# Comparisons of growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings fed different inclusion levels of black soldier fly (*Hermetia illucens*) pre-pupae meal diets and its effect on the physical characteristics of the feed

**by**

**NIKLAAS JAKOBUS GROENEWALD**

*Thesis presented in fulfilment of the requirements for the degree of*

***Master of Science in Agriculture (Aquaculture)***

at

Stellenbosch University

Supervisor: Dr Elsje Pieterse

Co-supervisor: Dr Khalid Salie

Department of Animal Science, Faculty of AgriSciences

**March 2018**

## Declaration

By submitting this thesis electronically, I declare that the entirely of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any party rights and that I have not previously in its entirely or in part submitted it for obtaining any qualification.

Date: March 2018

## Summary

The nutritional composition of fish and the health benefit associated with the consumption thereof makes it a valuable food and feed source for both humans and animals. However, with the increasing size of the world's population and the demand for fish and other fishery products, an increased amount of pressure is being placed on wild catch fisheries. As a result, the latter are becoming stagnant, or declining, in many regions around the world. Aquaculture has, to some extent, been able to relieve the pressure placed on wild catch fisheries. Yet, a major downfall is that it still relies on wild caught fish to be used as a protein source for many aquaculture fish species. Various alternative protein sources has been investigated for use in aquaculture feeds to replace conventional and unsustainable protein sources, such as fishmeal. Among the various alternative animal protein sources, the use of insects are gaining popularity as a promising and sustainable solution. Insects are a natural food source for many fish species and have relatively balanced amino- and fatty acid profiles, while it is also highly effective in integrated waste management (IWM) systems. The objective of the study was to investigate the effects of different inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal in the diets of Nile tilapia (*Oreochromis niloticus*) fingerlings (n = 630) on the growth performance, fillet yield and feed quality. Six diets were compared: BSF0 (0 %; the control diet), BSF5 (5 %), BSF10 (10 %), BSF15.6 (15.6 %), BSF20 (20 %) and BSF25 (25 %; the summit diet). There were no significant differences in the final average body weight, feed intake, average daily gain (ADG) and conditioning factor (CF) between the different treatments. There was no significant difference for the feed conversion ratio (FCR) of BSF0 (1.35) and BSF5 (1.50), however FCR was lower ($P \leq 0.05$) for BSF10 (1.63) relative to BSF0, and worsened with increasing inclusion levels. The FCR was not significantly different between BSF10 and BSF15.6 (1.79), but BSF10 did have a significantly lower FCR than BSF20 (1.97) and BSF25 (1.91), whilst the latter two did not differ ($P > 0.05$) from each other. During the preparation of the experimental feed, the inclusion of a binder was required in BSF20 and BSF25 in order to prevent complete crumbling with the extrusion process. The use of a binder changed the hardness and floating characteristics of diets BSF20 and BSF25, which may have had an influence on differences in FCR. There was no significant difference observed for specific growth rate (SGR) between BSF0 (2.05), BSF5 (1.91) and BSF10 (1.73), or between diets BSF10 through to BSF25 (1.62). Treatment BSF10 was thus not significantly different from any treatment diets. Treatment BSF0 was the only diet with a SGR significantly higher than BSF15 (1.68), BSF20 (1.60) and BSF25 (1.62). The protein efficiency ratio (PER) of BSF0 (2.02) and BSF5 (1.82) did not differ significantly from each other. The PER of BSF0 differed significantly from BSF10 (1.68), BSF15.6 (1.55), BSF20 (1.40) and BSF25 (1.43). The difference could be linked to the increasing lipid content of the feeds which is known to adverse effect the digestibility of the feeds. The carcass yield was not significantly different between any of the treatments, where all the yields (percentage body weight) ranged from 81.63 g (BSF20) to 83.5 g (BSF0). The fillet yield did show some variation, but the results are not very accurate ($R^2=0.094$) due to the methodology of filleting. Furthermore, no significant differences were observed for the proximate composition (moisture, crude protein, lipid and ash) of the fillets. The inclusion

level of the BSF pre-pupae meal showed a statistically significant influence on the unit density, sinking velocity, water uptake and leaching rate of the pellets. However, the effect of binder used in BSF20 and BSF25 may have had an influence on these results. All unit densities differed significantly from each other, increasing with higher BSF pre-pupae inclusion levels. The control (BSF0) and BSF5 had a sinking velocity of 0 (floating pellets), while the feed only started sinking with inclusion levels of 10 % BSF pre-pupae meal (BSF10, 4.44 cm/s). Faster sinking velocities were observed with increasing inclusion levels of BSF pre-pupae meal. Water uptake was generally higher in BSF0 compared to other treatments for all the timeframes, except for the shortest submersion time (5 min), where BSF15.6 (288.60 %) had a significantly higher water uptake than all the other diets. Due to the disintegration of the pellets over time, the water uptake was expressed as a percentage of feed remaining rather than initial quantity used. The feed remaining was used to determine disintegration rate. Factors such as lipid content and interactions between ingredients may have indirectly influenced feed quality parameters. The study did not generate enough evidence to verify the claim that BSF pre-pupae meal can be used as a viable alternative protein source to conventional sources in Nile tilapia feeds, due to variability in water stability and inclusion of a binder that were not accounted for. However, it was found that inclusion levels of up to 25 % can be used without any effect on the body composition, and up to 5 % without compromising the growth parameters. Therefore, it is recommended to use an inclusion level of 5 % BSF pre-pupae meal to maintain the growth performance. For future studies, it is suggested to use defatted BSF pre-pupae meal for potentially higher inclusion levels of the meal for comparative growth results relative to conventional protein sources – as the higher lipid content may adversely affect the binding ability and the feed quality.

## Opsomming

Die voedingswaarde van vis en die gesondheidsvoordele wat met die verbruik daarvan gepaard gaan, maak dit 'n waardevolle voedsel- en voedingsbron vir beide mens en dier. Alhoewel, met die toename in die wêreld se bevolking en die vraag na vis en ander visseryprodukte word daar toenemend druk op wildvang vissery geplaas. Gevolglik stagneer of verminder wildvang vissery in baie streke regoor die wêreld. Akwakultuur het tot 'n mate die druk op wildvang vissery ligter gemaak. 'n Groot tekortkoming is egter dat akwakultuur nogsteeds staatmaak op die gebruik van wilde vis as 'n proteïenbron vir baie akwakultuurvisse. Navorsing word voordurend gedoen op die gebruik van verskeie alternatiewe proteïenbronne in akwakultuurvoere om konvensionele en nie-volhoubare proteïenbronne, soos vismeel, te vervang. Die gebruik van insekte as 'n belowende en volhoubare oplossing raak egter meer gewild in vergelyking met verskeie ander alternatiewe dierlike proteïenbronne. Insekte is uiteraard 'n natuurlike voedselbron vir baie visspesies en het 'n gebalanseerde amino- en vetsuurprofiel, terwyl dit ook hoogs effektief is in geïntegreerde afvalbestuurstelsels. Die doel van die studie was om die effek van verskillende Swart Soldaat Vlieg (SSV) (*Hermetia illucens*) pre-papie meel insluitingsvlakke in die diëte van Nyl tilapia (*Oreochromis niloticus*) vingervissies (n = 630) op die groeiprestasie, filet opbrengs en voergehalte te ondersoek. Ses diëte is met mekaar vergelyk: SSV0 (0 %; die kontrole dieet), SSV5 (5 %), SSV10 (10 %), SSV15.6 (15.6 %), SSV20 (20 %) en SSV25 (25 %; die toppunt dieet). Daar was geen betekenisvolle verskille in die finale gemiddelde liggaamsgewig, voerinname, gemiddelde daaglikse toename en kondisioneringsfaktor tussen die verskillende behandelings nie. Daar was geen beduidende verskil vir die voer omskakeling verhouding (VOV) van SSV0 (1.35) en SSV5 (1.50) nie, maar die VOV was laer ($P \leq 0.05$) vir SSV10 (1.63) relatief tot SSV0, en het versleg met die toename in insluitingsvlak. Die VOV het nie betekenisvol tussen SSV10 en SSV15.6 (1.79) verskil nie, maar SSV10 het 'n beduidende laer VOV as SSV20 (1.97) en SSV25 (1.91) gehad, terwyl die laaste twee nie van mekaar verskil ($P > 0.05$) het nie.

Tydens die voorbereiding van die eksperimentele voer is die insluiting van 'n bindmiddel benodig in BSF20 en BSF25 om te verhoed dat die ekstrusieproses volledig verkrummel. Die gebruik van bindmiddel het egter die hardheid en drywende eienskappe van diëte BSF20 en BSF25 verander, wat die verskille van FCR beïnvloed het. Daar was geen betekenisvolle verskille waargeneem vir die spesifieke groeikoers tussen SSV0 (2.05), SSV5 (1.91) en SSV10 (173) nie, of tussen diëte SSV10 tot SSV25 (1.62) nie. Behandeling SSV10 was dus nie betekenisvol anders as enige ander behandelingsdieet nie. SSV0 was die engiste dieet wat ‘n spesifieke groeikoers beduidend hoër as SSV15 (1.68), SSV20 (1.60) en SSV25 (1.62) gehad het. Die proteïen doeltreffendheid verhouding van SSV0 (2.02) en SSV5 (1.82) het nie betekenisvol van mekaar verskil nie. Die proteïen doeltreffendheid verhouding van SSV0 het betekenisvol van SSV10 (1.68), SSV15.6 (1.55), SSV20 (1.40) en SSV25 (1.43) verskil. Hierdie verskil kan egter aan die toenemende lipiedinhoud van die voer gekoppel word, aangesien dit bekend is dat die lipiedinhoud die verteerbaarheid van die voer nadelig kan beïnvloed. Die karkasopbrengs het nie betekenisvol tussen enige van die behandelings verskil nie, waar al die opbrengste (persentasie liggaamsgewig) van 81.63 g (SSV20) tot 83.5 g (SSV0) gewissel het. Die opbrengs

van die filet het 'n mate van variasie getoon, maar die resultate is nie baie akkuraat nie ($R^2$=0.094), moontlik as gevolg van metodologie van filetering. Daar is verder geen betekenisvolle verskille vir die proksimale samestelling (vog, ruwe proteïen, lipied en as) waargeneem nie. Die insluitingsvlakke van SSV pre-papie meel het 'n statistiese betekenisvolle invloed op die eenheidsdigtheid, sinksnelheid, wateropname en uitlogsyfer van die voer gehad. Die effek van die bindmiddel wat in BSF20 en BSF25 gebruik word, kon egter die resultate beïnvloed het. Al die eenheidsdigthede het betekenisvol van mekaar verskil, waar digtheid met elke hoër insluitingsvlak van SSV pre-papie toegeneem het. Die kontrole (SSV0) en SSV5 het ‘n sinksnelheid van 0 (drywende korrels) gehad. Die voer het egter eers begin sink met insluitingsvlakke vanaf 10 % SSV pre-papie meel (SSV10, 4.44 cm/s). Vinniger sinkingsnelhede was waargeneem met toenemende insluiting vlakke. Wateropname was oor die algemeen hoër in SSV0 in vergelyking met ander behandelings van al die tye, behalwe vir die kortste onderdompelingstyd (5 min), waar SSV15.6 (288.60 %) ‘n betekenisvolle hoër wateropname as al die ander diëte gehad het. As gevolg van die disintegrasie van korrels met die verloop van tyd, was die wateropname uitgedruk as 'n persentasie van die oorblywende voer eerder as die aanvanklike hoeveelheid wat gebruik is. Die oorblywende voer was gebruik om die desintegrasietempo te bepaal. Faktore soos die lipiedinhoud en interaksies tussen bestanddele kon indirek die voergehalteparameters beïnvloed het. Die studie het nie genoeg bewyse gelewer om die eis te verifieer dat SSV pre-papie meel as 'n lewensvatbare alternatiewe proteïenbron gebruik kan word vir konvensionele bronne in Nyl tilapia voer nie,as gevolg van veranderlikheid in waterstabiliteit en insluiting van 'n bindmiddel wat nie in ag geneem is nie. Daar is egter gevind dat insluitingsvlakke van tot 25 % gebruik kan word sonder enige effek op die liggaamsamestelling en tot 5 % sonder om die groeiparameters in gedrang te bring. Daarom kan die aanbeveling gemaak word om 'n insluiting vlak van 5 % SSV pre-papie meel te gebruik om die groeiprestasie te handhaaf. Vir toekomstige studies word dit aanbeveel om ontvette SSV pre-papie meel te gebruik vir moontlike hoër insluitingvlakke van die meel vir vergelykbare groeiverslae relatief tot konvensionele proteïenbronne – aangesien die hoër lipiedinhoud die bindingsvermoë en gehalte van die voer nadelig kan beïnvloed.

**Acknowledgements**

I would like to thank everyone who contributed to the completion of my thesis, both directly and indirectly. Firstly my supervisors Dr Elsje Pieterse and Dr Khalid Salie for your patience in guiding me through these unchartered waters. A big thanks goes out to the support staff at the Department of Animal Sciences for assisting with lab work where necessary, as well as towards Gail Jordaan with regards to helping out with my statistics. I would specifically like to thank my mother, Helene Kuntzsch, for always being there for me when times got tough and encouraging me to push on in the face of adversity.

This project was made possible with funding from the NRF (National Research Fund) and AgriProtein. Thank you to all that made this possible, this has been a truly unforgetfull experience.

## Notes

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

# Abbreviations

| | |
|---|---|
| AA | Amino acid |
| AGD | Average daily gain |
| ANOVA | Analysis of variance |
| BM | Bone meal |
| BSF | Black soldier fly |
| CF | Conditioning factor |
| COS | Chicken offal silage |
| CP | Crude protein |
| CSM | Cottonseed meal |
| DO | Dissolved oxygen |
| EAA | Essential amino acids |
| EFA | Essential fatty acids |
| ER | Expansion ratio |
| FI | Feed intake |
| FW | Final weight |
| GLM | The general linear model |
| HFM | Hydrolysed feather meal |
| HUFA | Highly unsaturated fatty acids |
| ICLARM | The International Center for Living Aquatic Resources Management |
| IW | Initial weight |
| IWM | Integrated waste management |
| MUSFA | Mono-unsaturated fatty acids |
| NEAA | Non-essential amino acids |
| NRC | National Research Council |
| PBM | Poultry by-product meal |
| PUFA | Polyunsaturated fatty acids |
| RAS | Recirculatory aquaculture systems |
| SFA | Saturated fatty acids |
| SBM | Soy bean meal |
| SGR | Specific growth rate |

## Contents

# Chapter 1

## General introduction

Fish and fishery products are important contributors to food security, while it also provides a healthy source of protein, minerals and fatty acids (FAO, 2016). The annual *per capita* consumption of fish has shown a steady growth in developing regions (5.2 kg in 1961 to 18.8 kg in 2013). Developed countries have a sizeable and growing share of imported fish relative to domestic fish due to static and declining domestic fishery production. In 2013, 17 % of global animal protein consumption was fish, which accounted for 6.7 % of all protein consumed the same year (FAO, 2016). The total world capture fisheries increased from 90.2 million tons to 93.4 million tons in 2009 to 2014, whilst total aquaculture fisheries increased from 55.7 million to 73.8 million tons (FAO, 2016). Whether the *per capita* consumption of fish increases or not, the demand for fish and related products is expected to increase due to the growing world's population (FAO, 2012). In 2014, the world aquaculture production of fish (which includes its non-food uses) accounted for 44.1 % of the total production, which is more than the 31.1 % reported in 2004 (FAO, 2016). Aquaculture is a recognized method of animal production which contributes to the global protein requirement for the growing world's population (Lucas & Southgate, 2012).

The high protein and fatty acid content are some of the factors that make fishmeal the conventional protein source used in aquaculture (El-Sayed, 2006). A major problem faced by the aquaculture industry is the cost of nutrition, of which protein (specifically fishmeal) is the most expensive ingredient (El-Sayed, 2006; Webster & Chhorn, 2006). Apart from the cost of fishmeal, the sustainability of its use as a protein source has become a major concern (Lucas & Southgate, 2012). In response, various alternative protein sources have been investigated with the intent to substitute, at least to some extent, the use of fishmeal in fish feed formulations (Tacon *et al*., 1983; Viola & Zohar, 1984; Tacon & Jackson, 1985)

In countries where animal protein is limited, insects have regularly been used as an alternative protein source (Riggi *et al.,* 2013). Insects are effective in recycling and utilizing organic waste for growth. They are also high in protein and fat (Pretorius, 2011; Nijdam *et al*., 2012) and therefore, promising for the replacement of conventional proteins such as fish meal and soya oilcake meal (Sealey *et al.,* 2011; Kroeckel *et al.,* 2012).

This study investigated the suitability and nutritional value of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal as an alternative protein source and supplement to fishmeal for the production of Nile tilapia (*Oreochromis niloticus*). Various inclusion levels of BSF pre-pupae meal were formulated into Nile tilapia diets to investigate its effect on growth performance, fillets and feed quality. The protocol for this trial (SU-ACUD15-00035) was approved by the Animal Ethics Committee of the University of Stellenbosch

## 1.1 References

El-Sayed, A.-F.M., 2006. Tilapia culture. In : Aquaculture. CABI publishing, UK.

Food and Agriculture Organization of the United Nations (FAO), 2012. *The State of World Fisheries and Aquaculture 2012*. http://www.fao.org/3/a-i2727e.pdf. Rome.

Food and Agriculture Organization of the United Nations (FAO), 2016. *The State of World Fisheries and Aquaculture 2016*. http://www.fao.org/3/a-i5555e.pdf. Rome.

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*). Aquac. 364, 345-352.

Lucas, J.S. & Southgate, P.C., 2012. Aquaculture: Farming aquatic animals and plant. Blackwell Publishing Ltd., UK.

Nijdam, D., Rood, T. & Westhoek, H., 2012. The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. Food. Policy. 37(6), 760-770.

Pretorius, Q., 2011. The evaluation of larvae of *Musca domestica* (common house fly) as protein source for broiler production. Stellenbosch: Stellenbosch University.

Riggi, L., Veronesi, M., Verspoor, R., MacFarlane, C. & Tchibozo, S., 2013. Exploring entomophagy in Northern Benin-practices, perceptions and possibilities. In: Bugs for life. London, UK.

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 pre-pupae, *Hermetia illucens*. J. World. Aquac. Soc. 42(1), 34-45.

Tacon, A.G.J. & Jackson, A.J., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. Nutr. Feed. Fish. 119-145.

Tacon, A.G.J., Jauncey, K., Falaye, A., Pantha, M., MacGowan, I. & Stafford, E.A., 1983. The use of meat and bone meal and hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In: Proceedings of the First International Symposium on Tilapia in Aquaculture. pp. 356-365.

Viola, S. & Zohar, G., 1984. Nutritional study with market size tilapia hybrid *Oreochromis* in intensive culture. Protein levels and sources. Isr. J. Aquac. 36, 3-15.

Webster, C.D. & Chhorn, L., 2006. Tilapia: biology, culture and nutrition. CRC Press.

# Chapter 2

## Literature review

### 2.1 Introduction

Nutrition represents more than 50% of operating costs in intensive aquaculture operations, with protein being the most expensive dietary component (El-Sayed, 2006; Webster & Chhorn, 2006). This makes well-balanced and affordable diets an important focus for the success and sustainability of tilapia cultures. To reduce production costs, detailed and accurate information on nutrient requirements are necessary (Webster & Chhorn, 2006). The challenge faced by tilapia farmers and nutritionists (particularly in developing countries) is the development of commercial, cost effective tilapia feeds using local, cheap and unconventional resources and ingredients (El-Sayed, 2006). This review focusses on the nutritional profile and the use of black soldier fly (BSF) (*Hermetia illucens*) as an alternative protein source in aquaculture feed. Furthermore, the nutritional and water quality requirements of Nile tilapia (*Oreochromis niloticus*) for optimum growth, feed quality parameters, requirement of fish feed and its production, and the impact of physical pellet quality on the biological response of fish will be discussed.

### 2.2 Protein sources in aquaculture

Feed production is a major contributor towards the occupation of land, dependence of water, acidification and climate change, where fishmeal and soy meal are mainly linked to these impacts (Mungkung *et al*., 2013; Sánchez-Muros *et al*., 2014). Hence, there is a drive to reduce the use of the above-mentioned meals. Various investigations have been conducted to determine the suitability of alternative protein sources in aquaculture feeds. However, the alternative protein sources should not have adverse effects on the growth and health of farmed aquatic species (Webster, 2006). Several animal by-products have been evaluated as a potential substitute for fish meal in tilapia diets (Webster & Chhorn, 2006). El-sayed (1998) investigated poultry by-product meal (PBM), shrimp meal, meat meal (M) and bone meal (BM) as alternative protein sources for Nile tilapia. Hydrolysed feather meal (HFM) was also investigated in previous studies as a protein source in fish feed (Tacon & Metian, 2013).

Various studies have also been conducted on the assessment of plant protein in tilapia diets. Some plant sources include oilseed plants such as soy bean meal (SBM) (Tacon *et al.,* 1983; Viola & Zohar, 1984; Abdelghany, 1997), cottonseed meal (CSM) (Jackson *et al*., 1982; El-Sayed, 1987; El-Sayed, 1990) rapeseed meal (Jackson *et al.*, 1982), copra, peanut and sunflower meals (Jackson *et al.,* 1982). Spiruline (Olvera-Novoa *et al.*, 1998), azolla meal (Santiago & Lovell, 1988) and duckweed (Essa, 1997) are among the aquatic plants that have been investigated for use in aquaculture feed. Cssava leaf meal (Ng & Wee, 1989), maize (gluten) (Wu *et al.,* 1995), lucerne (Olvera-Novoa *et al.,* 1990) and coffee pulp (Ulloa Rojas & Van Weerd, 1997) have also been investigated.

A deficiency in certain essential amino acids (EAA) is one of the major issues with plant protein sources as it requires supplementation with other feedstuffs (Ogunji *et al.,* 2008a). However, blending certain oilseed cakes or by-products and vegetable feedstuffs can provide a balanced amino acid (AA) profile. Yet, such a blend may contain anti-nutrients which either limit their use in compound feeds or require further processing – increasing feed production costs (El-Sayed, 2006; Sánchez-Muros *et al.,* 2014). Other issues related to the use of vegetable feedstuffs include low palatability and a high proportion of fibre and non-starch polysaccharides which limits inclusion levels. This requires the addition of AA or high value protein sources with a better digestibility and AA balance to mitigate the effects and/or nutritional shortcoming of vegetable based feedstuffs (Sánchez-Muros *et al.*, 2014).

Insects are generally seen as pests, but the fact is that many are considered an important food source (Womeni *et al.,* 2009). The role of insects as a food source is particularly important in developing and poorer countries where animal protein is limited (Riggi *et al.,* 2013). Although the evaluation of insects as a potential foodstuff for animals started in the early 1900's it only recently started receiving attention for its use in fish feeds with increased attention given from 2006 onward (Calvert *et al.,* 1969; Ogunji *et al.,* 2006). Insect farming has a relatively low carbon footprint and land use requirement (Blonk *et al.,* 2008). Furthermore, insects are poikilothermic (Nijdam *et al.,* 2012) which make them effective food converters as they do not use energy to produce body heat.

Insects have been usefully implemented in the agricultural sector as recyclers of organic waste. They are capable of utilizing and converting waste for their requirements, thereby developing these wastes into high protein and fat sources to potentially replace more expensive fish meal and soya oilcake meal (Sealey *et al.,* 2011; Kroeckel *et al.,* 2012). Furthermore, food (i.e. biological) waste is becoming an increasingly important aspect of integrated waste management (IWM) systems. Currently, one third of all food produced (1.3 billion metric tons) is wasted or lost, having consequential negative environmental and economic effects (Gustavsson *et al.,* 2011). The majority of waste management is orientated around composting (Bauhus & Meiwes, 1994). By making use of the insect's ability of bioconversion, an alternative waste management system can be utilized to convert waste into valuable biomass and by-products.

Recent feeding experiments using insect based diets have been performed on fish species such as Mozambique tilapia *(Oreochromis mossambicus),* catfish (*Clarias gariepinus*) (Alegbeleye *et al.,* 2012), channel catfish (*Ictalurus punctatus*) (Bondari & Sheppard, 1987), rainbow trout (*Oncorhunchus mykiss*) (St-Hilaire *et al.*, 2007a; Sealey *et al.,* 2011), Atlantic salmon (*Salmo salar*) (Lock *et al.,* 2014) tilapia (*Oreochromis niloticus*) (Ogunji *et al.,* 2008b), blue tilapia (*Oreochromis aureus*) (Bondari & Sheppard, 1987) and turbot (*Psetta maxima*) (Kroeckel *et al.,* 2012). Depending on the fish species, substitutions above 30 % mostly reduced their growth rate. Ogunji *et al.* (2008a) partially replaced fishmeal with house fly (HF) larvae meal as a protein supplement in Nile tilapia diets at 15%, 25%, 35%, 45%, 55% and 68%. They found HF larvae meal to be a suitable replacement.

BSF pre-pupae meal has a good feed value and an amino acid composition which is comparable to commercial proteins sources (e.g., soy and fish) (Newton *et al.,* 2005a; Barroso *et al.,* 2014; Surendra *et al.,* 2016), while its bioconversion ability can contribute to aquaculture's sustainability factor and the production of valuable by-products (Zhu *et al.,* 2015). BSF pre-pupae meal has the potential to function as an alternative protein source, while it is also a solution for the implementation of more sustainable and green practices. Hence, this study selected BSF pre-pupae meal as a potential protein replacement for commercial protein sources in fish diets. Furthermore, the self-harvesting action of BSF pre-pupae can aid in commercial production of the product.

## 2.3 The black soldier fly (*H. illucens*)

The black soldier fly (BSF) (*H. illucens*) was first observed in 1930 in the sugarcane fields of the *Hilo Sugar Company* in Hawaii (Hardy, 1960). It has a wasp-like appearance, and is distributed worldwide, primarily throughout tropical and subtropical regions (James, 1935; Sheppard *et al.,* 1994). BSF is considered a non-pest species (Sheppard *et al.,* 1994), as it rarely enter homes during its adult stage (Furman *et al.,* 1959). In fact, it lives off stored fat reserves (Newton *et al.,* 2005a) and eliminates/minimises house fly breeding (McCallan, 1974). It can be differentiated from other stratiomyids by its large size, the two translucent spots on the second tergum (abdominal segment) and a clear spot on the second sternum (James, 1947; Hardy, 1960).

BSF larvae are found naturally in manures, decaying fruits and vegetables as well as decomposing animals, thereby presenting a wide range of suitable habitats (James, 1947; Sheppard *et al.,* 1994). BSF utilize food waste to support its life cycle (Surendra *et al.,* 2016). The females deposit about 500 eggs at a time, generally in dry crevices close to the larval feed source, and hatch after approximately four days at 24 °C (Booth & Sheppard, 1984). Depending on feed availability, the metamorphosis of the larvae to pre-pupae and then pupae happens within two weeks (Furman *et al.,* 1959). During the pre-pupae stage they migrate in an attempt to pupate. This is the most desired harvest stage due to a self-harvesting action that can be implemented using the flies natural migratory instinct (Sheppard *et al.,* 1994).

### *2.3.1 Nutritional composition of BSF*

BSF maggot meal nutrient composition influences the way it is utilized by fish (Ogunji *et al.,* 2008a). The mineral and proximate composition of BSF at different life stages and substrates used are shown in Table 2.1 and Table 2.2, respectively. Crude protein and fat content differ with the life stage and substrate used for BSF (St-Hilaire *et al.,* 2007b; Sánchez-Muros *et al.,* 2014). Processing (Fasakin *et al.,* 2003), drying (Aniebo & Owen, 2010), harvesting time (Atteh & Ologbenla, 1993) and age (Aniebo & Owen, 2010) are also factors that influence their nutritional value. Newton *et al.* (2005a) suggest that fractioning (separation of components, such as oil) could increase the protein content up to 40 %. In support, Surendra *et al.* (2016) reported an increase and decrease in protein and lipid content by 18 % and 38 %, respectively, by making use of a

mechanical press, and 46 % and 90 % by making use of chemical extraction, respectively (Table 2.2.2). Mechanically defatted pre-pupae meal used by Kroeckel *et al.* (2012) also showed a higher and lower protein and fat content, respectively.

