015. Salmon Farming Industry Handbook 2015.

Mariod, A.A., 2013. Insect oil and protein: Biochemistry, food and other uses: Review. Agric. Sci. 04, 76–80. doi:10.4236/as.2013.49B013

Martínez-Llorens, S., Vidal, A.T., Cerdá, M.J., 2012. A new tool for determining the optimum fish meal and vegetable meals in diets for maximizing the economic profitability of gilthead sea bream (*Sparus aurata*, L.) feeding. Aquac. Res. 43, 1697–1709. doi:10.1111/j.1365-2109.2011.02977.x

Maurice, J.T., 1960. The soldier flies or Stratiomyidae of California. Bull. Callifornia Insect Surv. 6, 72–122.

Maynard, L.A., Loosli, J.K., Hintz, H.F., Warner, R.G., 1969. Animal Nutrition, 7th ed. McGraw-Hill Book Co.

Merino, G., Barange, M., Mullon, C., Rodwell, L., 2010. Impacts of global environmental change and aquaculture expansion on marine ecosystems. Glob. Environ. Chang. 20, 586–596. doi:10.1016/j.gloenvcha.2010.07.008

Metcalfe, P., 2007. Large scale combustion of animal manure in the United Kingdom, in: Plantekongres 2007, Afbrænding Af Husdyrgødning - Hvad Er Mulighederne. pp. 314–317.

Michael, K.G., Sogbesan, A.O., 2015. Evaluation of Maggot Meal (*Musca domestica*) and Single Cell Protein (Mushroom) in the Diet of *Clarias gariepinus* Fingerlings (Burchell, 1822). Fish. Aquac. J. 06, 6–10. doi:10.4172/2150-3508.1000133

Miller, B.F., Teotia, J.S., Thatcher, T.O., 1974. Digestion of poultry manure by *Musca domestica*. Br. Poult. Sci. 15, 231–234.

Ming, J., Ye, J., Zhang, Y., Yang, X., Wu, C., Shao, X., Liu, P., 2013. The Influence of Maggot Meal and L-Carnitine on Growth, Immunity, Antioxidant Indices and Disease Resistance of Black Carp (*Mylopharyngodon Piceus*). J. Chinese Cereal. Oils Assoc. 28, 80–86.

Morales-Ramos, J., Rojas, G., Shapiro-Ilan, D.I., 2014. Mass Production of Beneficial Organisms, 1st. ed. Elsevier. doi:10.1016/B978-0-12-391453-8.01001-3

Morgan, N.O., Eby, H.J., 1975. Fly protein production from mechanically mixed animal wastes. Isr. J. Entomol. 10, 73–81.

Munro, L., Wallace, I., 2014. Scottish Fish Farm Production Survey 2013 . Marine Science Scotland.

Myers, H.M., Tomberlin, J.K., Lambert, B.D., Kattes, D., 2008. Development of Black Soldier Fly (Diptera: Stratiomyidae) Larvae Fed Dairy Manure. Environ. Entomol. 37, 11–15. doi:10.1603/0046-225X(2008)37[11:DOBSFD]2.0.CO;2

Nasr-Allah, A.M., Dickson, M.W., Al-Kenawy, D.A.R., Ahmed, M.F.M., El-Naggar, G.O., 2014. Technical characteristics and economic performance of commercial tilapia hatcheries applying different management systems in Egypt. Aquaculture 426-427, 222–230. doi:10.1016/j.aquaculture.2014.02.004

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiua, A., Elliottd, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Correction for Naylor *et al*., Feeding aquaculture in an era of finite resources, in: Proceedings of the National Academy of Sciences. pp. 15104–15110. doi:10.1073/pnas.0910577106

NC State University, 2006. Technology Report: Black Black Soldier Fly (SF).

New, M.B., Tacon, A.G.J., Csavas, I., 1994. Farm-made aquafeeds, FAO Fisheries Technical Paper. No. 343. FAO, Rome.

New, M.B., Wijkström, U.N., 2002. Use of Fishmeal and Fish Oil in Aquafeeds - Further thoughts on the fishmeal trap, FAO Fisheries Circular No. 975. FAO,

Rome.

Newton, G.L., Booram, C.V., Barker, R.W., Hale, O.M., 1977. Dried *Hermetia illucens* larvae meal as a supplement for swine. J. Anim. Ecol. 44, 395–400.

Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K., Thelen, E.E., 2005a. The Black Soldier Fly, *Hermetia Illucens*, as a Manure Management / Resource Recovery Tool, in: Symposium on the State of the Science of Animal Manure and Waste Management. San Antonio, Texas.

Newton, G.L., Sheppard, D.C., Watson, W.D., Burtle, G., Dove, R., 2005b. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure.

Ng, W.-K.K., Romano, N., 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. Rev. Aquac. 5, 220–254. doi:10.1111/raq.12014

Nguyen, T.T.X., Tomberlin, J.K., Vanlaerhoven, S., 2015. Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. Environ. Entomol. 44, 406–410. doi:10.1093/ee/nvv002

Nicholson, F., Rollett, A., Chambers, B., 2011. Review of pollutant losses from solid manures stored in temporary field heaps. Report for Defra Project: WT1006. Mansfield, UK.

NRC, 2011. Nutrient requirements of fish and shrimp. National Research Council, National Academy Press, Washington DC.

N-TOOLBOX, 2011. Innovative and cost-effective technologies to reduce N losses to water from agriculture in the EU: a catalogue of farm level strategies.

Obirikorang, K.A., Amisah, S., Fialor, S.C., Skov, P. V., 2015. Local agro-industrial by-products with potential use in Ghanaian aquaculture: a review. Aquac. Int. 23, 403–425. doi:10.1007/s10499-014-9831-1

Odesanya, B.O., Ajayi, S.O., Agbaogun, B.K.O., Okuneye, B., 2011. Comparative evaluation of nutritive value of maggots. Int. J. Sci. Eng. Res. 2, 1–5.

Ogello, E.O., Munguti, J.M., Sakakura, Y., Hagiwara, A., 2014. Complete Replacement of Fish Meal in the Diet of Nile Tilapia (*Oreochromis niloticus* L.) Grow-out with Alternative Protein Sources . A review. Int. J. Adv. Res. 2, 962–978.

Ogunji, J.O., Kloas, W., Wirth, M., Neumann, N., Pietsch, C., 2008a. Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of *Oreochromis niloticus* fingerlings. J. Anim. Physiol. Anim. Nutr. (Berl). 92, 511–518. doi:10.1111/j.1439-0396.2007.00745.x

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C., Rennert, B., 2008b. Housefly Maggot Meal (Magmeal) as a Protein Source for *Oreochromis niloticus* (Linn.). Asian Fish. Sci. 21, 319–331.

Ogunji, J.O., Toor, R.U.-A.S., Schulz, C., Kloas, W., 2008c. Growth performance,

nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turkish J. Fish. Aquat. Sci. 8, 141–147.

Ogunji, J.O., Nimptsch, J., Wiegand, C., Schulz, C., 2007. Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of *Oreochromis niloticus* fingerling. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 147, 942–947. doi:10.1016/j.cbpa.2007.02.028

Ogunji, J.O., Pagel, T., Schulz, C., Kloas, W., 2009. Apparent Digestibility Coefficient of Housefly Maggot Meal (magmeal) for Nile Tilapia (*Oreochromis niloticus* L.) and Carp (Cyprinus carpio). Asian Fish. Sci. 22, 1095–1105.

Olsen, R.E., Suontama, J., Langmyhr, E., Mundheim, H., Ringo, E., Melle, W., Malde, M.K., Hemre, G.-I., 2006. The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. Aquac. Nutr. 12, 280–290. doi:10.1111/j.1365-2095.2006.00400.x

Olsen, R.L., Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends Food Sci. Technol. 27, 120–128. doi:10.1016/j.tifs.2012.06.003

Omoyinmi, G.A.K., Olaoye, O.J., 2012. Growth Performance of Nile Tilapia-*Oreochromis niloticus* Fed Diets Containing Different Sources of Animal Protein. Libyan Agric. Res. Cent. J. Int. 3, 18–23.

Oonincx, D.G.A.B., van Itterbeeck, J., Heetkamp, M.J.W., van den Brand, H., van Loon, J.J.A., van Huis, A., 2010. An Exploration on Greenhouse Gas and Ammonia Production by Insect Species Suitable for Animal or Human Consumption. PLoS One 5. doi:10.1371/journal.pone.0014445

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. PLoS One 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. doi:10.1371/journal.pone.0144601

Owusu-Bennoah, E., Visker, C., 1994. Organic wastes hijacked. ILEIA Newsl. 10, 12–13.