The data illustrates that BSF pre-pupae meal has a high enough protein content to fulfil dietary protein requirements of Nile tilapia in all life stages, which ranges from 45 % for the first feeding to 28 % in brood stock (El-Sayed, 2006). A difference in proximate composition reflects variation in diets.

#### *2.3.1.1 Protein and amino acids*

The crude protein content of untreated BSF can range from 36 % to 44 % (Table 2.2.2). BSF pre-pupae meal has a good feed value and amino acid composition comparable to that of commercial feed ingredients such as soy and fish meal (Newton *et al.,* 2005a*;* Barroso *et al.*, 2014; Surendra *et al.,* 2016).

Table **2.3** compares the amino acid composition of different life stages and substrates used to the requirements of Nile tilapia. Although most of the EAA requirements will be met, there will still be a deficiency in some which can be balanced using pure amino acids, these differences are however smaller or larger than that for fishmeal and soyabean meal.

#### *2.3.1.2 Mineral content*

As previously mentioned, various factors such as processing, drying and age influence the nutritional values, including mineral content, of BSF. Poultry manure has been found to provide pre-pupae with a higher level of phosphorous than that fed on swine manure, whilst swine manure provides pre-pupae with a higher level of calcium and potassium compared to those fed on poultry manure (Newton *et al*., 2005a). Newton *et al.* (1977) found that pre-pupae grown on cattle manure had similar calcium and phosphorous levels when compared to those grown on poultry manure at 5 % and 1.5 %, respectively.

**Table 2.1** The mineral composition of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae raised on swine and poultry manure as a percentage of dry mass(Newton *et al*., 2005b)

| Mineral | Swine manure | Poultry manure |
|---|---|---|
| Ca (%) | 5.36 | 5.00 |
| K (%) | 1.16 | 0.69 |
| Mg (%) | 0.44 | 0.39 |
| P (%) | 0.88 | 1.51 |
| Fe (ppm) | 766 | 1370 |
| Mn (ppm) | 348 | 246 |
| Zn (ppm) | 271 | 108 |

ppm = parts per million.

**Table 2.2** The proximate composition (%) of black soldier fly (BSF) (*Hermetia illucens*) larvae, pre-pupae and pupae grown on different substrates

| **Feed** | **Swine manure [a]** | **Beef manure [b]** | **Food waste [c]** | **Food waste [c]** | **Food waste [c]** | **Commercial [d]** | **Commercial [d]** |
|---|---|---|---|---|---|---|---|
| **Life stage** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Larvae** | **Pre-pupae** |
| **Processing** | **-** | **-** | **Untreated** | **Mechanical extraction** | **Chemical extraction** | **Defatted** | **Defatted** |
| Crude protein (%) | 43.2 | 42.1 | 43.7 | 53.1 | 63.9 | 36.2 | 40.7 |
| Fat (%) | 28 | 34.8 | 31.8 | 19.7 | 3.4 | 18 | 15.6 |
| Fibre (%) | N/A | 7.0 | 10.1 | 10.9 | 13.2 | 36.5 | 24 |
| Ash (%) | 16.6 | 14.6 | 6 | 8.5 | 10.7 | 9.3 | 19.7 |

[a](Newton *et al*., 2005b); [b](Newton *et al*., 1977); [c](Surendra *et al*., 2016); [d](Santiago & Lovell, 1988); N/A = Not available.

**Table 2.3** Black soldier fly (BSF) (*Hermetia illucens*) essential amino acid (EAA) composition requirements of Nile tilapia (*Oreochromis niloticus*) as a percentage (%) of lysine

| **Feed** | **Swine manure [a]** | **Beef manure [b]** | **Food waste [c]** | **Dairy manure [d]** | **Fish offal enriched [d]** | **Commercial [e]** | **Commercial [f]** | **EAA Requirement [e]** |
|---|---|---|---|---|---|---|---|---|
| Life stage | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** | **Larvae** | |
| Lysine | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Arginine | 80.09 | 66.47 | 101.37 | 86.83 | 89.15 | 82.82 | 108.42 | 82.52 |
| Histidine | 43.44 | 56.68 | 77.17 | 37.07 | 38.68 | 29.19 | 69.61 | 33.57 |
| Isoleucine | 68.33 | 58.16 | 68.95 | 89.27 | 89.15 | 55.59 | 75.79 | 60.84 |
| Leucine | 118.55 | 104.75 | 106.85 | 129.76 | 128.30 | 99.16 | 90.39 | 66.43 |
| Methionine | 37.56 | 25.52 | 40.18 | 37.56 | 37.26 | 36.87 | 19.74 | 52.45 |
| Phenylalanine | 67.42 | 65.28 | 72.60 | 89.27 | 85.85 | 55.73 | 90.53 | 73.43 |
| Threonine | 63.80 | 16.32 | 67.58 | 77.07 | 75.47 | 57.54 | 70.92 | 73.43 |
| Tryptophan | 26.70 | 5.93 | N/A | N/A | N/A | N/A | N/A | 19.58 |
| Valine | 100.90 | 101.19 | 110.50 | 145.85 | 144.34 | 67.18 | 83.03 | 54.55 |

[a](Newton *et al*., 2005b); [b](Newton *et al*., 1977); [c](Surendra *et al*., 2016); [d](Sealey *et al*., 2011); [e](Santiago & Lovell, 1988); [f](Kroeckel *et al*., 2012); N/A = Not available.

**Table 2.4** The fatty acid composition of black soldier fly (BSF) (*Hermetia illucens*) grown on various feed sources, presented as a percentage of totals

| **Feed** | **Food waste [a]** | **Commercial [b]** | **Commercial [c]** | **Swine manure [d]** | **Dairy cow manure [e]** | **Fish offal enriched [e]** |
|---|---|---|---|---|---|---|
| **Life Stage** | **Pre-pupae** | **Pre-pupae** | **Larvae** | **Pre-pupae** | **Pre-pupae** | **Pre-pupae** |
| **Saturated fatty acids** | | | | | | |
| Lauric | 44.90 | 47.00 | 43.40 | 49.34 | 23.60 | 37.10 |
| Myristic | 8.30 | 6.50 | 7.90 | 6.83 | 5.10 | 6.30 |
| Palmitic | 13.50 | 15.00 | 13.20 | 10.48 | 9.80 | 17.30 |
| Stearic | 2.10 | 2.20 | 2.80 | 2.78 | 6.50 | 2.00 |
| **Unsaturated fatty acids** | | | | | | |
| **Monounsaturated fatty acids** | | | | | | |
| Palmitoleic | 2.40 | N/A | 2.30 | 3.45 | 6.30 | 7.60 |
| Oleic | 12.00 | 14.00 | 14.60 | 11.81 | 22.70 | 18.80 |
| **Polyunsaturated fatty acids** | | | | | | |
| Linoleic | 9.90 | 9.40 | 15.20 | 3.68 | 6.80 | 5.90 |
| α-Linolenic | 0.10 | 0.80 | 0.70 | 0.08 | 0.00 | 0.50 |
| Stearidonic | - | <0.10 | 0.00 | - | 0.00 | 0.50 |
| **Highly unsaturated fatty acids** | | | | | | |
| Eicosapentanoic | 0.00 | 0 .00 | 0.00 | 0.00 | 0.10 | 3.50 |
| Docosapentaenoic | 0.00 | <0.10 | 0.00 | 0.00 | 0.00 | 0.35 |
| Docosahexaenoic | 0.00 | <0.10 | 0.00 | 0.00 | 0.00 | 1.70 |
| **Total fatty acids** | | | | | | |
| Saturated fatty acids | 69.90 | 71.60 | 67.10 | N/A | N/A | N/A |
| Monounsaturated fatty acids | 14.90 | 17.30 | 16.90 | N/A | N/A | N/A |
| Omega-6 fatty acids | - | - | 15.20 | - | - | - |
| Omega-3 fatty acids | - | - | 0.70 | - | - | - |
| Polyunsaturated fatty acids | 12.50 | 10.20 | 15.90 | - | - | - |

[a](Surendra *et al*., 2016); [c](Kroeckel *et al*., 2012); [c](Barroso *et al*., 2014); [d](St-Hilaire *et al*., 2007b); [e](Sealey *et al*., 2011); N/A = Not applicable.

#### *2.3.1.3 Lipids and fatty acids*

The total lipid content of BSF far exceeds the requirements of Nile tilapia, which generally varies from 10-15 % (El-Sayed, 2006). The essential fatty acid composition of BSF, however, may vary depending on the substrate used as growth medium (Table 2.4). Various studies (St-Hilaire *et al.*, 2007b; Sealey *et al.*, 2011; Kroeckel *et al.*, 2012; Barroso *et al.*, 2014; Surendra *et al.*, 2016) have confirmed that BSF contains the fatty acids which are deemed essential for fish feeds, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Some have also reported negligible amounts of highly unsaturated fatty acids.

## 2.4 The Nile tilapia (*Oreochromis niloticus*)

Tilapia are tropical fresh water fish species that belong to the *Cichlidae* family, endemic to Africa, Jordan and Israel. They are the most adaptable and successfully cultured species worldwide (Webster & Chhorn, 2006). Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and hybrid combinations of these species are the most important tilapia species in aquaculture (Webster & Chhorn, 2006). The *Cichlidae* family can be distinguished from other bony fishes by an interrupted lateral line running superior along the anterior part of the fish, and inferior along the posterior portion, as well by a single nostril on either side of the snout. They have smooth, large scales on their back and flanks, with smaller scales on their chest and their belly area. Furthermore, they have a dark spot on the upper posterior corner of the operculum, and one on the anterior basal corner of the soft dorsal fin, dubbed the “tilapia mark” (Webster & Chhorn, 2006). A vertical banding in the caudal fin of both sexes of Nile tilapia, and the grey-pink pigmentation of the gular region, is what can be used to distinguish Nile tilapia from blue tilapia (Webster & Chhorn, 2006).

Nile tilapia is the fastest growing (Lucas & Southgate, 2012) and most popular cultured (Halver & Hardy, 2002) of the tilapia species, found in many countries around the world. In the past two decades, Nile tilapia represented more than 60% of the total market value of farmed tilapia (El-Sayed, 2006).

Technological development and improvements in the past three decades regarding reproduction, system designs, disease prevention and control, water management and feeding practices is what fuelled their rapid global expansion. The continuous technological improvements and expansion in the industry is resulting in the replacement of traditional extensive systems with semi-intensive to intensive systems (Webster & Chhorn, 2006). Increasing domestic and international market demand for tilapia is expected to maintain this trend (Webster & Chhorn, 2006).

Generally, tilapia can start reproducing at 5-6 months of age, spawning every 6-8 weeks at 25-32 °C. Nile tilapia are mouth brooders where the number of eggs depend on the size of the fish and can be up to 2000 at a time. However, overpopulation in ponds can become problematic when most of the fish do not reach market size. The latter can be due to early reproductive capability, high breeding frequency and high larval

survival rate. Monosex culture, farming with only the faster growing males, is a practice used to overcome this issue. This practise can make use of manual sexing, hybridization or sex reversal (Halver & Hardy, 2002).

In their juvenile tilapia stage, Nile tilapia will feed on phytoplankton and detritus, and become more omnivorous and filter feed using gill rakers as they develop into adults (Wohlfarth & Hulata, 1983). When stocked at lower densities, tilapia are efficient in utilizing natural food sources from where they obtain a significant amount of protein. Generally, lower protein diets are used under these circumstances (Webster & Chhorn, 2006). Semi-intensive systems make use of affordable, locally available feedstuffs such as rice bran, corn meal, copra meal, coffee pulp, brewery by-products and combinations thereof to supplement natural food (Webster & Chhorn, 2006). These feedstuffs are often deficient in protein, vitamins and minerals, and are mainly utilized as an energy source. Increased stocking densities require the use of more nutritionally complete feeds as the contribution of natural food decreases (Webster & Chhorn, 2006). When raised in intensive systems (i.e. raceways and cages), tilapia rely solely on prepared feeds for all their nutrient requirements. The protein content of feeds used in intensive systems are usually 32 %. Highly concentrated feeds may be desired in recirculatory aquaculture systems as operating costs are high and excess organic material are typically avoided (Halver & Hardy, 2002).

Sinking, floating and non-pelleted forms of feed are all accepted by tilapia. Even though tilapia are capable of utilizing non-pelleted feed effectively, it is not consumed efficiently. When using high quality feeds, the processing of the feed into pellets is recommended to reduce waste and costs. Non-pelleted feeds (crumble and meal forms) are used for fry and fingerlings. For growing out tilapia to a marketable size (500 g), farmers usually make use of a one size pellet of approximately 3-4 mm in diameter and 6-10 mm in length. Unlike most fin-fish, tilapia tend to chew pellets rather than to swallow them immediately (Halver & Hardy, 2002). Feeding rates depend on factors such as natural food availability, size, species, digestible energy/protein (DE/P) and water quality, and are inversely related to the size of the fish (Halver & Hardy, 2002). Hence, these factors should be taken into account when feeding rates are investigated.

### *2.4.1 Nutritional requirements*

#### *2.4.1.1 Protein and amino acids*

Protein is the most expensive dietary component in intensive aquaculture, representing roughly 50 % of the total feed costs (El-Sayed, 2006; Webster & Chhorn, 2006). The protein requirements for tilapia in freshwater are show in Table 2.6. The protein requirement varies, depending on the life stage of the fish where brood stock, juveniles and adults require 35-45 %, 30-40 % and 20-30 %, respectively (El-Sayed, 2006). Protein requirements are higher for younger fish with a low body weight. For example, fish of 0.8 g have a protein requirement of 40 %, whereas 40 g fish have a requirement of only 30 % (Siddiqui *et al.,* 1988). Experimental data indicates that small and grow-out fish should be fed 36 % and 30-32 % crude protein as part of their balanced diets, respectively (Halver & Hardy, 2002). Protein requirements for fish can also vary from 30-50 %, depending on factors such as the protein quality, water quality (i.e. water salinity, water

temperature etc.), dietary energy (non-protein energy levels), feeding rate, feed allowance, presence of natural food and fish size (NRC, 1983).

**Table 2.5** Nile tilapia (*Oreochromis niloticus*) dietary requirements according to size (FAO, 2015)

| Fish size | Fry (<10g) | Fingerling (10-30g) | Grow out (>30 g) | >300g | Breeding |
|---|---|---|---|---|---|
| **Moisture** | <10 | <10 | <10 | <10 | <10 |
| **Crude protein** | 40-50 | 28-35 | 25-30 | 20-25 | >40 |
| **Crude lipid** | 6-13 | 6-13 | 4-12 | 4-12 | >6 |
| **Crude fibre** | <4 | <8 | <8 | <8 | <10 |
| **Ash** | <16 | <16 | <16 | <16 | <16 |
| **Carbohydrate** | >25 | >25 | >25 | >25 | >25 |
| **Moisture** | <10 | <10 | <10 | <10 | <10 |

Inadequate levels of non-protein energy in the diets lead to a higher dietary protein requirement, due to the fish utilizing protein as an energy source to meet their metabolic energy needs. Inadequate protein levels results in retardation or cessation of growth or anabolic activity in muscles as the animals use protein from less vital tissues to maintain the functionality of more vital tissues, resulting in weight loss (Webster & Chhorn, 2006). Conversely, excess protein is metabolised for energy and essentially wasted (NRC, 1983).

**Table 2.6** Tilapia protein requirements in freshwater (El-Sayed, 2006)

| Life stage | Weight (g) | Requirement (%) |
|---|---|---|
| First feeding larvae | - | 45-50 |
| Fry | 0.02-1 | 40 |
| Fingerlings | 1-10 | 35-40 |
| Juveniles | 10-25 | 30-35 |
| Adults | 25-200 | 30-32 |
| | >200 | 28-30 |
| Broodstock | - | 40-45 |

Tilapia, like other fish, require a well-balanced mixture of essential and non-essential amino acids (Webster & Chhorn, 2006). Fish meal is the traditional protein source used in fish feeds due to its balanced EAA profile and high digestibility (El-Sayed, 2006). Protein quality is based on the EAA ratio, and it's digestibility and bioavailability (Webster & Chhorn, 2006). The closer the EAA content of the protein sources to the requirement of Nile tilapia, the better the quality (Webster & Chhorn, 2006). The EAA requirement for Nile tilapia are the same as that of other fish and terrestrial animals (which includes lysine, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine), represented in Table 2.7 (Webster & Chhorn, 2006).

**Table 2.7** Essential amino acid (EAA) requirement for Nile tilapia (*Oreochromis niloticus*) expressed as percentage (%) of lysine (Santiago & Lovell, 1988)

| Life stage | Percentage of lysine | Percentage of protein |
|---|---|---|
| Lysine | 100.00 | 5.12 |
| Arginine | 82.52 | 4.20 |
| Histidine | 33.57 | 1.72 |
| Isoleucine | 60.84 | 3.11 |
| Leucine | 66.43 | 3.39 |
| Methionine | 52.45 | 2.68 |
| Phenylalanine | 73.43 | 3.75 |
| Threonine | 73.43 | 3.75 |
| Tryptophan | 19.58 | 1.00 |
| Valine | 54.55 | 2.80 |

The supplementation of nonessential amino acids (NEAA) has a sparing action, as cysteine and tyrosine (both NEAA) can only be synthesized from EAA precursors of methionine and phenylalanine, respectively. Similarly, the requirement for tyrosine (an aromatic amino acid) in diets reduce phenylalanine requirements (NRC, 1983). Generally, applied diets have sufficient levels of phenylalanine and tyrosine, up to where the requirements for the fish is exceeded (Webster & Chhorn, 2006). The requirement for sulphur-containing amino acids can be met by methionine alone, or by using a proper mixture of methionine and cysteine in a 50:50 ratio ( Halver & Hardy, 2002; El-Sayed, 2006).

However, studies on the EAA requirements of Nile tilapia, regarding their relevance in commercial application, are questionable as they were typically short-term, indoor studies (El-Sayed, 2006). Most studies used casein as a sole dietary protein source, which contains all EAA in the required amounts except for arginine. The latter requires higher inclusion levels to meet the requirements (El-Sayed, 1989). Santiago and Lovell (1988) determined the quantitative EAA requirements by feeding fish test diets containing graded levels of each EAA, with the basal diet consisting of a casein-gelatine protein combination – similar to those reported for other fish.

#### *2.4.1.2 Energy*

Given that diets are balanced, Nile tilapia eat to satisfy their energy requirements. If diets are not balanced they could, depending on the extent of the imbalance, attempt to rectify this through increased or decreased intake (NRC, 1983). Kubaryk (1980) reported that an increase in dietary digestible energy (DE) decreased the feed intake, while increased protein content did not affect the feed intake. Furthermore, he reported that small Nile tilapia grow optimally when provided with a diet containing a digestible energy/protein (DE/P) between 8.2 and 9.4 kcal/g or higher. The dietary protein-to-energy ratio's required for optimal growth decrease with an increase in tilapia size (Halver & Hardy, 2002). Hence, less protein and more energy is required for optimal growth of larger fish.

Traditionally, metabolisable energy (ME) is preferred above gross energy (GE) and digestible energy (DE), as it accounts for energy loss from protein metabolism – providing a more accurate estimate on their requirement for growth. Unfortunately, the determination of ME is difficult due to problems associated with collecting fish metabolites. Moreover, it is suspected that the ME offers little advantage over DE since energy lost from urine is low (El-Sayed, 2006). El-Sayed (2006) suggests that it may be more appropriate to use DE as a measurement of dietary energy as it can be easily determined. Commercial feedstuffs are digested relatively well by tilapia. The digestibility of various feedstuff are presented in Table 2.8. High fibre feedstuffs, such as brewers grain, is not easily digested for protein and energy needs.

**Table 2.8** Digestibility coefficient for protein, gross energy (GE) and digestible energy (DE) adapted from Hanley (1987)

| Ingredient | Protein | GE | DE (kcal/kg) |
|---|---|---|---|
| Fish meal | 86.50 | 79.80 | 3.840 |
| Poultry-offal meal | 73.90 | 58.80 | 3.626 |
| Soybean meal | 90.70 | 56.60 | 2.678 |
| Wheat middlings | 75.60 | 57.60 | 2.746 |
| Brewers grain | 62.60 | 30.50 | 1.416 |
| Ground corn | 83.30 | 76.00 | 3.099 |
| Gelatin | 73.80 | 50.70 | 2.187 |
| Animal oil | - | 93.00 | 8.676 |
| 20:5*n*-3 | N/A | N/A | N/A |
| 22:6*n*-3 | N/A | N/A | N/A |

N/A = Not applicable.

#### *2.4.1.3 Lipid and fatty acids*

Lipids are an essential dietary component required for physiological functions. The latter include functions such as being a source of EFA's, provide energy (protein saving action), maintain normal growth and development, absorption and carrying of fat-soluble vitamins, cell membrane maintenance, steroid precursor and improving feed texture and flavour (El-Sayed, 2006).

As previously stated, the dietary lipid requirement for Nile tilapia is 10-15 % (El-Sayed, 2006). Chou and Shiau (1996) reported that a dietary lipid content of 5 % appeared to be sufficient to meet the minimum requirements for hybrid tilapia (*O. niloticus* and *O. aureus*), but required 12 % for maximal growth. Overall, a good growth and feed efficiency was obtained with a 10-15 % dietary lipid inclusion. EFA requirements for fish depend on the species as cold-and marine fish require *n*-3 polyunsaturated fatty acids (PUFA), whilst fresh and warm water fish require higher levels of *n*-6 PUFA (El-Sayed, 2006).

Takeuchi *et al.* (1983) reported that diets supplemented with vegetable oils, rich in C18:2*n*-6, performed better than those supplemented with fish oil high in C20:5*n*-3 and C22:6*n*-3 and beef tallow high in C18:1*n*-9 PUFA. Similar results were reported by Santiago and Reyes (1993). These results indicate that *n*-6 fatty acids

(of the linoleic family) are essential lipid components in Nile tilapia diets. Optimum levels of *n*-6 fatty acids have been found to be around 0.5-1 % (Takeuchi *et al.,* 1983). However, lipid requirements of tilapia have not been thoroughly evaluated regarding the *n*-3 fatty acids, as soybean and most other vegetable oils are also high in linolenic (C18:3*n*-3) fatty acids (Webster & Chhorn, 2006). However, a recent study by Chen *et al.* (2016) reported that moderate levels (0.32-0.63 %) of C18:3*n*-3 could significantly enhance non-specific immunity and anti-inflammatory responses in Nile tilapia. Furthermore, Kanazawa *et al.* (1980) suggested that Nile tilapia are capable of desaturation and chain elongation of C18:2*n*-6 and C18:3*n*-6 fatty acids when supplemented in their diets. However, conversion rates are lower when adequate levels of C20 and C22 fatty acids are available in the diets, suggesting that the conversion rates are dependent on fatty acid composition of the diets (Olsen *et al.,* 1990). Olsen *et al.* (1990) also suggested that the enzymes required for conversion of C18 fatty acids to C20 and C22 fatty acids may be inhibited by longer chain PUFA. The information suggest that tilapia are likely to utilize all the fatty acids in

Table **2.9** as EFA.

**Table 2.9** Essential fatty acid requirements for Nile tilapia (*Oreochromis niloticus*) (Webster & Chhorn ,2006)

| **Fatty acid** | **Requirement (%)** |
|---|---|
| Lipids | 10.00-15.00 % |
| C18:2*n*-6 | 0.50-1.00 % |
| C18:3*n*-3 | 0.32-0.63 % |
| C20:4*n*-6 | N/A |
| C20:5*n*-3 | N/A |
| C22:6*n*-3 | N/A |

N/A = Not available.

#### *2.4.1.4 Carbohydrates*

Like other finfish, tilapia do not have specific requirements for carbohydrates. However, carbohydrates are always included in fish feeds as they are the most abundant and least expensive energy source. Carbohydrates also aid in pellet binding and serve as a precursor for various metabolic intermediates which are essential for growth. They also have a sparing effect on protein utilization for energy in a similar manner to that of lipids (NRC, 1983; Shimeno *et al.,* 1993). However, Shiau and Peng (1993) reported that the sparing effect in hybrid tilapia (*O. niloticus* and *O. aureus*) only takes place whilst sub-optimal dietary protein inclusion levels were used. Anderson *et al.* (1984) reported that growth improved for Nile tilapia when carbohydrates increased from 0 % to 40 %. Like other finfish, tilapia do not digest highly fibrous feedstuffs, such as alfalfa and coffee pulp, well for energy. Inclusion of dietary fibre levels above 5 % reduced diet digestibility in Nile tilapia, while protein utilization was reduced when inclusion levels surpassed 10 % (Anderson *et al.,* 1984).

*2.4.1.5 Minerals*

Minerals are required in fish diets for tissue formation and various metabolic functions such as osmoregulation, acid-base balance as well as muscle and nerve functions. Limited information is available on the mineral requirements of Nile tilapia, but it is suggested that they have similar minerals requirements as other fish and are capable of absorbing minerals from their environment (Webster & Chhorn, 2006).

Calcium (Ca) and phosphorous (P) is absorbed efficiently via the gills and gut, respectively, in *O. mossambicus*. However, P is usually the limiting mineral in most natural waters. This entails the inclusion of P in diets without providing it in excess (as it is a component of eutrophication) which could be problematic for water quality (Luquet, 1991). Robinson *et al.* (1987) reported that Ca should be supplemented at 5-7 g/kg in low-calcium water for adequate bone mineralization and growth of blue tilapia (*O. aureus*). Takeuchi *et al.* (1983) and Watanabe *et al.* (1988) recommended that an available P inclusion level of 0.8-1.0 % should be maintained in Nile tilapia diets. The availability of P is significantly influenced by the source as monocalcium, monoammonium and monosodium (water-soluble sources) have a higher availability than dicalcium and tricalcium phosphate. Furthermore, Watanabe *et al.* (1988) recommended a dietary manganese (Mn) inclusion of 12 mg/kg feed for Nile tilapia. Symptoms of Mn deficiency include high mortality, anorexia and poor growth. Dabrowska *et al.* (1989) reported that dietary Mn levels of 0.59-0.77 g are sufficient for optimum performance of Nile tilapia. Symptoms related to diets deficient in magnesium (Mg) include poor growth and abnormal tissue mineralisation. Furthermore, when supplied in an excess of 3.2 g/kg Mg, growth depression may result if fish are fed low (24%) protein diets. Like P, the dietary requirement of iron (Fe) for tilapia is affected by the source (Dabrowska *et al.,* 1989). Kleemann *et al.* (2003) reported that 60 mg/kg of available Fe is necessary to maintain normal bodily functions for Nile tilapia. For optimum growth, it is suggested that other minerals such as potassium, zinc and copper are included at 2-3 g/kg (*O. niloticus* x *O. aureus*) (Shiau & Hsieh ,2001), 30 mg/kg (*O. niloticus*) (Eid & Ghonim, 1994) and 2-3 mg/kg (*O. niloticus*) (Watanabe *et al.,* 1988), respectively. The mineral requirements for juvenile Nile tilapia are shown in Table 2.10.

**Table 2.10** Mineral requirement of juvenile Nile tilapia (*Oreochromis niloticus*) adapted from Webster & Chhorn, (2006)

| Mineral/species | Requirement (g.kg$^{-1}$ diet) | Reference |
|---|---|---|
| **Macroelements** | | |
| **Calcium (Ca)** | | |
| *O. aureus* | 7.00 | (Robinson *et al*., 1987) |
| **Phosphorous (P)** | | |
| *O. niloticus* | 0.80-1.10 | (Watanabe *et al*., 1988) |
| *O. aureus* | 5.00 | (Robinson *et al*., 1987) |
| **Magnesium (Mg)** | | |
| *O. niloticus* | 0.59-0.77 | (Dabrowska *et al*., 1989) |
| **Potassium (K)** | | |
| *O. niloticus x O. aureus* | 2.00-3.00 | (Shiau & Hsieh, 2001) |
| **Microelements** | | |
| **Manganese** | | |
| *O. niloticus* | 12.00 | (Watanabe *et al*., 1988) |
| **Iron** | | |
| Iron sulfate | 85.00 | - |
| Available iron | 60.00 | - |
| **Zinc** | | |
| *O. niloticus* | 30.00 | (Eid & Ghonim, 1994) |
| **Copper** | | |
| *O. niloticus* | 2.00-3.00 | (Watanabe *et al*., 1988) |

#### *2.4.1.6 Vitamins*

Although vitamins are required in small amounts for functions such as growth, reproduction and general health it is often not included in feeds used for tilapia stocked at moderate densities in fertilised ponds as the phyto- and zooplankton provide sufficient quantities of the vitamins. However, when higher stocking densities or enclosed systems are used, it is necessary to use a more nutritionally complete feed that includes vitamin supplementation. Halver and Hardy (2002) and Webster and Chhorn (2006) recommends that a complete vitamin supplement should be included in tilapia feeds where natural food is absent or limited.

Vitamins can be divided into water-soluble and fat-soluble vitamins (

Table **2.11**). From most of the general symptoms, mortalities, poor growth and poor feed efficiency appear to be among the most common symptoms among various vitamin deficiencies. Of the B-vitamins, tilapia shows to have a requirement for vitamin $B_1$ (thiamine), $B_2$ (riboflavin), and $B_6$ (pyridoxine). Lovell and Limsuwan (1982) reported that Nile tilapia are capable of producing vitamin $B_{12}$ (cyanocobalamin) in their intestinal tract via bacterial synthesis. Similarly, Roem *et al.* (1990) reported that *O. aureus* are capable of meeting their pantothenic acid, choline and potentially other vitamin requirements in recirculatory aquaculture systems (RAS) by feeding on bacteria. Inositol synthesis in the liver and kidney occurs to a degree in numerous fish species, but some species require supplementation to meet metabolic needs in culture systems (NRC, 1983). Furthermore, Peres *et al.* (2004) concluded that common feedstuffs should contain sufficient levels of required vitamins to meet the metabolic needs for Nile tilapia.

Poor growth, high mortalities and eye cataracts are among the various signs related to riboflavin deficiency. Soliman and Wilson (1992) reported that 6 mg/kg of riboflavin is required for freshwater juvenile *O. aureus*. Lim *et al.* (1995) reported that tilapia are highly sensitive to pyridoxine deficiencies. Furthermore, the requirement for riboflavin has been found to be affected by the dietary protein levels. Shiau *et al.* (1987) reported that optimum levels for maintaining growth were 1.7-9.55 mg/kg and 15-16.5 mg/kg for 28 % and 36 % protein content in diets, respectively. Deficiency symptoms include anorexia, convulsions, high mortalities and unusual neurological signs within 2-3 weeks of being deprived of riboflavin (Lim *et al.,* 1995). Pantothenic acid is a vital vitamin for tilapia. Soliman and Wilson (1992) reported high mortality, anaemia and severe hyperplasia of gill lamellae in blue tilapia fed diets deficient in pantothenic acid. Shiau and Hsieh (1992) reported that niacin requirements are influenced by dietary carbohydrates. Fish fed glucose and dextrin diets required 26 mg/kg and 121 mg/kg of niacin, respectively. Niacin deficiency symptoms included haemorrhages, gill edema and deformed snouts (Shiau & Hsieh, 1992). Furthermore, 10 mg/kg of calcium d-pantothenate was sufficient to meet minimum requirements. Shiau and Chin (1999) found that *O. niloticus* and *O. aureus* hybrids fed biotin deficient diets showed poor growth and low body biotin concentrations. A dietary inclusion of 0.06 mg/kg biotin was sufficient to inhibit the above-mentioned deficiency symptoms. Nile tilapia fed diets free of folic acid supplementation resulted in reduced weight gain, feed intake and feed efficiency relative to Nile tilapia fed diet supplemented with 0.5 mg/kg folic acid. However, they still produced less red blood cells than Nile tilapia fed diets with a 1 mg/kg folic acid inclusion level or higher. The suggested folic acid supplementation level is 0.5-1.0 mg/kg diet (Lim *et al.,* 2011). Choline requirements are influenced by methionine levels present in the diet. Roem *et al.* (1990) suggested that excess methionine in diets may provide sufficient methyl groups required for choline synthesis. Shiau and Lo (2000) reported that 1000 mg/kg choline is required in the diets of juvenile *O. niloticus* x *O. aureus* hybrid tilapia to maintain optimum growth and biological functions. Ascorbic acid (vitamin C) requirement for Nile tilapia is 50 mg/kg diet. Typical deficiency symptoms are shown when the vitamin C requirements are not met. Since vitamin C is relatively unstable, a sizable margin of allowance needs to be in place for large amounts lost during feed processing and storage (Stickney *et al.,* 1984).

In terms of the fat soluble vitamins, Saleh *et al.* (1995) reported that retinol (vitamin A) requirements for Nile tilapia are about 5000 UI/kg feed. High mortalities, blindness and abnormal swimming are among the symptoms reported in Nile tilapia diets without supplementation of vitamin A. Over supplementation (40000 UI/kg) may result in hypervitaminosis, causing high mortalities, reduced weight gain and impaired skeletal formation among other symptoms. Shiau and Hwang (1993) reported that the optimum inclusion level of vitamin D for juvenile hybrid tilapia is 375 IU/kg. Poor growth and food efficiency, and low haemoglobin and hepatosomatic index levels were reported for fish fed diets without vitamin D (O'Connell & Gatlin ,1994).

**Table 2.11** Vitamin requirement of juvenile Nile tilapia adapted from (Webster & Chhorn, 2006)

| Vitamin/species | Requirement (g/kg diet) | Reference |
|---|---|---|
| **Water soluble vitamins** | | |
| **Vitamin $B_1$ (thiamine)** | | |
| *O. mossambicus* x *O. niloticus* | 2.50[a] | (Lim *et al*., 1993) |
| **Vitamin $B_2$ (riboflavin)** | | |
| *O. mossambicus* x *O. niloticus* | 5.00 | (Lim *et al*., 1993) |
| *O. aureus* | 6.00 | (Soliman & Wilson, 1992) |
| **Vitamin $B_6$ (pyridoxine)** | | |
| *O. niloticus* x *O. aureus* | 1.70-9.50[b] | - |
| | 15.00-16.50[c] | - |
| **Pantothenic acid** | | |
| *O. aureus* | 10.00 | (Soliman & Wilson, 1992) |
| **Nicotinic acid (niacin)** | | |
| *O. niloticus* x *O. aureus* | 26[d]-121[e] | (Shiau & Suen, 1992) |
| **Biotin** | | |
| *O. niloticus* x *O. aureus* | 0.06 | (Shiau & Chin, 1999) |
| **Folic acid** | | |
| *O. niloticus* | 0.50 | (Lim & Klesius, 2001) |
| **Vitamin $B_{12}$ (cyanocobalamin)** | | |
| *O. niloticus* | (not required) | (Lovell & Limsuwan, 1982) |
| *O. niloticus* x *O. aureus* | (not required) | (Shiau & Lung, 1993) |
| **Inositol (myo-inositol)** | | |
| *O. niloticus* x *O. aureus* | (not required) | (Peres *et al*., 2004) |
| **Choline** | | |
| *O. aureus* | (not required) | (Roem *et al*., 1990) |
| *O. niloticus* x *O. aureus* | 1000 | (Shiau & Lo, 2000) |
| **Vitamin C (ascorbic acid)** | | |
| *O. niloticus* | 50 | (Stickney *et al*., 1984) |
| **Fat soluble vitamins** | | |
| **Vitamin A (retinol) (IU/kg)** | | |
| *O. niloticus* | 5000 | (Saleh *et al*., 1995) |
| **Vitamin D (cholecalciferol)** | | |
| *O. aureus* | (not required) | (O'Connell & Gatlin, 1994) |
| *O. niloticus* x *O. aureus* | 375 IU | (Shiau & Hwang, 1993) |
| **Vitamin E (tocopherol)** | | |
| | 50-100[f] | - |
| | 500[g] | - |
| *O. aureus* | 10h-25[h] | (Roem *et al*., 1990) |
| *O. niloticus* x *O. aureus* | 42-44[i] | (Shiau & Shiau, 2001) |

[a] In salt water at 32 ppt; [b] 28 % Protein; [c] 36 % Protein; [d] Glucose diets; [e] Dextrin diets; [f] 5 % Dietary lipid content; [g] 10-15 % Dietary lipid content; [h] 6 % Lipid content; [i] 5 % Dietary lipid content.

Dietary lipid requirements for Nile tilapia have been reported around 50-100 mg/kg for diets with 5 % lipid inclusion level, and increased to 500 mg/kg for diets containing 10-15 % lipid content (Satoh *et al.,* 1987). Furthermore, Roem *et al*. (1990) reported that 10 mg and 25 mg is required per kg of feed for 3 % and 6 % dietary lipid content, respectively. Similarly, an increase in vitamin E requirement was reported by Shiau and

Shiau (2001) with increased lipid content. The vitamin E requirements increased from 42-44 mg/kg to 60-66 mg/kg when lipid content increased from 5 % to 12 %.

### *2.4.2 Water quality requirements*

Water quality is a crucial aspect for successful aquaculture practices. The major water quality parameters and their interrelationships affect health and growth, and are among the determining factors relating to the success or failure of an aquaculture practice. The global introduction of tilapia for aquacultural purposes has introduced this fish species to countries where environmental conditions are outside their natural tolerance limits – resulting in conventional land-based systems being unsuitable for farming tilapia. Environmental parameters discussed in this chapter include dissolved oxygen (DO), water temperature, ammonia-, nitrate and nitrite and pH. (El-Sayed, 2006)

#### *2.4.2.1 Temperature*

One of the most important factors affecting physiology, growth and reproduction in fish is temperature. Tilapia are thermophilic fish, but known to tolerate a wide range of temperature fluctuations allowing them to be cultivated at various climates, including tropical, subtropical and temperate climates (El-Sayed, 2006; Webster & Chhorn, 2006). Due to natural selection, the further tilapia are from the equator, the more tolerant they become to the cold (Sifa *et al.,* 2002).

The way in which temperature affects tilapia depends on the size, strain, species, duration of exposure, culture systems and environmental factors (El-Sayed, 2006). Yashouv (1960) found that extended periods of exposure to low temperatures (6-7 °C) renders fish unable to maintain their body temperature The culture system and environmental factors has an effect on the response of tilapia to the water temperature. Rearing tilapia in 200-300 cm deep water gave significantly higher survival rates relative to 50 cm deep water. In fact, in the deeper water the fish are capable of evading sub-optimal temperatures towards the surface and bottom in winter and summer, respectively. (El-Sayed *et al.,* 1996)

Balarin and Haller (1982), Chervinski (1982), Philippart and Ruwet (1982) and Wohlfarth and Hulata (1983) summarizes the general effect and temperature requirements for tilapia. For normal development, reproduction and growth, tilapia (depending on the exact species) have a required range of 25-30 °C, but can tolerate 20-35 °C fairly well. Furthermore, reduced feeding activity and no reproduction occurs at temperatures below 20 °C. Feeding reportedly stops at 16 °C. The upper and lower lethal limits for tilapia are 42 °C and 8-12 °C, respectively. Similarly, Fukusho (1968) and Beamish (1970) reported that the lower and upper lethal limits for Nile tilapia are 10.5 °C and 42 °C, respectively, with an optimum range of 27-30 °C. Also, it is well known that temperature has an effect on levels of dissolved oxygen (DO) where higher water temperature results in lower DO levels. For the current study it was important to maintain the temperature of the water at the optimum range.

#### *2.4.2.2 Dissolved oxygen*

Factors such as photosynthesis, respiration and diet fluctuations affects the level of dissolved oxygen in the water, which in turn affects the feeding, growth and metabolism of the fish (El-Sayed, 2006). According to Tsadik and Kutty (1987), low DO levels limits respiration, growth and metabolic activities of the fish.

Tilapia can tolerate DO levels as low as 0.1-0.5 mg/l (Tsadik & Kutty, 1987), but can tolerate levels as low as 0 mg/l if they have access to surface air due to their capability of utilizing air from the air-water interface (El-Sayed, 2006). Furthermore, tilapia are better adapted to low DO levels than most teleost's, with fast saturation and offloads of dissolved oxygen from haemoglobin to tissues (Balarin & Haller, 1982). This functionality aids the fish in supplying oxygen to meet higher metabolic needs (due to respiration) in water with higher temperatures and lower DO (El-Sayed, 2006). Morgan (1972) reported that tilapia could also tolerate oxygen super saturation of up to 400 %, which may occur at times of high photosynthesis.

Stress, due to handling, can increase the oxygen consumption by up to 150-300 % relative to resting rate and can remain at this level for up to 3 h (Ross & Ross, 1983). El-Sayed (2006) recommends that after handling the fish should be returned to water with high levels of DO and not fed for at least one hour. Although tilapia can survive at sub-optimal DO levels, their water (especially in pond systems) should be maintained above 2 mg/l at dawn as prolonged and frequent exposure to low DO levels reduce their growth and metabolism, induce stress and lowers disease resistance (Teichert-Coddington & Green, 1993). However, proper care and aeration techniques should be used, as it could upsurge settled matter and increase suspension and turbidity in water (El-Sayed, 2006).

#### *2.4.2.3 Ammonia and nitrite and nitrate*

Exposure to ammonia has been found to reduce blood oxygen content, due to a reduction of red blood cells and haemolytic anaemia (Ahmed *et al.,* 1992). El-Shafai *et al.* (2004) reported that $NH_3$ levels at 0.07-0.14 mg/l was toxic to Nile tilapia, resulting in reduced growth rates. Furthermore, they recommend that $NH_3$ should be maintained below 0.1 mg/l (UAI-N mg/l). The oxidation of ammonia produces nitrite ($NO_2$), which, when further oxidized by nitrifying bacteria, turns into nitrate ($NO_3$). Nitrite is highly toxic to fish, which can lead to growth retardation and disruption of physiological functions (El-Sayed, 2006). Even though nitrate is relatively non-toxic to tilapia, prolonged exposure may decrease their immune systems and result in mortalities (Plumb, 1997).

Fish excrete nitrogenous waste in the form of ammonia. The ammonia exists as non-ionized ($NH_3$) and ionized ($NH_4^+$) compounds which are toxic and non-toxic to fish, respectively. The toxicity of ammonia correlates with the pH of the water and is influenced (to a lesser extent) by the water's temperature and DO concentration (Chervinski,1982). Water pH and the exposure period have a relationship with ammonia. Soderherg (1997) reports that the higher the pH levels (above neutral), the more non-toxic $NH_4^+$ is converted to the toxic $NH_3$. Furthermore, toxicity increases with increased temperature. Total toxic ammonia increased from <1 % to 80-90 % when the water's pH increases from 7 to 10, under similar conditions (temperature at

24-32 °C) (Webster & Chhorn, 2006). When exposed briefly, blue tilapia (*O. aureus*) was not affected by the $NH_3$ concentration of 0.91 mg/l at a pH of 9. However, a reduced specific growth rate (SGR) was experienced when $NH_3$ levels were increased to 1.81 mg/l (Hargreaves & Kucuk, 2001). Raud *et al.* (1988) reported lethal concentrations for red tilapia (*O. mossambicus* x *O. niloticus*) fry at 6.6 ppm, 4.07 ppm and 2.88 ppm for exposure periods of 48 h, 72 h and 96 h, respectively. The high tolerance was related to high levels of DO (7-10.1 mg/l).

#### *2.4.2.4 pH (hydrogen ion concentration)*

Tilapia can survive at pH ranges between 4-11 (Balarin & Hatton, 1979), but grow best in near neutral or slightly alkaline water (Webster & Chhorn, 2006). Symptoms of high or low water pH include damage of gill epithelial cells and a reduction in nitrogenous excretion efficiency (El-Sayed, 2006).

### *2.4.3 Feed parameters/measurements*

Along with meeting the nutritional requirements of animals, feed also needs to accommodate the behaviour of the animal. What differentiates aquaculture feed from land animal feeds is the requirement for it to be pelleted (except meal-type feeds for younger fish) (Webster & Chhorn, 2006), and to be water stable for maximum utilization (Webster & Chhorn, 2006; Ighwela *et al.,* 2013). It is well known that physical properties and the composition of feed as well as the processing method has an effect on the physical quality (i.e. water stability) of the pellet. As feed is the highest expense in aquaculture practices, efficient use is of prime importance for reducing costs. Obaldo *et al.* (2002) defines water stability of pellets as the degree of retention of physical integrity with minimal disintegration and nutrient leaching in water before it is consumed.

Due to the worsening effect of submersion time on water quality (Ighwela *et al.,* 2013), the feeding behaviour of the animal should be accounted for as it will influence the requirements relating to water stability. For faster eating finfish (i.e. catfish and trout) (Chen & Jenn, 1992), the feed should only be stable for a few minutes, whereas slower eating species (i.e. shrimp) may require several hours to consume their feed (Tacon, 1996). Therefore, the feed of the latter should be stable for a few hours. Animals may also manipulate feeds. Unlike most finfish, tilapia tend to chew pellets instead of swallowing then immediately, especially if they are slightly too big (Halver & Hardy, 2002).

Guerrero (1980) reported that smaller fish (fingerlings and juveniles) performed better when provided with a mash diet, while larger fish performed better on pelleted diets. Also, pellets with higher moisture content were better utilized than dryer feed, but resulted in higher leaching rates. The performance of tilapia may also be influenced by the feed colour, especially in intensive systems. El-Sayed (2004) reported that fish fed darker (red and dark blue) colour diets performed better than those fed lighter (green and yellow) colour diets. Red colour diets performed the best, and yellow the poorest. El-Sayed (2006) suggested that the effect of colour on Nile tilapia should be investigated further.

#### *2.4.3.1 Composition*

Functional properties of some feedstuff may be less desirable than others, and can only be included in limited quantities (Lim & Cuzon, 1994). Hard ingredients or those that have low binding abilities (e.g., rice, oat and hulls) may weaken pellets (Lim & Cuzon, 1994) and require a binding agent, as shown in Table 2.12, to improve water stability (Stivers, 1971). Hygroscopic ingredients (e.g., salt and sugar) absorb and increase the moisture content of stored feed and can reduce its quality even before it is added to the water (Hastings, 1970). Ingredients with a high lipid content may result in reduced expandability and binding ability of feed due to reduced compression resulting from over lubrication. Consequently, the pellets will break easily when handled or moistened by water. Furthermore, a high lipid content reduces starch gelatinization as it covers the particle surface of the carbohydrates. In cases where the feed requires a high fat content, oil can be added in the form of a spray to the finished diet (Hastings, 1970). Gelatinization improves the durability and digestibility of the feed and nutrients, respectively (Chang & Wang, 1998). Also, interactions can be disrupted as a result of protein denaturation at elevated temperatures, which may reduce expansion (Guy, 1994). Various studies have reported that protein has an inverse relationship with the expansion ratios (Kannadhason & Muthukumarappan, 2010; Kannadhason *et al.,* 2011; Sayed *et al.,* 2014).

#### *2.4.3.2 Manufacturing*

The manufacturing of feeds entails the mixing and processing of ground ingredients into uniform feed particles for the fish, which can be either meals, crumbles, sinking or floating pellets (Webster & Chhorn, 2006). Pelleting of fish feeds can be done either by steam pelleting or extrusion (Webster & Chhorn, 2006). However, it is important to compensate for the nutrients lost during the processing of the feed. Ingredients affect the nutritional value of the feed and the physical properties such as the water stability (Lim & Cuzon, 1994). Grinding increases surface area for improved gelatinization (Hastings, 1970), allows for homogenous mixing, uniform pellet texture, higher acceptability and digestibility (Stickney & Lovell, 1977). The expansion ratio (ER) of feed is dependent of the temperature and moisture content, and how they interact with the feed ingredients, and is directly related (inversely) to feed density (Chang, 1992).

Generally steam pelleting is used in developing countries due to its lower cost and production of sinking pellets (Webster & Chhorn, 2006). Extrusion, on the other hand, can be used to produce floating pellets, but is more expensive (Webster & Chhorn, 2006). Extrusion cooking in aquaculture feeds has the advantage that it increases feed digestibility and conversion, improves control over pellet density, improves water stability and durability, improves production efficiency and versatility (Chang & Wang, 1998). Hilton *et al.* (1981) reported improved feed efficiency, prolonged gastric emptying, and lower weight gain for trout that were fed extruded feed compared to steam-pelleted feed. The results were attributed to improved water and physical stability of extruded feed over steam-pelleted feed. Furthermore, extrusion helps with reducing residual fine particles, the improvement of water quality, feed consumption and growth performance of fish (Lucas & Southgate, 2012). Webster and Chhorn (2006) suggests that the ability of extruded feed to float allows for a better observation

during feeding, thereby preventing overfeeding and reducing wastage. Barrows *et al.* (2007) attributes the improved digestibility and reduction of thermolabile antinutrients to the heat produced during extrusion.

**Table 2.12** Different binders types used in fish feed

| **Binder** | **Type of binder** |
|---|---|
| **Natural substances** | |
| Gampro | Wheat Gluten |
| AP-520 | Plasma protein |
| Nutraflex 40 Mega | Collagen protein |
| EX-5819 | Xanthen and locust bean gum |
| EX-5820 | Xanthen and locust bean gum |
| RE-9556 | Carrageenan mix |
| RE-9556/9557 | Carrageenan mix |
| **Modified substances** | |
| Gampro-plus | Modified wheat gluten |
| Ameri-Bond 2000R | Lignin sulfonate |
| Ameri-Bond D-357 | Modified lignin sulfonate |
| Nutri-Binder | Modified sorghum |
| **Synthetic substances** | |
| Aqua-Firm 1A | Urea formaldehyde |
| Aqua-Firm 2A | Urea formaldehyde |
| Aquabind | Ethylene/vinyl acetate copolymer |
| BASFIN | Urea formaldehyde |
| Pel-Plus 100 | Mineral |
| Pel-Plus 200A | Mineral |

## 2.5 Conclusion

Full-fat BSF pre-pupae meal is a suitable candidate for replacing conventional protein sources (i.e. fishmeal and soya meal) in Nile tilapia feed. The ability of the larvae to convert food waste, agricultural and other bio-wastes into high protein and fat sources during propagation can potentially alleviate the pressure placed on natural fish stocks for the production of fish meal. Bioconversion through insects also has an economic advantage in that it aids in reducing environmental and economic footprints related to the removal of bio-wastes. Furthermore, implementing BSF farming in poor or third world countries could provide a feed protein source for aquaculture by reducing feed costs, specifically protein, which generally makes up 50% of the feed. Thus, the use of BSF in fish feed can indirectly alleviate hunger in specific parts of the world where feedstuff protein sources are scarce. Its amino acid and fatty acid profile relates well to the requirement of Nile

tilapia. Furthermore, previous studies have successfully replaced conventional protein sources in other fish feeds by up to 30 %, without having any negative effects on the growth parameters. An important factor to take into account is the relatively high lipid content of full-fat BSF pre-pupae meal. This may have adverse effects on the physical characteristics of fish feed due to its lubricating effect during the extrusion process. Based on previous studies, there shows to be potential for the use of BSF in Nile tilapia feeds.

## 2.6 References

Abdelghany, A.E., 1997. Optimum ratio between anchovy fish meal and soy protein concentrate in formulated diets for Nile tilapia (*Oreochromis niloticus L*.). In: Proceedings from the Fourth International Symposium on tilapia in Aquaculture. Ed: Fitzimmons, K. pp. 31-39.

Ahmed, N.A., El-Serafy, S.S., El-Shafey, A.A.M. & Abdel-Hamid, N.H., 1992. Effect of ammonia on some haematological parameters of *Oreochromis niloticus*. In: Proceedings of Zoological Society of Arab Republic of Egypt 23. Vol. 23. pp. 155-160.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K. & Jimoh, W., 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus L*.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. Aquac. Res. 43(3), 412-420.

Anderson, J., Jackson, A.J., Matty, A.J. & Capper, B.S., 1984. Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus* (Linn.). Aquac. 37(4), 303-314.

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). P. J. Nutr. 9(5), 485-487.

Atteh, J.O. & Ologbenla, F.D., 1993. Replacement of fish meal with maggots in broiler diets: effects on performance and nutrient retention. N. J. Anim. Prod. 20(1), 44–49.

Balarin, J.D. & Haller, R.D., 1982. The intensive culture of tilapia in tanks, raceways and cages. Rec. Adv. Aquac. 1, 265-355.

Balarin, J.D. & Hatton, J.P., 1979. Tilapia: A guide to their biology and culture in Africa.

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. Aquac. 422, 193-201.

Barrows, F.T., Stone, D.A. & Hardy, R.W., 2007. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). Aquac. 265(1), 244-252.

Bauhus, J. & Meiwes, K.J., 1994. Potential use of plant residue wastes in forests of northwestern Germany. For. Ecol. Manag. 66(1-3), 87-106.

Beamish, F.W.H., 1970. Influence of temperature and salinity acclimation on temperature preferenda of the euryhaline fish tilapia nilotica. J. Fish. B. Can. 27(7), 1209-1214.

Blonk, H., Kool, A., Luske, B., De Waart, S. & Ten Pierick, E., 2008. Milieueffecten van Nederlandse consumptie van eiwitrijke producten: gevolgen van vervanging van dierlijke eiwitten anno 2008. In: Blonk Milieu Advies.

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. Man. 18(3), 209-220.

Booth, D.C. & Sheppard, C., 1984. Oviposition of the black soldier fly, *Hermetia illucens* (*Diptera: Stratiomyidae*): eggs, masses, timing, and site characteristics. Environ. Entomol. 13(2), 421-423.

McCallan, E., 1974. *Hermetia illucens* (L.) (*Diptera, Stratiomyidae*), a cosmopolitan American species long established in Australia and New Zealand. Entomol. Mo. Magazine. 109, 232-234.

Calvert, C.C., Martin, R.D. & Morgan, N.O., 1969. House fly pupae as food for poultry. J. Econ. Entomol. 62(4).

Chang, C.N., 1992. Study of the Mechanism of Starchy Biopolymer Extrudate. Rutgers University.

Chang, Y.K. & Wang, S.S., 1998. Advances in Extrusion Technology. In: International Symposium on Animal and Aquaculture Feedstuffs by Extrusion Technology. Eds: Chang, Y.K. & Wang S.S. São Paulo: Technomic Pub. Co.

Chen, C., Sun, B., Guan, W., Bi, Y., Li, P., Ma, J., … Xie, Q., 2016. N-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: Effects of linolenic acid on non-specific immunity and anti-inflammatory responses in juvenile fish. Aquac. 450, 250-257.

Chen, H.Y. & Jenn, J.S., 1992. Increased pellet water stability by the supplementation of phospholipid and its effects on shrimp growth. A. Fish. Sci. 5, 211-217.

Chervinski, J., 1982. Environmental physiology of tilapias. In: The Biology and Culture of Tilapias. 7, 119-128.

Chou, B.S. & Shiau, S.Y., 1996. Optimal dietary lipid level for growth of juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquac. 143(2), 185-195.

Dabrowska, H., Meyer-Burgdorff, K. & Günther, K.D., 1989. Interaction between dietary protein and magnesium level in tilapia (*Oreochromis niloticus*). Aquac. 76(3-4), 277-291.

Eid, AE. & Ghonim, S.I., 1994. Dietary zinc requirement of fingerling Oreochromis niloticus. Aquac. 119(2-3), 259-264.

El-Sayed, A.-F.M., 1987. Protein and energy requirements of Tilapia zillii. Michigan State University, East Lansing, Michigan.

El-Sayed, A.-F.M., 1989. Evaluation of semipurified test diets for Tilapia zillii fingerlings. J. World. Aquac. Soc. 20(4), 240-244.

El-Sayed, A.-F.M., 1990. Long-term evaluation of cottonseed meal as a protein source for Nile tilapia, *Oreochromis niloticus* (Linn.). Aquac. 84(3-4), 315-320.

El-Sayed, A.-F.M., 2004. Feed colour affects growth, feed utilization of Nile tilapia. Glob. Aquac. Adv. 7(2), 76.

El-Sayed, A.-F.M., 2006. Tilapia culture. In : Aquaculture. CABI publishing, UK.

El-Sayed, A.-F.M., El-Ghobashy, A. & Al-Amoudi, M.M., 1996. Effects of pond depth and water temperature on the growth, mortality and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquac. Res., 27(9), 681-687.

El-Shafai, S.A., El-Gohary, F.A., Nasr, F.A., Van der Steen, N.P. & Gijzen, H.J., 2004. Chronic ammonia toxicity to duckweed-fed tilapia (*Oreochromis niloticus*). Aquac. 232(1), 117-127.