Oyelese, O.A., 2007. Utilization of compounded ration and maggot in the diet of *Clarias gariepinus.* Res. J. Appl. Sci. 2, 301–306.

PAF, 2011. Partnership of African Fisheries (PAF) Aquaculture working group: Strategic review on Aquaculture feeds and feed supply.

Paoletti, M.G., Dufour, D.L., 2002. Minilivestock. Encycl. Pest Manag. 487–492.

Parker, R., 2011. Aquaculture science, 3rd ed. Cengage learning.

Pastor, B., 2011. Biological parameters for the mass rearing of the housefly (*Musca*

domestica L.) and its use as pig manure biodegradation agent. Universidad de Alicante.

Pastor, B., Velasquez, Y., Rojo, S., Gobbi, P., 2015. Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. J. Insects as Food Feed 1, 179–193. doi:10.1007/s10811-012-9802-8

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). Zool. Africana 10, 123–132. doi:10.1080/00445096.1975.11447501

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.

Phelps, R.P., 2006. Hormone manipulation of sex, in: Lim, C., Webster, C.D. (Eds.), Tilapia: Biology, Culture, and Nutrition. CRC Press, pp. 211–252.

Popma, T.J., Green, B.W., 1990. Aquaculture production manual: Sex Reversal of Tilapia in Earthen Ponds, Research and Development Series No. 35. International Center for Aquaculture, Auburn University, Alabama.

Popma, T.J., Lovshin, L.L., 1995. Worldwide Prospects for Commercial Production of Tilapia. International Center for Aquaculture and Aquatic Environments, Auburn University, Alabama. doi:ternational Center for Aquaculture and Aquatic Environments.

Pratoomyot, J., Bendiksen, E.Å., Campbell, P.J., Jauncey, K.J., Bell, J.G., Tocher, D.R., 2011. Effects of different blends of protein sources as alternatives to dietary fishmeal on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.). Aquaculture 316, 44–52. doi:10.1016/j.aquaculture.2011.03.007

PROteINSECT, 2013. Deliverable 5.1: Mapping Exercise Report with regard to current Legislation & Regulation: Europe and Africa & China.

PROteINSECT, 2015. Deliverable 1.3: Report on the characteristics of all fly rearing techniques and modifications of production techniques tested in the project. CABI, Switzerland.

PROteINSECT, 2016a. Deliverable 4.5: Recommendations on insect rearing stations. University of Leuven, Belgium.

PROteINSECT, 2016b. Deliverable 4.3: Production scenario analysis. University of Leuven, Belgium.

Rana, K.J., Siriwardena, S., Hasan, M.R., 2009. Impact of rising feed ingredient prices on aquafeeds and aquaculture production, FAO Fisheries and Aquaculture Technical Paper No. 541. FAO, Rome.

Randall, J.E., 1967. Food Habits of Reef Fishes of the West Indies. Stud. Trop. Oceanogr. 5, 665–847.

Ravi, C., Jeyashree, A., Devi, K.R., 2011. Antimicrobial Peptides from Insects: An Overview. Res. Biotechnol. 2, 1–7.

Refstie, S., Storebakken, T., Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162, 301–312. doi:10.1016/S0044-8486(98)00222-1

Refstie, S., Olli, J.J., Standal, H., 2004. Feed intake, growth, and protein utilisation by post-smolt Atlantic salmon (*Salmo salar*) in response to graded levels of fish protein hydrolysate in the diet. Aquaculture 239, 331–349. doi:10.1016/j.aquaculture.2004.06.015

Richmond, A., 2004. Handbook of microalgae culture. Biotechnology and applied phycology. Blackwell Science, Oxford, UK.

Riddick, E.W., 2014. Insect Protein as a Partial Replacement for Fishmeal in the Diets of Juvenile Fish and Crustaceans, in: Mass Production of Beneficial Organisms. Elsevier, pp. 565–582. doi:10.1016/B978-0-12-391453-8.00016-9

Rojas, J.B.U., 2002. Use of coffee pulp as feed ingredient for tilapia culture. Wageningen University.

Rozendaal, J.A., 1997. Houseflies, in: Rozendaal, J.A. (Ed.), Vector Control: Methods for Use by Individuals and Communities. World Health Organization, Geneva, pp. 302–323.