Essa, M.A., 1997. Utilization of some aquatic plants in diets for Nile tilapia, *Oreochromis niloticus*, fingerlings. Egypt. J. Aquat. Biol. Fish. 1(2), 19-34.

Food and Agriculture Organization of the United Nations (FAO), 2015. Aquaculture Feed and Fertilizer Resources Information System.

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(9), 733-738.

Fukusho, K., 1968. The specific difference of temperature responses among Cichlid fishes genus tilapia. Bull. Jpn. Soc. Sci. Fish. 34, 103-111.

Furman, D.P., Young, R.D. & Catts, P.E., 1959. *Hermetia illucens* (Linnaeus) as a factor in the natural control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52(5), 917-921.

Guerrero, R.D., 1980. Studies on the feeding of tilapia nilotica in floating cages. Aquac. 20(3), 169-175.

Gustavsson, J., Cederberg, C., Sonesson, U., Van Otterdijk, R. & Meybeck, A., 2011. Global food losses and food waste. FAO. Rome, Italy.

Guy, R.C.E., 1994. Raw materials for extrusion cooking processes. In: The Technology of Extrusion Cooking. pp. 52-72.

Halver, J.E. & Hardy, R.W., 2002. In: Fish Nutrition. Academic press.

Hanley, F., 1987. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in tilapia, *Oreochromis niloticus* (L). Aquac. 66(2), 163-179.

Hardy, D., 1960. *Diptera*: *Nematocera-Brachycera* (except *Dolichopodidae*). In: Insects of Hawaii. Vol. 10.

Hargreaves, J.A. & Kucuk, S., 2001. Effects of diel un-ionized ammonia fluctuation on juvenile hybrid striped bass, channel catfish, and blue tilapia. Aquac. 195(1), 163-181.

Hastings, W.H., 1970. Study of pelleted fish foods stability in water. In: Report of the 1970 Workshop on Fish Feed Technology and Nutrition, Warmwater Fish Cultural Laboratories. Arkansas, USA. pp. 7-19.

Hilton, J.W., Cho, C.Y. & Slinger, S.J., 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.). Aquac. 25(2-3), 185-194.

Ighwela, K.A., Bin Ahmad, A. & Abol-Munafi, A.B., 2013. Water stability and nutrient leaching of different levels of maltose formulated fish pellets. Glob. Vet. 10(6), 638-642.

Jackson, A.J., Capper, B.S. & Matty, A.J., 1982. Evaluation of some plant proteins in complete diets for the tilapia, *Sarotherodon mossambicus*. Aquac. 27(2), 97-109.

James, M.T., 1935. The genus *Hermetia* in the United States (Diptera: Stratiomyidae). Bull. Brooklyn. Entomol. Soc. 30(4), 165-170.

James, M.T., 1947. The flies that cause myiasis in man. US Department of Agriculture.

Kanazawa, A.S., Teshima, M.S. & Awal, M.A., 1980. Requirements of tilapia zillii for essential fatty acids. Bull. Jpn. Soc. Sci. Fish. 46, 1353-1356.

Kannadhason, S. & Muthukumarappan, K., 2010. Effect of starch sources on properties of extrudates containing DDGS. Int. J. Food Prop. 13(5), 1012-1034.

Kannadhason, S., Muthukumarappan, K. & Rosentrater, K.A., 2011. Effect of starch sources and protein content on extruded aquaculture feed containing DDGS. Food. Bioproc. Tech. 4(2), 282-294.

Kleemann, G.K., Barros, M.M., Pezzato, L.E., Sampaio, F.G., Ferrari, J.C., Valle, J.B., … Zuanon, J., 2003. Iron requirement for Nile tilapia *Oreochromis niloticus*. World Aquac. 82.

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*). Aquac. 364, 345-352.

Kubaryk, J.M., 1980. Effect of Diet, Feeding Schedule and Sex on Food Consumption, Growth and Retention of Protein and Energy by Tilapia. Auburn University.

Lim, C. & Cuzon, G., 1994. Water stability of shrimp pellet: a review. Asian. Fish. Sci. 7(2-3), 115-126.

Lim, C. & Klesius, P.H., 2001. Influence of dietary levels of folic acid on growth response and resistance of Nile tilapia, *Oreochromis niloticus* to *Streptococcus iniae*. In: Sixth Asian Fisheries Forum. Book of Abstracts. Vol. 150.

Lim, C., Leamaster, B. & Brock, J.A., 1993. Riboflavin requirement of fingerling red hybrid tilapia grown in seawater. J. World Aquac. Soc. 24(4), 451-458.

Lim, C., LeaMaster, B.R. & Brock, J.A., 1995. Pyridoxine requirement of fingerling red hybrid tilapia grown in seawater. J. Appl. Aquac. 5(2), 49-60.

Lim, C., Yildirim-Aksoy, M., Barros, M.M. & Klesius, P., 2011. Thiamin requirement of Nile tilapia, *Oreochromis niloticus*. J. World Aquac. Soc. 42(6), 824-833.

Lock, E.J., Arsiwalla, T. & Waagbø, R., 2014. Insect meal: A promising source of nutrients in the diet of Atlantic salmon (*Salmo salar*). In: Insects to Feed The World. p. 74.

Lovell, R.T. & Limsuwan, T., 1982. Intestinal synthesis and dietary nonessentiality of vitamin $B_{12}$ for tilapia nilotica. Trans. Am. Fish. Soc. 111(4), 485-490.

Lucas, J.S. & Southgate, P.C., 2012. Aquaculture: Farming aquatic animals and plant. Blackwell Publishing Ltd., UK.

Luquet, P., 1991. Tilapia, *Oreochromis* spp. In: Handbook of Nutrient Requirement of Finfish. Ed: Wilson, R.P. CRC Press, Boca Raton, FL. pp. 169-179.

Morgan, P.R., 1972. Causes of mortality in the endemic tilapia of Lake Chilwa (Malawi). Hydrobiologia. 40(1), 101-119.

Mungkung, R., Aubin, J., Prihadi, T.H., Slembrouck, J., Van Der Werf, H.M.G. & Legendre, M., 2013. Life cycle assessment for environmentally sustainable aquaculture management: A case study of combined aquaculture systems for carp and tilapia. J. Clean. Prod. 57, 249-256.

National Research Council (NRC), 1983. Nutrient requirements of warmwater fishes and shellfishes. Vol. 12. National Academy of Science, Washington, USA. pp. 102.

Newton, G.L., Booram, C.V., Barker, R.W. & Hale, O.M., 1977. Dried larvae meal as a supplement for swine. J. Anim. Sci. 44(3), 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. Symposium on the State of the Science of Animal Manure and Waste Management, 5-7.

Newton, L., Sheppard, C., Watson, D.W., Burtle, G. & Dove, R., 2005b. Using the black soldier fly, *Hermetia illucens*, as avalue-added tool for management of swine manure. Journal of Chemical Information and Modeling.

Ng, W.K. & Wee, K.L., 1989. The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. Aquac. 83(1-2), 45-58.

Nijdam, D., Rood, T. & Westhoek, H., 2012. The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. Food. Policy. 37(6), 760-770.

O'Connell, J.P. & Gatlin, D.M., 1994. Effects of dietary calcium and vitamin $D_3$ on weight gain and mineral composition of the blue tilapia (*Oreochromis aureus*) in low-calcium water. Aquac. 125(1-2), 107-117.

Obaldo, L.G., Divakaran, S. & Tacon, A.G., 2002. Method for determining the physical stability of shrimp feeds in water. Aquac. Res. 33(5), 369-377.

Ogunji, J.O., Toor, R.U.A.S., Schulz, C. & Kloas, W., 2008a. Growth performance, nutrient utilization of Nile tilapia Oreochromis niloticus fed housefly maggot meal (magmeal) diets. Turk. J. Fish. Aquat. Sci. 8(1), 141-147.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B., 2006. Housefly maggot meal (Magmeal): An emerging substitute of fishmeal in tilapia diets. Bonn, Germany.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B., 2008b. Housefly maggot meal (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). 21(3), 319-331.

Olsen, R.E., Henderson, R.J. & McAndrew, B.J., 1990. The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by tilapia (*Oreochromis*) nilotica *in vivo*. Fish Phys. Biochem. 8(3), 261-270.

Olvera-Novoa, M.A., Campos, S.G., Sabido, M.G. & Palacios, C.A.M., 1990. The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus*). Aquac. 90(3-4), 291-302.

Olvera-Novoa, M.A., Domínguez-Cen, L.J., Olvira- castillo, L. & Martínez-Palacios, C.A., 1998. Effect of the use of the microalga Spirulina maxima as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquac. Res. 29, 709-715.

Peres, H., Lim, C. & Klesius, P.H., 2004. Growth, chemical composition and resistance to *Streptococcus iniae* challenge of juvenile Nile tilapia (*Oreochromis niloticus*) fed graded levels of dietary inositol. Aquac. 235(1), 423-432.

Philippart, J.C. & Ruwet, J.C. 1982., Ecology and distribution of tilapias. In: The Biology and Culture of Tilapias. Vol. 7. pp. 15–60.

Plumb, J.A. 1997., Infectious diseases of tilapia. In: Tilapia Aquaculture in the Americas. Vol. 1. pp. 212-228.

Raud, S.K., Hasbollah, D. & Law, Q.T., 1988. Effects of unionized ammonia on red tilapia (*Oreochromis mossambicus/O. niloticus hybrid*) fry. In: ICLARM Conference Proceedings.

Riggi, L., Veronesi, M., Verspoor, R., MacFarlane, C. & Tchibozo, S., 2013. Exploring entomophagy in Northern Benin-practices, perceptions and possibilities. In: Bugs for life. London, UK.

Robinson, E.H., LaBomascus, D., Brown, P.B. & Linton, T.L. 1987., Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. Aquac. 64(4), 267-276.

Roem, A.J., Stickney, R.R. & Kohler, C.C., 1990. Vitamin requirements of blue tilapias in a recirculating water system. Progr. Fish-Cultur. 52(1), 15-18.

Ross, B. & Ross, L.G., 1983. The oxygen requirements of *Oreochromis niloticus* under adverse conditions. In: Proceedings of the International Symposium on Tilapia Aquaculture. Israel. pp. 134-143.

Saleh, G., Eleraky, W. & Gropp, J.M., 1995. A short note on the effects of vitamin A hypervitaminosis and hypovitaminosis on health and growth of tilapia nilotica (*Oreochromis niloticus*). J. Appl. Ichth. 11(3-4), 382-385.

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.

Santiago, C.B. & Lovell, R.T., 1988. Amino acid requirements for growth of nile tilapia. The J. Nutr. 118(12), 1540-1546.

Santiago, C.B. & Reyes, O.S., 1993. Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) broodstock. J. Appl. Ichth. 9(1), 33-40.

Satoh, S., Takeuchi, T. & Watanabe, T. 1987., Requirement of tilapia for alpha-tocopherol. Bull. Jpn. Soc. Sci. Fish. 53, 119-124.

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 pre-pupae, *Hermetia illucens*. J. World. Aquac. Soc. 42(1), 34-45.

Sheppard, D.C., Newton, G.L., Thompson, S.A. & Savage, S., 1994. A value added manure management system using the black soldier fly. Biores. Tech. 50(3), 275-279.

Shiau, S.-Y. & Lung, C.-Q. 1993. No dietary vitamin $B_{12}$ required for juvenile tilapia *Oreochromis niloticus* x *O. aureus*. Comp. Biochem. Physiol. Part A: Physiol. 105(1), 147-150.

Shiau, S., Chuang, J.L. & Sun, C.L., 1987. Inclusion of soybean meal in tilapia (*Oreochromis niloticus* x *O. aureus*) diets at two protein levels. Aquac. 65(3-4), 251-261.

Shiau, S.Y. & Chin, Y.H., 1999., Estimation of the dietary biotin requirement of juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. Aquac. 170(1), 71-78.

Shiau, S.Y. & Hsieh, H.L., 1997. Vitamin $B_6$ requirements of tilapia *Oreochromis niloticus* x *O. Aureus* fed two dietary protein concentrations. Fish. Sci. 63, 1002-1007.

Shiau, S.Y. & Hsieh, J.F., 2001. Quantifying the dietary potassium requirement of juvenile hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). Brit. J. Nutr. 85(2), 213-218.

Shiau, S.Y. & Hwang, J.Y., 1993. Vitamin D requirements of juvenile hybrid tilapia *Oreochromis niloticus* x *O. aureus*. Nippon Suisan Gakkaishi. 59(3), 553-558.

Shiau, S.Y. & Lo, P.S., 2000. Dietary choline requirements of juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. The J. Nutr. 130(1), 100-103.

Shiau, S.Y. & Peng, C.Y., 1993. Protein-sparing effect by carbohydrates in diets for tilapia, *Oreochromis niloticus* x *O. aureus*. Aquaculture, 117(3-4), 337-334.

Shiau, S.Y. & Shiau, L.F., 2001. Re-evaluation of the vitamin E requirements of juvenile tilapia (*Oreochromis niloticus* x *O. aureus*). Anim. Sci. 72(3), 529-534.

Shiau, S.Y. & Suen, G.S., 1992. Estimation of the niacin requirements for tilapia fed diets containing glucose or dextrin. The J. Nutr. 122(10), 2030.

Shimeno, S., Ming, D. & Takeda, M., 1993. Metabolic response to dietary carbohydrate to lipid ratios in *Oreochromis niloticus*. Bull. Jpn. Soc. Sci. Fish. 59(5), 827-833.

Siddiqui, A.Q., Howlader, M.S. & Adam, A.A., 1988. Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. Aquac. 70(1-2), 63-73.

Sifa, L., Chenhong, L., Dey, M., Gagalac, F. & Dunham, R., 2002. Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China. Aquac. 213(1), 123-129.

Soliman, A.K. & Wilson, R.P., 1992. Water-soluble vitamin requirements of tilapia. 2. Riboflavin requirement of blue tilapia, *Oreochromis aureus*. Aquac. 104(3-4), 309-314.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., … Irving, S., 2007b. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World. Aquac. Soc. 38(2), 309-313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., … Sealey, W., 2007a. Fly pre-pupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World. Aquac. Soc. 38(1), 59-67.

Stickney, R.R. & Lovell, T., 1977. Nutrition and feeding of channel catfish. Alabama Agricultural Experiment Station.

Stickney, R.R., McGeachin, R.B., Lewis, D.H., Marks, J., Riggs, A., Sis, R.F., … Wurts, W., 1984. Response of tilapia aurea to dietary vitamin C. J. World. Aquac. Soc. 15(1-4), 179-185.

Stivers, T., 1971. Feed manufacturing. In: In Gaudet, JL Report of the 1970 workshop of fish feed technology and nutrition. Resour. Publ. Bur. Sport. Fish. New York. pp. 14-42.

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. & Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew. Energy. 98, 197-202.

Tacon, A.G.J., 1996. Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. Aquac. Nutri. 2(3), 165-174.

Tacon, A.G.J., Jauncey, K., Falaye, A., Pantha, M., MacGowan, I. & Stafford, E.A., 1983. The use of meat and bone meal and hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In: Proceedings of the First International Symposium on Tilapia in Aquaculture. pp. 356-365.

Tacon, A.G.J. & Metian, M., 2013. Fish matters: Importance of aquatic foods in human nutrition and global food supply. Rev. Fish. Sci. 21(1), 22-38.

Takeuchi, T., Satoh, S. & Watanabe, T., 1983. Requirement of tilapia nilotica for essential fatty acids. Bull. Jpn. Soc. Sci. Fish.

Teichert-Coddington, D. & Green, B.W., 1993. Tilapia yield improvement through maintenance of minimal oxygen concentrations in experimental grow-out ponds in Honduras. Aquac. 118(1-2), 63-71.

Tsadik, G.G. & Kutty, M.N., 1987. Influence of Ambient Oxygen on Feeding and Growth of the Tilapia, *Oreochromis niloticus* (Linnaeus).

Ulloa Rojas, J. & Van Weerd, J.H., 1997. The growth and feed utilization of *Oreochromis aureus* fingerlings fed diets with various coffee pulp levels.

Viola, S. & Zohar, G., 1984. Nutritional study with market size tilapia hybrid *Oreochromis* in intensive culture. Protein levels and sources. Isr. J. Aquac. 36, 3-15.

Watanabe, T., Satoh, S. & Takeuchi, T., 1988. Availability of minerals in fish meal to fish. Asian Fish. Sci. 1(2), 75-195.

Webster, C.D. & Chhorn, L., 2006. Tilapia: biology, culture and nutrition. CRC Press.

Wohlfarth, G.W. & Hulata, G., 1983. Applied genetics of tilapias. ICLARM studies and review. Philippines.

Womeni, H.M., Linder, M., Tiencheu, B., Mbiapo, F.T., Villeneuve, P., Fanni, J. & Parmentier, M., 2009. Oils of insects and larvae consumed in Africa: potential sources of polyunsaturated fatty acids. Oléagineux. Corps. Gras. Lipides. 16(4), 230-235.

Wu, Y.V., Rosati, R., Sessa, D.J. & Brown, P., 1995. Utilization of corn gluten feed by Nile tilapia. Progr. Fish. Cult. 57(4), 305-309.

Yashouv, A., 1960. Effect of low temperatures on tilapia nilotica and tilapia galilaea. Bamidgeh. 12(3), 62-66.

Zhu, F.X., Yao, Y.L., Wang, S.J., Du, R.G., Wang, W.P., Chen, X.Y., … Yang, H.Q., 2015. Housefly maggot-treated composting as sustainable option for pig manure management. Waste Management, 35, 62-67.

# Chapter 3

## The effect of varying inclusion levels of black soldier fly (*Hermetia illucens*) pre-pupae on Nile tilapia (*Oreochromis niloticus*) feed characteristics

**Abstract**

The effects of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal on bulk density, sinking velocity, water absorption and disintegration were investigated for six treatments with increasing BSF inclusion levels. The six inclusion levels were 0 % (control diet) (BSF0), 5 % (BSF5), 10 % (BSF10), 15.6 % (BSF15.6), 20 % (BSF20) and 25 % (BSF25). Pellet density was significantly different among all treatments, with higher inclusion levels of BSF resulting in increased unit densities. The control (BSF0) and BSF5 had a sinking velocity of zero (floating pellets). The pellets only started sinking at inclusion levels of 10 % and higher which may be attributed to increased unit density. Water uptake was significantly different among most treatments, with BSF0 having the highest degree of water uptake for all time frames (stability of pellets were tested over time) except for the shortest time frame of 5 min. BSF15.6 had the highest absorption rate and degree of disintegration. The latter was significantly higher than all other treatments, which may be attributed to BSF15.6 having the highest lipid content of diets without the use of a binder. When attempting to extrude BSF20 and BSF25 little to no binding was observed, which prompted the inclusion of a pellet binder to prevent complete loss of feed. BSF20 and BSF25 were the only diets where pellet binders were used due to excessive crumbling observed during extrusion. There appears to be a relationship between lipid content and bulk density, with bulk density increasing with higher lipid contents (attributed to a lubrication effect). Lubrication during extrusion reduces gelatinization, and thus the expandability and binding ability of the feed, resulting in a more compact pellet with weaker structural stability which requires a binding agent to stay intact. The use of defatted BSF pre-pupae meal should be investigated, and binders should be included in similar levels for feed with a lower degree of physical differences.

Key words: Black soldier fly, pre-pupae, Nile tilapia, feed characteristics, bulk density, sinking velocity, water absorption

## 3.1 Introduction

The most expensive component in the aquaculture industry is feed, making up more than 50 % of its operating costs (El-Sayed, 2006). With this in mind, it goes without saying that optimization of nutritional components, such as the production thereof, is of high importance. Aquaculture feeds requires pelleting (except meal-type feeds for younger fish) (Webster & Chhorn, 2006), and must be water stable (Webster & Chhorn, 2006; Keri Alhadi Ighwela *et al*., 2013) to ensure efficient utilization. Furthermore, animal behaviour (e.g., feeding rate) needs to be accounted for due to the worsening effect of submersion on water quality. For example, tilapia chew pellets (Halver & Hardy, 2002), which is a factor to consider when feed is manufactured to meet certain water quality parameters. Low quality feeds are characterized by immediate dispersion when immersed, disintegration during transportation or sinking velocities outside specifications (Haubjerg *et al*., 2015). It also raises farming costs by reducing uptake and results in pollution of water environments.

Inclusion levels of certain feedstuffs are limited by their functional properties. For example, the inclusion levels of rice and oat hulls are limited due to their hardness which may weaken the pellets (Lim & Cuzon, 1994). Inclusion levels of lipids are also limited due to their diminishing effect on compression and over lubrication during extrusion, in turn reducing expansion and binding ability of feed, thereby increasing breakability and reducing floatability of feed (Hastings, 1970). Over lubrication also reduces gelatinization during extrusion, which is an important reaction during extrusion as it improves the durability and digestibility of feedstuffs (Chang & Wang, 1998).

Also, the gelatinization of starch promotes molecular interlinking (binding) and is a major factor influencing the ability of starch to expand during the extrusion process (Chinnaswamy & Hanna, 1988). Starch consists of two chains of glucose molecules, i.e. amylose and amylopectin (Tharanathan, 1995). These molecules react differently during the extrusion process. Their ratio in starch determines the physical structural changes that occur (Davidson *et al*., 1984). Amylopectin is primarily responsible for expansion when exposed to high extrusion temperatures (Davidson *et al*., 1984). Sankaranandh Kannadhason *et al.* (2011) attributed lower expansion in corn-starch diets relative to cassava and potato starch due to the lower amylopectin proportions in corn. Increasing levels of amylopectin in diets produce a more homogenous and sticky texture, which is light and elastic, whilst amylose reduces expansion and produces a harder pellet (Mercier & Feillet, 1975). Kannadhason and Muthukumarappan (2010), Sayed *et al.* (2014) and Sankaranandh Kannadhason *et al*. (2011) reported that the source and inclusion level of starch has a significant influence on the binding capacity and expansion ratio of the feed during extrusion. In fact, cassava and potato starches were better suited for floating and sinking pellets, respectively, whilst corn starch improved the durability.

Unit density is useful in predicting what the floatability of extrudates are, especially for species such as Nile tilapia where floating feed is preferred (Ayadi *et al*., 2016). Increased protein levels in feed have also been reported to increase the unit density and sinking velocity of feed (Kannadhason *et al*., 2011), which may be due to its binding ability (Cheftel *et al*., 1985). Exposure of feed to elevated temperatures and shear pressure during extrusion could denature and alter the structure of proteins (Kannadhason *et al*., 2011). These actions

may disrupt interactions with other components, and may reduce expansion (Guy, 1994). Protein inclusion levels have an inverse relationship to the expansion ability of feed (Kannadhason & Muthukumarappan, 2010; Kannadhason *et al*., 2011; Khater *et al*., 2014) and increases unit density and sinking velocity with varying degrees at higher inclusion levels, depending on the protein source. Similarly, Sankaranandh Kannadhason *et al*. (2011) reported that the expansion ratio (ER) (a measure of the ability of feed to expand) decreased by 14.2 %, 5.98 % and 15.1 % for cassava, corn and potato starch sources, respectively, when distillers dried grains and solubles (DDGS) (a high protein and low starch feedstuff) were increased from 20 % to 40 %. Pellet durability is also inversely affected by increased protein content. Sankaranandh Kannadhason *et al*. (2011) reported that the pellet durability index (PDI) (a measure of pellet durability) decreased by 15.2 % for corn starch extrudates when protein content was increased from 28 % to 32 %. Furthermore, soy meal inclusion levels exceeding 42 % decreased (Lim & Dominy, 1989) and high inclusion levels of gluten wheat flower (Balazs *et al*., 1973) increased pellet water stability, a measurement of the stability of feeds when submerged in water, respectively.

These results indicate that protein does have an effect on the binding capacity of feed, but increases or decreases binding capacity depending on the source of the protein used. Protein also differentiates depending on the starch sources used in conjunction with other ingredients.

The two most common processing methods are extrusion and pelleting, involving varying degrees of heat, moisture and pressure (Hilton *et al*., 1981). Extruded feed are regarded as superior to steam pelleted feeds when it comes to water stability and floating properties, with the latter aiding in determination of feed consumption (Stickney & Lovell, 1977). Steam pelleted feeds are used for sinking feeds, and is the usual production method in developing countries as it is a less expensive method, using moisture, heat and pressure to turn ground ingredients into homogenous feed particles. The process generally entails increasing the moisture content to 15-16 % by adding steam and increasing the temperature to about 70-85 °C, which gelatinizes the starch and activates binding agents. The preconditioning is discharged into a rotating die ring, and then compressed through holes that shape the pellets. Once the pellets emerge from the exterior of the die, knives cut them into the desired length. Once in pellet form, the feed has a moisture content of 14-15 % and is then cooled and dried via evaporative cooling at lower temperatures until an ambient final temperature and a moisture content of 8-10 % is reached. Proper compaction, binding and production rates are maintained by upholding the optimum discharge speed, rotational speed of the die and rollers, and hole diameter and thickness. The compaction of the pellets is determined by the discharge speed and uniformity through the die (Webster & Chhorn, 2006).

Extrusion is mainly used for the production of floating pellets, making use of moisture, heat, shear force and friction (Webster & Chhorn, 2006). Firstly the feed ingredients are moistened and formed into a mash. The ingredients may be pre-conditioned in a pressurized container for 3-5 min where moisture is further increased to approximately 25 % by adding steam (Webster & Chhorn, 2006). Cooking during pre-conditioning improves the gelatinization, flavour development and digestibility of the feed (Webster & Chhorn, 2006). Hastings (1970)

reported that pre-conditioning considerably increased water stability of channel catfish feed. The mash then gets discharged into an extruder and forced through the barrel and die at the end with rotating screws, increasing temperatures to 120-150 °C as steam is added and friction increases (Webster & Chhorn, 2006). The restricted flow at the die (end of the barrel) provides the necessary shear pressure that shapes the feed. The extreme drop in pressure (once feed passed through die) results in vaporization of water within the pellets, creating air pockets and thus allowing the feed to float. However, sinking pellets can be produced with extrusion by lowering the pressure in the barrel (i.e. the expansion rate) (Webster & Chhorn, 2006). The pellets will have a moisture content of 18-19 % (higher than steamed pellet), thus, requiring it to be dried with heat until the moisture content is about 8-10 % to improve shelf life (Webster & Chhorn, 2006).

In certain cases, having control over diet composition and processing conditions are not enough to ensure production of a quality pellet. Consequently, a binding agent may be required. Binders are used to improve water stability, and are classified as natural, modified or synthetic, shown in Chapter 2 (Table 2.12). Binders have three actions, as indicated by Stivers (1971): acting as a filler (reducing void spaces to improve compaction), providing adhesion (physically binding particles together) and chemical actions (resulting action due to exposure to heat, pressure and moisture changes). By using extrusion processing for feed, a viable feedstuff should be produced with BSF included as an alternative protein source.

## 3.2 Materials and methods

### *3.2.1 Experimental procedures*

The following procedures were carried out on the experimental diets used to test the effect of BSF pre-pupae meal on the physical properties of pellets. Six diets were used with various inclusion levels ranging from 0 % (control diet) to 25 % BSF pre-pupae meal (summit diet). The diets will be referred to based on their inclusion levels as shown in Table  throughout the chapter.

**Table 3.1** Diets with varying inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal

| **Diet** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| BSF pre-pupae (%) | 0 | 5 | 10 | 15.6 | 20.0 | 25 |
| Code | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 |

### *3.2.2 Unit density*

Unit density was taken as a measurement of feed mass per unit volume of space occupied by feed, as described by Clementson and Ileleji (2010). After sieving feed through a 2 mm mesh (Figure 3.1 a), the pellets were poured into a glass container up to the 800 ml mark (Figure 3.1 b). Six replicates were measured for

each diet, and calculated with Equation 1 adapted from Sankaranandh Kannadhason *et al*. (2011). The bulk density was expressed as a unit of kg/m$^3$.

**Equation 1: UD = M / V**

UD = Unit Density of extrudates (kg/m$^3$); M = Mass (kg); V = Volume (m$^3$).


a) b)

**Figure 3.1** Sieving (a) and weighing (b) of feed to determine bulk density

### *3.2.3 Sinking velocity*

Sinking velocity was calculated as an average of pellets that were individually dropped in a 2000 mL glass cylinder for every diet containing 2000 mL tap water at room temperature (27 °C). The sinking velocity was expressed in cm/s for ease of comparison. The time in seconds it took the pellets to sink to the bottom of the glass cylinder was measured with a stopwatch, and used to calculate the sinking velocity of the pellets (Kannadhason *et al*., 2011):

**Equation 2:** SV = m / t

SV = Sinking Velocity; m = Height (mm) of the water column; t = Time (s) taken to reach the bottom of the container.