Rumpold, B.A., Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. Technol. 17, 1–11. doi:10.1016/j.ifset.2012.11.005

Rurangwa, E., Agyakwah, S.K., Boon, H., Bolman, B.C., 2015. Development of Aquaculture in Ghana Analysis of the fish value chain and potential business cases - IMARES report C021/15. Wageningen.

Russin, T.A., Boye, J.I., Arcand, Y., Rajamohamed, S.H., 2011. Alternative Techniques for Defatting Soy: A Practical Review. Food Bioprocess Technol. 4, 200–223. doi:10.1007/s11947-010-0367-8

Rust, M.B., 2002. Nutritional physiology, in: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. The Academic Press, New York, pp. 368–446.

Rust, M.B., Barrows, F.T., Hardy, R.W., Lazur, A., Naughten, K., Silverstein, J., 2011. The Future of Aquafeeds. NOAA/USDA Alternative Feeds Initiative.

Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.J., 2004. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J. Hum. Nutr. Diet. 17, 449–459. doi:10.1111/j.1365-277X.2004.00552.x

Sanchez-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 [WWW Document]. URL <http://creatures.ifas.ufl.edu>

Sánchez-Muros, M.-J., Barroso, F.G., Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod. 65, 16–27. doi:10.1016/j.jclepro.2013.11.068

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. Aquac. Nutr. doi:10.1111/anu.12313

Schank, J.C., Koehnle, T.J., 2009. Pseudoreplication is a pseudoproblem. J. Comp. Psychol. 123, 421–433. doi:10.1037/a0013579

Schroeder, G.L., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture 30, 203–210. doi:10.1016/0044-8486(83)90162-X

Scott, W.B., Crossman, E.J., 1973. Freshwater Fishes of Canada. Fish. Res. Board Canada Bulletin N, 966. doi:10.1002/iroh.19760610109

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*. J. World Aquac. Soc. 42, 34–45. doi:10.1111/j.1749-7345.2010.00441.x

Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119, 63–88. doi:10.1016/0044-8486(94)90444-8

Shearer, K.D., 2000. Experimental design , statistical analysis and modelling of dietary nutrient requirement studies for fish : a critical review. Aquac. Nutr. 6, 91–102. doi:10.1046/j.1365-2095.2000.00134.x

Sheppard, D.C., Newton, L.G., Thompson, S.A., Savage, S., 1994. A value added manure management system using the black soldier fly. Bioresour. Technol. 50, 275–279. doi:10.1016/0960-8524(94)90102-3

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). J. Med. Entomol. 39, 695–698. doi:10.1603/0022-2585-39.4.695

Sheppard, D.C., Newton, G.L., Burtle, G., 2007. Black Soldier Fly Prepupae A Compelling Alternative to Fish Meal and Fish Oil. A Public Comment Prepared in response to a request by the National Marine Fisheries Service. Tifton.

Shiau, S.-Y., Yu, Y.-P., 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus×O. aureus*. Aquaculture 179, 439–446. doi:10.1016/S0044-8486(99)00177-5

Shiau, S.-Y., 2002. Tilapia, *Oreochromis* spp., in: Webster, C.D., Lim, C.E. (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI Publishing, Wallingford, pp. 273–292.

Sing, K.-W., Kamarudin, M.S., Wilson, J.-J., Sofian-Azirun, M., 2014. Evaluation of Blowfly (*Chrysomya megacephala*) Maggot Meal as an Effective, Sustainable Replacement for Fishmeal in the Diet of Farmed Juvenile Red Tilapia

(*Oreochromis* sp.). Pak. Vet. J. 8318, 85–92. doi:10.1097/QCO.0b013e3283638104

Singer, S.M., Sawan, O.M., Abd-El-Mouty, M.M., Salman, S.R., 1998. Study of the effects of Delta mix TM and organic matter on growth and productivity of bean plants grown under calcareous soil condition. Egypt. J. Hortic. 25, 335–347.

Skidmore, P., 1985. The Biology of the Muscidae of the World. Dr W. Junk Publishers, Dordrecht.

Slade, R., Bauen, A., Gross, R., 2010. The UK bio-energy resource base to 2050: estimates, assumptions, and uncertainties (No. UKERC/WP/TPA/2010/002). London.

Sogbesan, A.O., Ajuonu, N., Musa, B.O., Adewole, A.M., 2006. Harvesting Techniques and Evaluation of Maggot Meal as Animal Dietary Protein Source for "Heteoclarias" in outdoor concrete tanks. World J. Agric. Sci. 2, 394–402.

Sogbesan, A.O., Ugwumba, A.A.A., 2008a. Nutritional Values of Some Non-Conventional Animal Protein Feedstuffs Used as Fishmeal Supplement in Aquaculture Practices in Nigeria. Turkish J. Fish. Aquat. Sci. 164, 159–164.

Sogbesan, A.O., Ugwumba, A.A.A., 2008b. Nutritional Evaluation of Termite (*Macrotermes subhyalinus*) Meal as Animal Protein Supplements in the Diets of *Heterobranchus longifilis* (Valenciennes, 1840) Fingerlings. Turkish J. Fish. Aquat. Sci. 157, 149–157.

Speedy, A.W., 2003. Animal Source Foods to Improve Micronutrient Nutrition in Developing Countries Global Production and Consumption of Animal Source Foods 1. J. Nutr. 133, 4048–4053.

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, Seattle, Washington, U.S.A., 9 October-15 December 1978. FAO, Rome, pp. 187–214.

Stafford, E.A., Tacon, A.G.J., 1984. Nutritive Value of the Earthworm, *Dendrodrilus subrubicundus*, Grown on Domestic Sewage, in Trout Diets. Agric. Wastes 9, 249–266. doi:10.1016/0141-4607(84)90084-2

Steffens, W., 1989. Principles of fish nutrition. Ellis Horwood Ltd., Chichester, UK.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, D.C., Irving, S., 2007a. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World Aquac. Soc. 38, 309–313. doi:10.1111/j.1749-7345.2007.00101.x

St-Hilaire, S., Sheppard, D.C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., Sealey, W., 2007b. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquac. Soc. 38, 59–67. doi:10.1111/j.1749-7345.2006.00073.x

Stirling, H.P., Beveridge, M.C., Ross, L.G., Phillips, M.J., 1985. Chemical and biological methods of water analysis for aquaculturists, 1st ed. Stirling, UK.

Tacon, A.G.J., Stafford, E.A., Edwards, C.A., 1983. A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 35, 187–199. doi:10.1016/0044-8486(83)90090-X

Tacon, A.G.J., 1988. The nutrition and feeding of farmed fish and shrimp - a training manual. 3. Feeding methods, FAO Field Document, Project GCP/RLA/075/ITA "Support to the Regional Aquaculture Activities for Latin America and the Caribbean", Field Document No. 7/E, Brasilia, Brazil, 208 pp.

Tacon, A.G.J., 1996. Feed formulation and evaluation for semi-intensive culture of fishes and shrimps in the tropics, in: Santiago, C.B., Coloso, R.M., Millamena, O.M., Borlongan, I.G. (Eds.), Feeds for Small-Scale Aaquaculture. Proceedings of the National Seminar- Workshop on Fish Nutrition and Feeds. Tigbauan, Iloilo, Philippines : SEAFDEC Aquaculture Department. pp. 29–43.

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. doi:10.1016/S0044-8486(96)01494-9

Tacon, A.G.J., Hasan, M.R., Subasinghe, R.P., 2006. Use of fishery resources as feed inputs to aquaculture development: trends and policy implications., FAO Fisheries Circular No. 1018. FAO, Rome.

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. doi:10.1016/j.aquaculture.2008.08.015

Tacon, A.G.J., Hasan, M.R., Metian, M., 2011. Demand and supply of feed ingredients for farmed fish and crustaceans : Trends and prospects, FAO Fisheries and Aquaculture Technical Paper No 564. FAO, Rome.

Tacon, A.G.J., Metian, M., 2015. Feed Matters: Satisfying the Feed Demand of Aquaculture. Rev. Fish. Sci. Aquac. 23, 1–10. doi:10.1080/23308249.2014.987209

Taylor, J.F., Preston, A.C., Guy, D., Migaud, H., 2011. Ploidy effects on hatchery survival, deformities, and performance in Atlantic salmon (*Salmo salar*). Aquaculture 315, 61–68. doi:10.1016/j.aquaculture.2010.11.029

Teotia, J.S., Miller, B.F., 1974. Nutritive content of house fly pupae and manure residue. Br. Poult. Sci. 15, 177–182. doi:10.1080/00071667408416093

Thakur, D.P., Yang, Y., Diana, J.S., Lin, C.K., 2004. Effects of fertilisation and feeding strategy on water quality, Growth performance, nutrient utilization and economic return in Nile tilapia (*Oreochromis niloticus*) ponds, in: Bolivar, R.B., Mair, G.C., Fitzsimmons, K. (Eds.), Proceedings of the 6th International Symposium on Tilapia Aquaculture, September 12-16, 2004. Manilla, Philippines.