### *3.2.4 Water uptake and disintegration*

A modified version of the method used by Obaldo *et al.* (2002) to test water uptake and disintegration of the feed was used. The feed was submerged into static water for specific time frames. The trial was carried out in a climate controlled laboratory where constant water temperature was maintained (27 °C). Feed was sieved through a mesh size of 2 mm (to remove fine particles) (Figure 3.1 a), before 5 g was placed into marked circular steel containers (Figure 3.2 and Figure 3.3). The procedure was replicated six times for all diets at each time interval. The steel containers had a diameter of 83 mm, height of 35 mm, and a mesh size of 1 mm. The steel containers containing the feed was placed into 1 L glass flasks containing 800 mL tap water

and positioned centrally on top of a circular PVC pipe, with a diameter of 32 mm and length of 20 mm (Figure 5.3d), to suspend the feed from the bottom surface of the glass container.

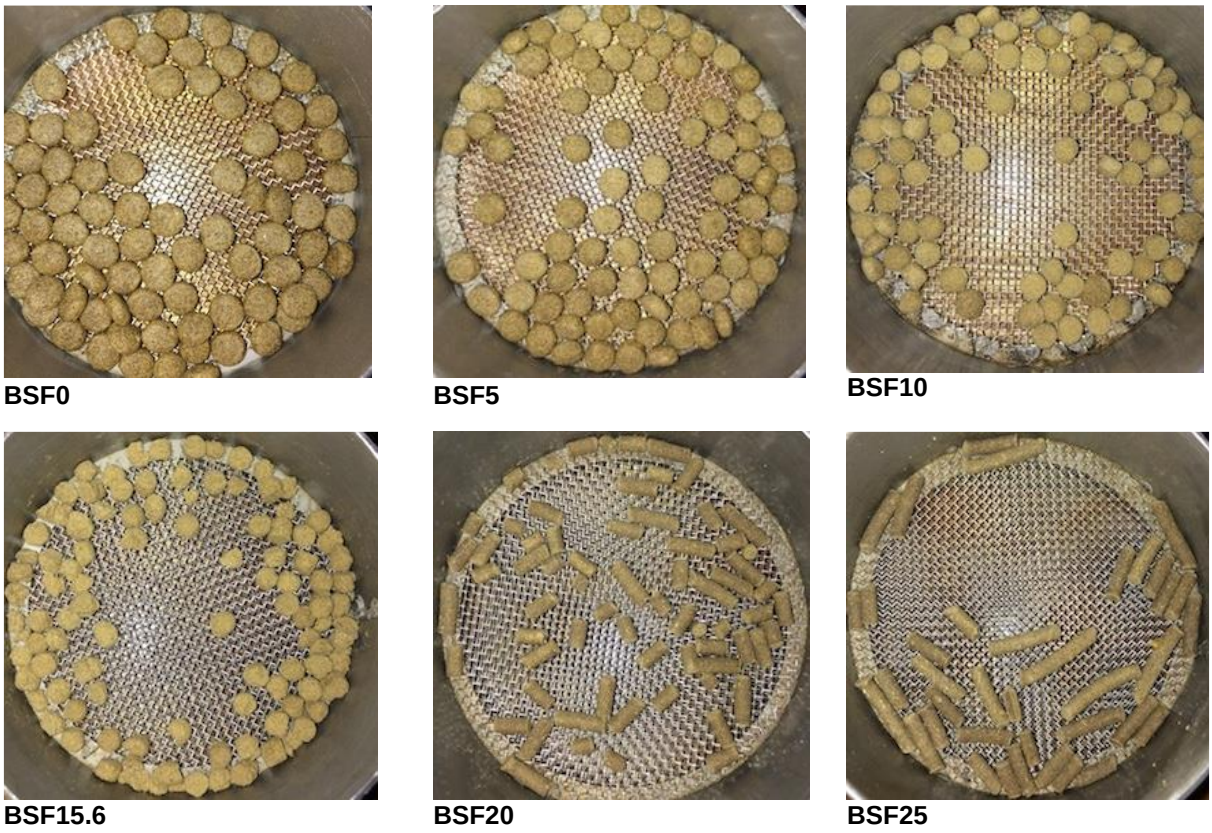



**Figure 3.2** The experimental diets used in the trial before being submerged in water for testing the water quality, showing irregular shapes of pellets due to effects of increasing lipid content and requirement of binder in BSF20 and BSF25

Each diet (Figure 3.2) was inspected individually after submersion for 5 min, 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h and 20 h. The 5 min, 10 min and 30 min time frames represent the longest time frame that feed was visible in the tanks. At each specific submersion time period, the feed was lifted out of the glass container with the black netting used to lower it into the glass jar (Figure 3.3d) and allowed to drip dry for 20 min at an angle (Figure 3.3e) before patting dry excess moisture droplets on the metal surface with a paper towel (Figure 3.3 f) (Baeverfjord *et al*., 2006). The metal containers were then placed on drying racks in a drying oven at 60°C for 48 h, and then allowed to cool down in desiccators for 30 min. The containers with feed were then weighed to calculate the amount of dry matter remaining. After weighing, the steel containers were thoroughly cleaned in warm water to remove any remaining debris, placed in a drying oven at 60 °C for 4 h to remove all moisture and cooled at room temperature for 30-45 min for the next set of analyses. Photos were taken and weights were measured on a Mettler AE 200 (Mettler-Toledo, Switzerland) scale accurate to ±0,0001 g.


**Figure 3.3** Steel container used for water immersion of feeds: a) internal view of metal container used; b) external view of metal container used; c) submerged containers on 2 cm high circular pvc pipe; d) black netting used to place and lift metal containers; e) dripping of metal containers for 20 min; f) drying of metal container before weighing for water uptake

### *3.2.5 Disintegration rate*

After the required submersion time, disintegration was calculated as the feed lost (on an as-fed basis) after immersion and expressed as a percentage of total feed used. Equation 3 was used to determine disintegration and is derived from a formula used to calculate leaching rate by Ruscoe *et al.* (2005).

**Equation 3:** $$\boldsymbol{DMR = \frac{W_o*(1-M)-W_t}{W_o*(1-M)}\ x\ 100}$$

$W_o$ = Feed weight as fed; Wt = Weight after immersion and drying; M = Moisture content of diet as a proportion.

### *3.2.6 Water uptake*

The water uptake by the feed pellets was expressed as a percentage of weight gain for feed remaining after the immersion time, adapted from Hilton *et al*. (1981). Following the 20 min drip-drying period, the containers were patted dry with a paper towel to remove any visible droplets on and inside the container without direct contact to feed (Figure 3.1 f). The container, containing feed remaining and water absorbed by

the remaining feed, was then weighed and collectively expressed as wet weight (Fw), Equation 4. After recording Fw, the containers containing the wet feed was placed in a drying oven at 60 °C for 48 h. After the drying period the steel containers, containing the moisture free feed, was placed in a desiccator and allowed to cool for 30 min before weighing again. The weight of the moisture free feed remaining post immersion was calculated by subtracting the container weight (Cw) from the total dry weight, and corrected for moisture (Equation 5) to express water uptake (Equation 6) as a percentage of feed remaining post immersion rather than as a percentage of feed used pre-immersion to correct for mass loss. By using the post immersion as-fed weight, in comparison to pre-immersion as-fed weight, it was possible to correct for mass and leaching loss (related to water stability) as well as moisture content for post immersion moisture free weight (Wt) (Equation 5). Equation 6 was then used to express water uptake as a percentage of feed weight remaining post immersion on an as-fed basis (Wo).

**Equation 4:** $\boldsymbol{Fw = Cw + Fw - Cw}$

Fw = Feed remaining + Water uptake post immersion; Cw = Container weight.

**Equation 5:** $\boldsymbol{Wo = \frac{Wt}{M}}$

Wo = Remaining feed weight post immersion corrected for moisture content after drying; Wt = Moisture free feed weight (post immersion); M = moisture content of diet as a proportion.

**Equation 6:** $\boldsymbol{Water\ Uptake = \left(\frac{Fw - Wo}{Wo}\right) * 100}$

## 3.3 Statistical analysis

The general linear model (GLM) and analysis of variance (ANOVA) using SAS™ statistical software (2015) were performed on the experimental feed data with treatment as the main effect. Homoscedasticity and normality was tested on all parameters. Significance levels were declared at P≤0.05 (5 % significance level). The Bonferroni;s *post hoc* test (SAS, 2015) was used to separate means.

## 3.4 Results and discussion

### *3.4.1 Unit density*

The densities of extruded feeds are directly related to expansion during extrusion (Colonna *et al*., 1984). The individual unit densities reported for diets used in the trial are shown in Table . All diets have unit densities with significant differences (Table 3.2). The density for BSF5 was higher (P≤0.05) than BSF0 by 27.1 %, and BSF10 was higher (P≤0.05) than BSF5 by 32.1 % - showing an increase in density with increasing BSF pre-pupae meal inclusion levels. However, the density for BSF15.6 was significantly lower than BSF10, by 14.7 %. This could be attributed to the irregular shape of the pellets, which was a result of poor binding during extrusion. BSF20 had a density higher (P≤0.05) than BSF15.6, by 55.1 %, illustrating the binding effects of the binder which, along with the reduced expansion (likely due to higher lipid content; Table 5.2) provided a much

denser pellet. However, BSF25 had a lower (P≤0.05) density than BSF20 (9.9 % lower), but was still higher (P≤0.05) than BSF15.6 (by 39.7 %). The highest unit density and sinking velocity were observed for BSF20. Sankaranandh Kannadhason *et al*. (2011) reported a strong negative correlation between expansion ratio and unit density, showing that unit density increased with decreasing expansion ratios. These findings support the report by Hastings (1970) stating that an increasing lipid content reduces expansion of feed during extrusion, which will thus produce a feed of higher density.

**Table 3.2** Unit densities and lipid content for the experimental diets using different black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal inclusion levels

| Diet | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 | SE |
|---|---|---|---|---|---|---|---|
| Density (kg/m$^3$) | 304.68$^f$ | 387.29$^e$ | 511.80$^c$ | 436.43$^d$ | 676.98$^a$ | 609.84$^b$ | 1.80 |
| Lipid content (%) | 7.32 | 7.77 | 9.32 | 11.23 | 11.98 | 11.10 | |

($^{a,b,c,d,e,f}$) Means with different superscripts within the same row differ significantly (P≤0.05); BSF = Black soldier fly; BSF0 = Control diet, 0% BSF pre-pupae meal inclusion level; BSF5 = 5% BSF pre-pupae meal inclusion level; BSF10 = 10% BSF pre-pupae meal inclusion level; BSF15.6% = 15.6% BSF pre-pupae meal inclusion level; BSF20 = 20% BSF pre-pupae meal inclusion level; BSF25 = 25% BSF pre-pupae meal inclusion level.

The density of all dietary feeds used were significantly different from each other. The density of the feeds appeared to increase up to BSF10, decrease slightly at BSF15.6, and then increased for diet BSF20 and BSF25 where binders were used. The densities correlate well with the lipid content of the feeds. However, as previously mentioned, BSF15.6 does not fit well into the trend, and may be attributed to the irregular shape of the pellet.

### *3.4.2 Sinking velocity*

The sinking velocities of the experimental diets are shown in Table 3.3. BSF0 and BSF5 had a sinking velocity of zero cm/s. Feeds sank when BSF pre-pupae meal inclusion levels exceeded 10 % (BSF10). BSF20 reported the highest sinking velocity (and lipid content) of all the diets. As discussed, the lipid content reduces expansion, which in turn increases the unit density. It is well known that a higher densities results in a heavier relative weight, which in turn will result in a higher sinking due to an heavier weight per unit mass ratio.

**Table 3.3** Sinking velocities of the experimental diets

| Diet | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 |
|---|---|---|---|---|---|---|
| **Sinking velocity (cm/s)** | 0 | 0 | 4.44 | 7.07 | 11.38 | 10.75 |
| **SE** | 0 | 0 | 0.40 | 0.29 | 0.25 | 0.34 |

BSF = Black soldier fly; BSF0 = Control diet, 0% BSF pre-pupae meal inclusion level; BSF5 = 5% BSF pre-pupae meal inclusion level; BSF10 = 10% BSF pre-pupae meal inclusion level; BSF15.6% = 15.6% BSF pre-pupae meal inclusion level; BSF20 = 20% BSF pre-pupae meal inclusion level; BSF25 = 25% BSF pre-pupae meal inclusion level.

Figure 3.4 illustrates the relationship between bulk density, sinking velocity and lipid content. BSF15 had the highest values for all parameters (Figure 3.4). As previously discussed, lipids lubricate feeds during the extrusion process, resulting in reduced expansion. Lower expansion sequentially results in higher density, which in turn provides a higher sinking velocity. Similarly, Sankaranandh Kannadhason *et al*. (2011) reported a strong negative correlation between expansion ratio and sinking velocity.

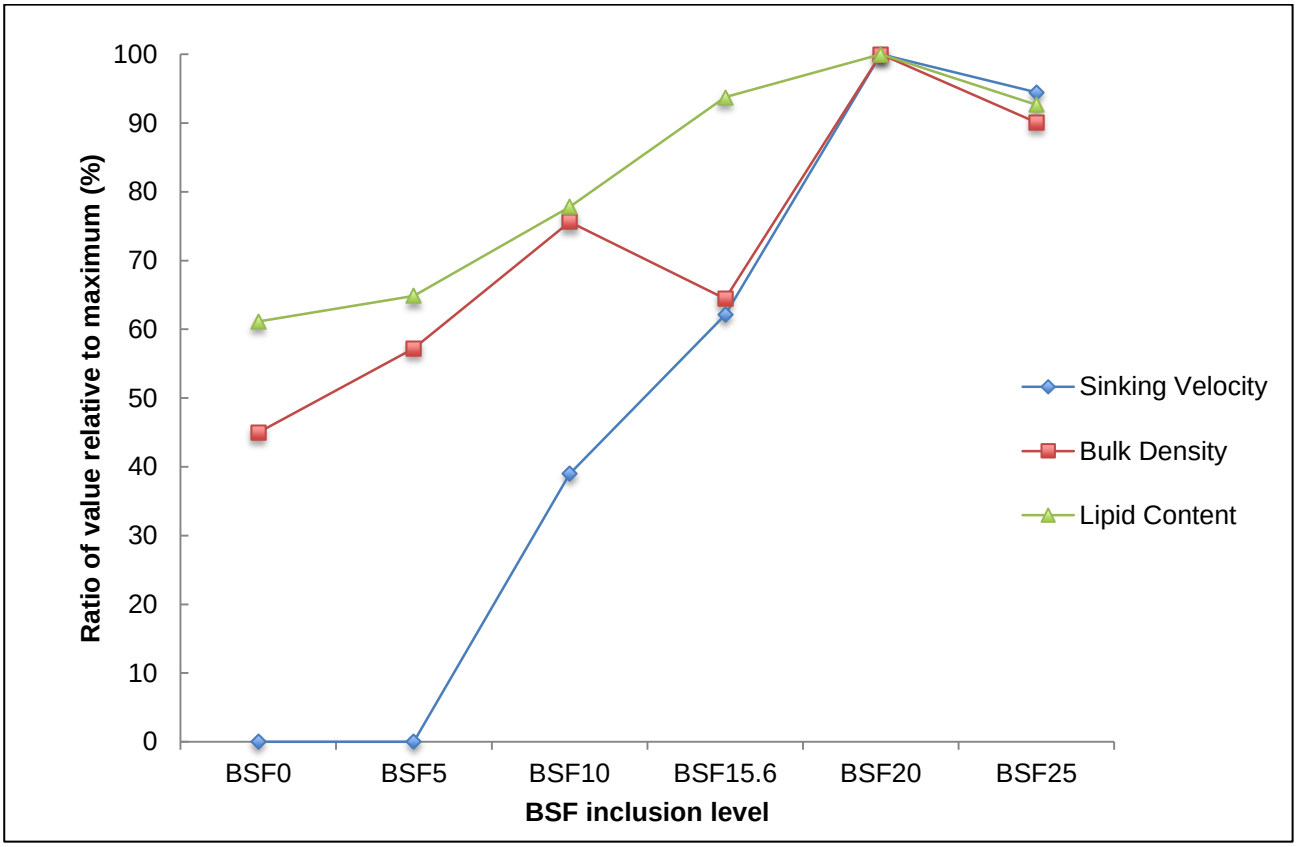



**Figure 3.4** The relationship between bulk density, sinking velocity and lipid content expressed as a percentage of highest value (BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level)

### *3.4.3 Water uptake and disintegration rate*

At 5 min, BSF15.6 absorbed more water (P≤0.05) than all other treatments (

Table 3.4). This may be due to void spaces in the pellet caused by lack of binding ability thereby increasing the surface area available for moisture absorption. For all other time frames, BSF0 absorbed the most water and BSF20 and BSF25 absorbed the least. The use of a binder in BSF20 and BSF25 appeared to have a reducing effect on the water uptake of the feed. The highest percentage water uptake for BSF20 and BSF25, at 20 h, was 150.37 % and 137.59 %, respectively, whilst all other diets exceeded 200 % after just 5 min (Table 3.4).

**Table 3.4** Water uptake of the experimental diets expressed as a percentage (%) of feed remaining for diets containing varying levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal

| | Diet | | | | | | |
|---|---|---|---|---|---|---|---|
| **Time** | **BSF0** | **BSF5** | **BSF10** | **BSF15.6** | **BSF20** | **BSF25** | **SE** |
| 5 min | 256.71$^{b}$ | 203.40$^{c}$ | 212.46$^{c}$ | 288.60$^{a}$ | 45.60$^{d}$ | 41.42$^{d}$ | 6.41 |
| 10 min | 337.26$^{a}$ | 296.17$^{b}$ | 286.77$^{b}$ | 292.82$^{b}$ | 56.81$^{c}$ | 45.77$^{c}$ | 4.30 |
| 30 min | 387.43$^{a}$ | 351.98$^{b}$ | 335.09$^{b}$ | 326.22$^{b}$ | 75.15$^{c}$ | 65.95$^{c}$ | 6.79 |
| 1 h | 415.17$^{a}$ | 371.34$^{c}$ | 392.38$^{b}$ | 402.68$^{ab}$ | 89.32$^{d}$ | 84.97$^{d}$ | 3.04 |
| 2 h | 431.03$^{a}$ | 400.10$^{b}$ | 407.96$^{b}$ | 409.07$^{b}$ | 108.85$^{c}$ | 98.44$^{c}$ | 2.25 |
| 4 h | 454.93$^{a}$ | 424.48$^{b}$ | 419.13$^{b}$ | 412.60$^{b}$ | 122.14$^{c}$ | 122.77$^{c}$ | 3.86 |
| 8 h | 444.07$^{a}$ | 402.97$^{bc}$ | 409.81$^{c}$ | 385.84$^{c}$ | 124.76$^{d}$ | 124.29$^{d}$ | 4.07 |
| 12 h | 433.71$^{a}$ | 407.25$^{b}$ | 422.72$^{ab}$ | 421.59$^{ab}$ | 137.02$^{c}$ | 128.49$^{c}$ | 4.66 |
| 20 h | 392.64$^{a}$ | 408.41$^{a}$ | 398.79$^{a}$ | 314.69$^{b}$ | 150.37$^{c}$ | 137.59$^{c}$ | 10.05 |

($^{a,b,c,d,}$) Means with different superscripts within the same row differ significantly (P≤0.05); BSF = Black soldier fly; BSF0 = Control diet, 0% BSF pre-pupae meal inclusion level; BSF5 = 5% BSF pre-pupae meal inclusion level; BSF10 = 10% BSF pre-pupae meal inclusion level; BSF15.6% = 15.6% BSF pre-pupae meal inclusion level; BSF20 = 20% BSF pre-pupae meal inclusion level; BSF25 = 25% BSF pre-pupae meal inclusion level.

Figure 3.5 provides a visual comparison of water uptake between experimental diets over time, clearly illustrating the difference in water uptake between BSF20 and BSF25, and all other diets.

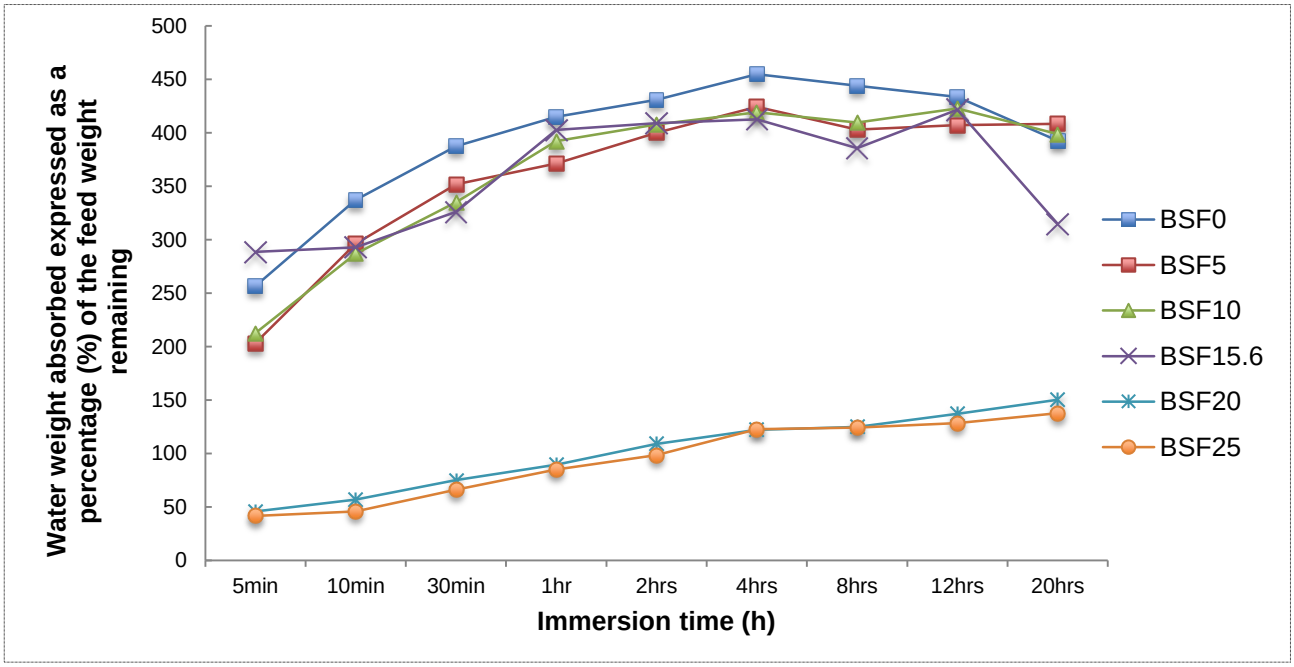



**Figure 3.5** Comparison of water uptake, expressed as a percentage of feed weight remaining for diets containing varying levels of BSF pre-pupae meal (BSF = Black soldier fly; BSF0 = Control diet, 0% BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level)

Hilton *et al.* (1981) suggested that lower density results in higher absorption rates. The findings in this experiment supports this statement, based on the fact that BSF0, having the lowest density (304.68 kg/m$^3$), had the highest average absorption rate over all time intervals. While, BSF15.6 and BSF25, which had the highest densities of 676.98 kg/m$^3$ and 609.84 kg/m$^3$, respectively, had the lowest absorption rates.

**Table 3.5** Disintegration rate for diets containing varying levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal expressed as a percentage (%) of total initial weight lost

| Diet | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 | SE |
|---|---|---|---|---|---|---|---|
| 5 min | 0.68$^{b}$ | 0.50$^{b}$ | 1.14$^{b}$ | 47.76$^{a}$ | 0.53$^{b}$ | 0.12$^{b}$ | 0.73 |
| 10 min | 2.55$^{b}$ | 2.26$^{b}$ | 5.56$^{b}$ | 43.33$^{a}$ | 1.30$^{b}$ | 0.82$^{b}$ | 1.49 |
| 30 min | 7.66$^{c}$ | 7.94$^{c}$ | 13.66$^{b}$ | 49.80$^{a}$ | 4.01$^{c}$ | 3.53$^{c}$ | 1.06 |
| 1 h | 11.03$^{bc}$ | 11.17$^{bc}$ | 16.39$^{b}$ | 55.28$^{a}$ | 6.26$^{c}$ | 5.86$^{c}$ | 1.28 |
| 2 h | 11.79$^{c}$ | 12.64$^{bc}$ | 17.63$^{b}$ | 60.05$^{a}$ | 7.63$^{c}$ | 8.69$^{c}$ | 1.13 |
| 4 h | 13.40$^{b}$ | 15.04$^{b}$ | 18.28$^{b}$ | 58.37$^{a}$ | 14.11$^{b}$ | 14.06$^{b}$ | 1.11 |
| 8 h | 14.53$^{c}$ | 15.56$^{c}$ | 18.74$^{b}$ | 62.98$^{a}$ | 15.63$^{c}$ | 15.85$^{bc}$ | 0.65 |
| 12 h | 17.65$^{b}$ | 17.36$^{b}$ | 21.97$^{b}$ | 66.64$^{a}$ | 17.54$^{b}$ | 16.77$^{b}$ | 1.23 |
| 20 h | 24.47$^{bc}$ | 17.01$^{c}$ | 31.04$^{b}$ | 75.12$^{a}$ | 18.18$^{c}$ | 17.29$^{c}$ | 2.61 |

($^{a,b,c,d}$) Means with different superscripts within the same row differ significantly (P≤0.05); BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

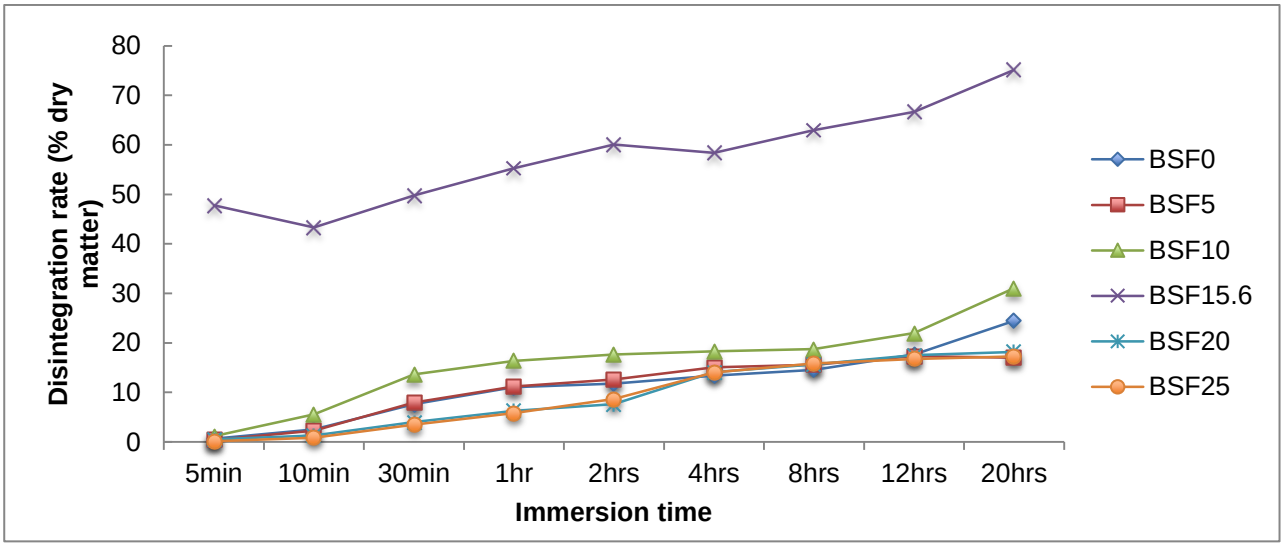



**Figure 3.6** Disintegration of leaching rate between experimental diets (BSF = black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae inclusion; BSF5 = 5 % BSF pre-pupae inclusion; BSF10 = 10 % BSF pre-pupae inclusion; BSF15.6 = 15.6 % BSF pre-pupae inclusion; BSF20 = 20 % BSF pre-pupae inclusion; BSF25 = 25 % BSF pre-pupae inclusion)

At all time frames BSF15.6 had a significantly higher disintegration rate than other diets, and lost 75.12 % of its initial weight after 20 h (Table 3.5). Of the remaining diets, BSF10 lost the most weight (at 20 h), 31.04 %, which was still exceeded by the lowest weight loss of BSF15.6, which was 43.33 % at 10 min. This is mainly attributed to the poor water stability resulting from lack of binding, likely due to higher lipid content (as previously discussed). Figure 3.6 illustrates how BSF15.6 lost significantly more weight than all other diets through all of the time frames.