Thompson, K.R., Rawles, S.D., Koch, J.F.A., Gannam, A.L., Twibell, R.G., Webster, C.D., 2016. Use of distiller's dried grains with solubles, which had been used as a substrate for Black Soldier Fly larvae, in diets for Nile tilapia (*Oreochromis niloticus*), in: Proceedings of the Asian-Pacific Aquaculture 2016, Surabaya, Indonesia.

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 Physiol. Biochem. 5, 229–39. doi:10.1007/BF01874800

Tomberlin, J.K., Sheppard, D.C., Joyce, J. a., 2002. Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. Ann. Entomol. Soc. Am. 95, 379–386. doi:10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2

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., Crippen, T.L., 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 means to produce protein for use as livestock, poultry, and aquaculture feed. J. Insects as Food Feed 1, 307–309. doi:10.3920/JIFF2015.0098

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, Å., 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? Proc. Natl. Acad. Sci. 111, 13257–13263. doi:10.1073/pnas.1404067111

Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57. doi:10.1111/j.1753-5131.2008.01001.x

Turner, W.A., 2015. Nam Sai Farms [WWW Document]. Tilapia Prod. URL <http://www.tilapiathai.com/product-tilapia.html>

Turner, W.A., 2016. Nam Sai Farms, Farm-gate prices [WWW Document]. URL <http://www.tilapiathai.com/price-farmgate.html>

Tylko, G., Banach, Z., Borowska, J., Niklińska, M., Pyza, E., 2005. Elemental changes in the brain, muscle, and gut cells of the housefly,*Musca domestica*, exposed to heavy metals. Microsc. Res. Tech. 66, 239–247. doi:10.1002/jemt.20164

United Nations Department of Economic and Social Affairs, 2015. World Population Prospects: Key findings and advance tables. 2015 Revision (ESA/P/WP.241) 1–59. doi:Working Paper No. ESA/P/WP.241.

Uphouse Farm Ltd., n.d. Uphouse Farm Facts Booklet, Norfolk, UK. [WWW Document]. URL < http://www.face-online.org.uk/docman/resources/poultry-education-initiative/uphouse-farm-booklet/view>

Valente, D.S.M., de Queiroz, D.M., de Assis de Carvalho Pinto, F., Santos, N.T., Santos, F.L., 2012. The relationship between apparent soil electrical conductivity and soil properties. Rev. Ciência Agronômica 43, 683–690. doi:10.1590/S1806-66902012000400009

van Huis, A., Itterbeeck, J. Van, Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P., 2013. Edible insects: Future prospects for food and feed security, FAO Forestry Paper No.171. FAO, Rome.

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. J. Clean. Prod. 102, 362–369. doi:10.1016/j.jclepro.2015.04.106

van Zwieten, P.A.M., Béné, C., Kolding, J., Brummett, R., Valbo-Jørgensen, J., 2011. Review of tropical reservoirs and their fisheries – The cases of Lake Nasser, Lake Volta and Indo-Gangetic Basin reservoirs., FAO Fisheries and Aquaculture Technical Paper No.557. FAO, Rome.

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. Report 638. Wageningen UR Livestock Research.

Vera Cruz, E.M., Mair, G.C., 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). Aquaculture 122, 237–248.

Wang, H., Zhang, Z., Czapar, G.F., Winkler, M.K.H., Zheng, J., 2013. A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. Waste Manag. Res. 31, 223–31. doi:10.1177/0734242X12469431

Warburton, K., Pillai-McGarry, U., Ramage, D., 2002. Integrated biosystems for sustainable development, Proceedings of the InFoRM 2000 National Workshop on Integrated Food Production and Resource Management. Rural Industries Research and Development Corporation.

Westerman, P.W., Bicudo, J.R., 2005. Management considerations for organic waste use in agriculture. Bioresour. Technol. 96, 215–221. doi:10.1016/j.biortech.2004.05.011

Wiegmann, B.M., Yeates, D.K., 2007. Tree of Life Web Project [WWW Document]. Diptera. True flies. URL <http://tolweb.org/Diptera/8226/2007.11.29>

Wilson, J.B., 1988. A review of evidence on the control of shoot:root ratio, in relation to models. Ann. Bot. 61, 433–449.