## 3.5 Conclusions

The results show that the BSF pre-pupae meal content has an influence on the feed unit density, sinking velocity, water uptake and leaching rate. Reduced expansion may have resulted during extrusion due to lubrication from increasing lipid content with increasing inclusion levels of BSF pre-pupae meal. This may in turn have resulted in an increased unit density as well as sinking velocity. Furthermore, sinking velocity may also have been influenced by the rate of water uptake. The findings support the suggestion by Hilton *et al.* (1981) that lower densities result in higher absorption rates. Higher water uptake of lower density diets may be attributed to the larger surface area resulting from void spaces in pellets with lower densities. The lipid content may thus have contributed indirectly to the results. A higher lipid content reduces expansion, resulting in fewer void spaces and lower surface area available for water uptake. However, due to the varying shapes of the pellets, and the requirement of a binder in BSF20 and BSF25 to prevent crumbling, the findings cannot provide conclusive results. Also, the variation in ratios of feed ingredients (in order to maintain nutrient balance) may have influenced the binding efficacy of the starch in the feed as different protein sources for example, have different binding abilities.

For future trials, the dietary lipid content should be identical among all diets, as well as non-nutritive feed additives such as pellet binders, and the shape and size of the pellets. Other ingredients should also be investigated that may potentially improve consistency of the pellets based on how the ingredients interact with the process used during manufacturing and with each other. Furthermore, if binders are included it should be in similar concentrations across all diets to ensure uniformity in the physical properties of the feed.

## 3.6 References

Ayadi, F., Rosentrater, K.A., Muthukumarappan, K. & Kannadhason, S., 2016. Effects of amylose-to-amylopectin ratios on binding capacity of DDGS/Soy-Based Aquafeed Blends. J. Food. Res. 5(5), 43.

Baeverfjord, G., Refstie, S., Krogedal, P. & Åsgård, T., 2006. Low feed pellet water stability and fluctuating water salinity cause separation and accumulation of dietary oil in the stomach of rainbow trout (*Oncorhynchus mykiss*). Aquac. 261(4), 1335-1345.

Balazs, G.H., Ross, E. & Brooks, C.C., 1973. Preliminary studies on the preparation and feeding of crustacean diets. Aquac. 2, 369-377.

Chang, Y.K. & Wang, S.S., 1998. Advances in Extrusion Technology. In: International Symposium on Animal and Aquaculture Feedstuffs by Extrusion Technology. Eds: Chang, Y.K. & Wang S.S. São Paulo: Technomic Pub. Co.

Cheftel, J.C., Cuq, J.L. & Lorient, D., 1985. Amino acids, peptides, and proteins. Food Chem. 2, 245-369.

Chinnaswamy, R. & Hanna, M.A., 1988. Optimum extrusion-cooking conditions for maximum expansion of corn starch. J. Food Sci. 53(3), 834-836.

Clementson, C.L. & Ileleji, K.E., 2010. Variability of bulk density of distillers dried grains with solubles (DDGS) during gravity-driven discharge. Biores. Tech. 101(14), 5459-5468.

Davidson, V.J., Paton, D., Diosady, L.L. & Larocque, G., 1984. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. J. Food Sci. 49(2), 453-458.

El-Sayed, A.-F.M., 2006. Tilapia culture. In : Aquaculture. CABI publishing, UK.

Guy, R.C.E., 1994. Raw materials for extrusion cooking processes. In: The Technology of Extrusion Cooking. pp. 52-72.

Halver, J.E. & Hardy, R.W., 2002. In: Fish Nutrition. Academic press.

Hastings, W.H., 1970. Study of pelleted fish foods stability in water. In: Report of the 1970 Workshop on Fish Feed Technology and Nutrition, Warmwater Fish Cultural Laboratories. Arkansas, USA. pp. 7-19.

Haubjerg, A.F., Veje, C.T., Jørgensen, B.N., Simonsen, B. & Løvgreen, S., 2015. Structural properties and mechanical durability of extruded fish feed. J. Food Proc. Eng. 38(6), 621-631.

Hilton, J.W., Cho, C.Y. & Slinger, S.J., 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.). Aquac. 25(2-3), 185-194.

Ighwela, K.A., Bin Ahmad, A. & Abol-Munafi, A.B., 2013. Water stability and nutrient leaching of different levels of maltose formulated fish pellets. Glob. Vet. 10(6), 638-642.

Kannadhason, S. & Muthukumarappan, K., 2010. Effect of starch sources on properties of extrudates containing DDGS. Int. J. Food Prop. 13(5), 1012-1034.

Kannadhason, S., Muthukumarappan, K. & Rosentrater, K.A., 2011. Effect of starch sources and protein content on extruded aquaculture feed containing DDGS. Food. Bioproc. Tech. 4(2), 282-294.

Khater, E.S.G., Bahnasawy, A.H. & Ali, S.A., 2014. Physical and mechanical properties of fish feed pellets. J. Food Proc.Tech. 5(10).

Lim, C. & Cuzon, G., 1994. Water stability of shrimp pellet: a review. Asian. Fish. Sci. 7(2-3), 115-126.

Lim, C. & Dominy, W., 1989. Utilization of plant protein by warmwater fish. In: Proceedings of World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs. Ed: Applewhite, T.H. pp. 245-251.

Mercier, C. & Feillet, P., 1975. Modification of carbohydrate components by extrusion-cooking of cereal products. Cereal Chem. 52, 283-297.

Obaldo, L.G., Divakaran, S. & Tacon, A.G., 2002. Method for determining the physical stability of shrimp feeds in water. Aquac. Res. 33(5), 369-377.

Ruscoe, I.M., Jones, C M., Jones, P.L. & Caley, P., 2005. The effects of various binders and moisture content on pellet stability of research diets for freshwater crayfish. Aquac. Nutr. 11(2), 87-93.

Stickney, R.R. & Lovell, T., 1977. Nutrition and feeding of channel catfish. Alabama Agricultural Experiment Station.

Stivers, T., 1971. Feed manufacturing. In: In Gaudet, JL Report of the 1970 workshop of fish feed technology and nutrition. Resour. Publ. Bur. Sport. Fish. New York. pp. 14-42.

Tharanathan, R.N., 1995. Starch: The polysaccharide of high abundance and usefulness. J. Sci. Ind. Res. 54(8), 452-458.

Webster, C.D. & Chhorn, L., 2006. Tilapia: biology, culture and nutrition. CRC Press.

# Chapter 4

## Influence of black soldier fly (*Hermetia illucens*) pre-pupae meal on growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*)

**Abstract**

The effect of different inclusion levels of full-fat black soldier fly (BSF) pre-pupae meal on the growth and feed utilization of Nile tilapia was investigated in a recirculatory aquaculture system (RAS). A summit diet with 25 % BSF pre-pupae (BSF25), and a dilution with 0 % BSF pre-pupae (BSF0) was formulated according to the ideal amino acid profile for Nile tilapia. The diets were blended to contain 0 % (control) (BSF0), 5 % (BSF5), 10 % (BSF10), 15.60 % (BSF15), 20 % (BSF20) and 25 % (BSF25) BSF pre-pupae meal, respectively. A total of 630 juvenile Nile tilapia (45-95 g) were randomly allocated to treatment diets with seven replicates per treatment. Fish were hand fed twice per day to apparent satiation over a 30 day period. The initial and final weights were not significantly different between treatments. However, feed utilization (specific growth rate, protein efficiency ratio and feed conversion ratio) differed ($P \leq 0.05$) among diets. Lipid content and pellet water stability had a direct and inverse relationship with increasing inclusion levels of BSF pre-pupae meal, respectively. The study concluded that full-fat BSF pre-pupae meal should not exceed 5% without assessing additives for increasing pellet water stability. It is suggested to use defatted BSF pre-pupae meal when higher inclusion levels are to be attempted, as the high lipid content of full-fat BSF pre-pupae may have adverse effects on the characteristics of the feed used.

Key words: Black Soldier Fly, growth performance, feed utilization, Nile tilapia, alternative protein sources, pre-pupae meal

### 4.1 Introduction

The aquaculture industry has conventionally used fishmeal as the main protein source in the aqua feed industry due its high protein content, balanced essential amino acid (EAA) profile, inclusion of essential fatty acids (EFA), highly digestible energy, minerals and vitamins (El-Sayed, 2006). These advantages, along with laws preventing the use of many meat meals (due to disease risk), has resulted in fish being an indispensable protein source in fish feeds (Sánchez-Muros *et al.,* 2014). The increase in demand and competition for fishmeal has resulted in fishmeal being the most expensive protein commodity in aquaculture feeds (El-Sayed, 2006). As a result, the affordability of fish feeds may be problematic for developing countries; the price of fishmeal almost tripled in a period of eight years, increasing from around US$500/t in 2000 to around US$1200/t in 2008 (Lucas & Southgate, 2012). Hence, developing countries will be unable to afford fishmeal as a major protein source in commercial fish feeds, and it will have to be replaced by a less expensive, locally available

protein source (El-Sayed, 2006). Another major concern with the use of fishmeal as a protein source is its sustainability; the aquaculture industry consumed 3.06 million tons (56 %) of world fishmeal production in 2006 (Lucas & Southgate, 2012).

Some alternative animal protein sources investigated include hydrolysed feather meal (HFM), poultry by product meal (PBM), shrimp meal, chicken offal silage (COS) and meat (M) and bone meal (BM). These protein sources have relatively high protein contents and good amino acid profiles, but may be deficient in certain amino acids. NRC (1983) and Tacon and Jackson (1985) reported that the most limiting amino acids for alternative terrestrial protein sources are methionine (M, BM and HFM), isoleucine (BM) and lysine (PBM and HFM). Viola and Zohar (1984) attributed poor performance of HFM diets to deficiency in amino acids and energy. However, Tacon *et al.* (1983) found that only 30 % of fish meal protein could be effectively replaced by HFM in Nile tilapia diets, even with the supplementation of lysine, methionine and histidine. El-Sayed (1998) reported significantly poorer feed and protein efficiency ratios and growth performance when PBM, shrimp meal and M and BM replaced a 30 % fishmeal diet by 47 %, 50 % and 40 %, respectively. Wu *et al.* (1999) reported that an inclusion of 6 % M and BM as a fishmeal replacement had no effect on the growth performance for Nile tilapia.

Nile tilapia diets can be formulated to contain high percentages of plant protein (Ogunji *et al.,* 2008a; FAO, 2015). Soy bean meal is a common protein source used in fish feed as it has the most balanced amino acid profile among plant protein sources available required by most fish (El-Sayed, 2006). It's relatively high digestibility and protein content has made it a readily accepted protein source in fish feeds (Sánchez-Muros *et al.,* 2014). Lim and Dominy (1989) suggested that soya is the most studied plant protein source due to it being readily available, having consistent quality and being more affordable than most other protein sources. Shiau *et al.* (1987) reported that SBM can partially replace fish meal in diets with protein levels below optimum (24 %), but requires supplementation of methionine to prevent depressed growth when replacing 30 % of fish meal in diets with a 32 % protein content. In support, Jackson *et al.* (1982) reported growth reduction when 50 % or more fishmeal was replaced with SBM, which was attributed to the methionine deficiency and the presence of anti-nutritional factors such as trypsin inhibitors and lectin. However, the diets used by Jackson *et al.*(1982) were formulated for protein content (iso-nitrogenous) and not amino acid composition and digestible energy, which may have influenced growth parameters. Rapeseed and canola meal have carbohydrates that are highly indigestible and have anti-nutritional factors limiting its use in fish feeds, but is a readily used feedstuff in livestock and poultry diets (Webster & Chhorn, 2006). In support, Jackson *et al.* (1982) reported a significant reduction in weight gain with rapeseed inclusion levels of 63 % and higher. Similarly, inclusion levels of cottonseed meal (CSM) are also limited by anti-nutritional factors (i.e. gossypol) and low lysine content (Webster & Chhorn, 2006). However, pre-press solvent-extracted CSM has proved to be acceptable in *O. mossambicus* diets by up to 50 %. CSM with low gossypol levels can be included in the same quantities as SBM in *O. niloticus* and *O. aureus* hybrid diets (Viola & Zohar, 1984). Cottonseed cake at 19.4 % inclusion levels have been reported to reduce weight gain and feed efficiency in Nile tilapia (Ofojekwu & Ejike, 1984).

Similarly, El-Sayed (1990) reported that an inclusion level of 65 % CSM reduced weight gain and feed efficiency by 24 % and 35 %, respectively.

BSF has been found to be useful in managing manure. It can reduce dairy manure by up to 58 % (Myers *et al.,* 2008), laying hen manure by up to 50 % (Sheppard *et al.,* 1994) and pig manure by up to 56 % (Newton a *et al.,* 2005). Similarly, Diener *et al.* (2009) reported that BSF is capable of converting large amounts of organic waste into a protein-rich biomass which can be used as a potential substitute for commercial protein sources such as fish and soya oilcake meal in animal feeds. Bondari and Sheppard (1981), Ogunji *et al.* (2008a) and Ogunji *et al.* (2008b) showed that BSF larvae of animal origin has great potential to replace commercial protein sources in fish feed production alone or in combination with other ingredients for channel catfish and tilapia. In support, Zhu *et al.* (2015) reported that BSF is capable of converting pig manure into a quality protein source.

The nutritive value of the larvae and pre-pupae can be optimised by rearing them under different controlled environments (Sánchez-Muros *et al.,* 2014). St-Hilaire *et al.* (2007) found that inclusion of 10 % fish offal as a fly larval feed source increased lipid content by 43 %, and omega-3 levels from negligible amounts to 3 %, relative to being fed cow manure only. Furthermore, Barroso *et al.* (2014), Newton *et al.* (2005a) and Surendra *et al.* (2016) reported that the protein has an amino acid composition comparable to fish meal (Table 3.1).

**Table 4.1** Amino acid composition of BSF-larvae, pre-pupae and fishmeal represented as a percentage of total amino acids adapted from Barroso *et al.* (2014)

| Amino acid | Larvae | Pre - pupae | Fishmeal |
|---|---|---|---|
| Arginine | 8.24 | 8.05 | 7.42 |
| Histidine | 5.29 | 5.16 | 7.86 |
| Isoleucine | 5.76 | 5.34 | 5.04 |
| Leucine | 6.87 | 6.83 | 7.81 |
| Lysine | 7.60 | 7.31 | 8.78 |
| Methionine | 1.50 | 3.26 | 2.93 |
| Phenylalanine | 6.88 | 6.22 | 5.38 |
| Threonine | 5.39 | 4.95 | 6.26 |
| Tryptophan | N/A | N/A | N/A |
| Valine | 6.31 | 6.34 | 5.56 |

N/A = Not available.

Additionally, bioconversion rates of up to 55 % have been reported on fresh human waste and can reduce harmful microorganisms such as *Salmonella* spp. found in it (Lalander *et al.,* 2013). This may provide a solution to health problems associated with poor sanitation and waste management in developing countries (Banks *et al.,* 2014). Black soldier flies also aids in controlling house flies by competing for larval habitat whilst producing economically attractive quantities of larval feedstuff (Sheppard *et al.,* 1994). Black Soldier Fly is a

good potential candidate for alternative protein source to conventional sources considering it being a high protein and fat source, and it's amino acid profile being comparable to that of fishmeal.

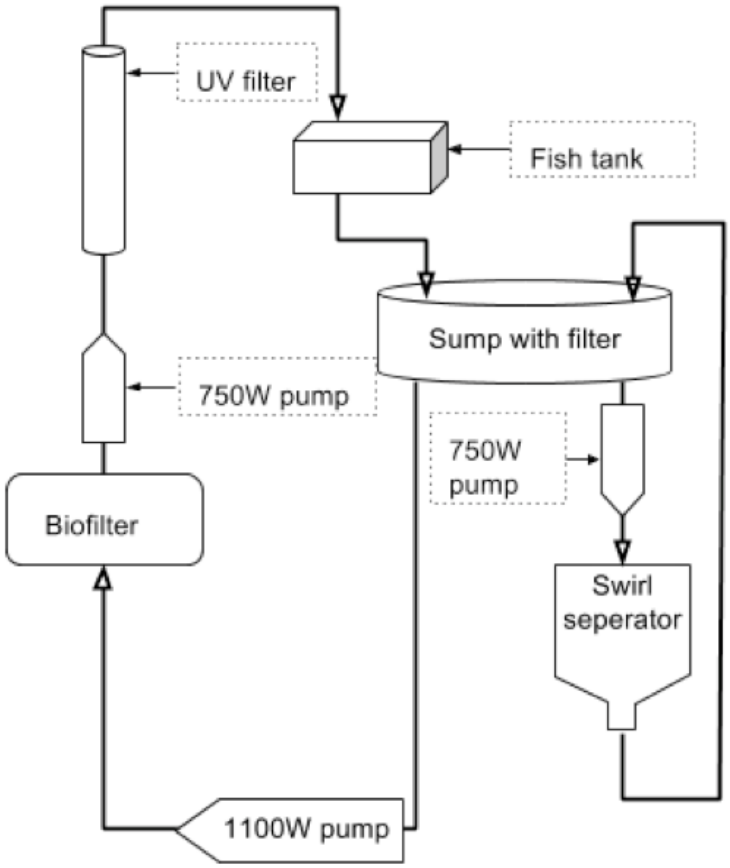



**Figure 4.1** Diagram representation for water flow of recirculatory aquaculture system used for experimental trial

## 4.2 Material and methods

### *4.2.1 Experimental procedure*

A recirculatory aquaculture system (RAS), demonstrated in

Figure **4.1**, was used to carry out the trial at the Welgevallen experimental farm of Stellenbosch University in Stellenbosch, Western Cape, South Africa, with Animal ethics clearance number, protocol number sSU-ACUD15-00035. The RAS system consisted of 42 glass tanks, a 400 L sump, 250 L swirl separator, a two trickle bio-filters (0.8 m x 0.7 m x 3 m with a 400 L gathering tank on the bottom), a 450 W pump, 750 W pump, 1100 W pump, a 1.1 kW blower and a UV filter. The 42 glass tanks had a total volume of 120 L and usable volume of 100 L each, as determined by the outflow level. The pump supplied water to tanks once it passed through the bio-filters. The outflow went directly through a filter in the sump, and then the water was partitioned towards the swirl separator with a 450 W pump (flowing back into sump) and bio-filters with an 1100 W pump. Once water passed through the bio-filters, it was redirected back to the glass tanks with a 750 W pump after

passing through the UV filter. A 1.1 kW blower (FPZ Effepizeta, SRL, Model SCL V4, Incorezzo, Milano, Italy) supplied aeration via air stones. A complete volume exchanged occurred every 12 min; thus the replacement rate per tank was 138 ml/s.

Temperature was automatically controlled through a central control unit with extraction fans. A heating system was not required as the trial was conducted during mid-summer months, allowing water to maintain elevated temperature in the temperature controlled unit. The tanks were aligned in rows on top of each other. Figure 4.2a shows the shading/netting that was used to mitigate direct exposure to sunlight of the top level tanks to keep temperatures constant between top and bottom level tanks. The sump, Figure 4.2b, was used for the collection and removal of physical particles (waste) from in the water. The swirl separator, Figure 4.2c, helped to remove sediment from the water as it settled at the bottom of the swirl. The UV filter, Figure 4.2d, was used to help keep microbial loads down in the water by the use of a UV light.

a)

b)

c)


d)

**Figure 4.2 (a-d)** Components of aquaculture system used for the trial: a) Shading used over tanks; b) Sump with filter; c) Swirl separator; d) UV filter

Nile tilapia fingerlings were obtained from Envirocin Aquaculture (Johannesburg). The fish were transported and delivered the same day by aeroplane from Pretoria to Cape Town. The fish were transported from Cape Town international airport to Welgevallen experimental farm in polystyrene boxes containing two double bags with 5 L water and 5 L oxygen, each bag containing 50 fish. Weights varied from 4-8 g upon arrival. Fish were kept in the system used for the trial until the trial commenced. At the beginning of the trial

the fish had a recorded starting weight of 45-95 g. At the start of the trial a total of 630 fish were recorded, weighed and measured before being placed into tanks (15 fish per tank). In an effort to mitigate dominance and size related competition, the fish were grouped into weight intervals varying no more than 5 g. One of each weight group was randomly allocated to each treatment, and were randomly and evenly distributed between treatments.

Performance was expressed with the feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), feed intake (FI), hepatosomatic index (HSI), conditioning factor ($C_f$), percentage weight gain (WG) and average daily gain (ADG) as indicated:

**Equation 7:** FCR = Food fed (g) / Live weight gain (g)

**Equation 8:** SGR = (ln W2-ln W1 / T2-T1) x 100

**Equation 9:** PER = Live weight gain (g) / Protein fed (g)

**Equation 10:** FI = Total feed calculated / (Initial + Final number of animals / 2)

**Equation 11:** HSI = (Liver weight (g) / Total fish weight (g)) x 100

**Equation 12:** $C_f = W2 / (\mathbf{L}_2)^3 \times 100$

**Equation 13:** WG (%) = mean final weight / mean initial weight x 100

**Equation 14:** SR (%) = F2 / F1 x 100

For equation one to eight, abbreviations below apply:

W1 = Initial weight of fish; W2 = Final weight of fish; T1 = Begin of experiment (day); T2 = End of experiment (day); F1 = Number of fish at end of experiment; F2 = Number of fish at beginning of experiment; $L_2$ = Standard length.

Average daily gain (ADG) was calculated by means of fitting a simple linear regression of weight over time (days) for each tank, the slope of the regression represents the rate of change and as such ADG. Other parameters investigated at the end of the trial included survival rate (SR). All the experimental fish were weighed weekly.

### *4.2.2 Experimental feed*

The fish were fed a commercial tilapia diet for adaption and holding period supplied by Montego pet foods (product code T3206i). The feed was in granular form with a length of 1-2 mm, and had a protein and lipid content of 32 % and 6 %, respectively.

Two diets, a summit (BSF25) and dilution diet (BSF0) were formulated according to the ideal amino acid profile of Nile tilapia. BSF0 contained no BSF pre-pupae meal while BSF25 contained 25% BSF meal. These diets were blended in specific ratios to yield six treatment diets (A protein percentage and digestible energy/protein (DE/P) ratio of 36 % and 8.3 kcal/g have been shown to provide maximum growth for Nile tilapia (Halver & Hardy, 2002). The diets were formulated for 36 % protein and 15 % fat. However, in order to maintain a balanced amino acid profile in both diets, the dilution and summit diets were formulated to have a 36 %

protein and 14.6 % fat, and 38.45 % protein and 15.47 % fat content, respectively. The formulated protein and fat contents for treatment diets are shown in **Error! Not a valid bookmark self-reference.**. The protein and fat contents of diets 2-6 were dependent of the ratios of the summit and dilution diet of which they were made up of.

**Table 4.3** The ratio of summit and dilution diets used to formulate experimental feeds

| | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 |
|---|---|---|---|---|---|---|
| Dilution diet | 100.00 | 80.00 | 60.00 | 40.00 | 20.00 | 0.00 |
| Summit diet | 0.00 | 20.00 | 40.00 | 60.00 | 80.00 | 100.00 |
| BSF % | 0.00 | 5.00 | 10.00 | 15.60 | 20.00 | 25.00 |
| Protein % | 36.00 | 36.49 | 36.98 | 37.47 | 37.96 | 38.45 |
| Lipid | 14.60 | 14.77 | 14.95 | 15.12 | 15.30 | 15.47 |

BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

The feed was prepared via extrusion through a 4 mm die, with the intent to produce floating pellets. After extrusion the pellets were oven dried and bagged in breathable polythene bags until used. Of the experimental diets used, only diet BSF0 and BSF5 resulted in floating pellets. Increasing inclusion levels of full-fat BSF pre-pupae meal resulted in an increased crumbling effect. The BSF20 and BSF25 diets required the addition of a binder, Aquacube at 0.25 % dietary inclusion levels, in order to prevent complete crumbling during extrusion.

3). The diets consisted of varying ratios of dilution and summit diet, with the summit diet containing a maximum inclusion level of 25% BSF pre-pupae meal and dilution diet 0 % BSF pre-pupae meal. The ingredients of the dilution and summit diets are shown in

Table **4.**. The ratios increased in increments of 5 %, i.e. BSF0, BSF5, BSF10, BSF15.6, BSF20 and BSF25 and had a dilution diet:summit diet ratio of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0, respectively (A protein percentage and digestible energy/protein (DE/P) ratio of 36 % and 8.3 kcal/g have been shown to provide maximum growth for Nile tilapia (Halver & Hardy, 2002). The diets were formulated for 36 % protein and 15 % fat. However, in order to maintain a balanced amino acid profile in both diets, the dilution and summit diets were formulated to have a 36 % protein and 14.6 % fat, and 38.45 % protein and 15.47 % fat content, respectively. The formulated protein and fat contents for treatment diets are shown in **Error! Not a valid bookmark self-reference.**. The protein and fat contents of diets 2-6 were dependent of the ratios of the summit and dilution diet of which they were made up of.

**Table 4.3** The ratio of summit and dilution diets used to formulate experimental feeds

| | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 |
|---|---|---|---|---|---|---|
| Dilution diet | 100.00 | 80.00 | 60.00 | 40.00 | 20.00 | 0.00 |
| Summit diet | 0.00 | 20.00 | 40.00 | 60.00 | 80.00 | 100.00 |
| BSF % | 0.00 | 5.00 | 10.00 | 15.60 | 20.00 | 25.00 |
| Protein % | 36.00 | 36.49 | 36.98 | 37.47 | 37.96 | 38.45 |
| Lipid | 14.60 | 14.77 | 14.95 | 15.12 | 15.30 | 15.47 |

BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

The feed was prepared via extrusion through a 4 mm die, with the intent to produce floating pellets. After extrusion the pellets were oven dried and bagged in breathable polythene bags until used. Of the experimental diets used, only diet BSF0 and BSF5 resulted in floating pellets. Increasing inclusion levels of full-fat BSF pre-pupae meal resulted in an increased crumbling effect. The BSF20 and BSF25 diets required the addition of a binder, Aquacube at 0.25 % dietary inclusion levels, in order to prevent complete crumbling during extrusion.

4.3).

**Table 4.2** Dilution and summit diet ingredients and nutrient composition for dilution and summit diet

| **Ingredients (%)** | **Dilution diet (0 %)** | **Summit diet (25 %)** |
|---|---|---|
| BSF larvae | - | 25.00 |
| Yellow maize | 10.00 | 10.00 |
| Maize gluten 60 | 21.27 | 21.94 |
| Soybean full-fat | - | 39.00 |
| Soybean 46 | 47.51 | - |
| DL-methionine | 0.11 | - |
| Vitamin and mineral premix | 0.15 | 0.15 |
| Limestone | 4.85 | - |
| Monocalcium phosphate (MCP) | 3.11 | 2.61 |
| Oil (sunflower) | 13.00 | 1.30 |
| **Total** | 100.00 | 100.00 |

| **Nutrient** | **Units** | **Total** | |
|---|---|---|---|
| AME | MJ/kg | 12.48 | 10.25 |
| DE (pig) | MJ/kg | 17.52 | 13.00 |
| Crude protein | % | 36.00 | 38.49 |
| Dry matter | % | 91.47 | 90.31 |

| | | | |
|---|---|---|---|
| Lysine | % | 1.63 | 1.83 |
| Methionine | % | 0.75 | 0.75 |
| Cysteine | % | 0.57 | 0.56 |
| Methionine + Cystine | % | 1.32 | 1.23 |
| Threonine | % | 1.35 | 1.43 |
| Tryptophan | % | 0.37 | 0.31 |
| Arginine | % | 2.10 | 2.09 |
| Isoleucine | % | 1.69 | 1.82 |
| Leucine | % | 4.01 | 4.27 |
| Histidine | % | 0.89 | 0.99 |
| Phenylalanine | % | 1.84 | 2.01 |
| Valine | % | 1.80 | 2.21 |
| Crude fibre | % | 2.91 | 4.29 |
| Crude fat | % | 14.60 | 15.47 |
| Calcium | % | 2.40 | 2.27 |
| Phosphorous | % | 1.20 | 1.20 |

A protein percentage and digestible energy/protein (DE/P) ratio of 36 % and 8.3 kcal/g have been shown to provide maximum growth for Nile tilapia (Halver & Hardy, 2002). The diets were formulated for 36 % protein and 15 % fat. However, in order to maintain a balanced amino acid profile in both diets, the dilution and summit diets were formulated to have a 36 % protein and 14.6 % fat, and 38.45 % protein and 15.47 % fat content, respectively. The formulated protein and fat contents for treatment diets are shown in **Error! Not a valid bookmark self-reference.**. The protein and fat contents of diets 2-6 were dependent of the ratios of the summit and dilution diet of which they were made up of.