World Bank, 2013. Fish to 2030: Prospects for fisheries and aquaculture, World Bank Report Number 83177-GLB. International Bank for Reconstruction and Development / International Development Association or The World Bank, Washington.

WRAP, 2013. Estimates of Food and Packaging Waste in the UK Grocery Retail and Hospitality Supply Chains.

Wurts, W.A., Masser, M.P., 2013. Liming Ponds for Aquaculture, Southern Regional Aquaculture Centre Publication No.4100.

WWF, 2014. The Growth of Soy Impacts and Solutions, WWF Report. Gland,

Switzerland.

Yi, Y., Lin, C.K., Diana, J.S., 2008. A manual of fertilization and supplemental feeding strategies for small-scale Nile tilapia culture in ponds. Corvallis, Oregon, USA.

Žáková, M., Borkovcová, M., 2013. *Hermetia illucens* application in management of selected types of organic waste, in: The 2nd Electronic International Interdisciplinary Conference, September, 2-6. EIIC, pp. 367–370.

Zhang, J., Huang, L., He, J., Tomberlin, J.K., Li, J., Lei, C., Sun, M., Liu, Z., Yu, Z., 2010. An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. J. Insect Sci. 10, 202. doi:10.1673/031.010.20201

Zhang, Z., Wang, H., Zhu, J., Suneethi, S., Zheng, J., 2012. Swine manure vermicomposting via housefly larvae (*Musca domestica*): The dynamics of biochemical and microbial features. Bioresour. Technol. 118, 563–571. doi:10.1016/j.biortech.2012.05.048

Zhao, W., Lu, L., Tang, Y., 2010. Research and Application Progress of Insect Antimicrobial Peptides on Food Industry. Int. J. Food Eng. 6. doi:10.2202/1556-3758.1943

Zheng, L., Li, Q., Zhang, J., Yu, Z., 2012. Double the biodiesel yield: Rearing black soldier fly larvae, *Hermetia illucens*, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. Renew. Energy 41, 75–79. doi:10.1016/j.renene.2011.10.004

Zhu, F.X., Wang, W.P., Hong, C.L., Feng, M.G., Xue, Z.Y., Chen, X.Y., Yao, Y.L., Yu, M., 2012. Rapid production of maggots as feed supplement and organic fertilizer by the two-stage composting of pig manure. Bioresour. Technol. 116, 485–491. doi:10.1016/j.biortech.2012.04.008

Zhu, F.X., Yao, Y.L., Wang, S.J., Du, R.G., Wang, W.P., Chen, X.Y., Hong, C.L., Qi, B., Xue, Z.Y., Yang, H.Q., 2015. Housefly maggot-treated composting as sustainable option for pig manure management. Waste Manag. 35, 62–67. doi:10.1016/j.wasman.2014.10.005

Appendix. Publications and presentations from the project

Ia. Publications in peer-reviewed journals •

Devic, E., Little, D.C., Leschen, W., Jauncey, K., 2013. A Model for Substitution of Fishmeal with Maggot- Meal in Tilapia Feeds - A Commercial Production Farm in West Africa. Isr. J. Aquac. - BAMIGDEH.

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 2, 107–114.

Devic, E., Maquart, P.-O., 2015. *Dirhinus giffardii* (Hymenoptera: Chalcididae), parasitoid affecting Black Soldier Fly production systems in West Africa. Entomologia 3, 25–27.

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R., Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. J. Insects as Food Feed 1, 7–16.

Ib. In progress (publications in peer-reviewed journals)

Devic, E.D., Stanford, R.J., Leschen, W., Little, D.C., Sprague, M. Assessing the suitability of housefly larvae meal (*Musca domestica*) as a substitute for fishmeal in the diet of Atlantic salmon (*Salmo salar*) parr. *To be submitted for publication.*

Devic, E.D., Leschen, W., Murray, F., Little, D.C Partial replacement of fish meal with Black Soldier Fly (*Hermetia illucens*) larvae meal in commercial diets for advanced nursing Nile tilapia (*Oreochromis niloticus*). *Submitted to Aquaculture Nutrition Journal, May 2016. Under review.*

Devic, E.D., Turner, W., Little, D.C. Cost-efficiency of maggot meals used as dietary substitutes to fish meal in Nile tilapia (*Oreochromis niloticus*) sex-reversal process. *To be submitted for publication*.