**Table 4.3** The ratio of summit and dilution diets used to formulate experimental feeds

| | **BSF0** | **BSF5** | **BSF10** | **BSF15.6** | **BSF20** | **BSF25** |
|---|---|---|---|---|---|---|
| Dilution diet | 100.00 | 80.00 | 60.00 | 40.00 | 20.00 | 0.00 |
| Summit diet | 0.00 | 20.00 | 40.00 | 60.00 | 80.00 | 100.00 |
| BSF % | 0.00 | 5.00 | 10.00 | 15.60 | 20.00 | 25.00 |
| Protein % | 36.00 | 36.49 | 36.98 | 37.47 | 37.96 | 38.45 |
| Lipid | 14.60 | 14.77 | 14.95 | 15.12 | 15.30 | 15.47 |

BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

The feed was prepared via extrusion through a 4 mm die, with the intent to produce floating pellets. After extrusion the pellets were oven dried and bagged in breathable polythene bags until used. Of the experimental diets used, only diet BSF0 and BSF5 resulted in floating pellets. Increasing inclusion levels of full-fat BSF pre-pupae meal resulted in an increased crumbling effect. The BSF20 and BSF25 diets required the addition of a binder, Aquacube at 0.25 % dietary inclusion levels, in order to prevent complete crumbling during extrusion.

#### *4.2.2.1 Feeding regime*

The juvenile tilapia were hand-fed twice a day at 09:00 and 17:00 until apparent satiation, based on observation of feeding behaviour. The fish were sampled every five days, and only fed in the evenings due to handling stress in the mornings. The amount of feed dispensed was minimum 4% and maximum 10% of body weight of the fish.

Feeding rations were adjusted after every sampling. Feeding sessions consisted of 3-5 rounds of feeding small amounts of feed at a time. Roughly 25 % of total daily feed allocation provided in first round, thereafter the fish were fed a small amount of feed until apparent satiation. Diets 5 and 6 were given feed 10 min prior to feeding the other diets to allocated tanks, as the binder in these feeds called for submersion time to soften. Losses of uneaten food due to fragmentation and dissolution of pellets were not quantified; thus food consumption is based on virtual feed intake. Specimens appeared to have a higher feeding rate at the 17:00 feeding time than 09:00; roughly 40% of allotted feed was consumed in the morning and the remaining 60 % during the later feeding. The feed allotted was recorded daily to determine growth performance and feed utilization at the end of the trial.

### ***4.2.3 Water quality***

Daily measurements were taken of water temperature, dissolved oxygen, pH and ammonia. The pH, ammonia and dissolved oxygen were monitored using a water quality meter, model 8603 Handheld IP67 Combo PH/COND./D.O (Figure 4.3.3a). Ammonia and nitrate was measured using a handheld calorimeter, HACH DR/850, shown in Figure 4.3.3b.


a


b

c

**Figure 4.3** Water quality and exchange rate instruments: a) Hand-held pH, dissolved oxygen and water temperature meter (8603 Handheld IP67 Combo PH/COND./D.O); b) Hand held calorimeter meter (HACH DR/850); c) Bucket of known volume and siphoning pipe.

Fish excrete nitrogenous waste via gills in the form of ammonia (El-Sayed, 2006). Ammonia levels were monitored daily, when levels started approaching upper tolerance levels, water was partially siphoned out during the day to minimize the stress related to water quality on the fish. The water was siphoned at between 0.5 L/s and 1 L/s directly from the tank outflow water as it flows into the sump (Figure 4.3c), which effectively lowered the nitrogenous wastes and improved the water quality to within optimal ranges required for Nile tilapia. During siphoning, the lost water was automatically replaced by a floating ball-valve mechanism to maintain same water level at all times

#### *4.2.3.1 Sampling procedures*

All the fish were individually blotted dry with a wet cloth after they were anaesthetised with clove oil for stress mitigation during handling. Their standard and total lengths were recorded in millimetres using a measuring board with a ruler fixed to it. A digital weighing scale (UWE, HGS-1500) was used to measure fish to the nearest 0.01 g. Every fish was sampled individually in every tank. The tanks were completely drained and cleaned and replaced with when sampling took place. On the final sampling day, 10 fish were randomly selected from each tank for proximate analysis. Four fish from the 10 randomly selected fish which were close to the average weight class of the fish in the tank were selected for proximate analysis. After fish were humanely euthanatized using clove oil, they were kept on ice until they were frozen at –20 °C until analysis.

### *4.2.4 Analytical procedures*

A proximate composition analysis was carried out on the experimental feeds at the Department of Animal Sciences (Stellenbosch University, South Africa). The samples were analysed for moisture, ash, crude protein, crude fibre and crude fat. All measurements were carried out in duplicate.

#### *4.2.4.1 Moisture content*

Two clean and dry crucibles containing 2 g of feed sample were dried at 105 °C in an oven for 24 h. The methodology of analysis was according to the Association of Official Analytical Chemist International (AOAC, 2002), official method 934.01.

**Equation 15:** % Moisture = ((A+B)-C / B x 100)

A = Weight of empty and dry crucible; B = Weight of empty dried test sample; C = Weight of crucible and moisture free test sample.

#### *4.2.4.2 Crude protein content*

The Dumas combustion method was used to determine crude protein (CP) content with the use of a LECO FP 528 (LECO FP 528, USA). Methodology was carried out according to AOAC (2002), official method 4.2.07. A sample of known mass (± 0.1g) was placed in a folded foil cup and combusted at ± 900 °C in the presence of oxygen. The gasses released were absorbed in special columns, where nitrogen was separated and the concentration thereof used to calculate crude protein content with the conversion factor of 6.25. Total crude protein was calculated using Equation 16.

**Equation 16:** CP (%) = Nitrogen (%) x 6.25

#### *4.2.4.3 Crude fat content*

The crude fat content was determined using acid hydrolysis. The process consists of acid hydrolysis using hydrochloride followed by extraction of hydrolysed lipids with mixed ethers. A sample of 2 g was placed in a test tube and mixed with 2 ml ethanol and 10 ml hydrochloride, and boiled for 30 min in a water bath. After cooling to room temperature (30 min), the sample was poured into a separating funnel and rinsed with 10 ml ethanol. Then 25 ml of diethyl ether and petroleum ether was added to the test tube and shaken for 1 min. The upper portion of the solution was then poured off after which 15 ml diethyl ether and 25 ml petroleum ether was added and the proses repeated. After the second portion was poured off, the cup containing the solution was placed in a sand bath at 30°C until all the ether evaporated. After evaporation, Equation  was used to calculate crude fat content. The methodology was carried out according to official method 954.02 (AOAC, 2000).

**Equation 17:** % Fat = ((A-B)/Sample mass) x 100)

A = Collective mass of cup and fat; B= Mass of cup.

#### *4.2.4.4 Crude fibre content*

The crude fibre content was determined with the Fibretec system. After the 1 g sample was weighed off and placed in its crucible, 150 ml of preheated sulphuric acid was added to the tube. Once the acid, containing the sample, was brought to boil at 100 °C, the temperature was lowered to 65 °C and maintained at this temperature for 30 min. The solution was then extracted with a suction pump and the sample washed (three

times) with 150 ml hot distilled water. The sample was then rinsed (three times) with 20 ml acetone to remove any traces of water, and placed in a drying oven at 100 °C for 48 h. After drying, the sample was cooled for 30 min and then placed in ash oven at 500 °C for 6 h. The percentage crude fibre was calculated using Equation 7.

**Equation 7:** $\%\ \text{Crude fibre} = \frac{\text{A} - \text{B}}{\text{Mass of sample}}\ \text{x}\ 100$

A = Mass (g) of residue in crucible after drying; B = Mass (g) of residue in crucible after ashing.

The nitrogen free extract (NFE) was determined with Equation 8.

**Equation 8:** NFE = 100 – (moisture + ash + protein + fat + fibre)

*4.2.4.5 Ash Content*

The 2 g sample used for moisture determination was used to calculated values for ash content. The sample and original crucible was placed in a furnace at 500 °C (temperature gradually increased) for 6 h. The samples were allowed to cool for 2 h, and then placed in desiccators to cool for 30 min. The ash content was calculated with Equation .

**Equation 20:** %Ash = ((D-A) / B) x 100

D = Mass (g) of crucible and sample after ashing; A = Mass (g) of dry crucible; B = Mass (g) of sample.

### *4.2.5 Statistical analysis*

The general linear model (GLM) and analysis of variance (ANOVA) procedures using SAS™ statistical software (2015) were performed on tanks with treatment as the main effect. Homoscedasticity and normality were tested on all parameters. Significance levels were declared at P≤0.05 (5 % significance level). The Bonferroni's *post hoc* test (SAS, 2015) was used to separate means. The ADG was determined by fitting a simple linear regression of weight over time. The proximate compositions of the diets used are represented in Table 3.4. Initial and final weights were used to compare growth parameters. All weight classes were well represented in all treatments at the start of the trial, thus they were not included as a covariate in the final models.

**Table 4.4** Proximate composition of experimental diets (DM basis) with different inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal

| Proximate analysis (%) | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 |
|---|---|---|---|---|---|---|
| Moisture | 5.8 | 6.4 | 6.7 | 6.8 | 4.2 | 4.9 |
| Crude protein | 34.6 | 33.2 | 31.5 | 31.2 | 33.7 | 32.0 |
| Crude lipid | 7.3 | 7.8 | 9.3 | 11.2 | 12.0 | 11.1 |
| Crude fibre | 1.7 | 2.1 | 2.9 | 3.6 | 3.5 | 3.2 |
| NFE | 44.1 | 44.7 | 44.0 | 41.8 | 40.5 | 42.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ash | 6.5 | 5.9 | 5.6 | 5.5 | 6.2 | 6.1 |

BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

## 4.3 Results and discussion

The differences in the diets observed for crude protein and fat content differed to that formulated for (Table 4.4). The crude protein was formulated to increase by 0.49 % with the increasing inclusion levels of BSF25. Instead, the increasing ratios of the summit diet resulted in a decrease in the crude protein content. Crude protein content ranged from 31.5 % to 34.56 %, and crude fat ranged from 7.32 % to 11.98 % (Table 4.4). The increased levels of fat content appear to be related to the increase in inclusion levels. Hence, the more BSF pre-pupae meal added, the higher the crude lipid content. Similar problems were reported by Talamuk (2016) and Newton *et al.* (2005b).

Growth parameters, feed utilization and conditioning factor (CF) are shown in Table . There were no differences between initial weight (IW), final weight (FW), feed intake (FI), average daily gain (AGD), initial conditioning factor (CF) and final CF between any of the treatments. The only significant differences observed with regards to growth performance was that of feed conversion ratio (FCR), specific growth rates (SGR) and protein efficiency ratio (PER) (Table ). The FCR of BSF0 (1.35) did not differ significantly ($P>0.05$) from FCR of BSF5 (1.50), but did differ significantly from BSF10 (1.63). BSF5 and BSF10 did not differ significantly from each other with regards to FCR, but FCR of BSF5 differed ($P\leq0.05$) from FCR of BSF15.6 (1.79). There was no significant difference between the FCR of BSF10 and BSF15.6. However, BSF10 did have a lower ($P\leq.05$) FCR from BSF20 (1.97) and BSF25 (1.91). The SGR were not significantly different between diets BSF0 (2.05), BSF5 (1.91) and BSF10 (1.73). The only significant difference in SGR was between BSF0 and BSF15.6 (1.68), BSF20 (1.60) and BSF25 (1.62), respectively. There was no significant difference in SGR through diet BSF5 to BSF25. The PER of BSF0 (2.02) and BSF5 (1.82) did not differ significantly from each other. The PER of BSF0 (2.02) did however differ significantly from BSF10 (1.68), BSF15.6 (1.55), BSF20 (1.40) and BSF25 (1.43). The differences in PER may be attributed to decreased digestibility generally

**Table 4.5** The means and standard deviations for the growth performance of Nile tilapia (*Oreochromis niloticus*) fed different dietary inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal

| Parameter | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 | P value |
|---|---|---|---|---|---|---|---|
| IW (g) | 66.55 ± 4.60 | 68.24 ± 4.54 | 73.27 ± 4.73 | 68.95 ± 4.71 | 66.67 ± 4.46 | 69.68 ± 5.00 | 0.92 |
| FW (g) | 123.14 ± 8.52 | 120.84 ±7.58 | 123.02 ± 8.07 | 114.29 ± 8.34 | 107.53 ± 6.99 | 113.09 ± 7.33 | 0.66 |
| FI[1] (g) | 76.09 ± 4.75 | 78.51 ± 3.71 | 80.06 ± 4.46 | 79.50 ± 5.68 | 78.89 ± 3.80 | 82.34 ± 4.31 | 0.96 |
| FCR[2] | 1.35$^{a}$ ± 0.02 | 1.50$^{ab}$ ± 0.03 | 1.63$^{bc}$ ± 0.06 | 1.79$^{cd}$ ± 0.09 | 1.97$^{d}$ ± 0.10 | 1.91$^{d}$ ± 0.04 | <0.001 |
| SGR[3] | 2.05$^{a}$ ± 0.08 | 1.91$^{ab}$ ± 0.06 | 1.73$^{ab}$ ± 0.08 | 1.68$^{b}$ ± 0.09 | 1.60$^{b}$ ± 0.08 | 1.62$^{b}$ ± 0.06 | <0.001 |
| PER[4] | 2.02$^{a}$ ± 0.03 | 1.82$^{ab}$ ± 0.04 | 1.68$^{bc}$ ± 0.06 | 1.55$^{cd}$ ± 0.07 | 1.40$^{d}$ ± 0.07 | 1.43$^{d}$ ± 0.03 | <0.001 |
| ADG[5] | 0.95 ± 0.09 | 0.85 ± 0.09 | 0.80 ± 0.09 | 0.75 ± 0.09 | 0.83 ± 0.09 | 0.77 ± 0.09 | 0.68 |
| Initial CF[7] | 3.18 ±0.04 | 3.22 ± 0.03 | 3.34 ± 0.09 | 3.16 ± 0.03 | 3.21 ± 0.02 | 3.19 ± 0.03 | 0.15 |
| Final CF[9] | 3.47 ±0.04 | 3.52 ± 0.06 | 3.51 ± 0.02 | 3.49 ± 0.03 | 3.38 ± 0.04 | 3.42 ± 0.03 | 0.08 |

($^{a,b,c,d}$) Means with different superscripts within the same row differ significantly (P≤0.05); [1]FI = Feed intake; [2]FCR = Feed conversion ratio; [3]SGR = Specific growth rate; [4]PER = Protein efficiency ratio; [5]ADG = Average daily gain; [6]Initial CF = Initial conditioning factor; [7]Final CF = Final conditioning factor; BSF = Black soldier fly; BSF0 = Control diet, 0% BSF pre-pupae meal inclusion level; BSF5 = 5% BSF pre-pupae meal inclusion level; BSF10 = 10% BSF pre-pupae meal inclusion level; BSF15.6% = 15.6% BSF pre-pupae meal inclusion level; BSF20 = 20% BSF pre-pupae meal inclusion level; BSF25 = 25% BSF pre-pupae meal inclusion level.

associated with increased lipid content in feeds, but this statement cannot be confirmed due to variations in feed characteristics which may also have had an influence.

Although experimental diets had a similar nutritional composition and met all the nutritional requirements of Nile tilapia, differences were observed in feed utilization with regard to FCR, SGR and PER. A worsening trend was observed on FCR, SGR and PER with increasing inclusion levels of BSF pre-pupae meal, especially FGR and PER. Pellet water stability worsened with increasing inclusion levels of BSF pre-pupae meal. The growth parameters may have been influenced by reduced water stability due to reducing binding ability with increasing inclusion levels, and potentially reduced digestibility due to the use of a binder in BSF20 and BSF25. Diets BSF20 and BSF25 required a binder to avoid crumbling during extrusion, and displayed excellent water stability with near zero wastage during feeding, whilst BSF15.6 had very poor water stability. However, there were no significant differences between diet BSF15.6 (no binder and poor water stability) and BSF20 and BSF25 (with binder and excellent water stability) with regards to FCR, SGR and PER. It is well known that fish, like terrestrial animals, feed to satisfy their energy requirements. No significant difference in feed intake was observed between treatment diets, suggesting that protein to energy ratios (P/E) was similar between treatments and thus, did not affect intake. Variation in growth parameters may be related to digestibility and nutrient availability of feed.

Given the limitations around the differences between the diets relating to the influence of increasing lipid levels and the use of a binder in BSF20 and BSF25, the results may have been different if all diets had similar physical properties and inclusion levels of the feed binder. The effects of BSF pre-pupae meal, increasing lipid levels and binders related to feed quality are were discussed in Chapter 3.

## 4.4 Conclusion

This study demonstrates that BSF pre-pupae meal may be a viable alternative protein source for Nile tilapia. However, flaws related to feed characteristics may render the results inaccurate. Inclusion levels of up to 5% did not compromise growth parameters. However, due to limitations related to variation in feed stability and the use of a binder, the results may have been different if the binder was included equally in all diets. It is recommended that future studies make use of defatted BSF pre-pupae meal rather than full-fat meal. Also, to test the water stability on all diets/treatments prior to the commencement of the trial, so that the necessary measures can be put in place to maintain the uniformity of the feed quality. Furthermore, even though the summit diet had significantly poorer results for the growth parameters, they are still acceptable relative to industry standards and other diets.

## 4.5 References

Banks, I.J., Gibson, W.T. & Cameron, M.M., 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Trop. Med. Int. Health, 19(1), 14-22.

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. Aquac. 422, 193-201.

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

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

El-Sayed, A.-F.M., 1990. Long-term evaluation of cottonseed meal as a protein source for Nile tilapia, *Oreochromis niloticus* (Linn.). Aquac. 84(3-4), 315-320.

El-Sayed, A.-F.M., 1998. Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. Aquac. Res. 29(4), 275-280.

El-Sayed, A.-F.M., 2006. Tilapia culture. In : Aquaculture. CABI publishing, UK.

Food and Agriculture Organization of the United Nations (FAO), 2015. Aquaculture Feed and Fertilizer Resources Information System.

Halver, J.E. & Hardy, R.W., 2002. In: Fish Nutrition. Academic press.

Jackson, A.J., Capper, B.S. & Matty, A.J., 1982. Evaluation of some plant proteins in complete diets for the tilapia, *Sarotherodon mossambicus*. Aquac. 27(2), 97-109.

Lalander, C., Diener, S., Magri, M.E., Zurbrugg, C., Lindstrom, A. & Vinneras, B., 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) - From a hygiene aspect. Sci. Tot. Environ. 458, 312-318.

Lim, C. & Dominy, W., 1989. Utilization of plant protein by warmwater fish. In: Proceedings of World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs. Ed: Applewhite, T.H. pp. 245-251.

Lucas, J.S. & Southgate, P.C., 2012. Aquaculture: Farming aquatic animals and plant. Blackwell Publishing Ltd., UK.

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(1), 11-15.

National Research Council (NRC), 1983. Nutrient requirements of warmwater fishes and shellfishes. Vol. 12. National Academy of Science, Washington, USA. pp. 102.

Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K. & Thelen, E.E., 2005b. The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Symposium on the State of the Science of Animal Manure and Waste Management, 5-7.

Newton, L., Sheppard, C., Watson, D.W., Burtle, G. & Dove, R., 2005a. Using the black soldier fly, *Hermetia illucens*, as avalue-added tool for management of swine manure. Journal of Chemical Information and Modeling.

Ofojekwu, P.C. & Ejike, C., 1984. Growth response and feed utilization in the tropical cichlid *Oreochromis niloticus* (Linn.). fed on cottonseed-based artificial diets. Aquac. 42(1), 27-36.

Ogunji, J.O., Toor, R.U.A.S., Schulz, C. & Kloas, W., 2008a. Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turk. J. Fish. Aquat. Sci. 8(1), 141-147.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B., 2008b. Housefly maggot meal (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). 21(3), 319-331.

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.

Sheppard, D.C., Newton, G.L., Thompson, S.A. & Savage, S., 1994. A value added manure management system using the black soldier fly. Biores. Tech. 50(3), 275-279.

Shiau, S., Chuang, J.L. & Sun, C.L., 1987. Inclusion of soybean meal in tilapia (*Oreochromis niloticus* x *O. aureus*) diets at two protein levels. Aquac. 65(3-4), 251-261.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., … Irving, S., 2007. Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J. World. Aquac. Soc. 38(2), 309-313.

Surendra, K.C., Olivier, R., Tomberlin, J.K., Jha, R. & Khanal, S.K., 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew. Energy. 98, 197-202.

Tacon, A.G.J. & Jackson, A.J., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. Nutr. Feed. Fish. 119-145.

Tacon, A.G.J., Jauncey, K., Falaye, A., Pantha, M., MacGowan, I. & Stafford, E.A., 1983. The use of meat and bone meal and hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In: Proceedings of the First International Symposium on Tilapia in Aquaculture. pp. 356-365.

Talamuk, R., 2016. Comparisons of growth performance of African catfish (*Clarias gariepinus)* fingerlings fed different inclusion levels of black soldier fly (*Hermetia illucens*) larvae meal diets. Stellenbosch University.

Viola, S. & Zohar, G., 1984. Nutritional study with market size tilapia hybrid *Oreochromis* in intensive culture. Protein levels and sources. Isr. J. Aquac. 36, 3-15.

Webster, C.D. & Chhorn, L., 2006. Tilapia: biology, culture and nutrition. CRC Press.

Wu, Y.V., Tudor, K.Y., Brown, P. & Rosati, R.R., 1999. Substitution of plant protein and meat and bone meal for fish meal in diets for Nile tilapia. N. Am. J. Aquac. 61(1), 58-63.

Zhu, F.X., Yao, Y.L., Wang, S.J., Du, R.G., Wang, W.P., Chen, X.Y., … Yang, H.Q., 2015. Housefly maggot-treated composting as sustainable option for pig manure management. Waste Management, 35, 62-67.

# Chapter 5

## The effect of varying inclusion levels of black soldier fly (*Hermetia illucens*) (full-fat pre-pupae meal) in feed on Nile tilapia (*Oreochromis niloticus*) fillets

**Abstract**

The effects of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal on body composition and yield of Nile tilapia (*Oreochromis niloticus*) were investigated for six treatments with increasing BSF inclusion levels. The six inclusion levels were 0 % (control diet) (BSF0), 5 % (BSF5), 10 % (BSF10), 15.6 % (BSF15.6), 20 % (BSF20) and 25 % (BSF25). No significant difference in the final body weight and carcass yield was observed between the treatments. The only significant difference was for fillet yield between BSF5 (53.64 %) and BSF20 (50.72 %), but the yields may be inaccurate as indicated by the low $R^2$ value (0.094). Thawing loss, due to freezing, for BSF25 (5.36 %) was higher ($P \leq 0.05$) than that of BSF0 (3.91 %) and BSF10 (3.77 %). The hepatosomatic index (HSI) for BSF20 and BSF25 was significantly higher than BSF0, BSF5 and BSF10. There were no differences ($P > 0.05$) for body composition parameters measured (i.e. moisture, crude protein, lipid and ash content) for the fillets between any of the treatments. This study concluded that full-fat BSF pre-pupae meal can be included in Nile tilapia diets by up to 25 % of the "as fed" diets without any effect on the fillet composition, final body weight or carcass yield, whilst the HSI differed significantly when inclusion levels exceeded 20 % of the total diet.

Key words: Black soldier fly, pre-pupae meal, Nile tilapia, carcass yield

### 5.1 Introduction

Fish are rich in vitamins, minerals, fatty acids and proteins (including all essential amino acids). In fact, its high nutritional value makes it an important food source for humans (Murray & Burt, 2001). Fish make up more than 50 % of the total animal protein intake in underdeveloped countries (i.e. Africa and Asia), providing an important source of essential fatty acids (EFA) and other important micronutrients (FAO, 2016). Fish and mammals (e.g., beef) consist of the same principal constituents (Table ). The chemical composition of fish is affected by numerous factors such as species, age, environment and season (Huss, 1995; Murray & Burt, 2001). Other factors affecting the chemical composition of fish include feed intake, activity level and spawning. The chemical composition of fish is of importance to the processor, nutritionist, cook and consumer. Processors require information on the nature of the raw material in order to apply suitable processing methods, while nutritionists need to know its nutritional content (Murray & Burt, 2001). For example, knowing the lipid content and composition may be of importance to a cook or consumer due to its influence on taste (Johansson *et al.,* 2000) and the preparation required (Murray & Burt, 2001), and to a producer owing to its influence on shelf life (related to oxidative activity of lipids) (Kjær *et al.*, 2008).

**Table 5.1** Principal constituents of fish, adapted from Huss (1995)

| Constitution | Fish (Normal fillet variation) | Beef (Isolated muscle) |
|---|---|---|
| Protein (%) | 16-21 | 20 |
| Lipid (%) | 0.2-25 | 3 |
| Carbohydrate (%) | <0.50 | 1 |
| Ash (%) | 1.2-1.5 | 1 |
| Water (%) | 66-81 | 75 |

Fish protein is highly digestible, with small portions of connective tissues (Steffens *et al.,* 2006). Structural protein in fish have a similar amino acid composition to corresponding proteins in mammalian muscle (Table ). It consists of a combination of amino acids that are comparable to that of meat, milk and eggs, which is highly suited to the nutritional requirements of humans (Murray & Burt, 2001). To utilize diets for its full potential, amino acids must be present in the correct proportions (Murray & Burt, 2001). In contrast to cereal and grain proteins, fish proteins contain relatively high lysine and methionine concentrations (Murray & Burt, 2001). The use of fishmeal in grain based diets increases the biological value thereof by providing amino acids which would otherwise be insufficient (Huss, 1995).

**Table 5.2** General essential amino acid (EAA) composition of fish, milk, beef and eggs as a percentage (%) of lysine, adapted from Huss (1995).

| Amino-acid | Fish | Milk | Beef | Eggs |
|---|---|---|---|---|
| Lysine | 100.00 | 100.00 | 100.00 | 100.00 |
| Tryptophan | 11.36 | 19.75 | 11.83 | 27.94 |
| Histidine | 22.73 | 32.10 | 40.86 | 32.35 |
| Phenylalanine | 44.32 | 65.43 | 48.39 | 79.41 |
| Leucine | 95.45 | 125.93 | 88.17 | 123.53 |
| Isoleucine | 68.18 | 88.89 | 55.91 | 104.41 |
| Threonine | 52.27 | 54.32 | 45.16 | 80.88 |
| Methionine-cystine | 45.45 | 53.09 | 31.18 | 48.53 |
| Valine | 68.18 | 93.83 | 53.76 | 119.12 |

It has been shown that the lipid composition of fish, taking all species into account, has the highest level of variation compared to other constituents and an inverse relationship with water content (Murray & Burt, 2001). Furthermore, there may be a variation in the lipid distribution throughout the flesh of fish. For instance, the concentration of lipid may be double in muscles close to the head compared to that in the tail of Pacific salmon (Murray & Burt, 2001). Fish use their stored lipids as energy reserves during spawning and migration (Huss, 1995). Ighwela *et al.* (2014) reported an increased HSI with increased dietary energy levels in Nile tilapia diets. This may be attributed to the storing effect of excess energy from carbohydrates as lipids in the

liver. Assessment of the HSI is an useful and a widely practiced measurement tool in assessing food value. Lipids are located in subcutaneous tissue, belly flap muscle, as well as the general muscle structure of the fish (Huss, 1995). Fish muscle consists of light and dark muscle, with the latter containing a higher concentration of fat and certain minerals (Murray & Burt, 2001). Fats are normally concentrated close to myocommata and in-between the light and dark muscle (Kiessling *et al.,* 1991). Freshwater fish have a slightly lower content of polyunsaturated fatty acids (PUFA) (four to six double bonds) than corresponding lipids from marine fish (Stansby & Hall, 1967). Fish oils contain the linoleic and linolenic acids regarded as essential fatty acids (EFA) in human nutrition, as humans cannot synthesize these naturally. These EFA, along with other essential PUFA help to prevent arteriosclerosis, skin diseases and are associated with neurological benefits in growing children (Huss, 1995). The carbohydrates constitution in fish muscle are generally very low, usually less than 1%, but up to 2% in dark muscle of some species (Murray & Burt, 2001). The potential influence of BSF on the Nile tilapia fillets are unclear, and thus requires investigation.

## 5.2 Material and methods

### *5.2.1 Experimental system*

The trial was carried out as described in Chapter 4, Section 4.2.1.

### *5.2.2 Experimental feed*

Composition of experimental feed and inclusion levels were discussed in Chapter 4, Section 4.2.2.

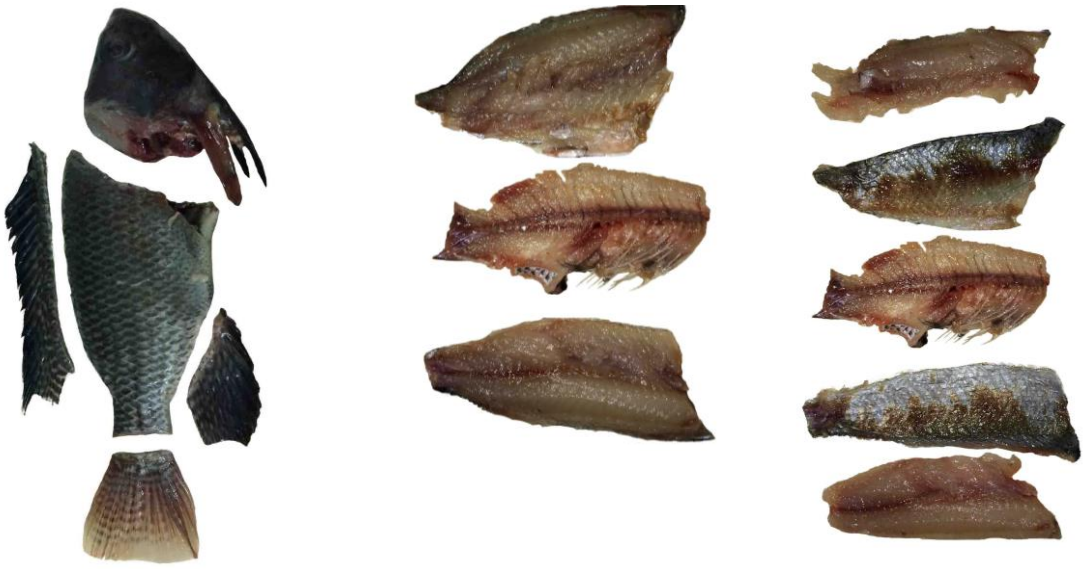
### *5.2.3 Experimental procedure*

The description of the treatment diets, quantity of Nile tilapia used and the feeding procedure are described in Chapter 4. On the final sampling day (day 30), 10 fish per tank were euthanized using clove oil, and immediately placed on ice in foam boxes, vacuum sealed and frozen at -20 °C. Four fish close to the average weight per tank were selected for proximate analysis from each tank. Hence, with seven tanks a total of 28 fish were selected to represent each diet (treatment). Weights were recorded before fish were euthanized, as well as after defrosting to take moisture loss into account. Fish designated for proximate analysis were defrosted overnight (12 h) in a fridge at 4 °C. Once defrosted, the fish were rinsed with freshwater for 2 seconds to remove excess mucus, and dried with a paper towel before weighing. Water loss was determined through the difference in weight and expressed as a percentage of the initial body weight (Vieira *et al.,* 2009; Ahamed Ali, 1988). All of the fish were gutted, dressed and individually filleted by hand. After removal of the head, dorsal, ventral and tail fins, fillets were removed from the skeletal structure and skin before being weighed to obtain the fillet yield (Figure .1). Livers were removed and weighed individually to determine the hepatosomatic index (HSI). The fillet yield was calculated as a percentage of the initial body weight before freezing (Clement & Lovell, 1994). One person carried out filleting in a standardized manner for comparison between treatment diets to attempt to maintain uniformity during the procedure. After the weight

was recorded, the fillets were homogenized, vacuum packed and frozen immediately at -20 °C until analysis. Between each sample the mixer bowl was cleaned to prevent cross-contamination of samples.

## 5.3 Proximate analysis

The proximate analysis for the body composition of the Nile tilapia was carried out at the Department of Animal Sciences (Stellenbosch University, South Africa). The frozen homogenized samples were defrosted overnight (12 h) in a fridge at 4 °C. Once defrosted, the homogenized sample was mixed within the vacuum bag before samples were taken for analysis. Samples were analysed in duplicate for moisture, protein, lipid and ash content.



a) b) c)

**Figure 5.1** Filleting of fish a) specimen after being dressed b) filleted fish c) removal of skin from fillet

### *5.3.1 Moisture content*

The AOAC official method 934.01, according to Association of Official Analytical Chemist International (AOAC, 2002) was used to measure moisture content as described in Chapter 4.

### *5.3.2 Protein content*

Protein content was determined with a LECO FP 528 using the Dumas combustion method, according to AOAC (2002), official method 992.15. A sample of 0.1 g was folded into a foil cup and incinerated in the LECO FP 528, after which the total nitrogen content was determined to calculate protein content by multiplying the nitrogen percentage (%) with 6.25.

### *5.3.3 Lipid content*

The determination of lipid content was carried out according to Lee *et al.* (1996), using a chloroform/methanol (2:1) solution. Homogenized fish fillet (5 g) was mixed/homogenized with 50 ml chloroform/methanol solution in a glass beaker. Once thoroughly mixed, the solution was filtered through Whatman no. 1 filter paper into a separation funnel. After filtration, the filter paper and residue was removed and dried for protein determination. Twenty millilitres of 0.5 % NaCl was added to the separation funnel, which was shaken four times and allowed to stand for 30 min. A 100 ml Erlenmeyer flask was used to collect the lower layer of liquid, of which 5 ml was extracted with a pipette and poured into a glass beaker of known weight. The beakers were placed onto a sand bath at 30 °C for 45 min which allowed total evaporation of the solvents, and then cooled in a desiccator for 30 min before weighing.

### *5.3.4 Ash content*

Method used for ash determination was described Chapter 4 under Section 4.2.4.

## 5.4 Statistical analysis

The general linear model (GLM) and analysis of variance (ANOVA) procedures using SAS™ statistical software (2015) were performed on tanks with treatment as the main effect. Homoscedasticity and normality was tested on all parameters. Significance levels were declared at P≤0.05 (5% significance level). The Bonferroni's *post hoc* test (SAS, 2015) was used to separate means. Initial and final weights were used to compare growth parameters. All weight classes were well represented in all treatments at the start of the trial and thus are not included as covariate in final models (fat and full weight were investigated and no correlations were found).

## 5.5 Results and discussion

Moisture loss was measured on the whole fish after defrosting overnight, prior to dissection. Carcass yield (visceral organs removed), water loss after freezing and HSI are illustrated in Table . There was no difference (P>0.05) between the treatments regarding final body weight (107.53 g-123.14 g) and carcass yield (81.63-83.50 %) (Table 5.3). However, fillet yields (50.72-53.64 %) showed significant differences between BSF5 (53.64 %) and BSF20 (50.72 %) (Figure ). Although significant, the difference in fillet yield may be inaccurate indicated by the low $R^2$ value (0.094) obtained, indicating that only 9.4 % of variability in the data is explained by the model. There appears to be a slight decrease in fillet yield with increasing BSF pre-pupae meal inclusion levels and could be attributed to increase in feed lipid content, the physical acceptability (shape, size and texture) of the feed, or potentially both.

Furthermore, the carcass and fillet yield was lower and higher than that reported by Dos Santos *et al.* (2011), respectively for Nile tilapia weighing 344.01-383.20 g (Table 5.5). Similarly, Rutten *et al*. (2004) reported fillet yields of 34.5-37.8 % for mixed sex Nile tilapia weighing 705-784 g (an average fillet yield of 35.8 % was reported for all males with an average body weight of 866 g). The difference in fish size for which fillet

yields were reported may have had an influence on the fillet yield. Rutten *et al*. (2004) reported fillet yield and body weight to have a regression coefficient of only 0.001. Meaning that for every 100 g of weight increase, fillet yield may only increase with 0.1 g. Furthermore, the correlation coefficient between fillet yield and body weight was 0.03. The results indicate that although body weight may have an effect on fillet yield, it is small enough to still compare fillet yield between different weight classes.

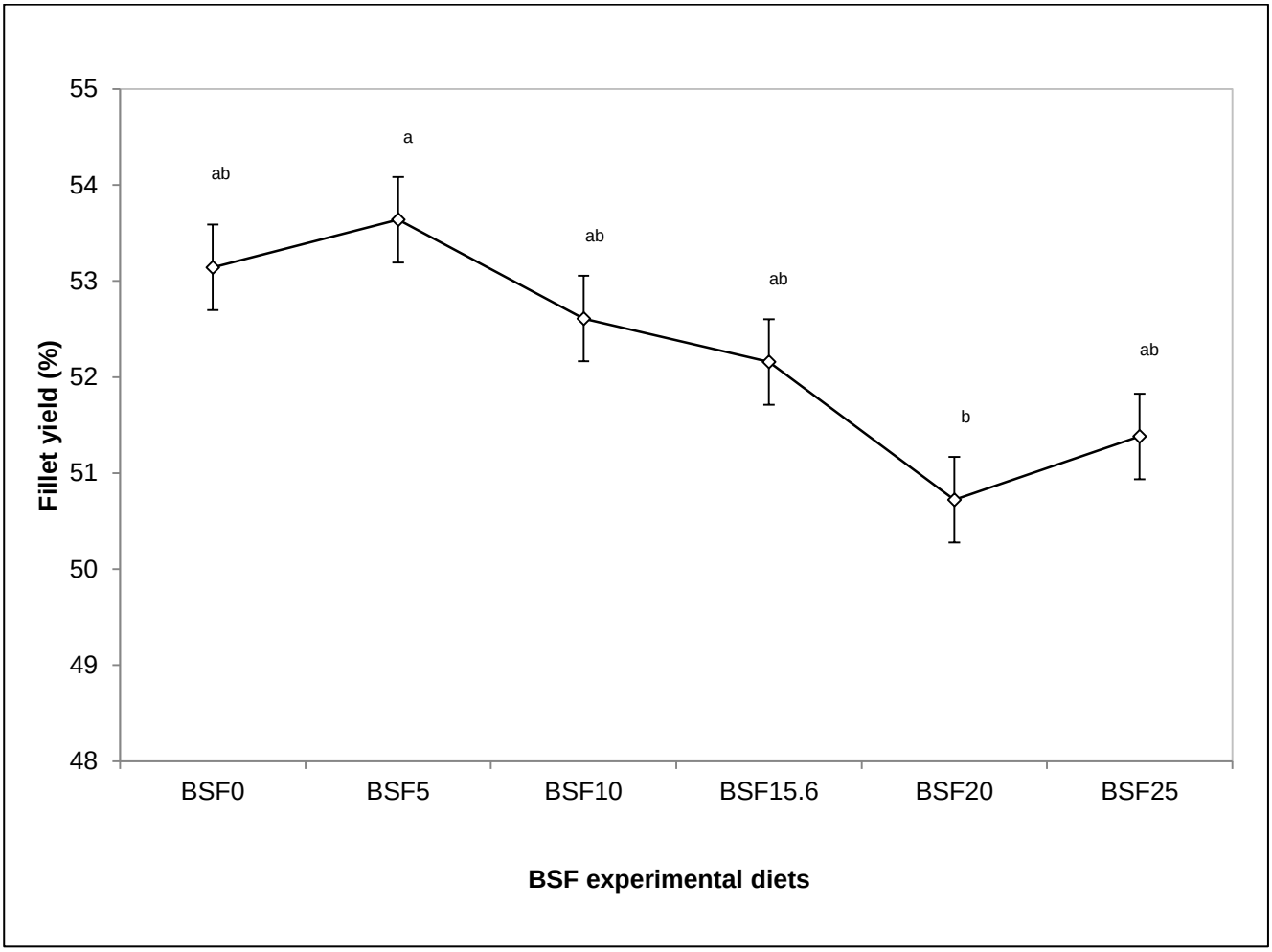



**Figure 5.2** Mean fillet yields of Nile tilapia (*Oreochromis niloticus*) receiving different inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal in treatment diets; ($^{a,b}$) Means with different superscripts differ significantly (P≤0.05) (BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level)

**Table 5.3** Means and standard deviations for dressing percentage, water loss and HSI values of six fish per experimental treatment with varying inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal in the diets of Nile tilapia (*Oreochromis niloticus*)

| Parameter | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 | P Value |
|---|---|---|---|---|---|---|---|
| Body weight | 123.14 ± 8.52 | 120.84 ± 7.58 | 123.02 ± 8.07 | 114.29 ± 8.34 | 107.53 ± 6.99 | 113.09 ± 7.33 | 0.659 |
| Carcass yield (%BW) | 83.50 ± 0.27 | 83.09 ± 0.26 | 82.51 ± 0.45 | 82.67 ± 0.33 | 81.63 ± 0.78 | 82.39 ± 0.37 | 0.080 |
| Fillet yield (%BW) | 53.14 $^{ab}$ ± 0.60 | 53.64$^{a}$ ± 0.65 | 52.61$^{ab}$ ± 0.61 | 52.16$^{ab}$ ± 0.65 | 50.72$^{b}$ ± 0.60 | 51.38$^{ab}$ ± 0.65 | 0.011 |
| Water loss (%BW) | 3.91$^{a}$ ± 0.27 | 4.33$^{ab}$ ± 0.29 | 3.77$^{a}$ ± 0.27 | 4.42$^{ab}$ ± 0.29 | 4.09$^{a}$ ± 0.27 | 5.36$^{b}$ ± 0.29 | 0.002 |
| $^{1}$HSI | 3.20$^{a}$ ± 0.14 | 3.09$^{a}$ ± 0.16 | 3.16$^{a}$ ± 0.14 | 2.81$^{ab}$ ± 0.10 | 2.47$^{b}$ ± 0.09 | 2.50$^{b}$ ± 0.10 | <0.001 |
| Feed lipid content (%) | 7.32 | 7.77 | 9.32 | 11.23 | 11.98 | 11.09 | - |

BSF = Black soldier fly; Carcass yield = (live weight – weight of visceral content)/body weight x 100; $^{1}$HSI = hepatosomatic index; ($^{a,b}$) Means with different superscripts within the same row differ significantly (P≤0.05); BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5% BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

**Table 5.4** Means and standard deviations for proximate fillet composition (%) of Nile tilapia (*Oreochromis niloticus*) fed different inclusion levels of black soldier fly (BSF) (*Hermetia illucens*) pre-pupae meal

| Parameter | BSF0 | BSF5 | BSF10 | BSF15.6 | BSF20 | BSF25 | P Value |
|---|---|---|---|---|---|---|---|
| Moisture (%) | 77.92 ± 0.19 | 77.70 ± 0.19 | 77.93 ± 0.19 | 78.06 ± 0.19 | 78.36 ± 0.19 | 78.25 ± 0.19 | 0.199 |
| Crude protein (%) | 19.22 ± 0.17 | 18.96 ± 0.17 | 18.94 ± 0.17 | 18.74 ± 0.17 | 18.77 ± 0.17 | 18.76 ± 0.17 | 0.339 |
| Fillet lipid (%) | 2.45 ± 0.17 | 2.92 ± 0.17 | 2.73 ± 0.17 | 2.84 ± 0.17 | 2.44 ± 0.17 | 2.67 ± 0.17 | 0.261 |
| Ash (%) | 1.22 ± 0.03 | 1.33 ± 0.03 | 1.25 ± 0.03 | 1.24 ± 0.03 | 1.28 ± 0.03 | 1.23 ± 0.03 | 0.115 |

BSF = Black soldier fly; BSF0 = Control diet, 0 % BSF pre-pupae meal inclusion level; BSF5 = 5 % BSF pre-pupae meal inclusion level; BSF10 = 10 % BSF pre-pupae meal inclusion level; BSF15.6 = 15.6 % BSF pre-pupae meal inclusion level; BSF20 = 20 % BSF pre-pupae meal inclusion level; BSF25 = 25 % BSF pre-pupae meal inclusion level.

**Table 5.5** Comparison of carcass and fillet yield ranges of Nile tilapia (*Oreochromis niloticus*)

| **Reference** | **Current trial** | **(Dos Santos *et al.*, 2011)** | **(Rutten *et al.*, 2004)** | **(Nguyen *et al.*, 2010)** |
|---|---|---|---|---|
| Final weight (g) | 107.53-123.14 | 344.01-383.20 | 705-784 | 527 |
| Carcass yield (%) | 81.63-83.50 | 89.98-90.73 | - | - |
| Fillet yield (%) | 50.72-53.64 | 34.7-35.66 | 34.5-37.8 | 33.6 |
| [1]HSI | 2.47-3.20 | 1.94-2.18 | - | - |

[1]HSI = hepatosomatic index

The significantly higher fillet yield obtained in this trial (50.72-53.64 %) relative to previous trials (34-38 %) is not necessarily due to the smaller size of the fish (Table 5.5). According to Rutten *et al.* (2004), other external attributions may also have an effect on fillet yield, namely the exact method used by person filleting (which may cause a variation of up to 2 %), environmental conditions during incubation or larval stage, genetic differences, tank effects, etc. On the other hand, the significant differences observed within the trial (between BSF5 and BSF20), may in fact be due to the small size of the fish resulting in handling difficulty during filleting. The only significant difference (P≤0.05) in water loss due to freezing was observed between BSF0 (3.91 %) and BSF25 (5.36 %) (Table 5.3).

The HSI for BSF20 (2.47) and BSF25 (2.50) was not significantly different, but was significantly lower (P≤0.001) than BSF0 (3.20), BSF5 (3.09) and BSF10 (3.16). Utilization of dietary carbohydrates by fish have been reported to be related to the complexity of the carbohydrate (example starch) used in their diets (Wilson, 1994). The carbohydrates influences liver size (and HSI), since they are stored as energy in the liver (Ighwela *et al.,* 2014). The effects of lipid content on starch gelatinization and feed are discussed further in Chapter 3, which are in support of assumptions related to this trail. Starch gelatinization decreased with increasing inclusion levels of lipid content in the feed. The increasing lipid content associated with increased BSF inclusion levels in this trial may have reduced gelatinization (resulting in a lower complex energy source) thereby reducing the ability of the fish to utilize starch as efficiently in BSF25 (the summit diet) as in BSF0 (the dilution diet). BSF0 showed the highest degree of expansion (starch gelatinization) during the extrusion process, which correlates with the current finding of the dilution diet having the highest HSI (Table 4.3).

There was no significant difference on the body composition between treatments regarding moisture, crude protein, lipid and ash content (Table ). The range for the fillet moisture (77.70-78.36 %), protein (18.74-19.22 %), lipid (2.44-2.93%) and ash (1.22-1.33 %) was in agreement with that reported by Dos Santos *et al.* (2011) and others (Table ). El-Saidy and Gaber (2003) reported similar ranges for protein, fat and ash content of 15.02-16.18 %, 1.95-2.38 % and 1.35-1.64 %, respectively. Al Hafedh (1999) and Ogunji *et al.* (2008) also reported similar results. However, higher initial lipid contents were reported, up to 12.63 % and 7.64 %, respectively, which may be due to whole body measurements – including the liver which stores excess energy in the form of lipids (Ighwela *et al.,* 2014).

The experimental diets had an increasing lipid content associated with increasing levels of BSF pre-pupae meal ranging from 7.32 % to 11.98 % (Chapter 3, Table 3.4). Hanley (1991) reported that increasing dietary lipid content, ranging from 5.1 % to 12.4 %, had a slight, but non-significant effect on whole body moisture (73-75 %) and protein (13.8-15.2 %) content. Yet, it significantly increased carcass (12 % in control to 7.9%) and visceral (1.3-18.5%) lipid levels. No variation in biomass indicates an inverse relationship between moisture, protein and lipid content, as moisture is displaced with increasing lipid and protein levels, and visa-versa. Hanley's (1991) findings supports the results from this trial. The Pearson's correlation coefficient's (r) and accompanying p-values showed that protein and fat had a moderately, but significant, negative correlation of r = -0.543 (P = <0.001) and r = -0.414 (P = 0.006), respectively. Due to considerable differences between fish tissues (Steffens *et al.,* 2006), proximate whole body composition cannot be compared to fillet composition. Furthermore, no literature was found on the effects of BSF pre-pupae meal on the proximate body composition of Nile tilapia.

## 5.6 Conclusions

Based on the findings from the analyses in this trial, it is unclear whether BSF pre-pupae meal has a distinctive influence (positively or negatively) on the fillet yield of Nile tilapia. However, there are signs that there might be a slight inverse relationship between the BSF pre-pupae meal inclusion level and fillet yield. The lack of significant difference in carcass yield demonstrates that the difference in fillet yield might be due to human error during the filleting process which is intensified by the small filleting size of the fish. The study demonstrate that BSF pre-pupae meal inclusion levels of up to 25% in Nile tilapia diets had no effect on body composition. Based on the results obtained, BSF pre-pupae meal could potentially be incorporated into Nile tilapia diets without changing the proximate composition of the fillet. However, this cannot be regarded as a conclusion due to external factors (the variation in feed characteristics) that could have influenced the fillet composition. Furthermore, it is recommended to harvest larger fish so as to mitigate the potential effects of human error during filleting. It is also advised to investigate how the ingredients affect the degree of gelatinization of the processed feed. Among other factors, the lipid content may have an effect on the degree of gelatinization. Gelatinization affects the availability of certain nutrients such as carbohydrates (making it more digestible with a higher degree of gelatinization), which affects the available energy and HSI of Nile tilapia. Therefore, the degree of gelatinization can have an indirect effect on HSI of Nile tilapia (higher degree of gelatinization can increase the HSI due to greater availability of digestible energy), since tilapia store excess available energy in their liver deposits. Also, the inconsistent inclusion of pellet binder may have influenced the result to a certain extend.

## 5.7 References

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E. & Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). Aquac. 298(3), 267-274.

Ahamed Ali, S., 1988. Water stability of prawn feed pellets prepared using different binding materials with special reference to tapioca. Indian J. Fish. 35(1), 46-51.

Al Hafedh, Y.S., 1999. Effects of dietary protein on growth and body composition of Nile tilapia, *Oreochromis niloticus* L. Aquac. Res. 30(5), 385-393.

Clement, S. & Lovell, R.T., 1994. Comparison of processing yield and nutrient composition of cultured Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*). Aquac. 119(2-3), 299-310.

Dos Santos, L.D., Furuya, W.M., Da Silva, L.C.R., Matsushita, M. & De Castro Silva, T.S., 2011. Dietary conjugated linoleic acid (CLA) for finishing Nile tilapia. Aquac. Nutr. 17(2).

El-Saidy, D.M.S.D. & Gaber, M.M.A., 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquac. Res. 34(13), 1119-1127.

Food and Agriculture Organization of the United Nations (FAO), 2016. *The State of World Fisheries and Aquaculture 2016*. http://www.fao.org/3/a-i5555e.pdf. Rome.

Hanley, F., 1991. Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion, and body composition of Nile tilapia, *Oreochromis niloticus* (L.). Aquac. 93, 323-334.

Huss, H.H., 1995. Quality and quality changes in fresh fish. Vol. 348. Rome.

Ighwela, K.A., Ahmad, A.B. & Abol-Munafi, A.B., 2014. The selection of viscerosomatic and hepatosomatic indices for the measurement and analysis of Oreochromis niloticus condition fed with varying dietary maltose levels. Int. J. Fauna Biol. Stud. IJFBS. 1, 18-20.

Johansson, L., Kiessling, A., Kiessling, K.-H. & Berglund, L., 2000. Effects of altered ration levels on sensory characteristics, lipid content and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). Food Qual. Pref. 11(3), 247-254.

Kiessling, A., Storebakken, T., Åsgård, T. & Kiessling, K.-H., 1991. Changes in the structure and function of the epaxial muscle of rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age: I. Growth dynamics. Aquac. 93(4), 373-387.

Kjær, M., Todorčević, M., Torstensen, B. & Vegusdal, A., 2008. Dietary *n*-3 HUFA affects mitochondrial fatty acid β-oxidation capacity and susceptibility to oxidative stress in Atlantic salmon. Lipids. 43(9), 813-827.

Lee, C.M., Trevino, B. & Chaiyawat, M., 1996. A simple and rapid solvent extraction method for determining total lipids in fish tissue. J. AOAC Int. 79(2), 487-492.

Murray, J. & Burt, J.R., 2001. The composition of fish.

Nguyen, N.H., Ponzoni, R.W., Abu-Bakar, K.R., Hamzah, A., Khaw, H.L. & Yee, H.Y., 2010. Correlated response in fillet weight and yield to selection for increased harvest weight in genetically improved farmed tilapia (GIFT strain), *Oreochromis niloticus*. Aquac. 305(1), 1-5.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B., 2008. Housefly maggot meal (magmeal) as a protein source for *Oreochromis niloticus* (Linn.). 21(3), 319-331.

Rutten, M.J., Bovenhuis, H. & Komen, H., 2004. Modeling fillet traits based on body measurements in three Nile tilapia strains (*Oreochromis niloticus* L.). Aquac. 231(1), 113-122.

Stansby, M.E. & Hall, A.S., 1967. Chemical composition of commercially important fish of the United States. Fish. 3(4), 29-46.

Steffens, W., 2006. Freshwater fish – wholesome foodstuffs. Bulg. J. Agric. Sci. 12(2), 320.

Vieira, C., Diaz, M.T., Martínez, B. & García-Cachán, M.D. 2009. Effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. Meat Sci. 83(3), 398-404.

Wilson, R.P., 1994. Utilization of dietary carbohydrate by fish. Aquac. 124(1-4), 67-80.

# Chapter 6

## General conclusion

The dependence on wild catch fisheries has been alleviated to a large extent through aquaculture practices over the past few decades. However, the reliance on fishmeal as a conventional protein source in aquaculture feeds present long-term sustainability issues. The world's population is growing and will require a higher output of fish as a protein source. The findings in this study showed that full-fat BSF pre-pupae meal has the potential to supplement fishmeal in the diets of Nile tilapia as inclusion levels of up to 5 % had no significant negative effect on any of the growth parameters investigated. Furthermore, there were no significant difference in the final weights between any of the treatment classes.

No significant difference in carcass yield was observed between treatments. A significant differences in fillet yield was observed for the BSF20 treatment, but this may be attributed to human error during the filleting due to the small size of the fish. There was no significant difference in the proximate composition of the fillets between treatments. The study concludes that carcass yield and the proximate composition of Nile tilapia fillets (moisture, protein, lipids and ash content) were unaffected by the inclusion of full-fat BSF pre-pupae meal.

Variation in feed stability and the necessary use of a binder at inclusion levels of 20 % and 25 % may have been different had all pellets been equal in terms of stability and overall quality. The BSF pre-pupae meal had an influence on the unit density, sinking velocity, water uptake and leaching rate of pellets. The reduced feed quality may be associated with the increasing lipid contents with increasing BSF pre-pupae meal.

**Future recommendations:**

It is recommended that future studies use defatted, instead of full-fat, BSF pre-pupae meal as a protein source to ensure optimal gelatinization and binding of the feed. The water stability should also be tested for all diets before carrying out an experimental trial. In addition, uniform experimental diets, with regard to quality parameters, should be used. Further studies should be carried out over a longer feeding period to ensure that the fish grow large enough for harvesting, thereby mitigating the potential influence of human error related to filleting. The current trial was conducted over 30 days and the period was sufficient to obtain six measuring points as measurements were taken every five days. However, the trial was ended at 30 days due to worsening water quality related to carrying capacity of the RAS. Therefore, future studies should take this into account during the experimental design stage.