COMPARISONS OF GROWTH PERFORMANCE OF AFRICAN CATFISH (*Clarias gariepinus* BURCHELL, 1822) FINGERLINGS FED DIFFERENT INCLUSION LEVELS OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL DIETS

BY

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DECLARATION

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SUMMARY

Fish food are a good source of quality protein and other essential nutrients required in human diets. The health benefits of eating fish have been extensively reported. Fish provide the global population with 17% of animal protein intake. However, fish supply from capture fisheries is believed to be stagnant or declining and is not likely to increase in the future. Aquaculture is widely acknowledged as having the potential to fill this gap in food fish production for the human population. One of aquaculture's drawbacks is heavy reliance on non-sustainable protein sources for aqua feed.

The search for alternative and sustainable protein sources has rendered insects as an attractive option in fish diets, since fish naturally consume insects. Insects have a satisfactory amino acid profile and are rich in fats, minerals and vitamins. This study investigated the effects of different inclusion levels of black soldier fly (BSF) larvae meal in the diet of African catfish (*Clarias gariepinus*) fingerlings. The production parameters and feed utilisation of fish that received the control diet and the BSF supplemented diets were compared. Moreover, the influence of these different diets on water quality parameters, gut histology and body composition of the experimental fish were investigated.

Four experimental diets were formulated containing 0% (control), 10%, 20% or 30% BSF larvae meal. A total of 720 fingerlings (5 – 6 g) were randomly allocated to four treatments with six replicates per treatment. The trial was terminated after a 91 day feeding period. Proximate analyses were performed on the experimental diets on a dry matter basis. Although the experimental diets were prepared to be similar iso-nitrogenous and iso-caloric, the proximate analysis showed crude protein content ranged from 41.50% - 44.24%, and crude fat ranged from 11.16% - 16.49%. The gross energy ranged from 19.10 – 20.06 MJ/kg.

For statistical analysis, the level of significance used was at p-value 0.05. Treatment effects with p-values less than 0.05 were considered significant. Alternatively, treatment effects with p-values more than 0.05 were not significantly different. If the ANOVA test was found less than 0.05, then Bonferroni *post hoc* test for least square means was used for multiple comparisons.

The results showed that water quality parameters fluctuated within the acceptable range for African catfish and did not compromise the growth of fish between treatments. The growth of fish that received the control diet and 10% BSF diet were not significantly different ($P > 0.05$); however, both were significantly different ($P < 0.05$) from fish that received BSF 30% larvae meal diet. The fish fed BSF 10% and BSF 20% larvae meal diets were not significantly different from each other. No significant difference in feed utilisation between treatments in terms of average daily gain, feed conversion ratio, protein efficiency ratio and specific growth rate were observed.

No differences ($P > 0.05$) were found in dressing percentages between treatments. The proximate composition of the catfish showed no difference ($P > 0.05$) in percentage moisture, protein, lipids and ash between treatments. This study shows that BSF larvae meal did not affect the proximate body composition of the African catfish at any tested inclusion levels compared to the control diet.

Gut histology of fish that fed on the control diet, 10% BSF, 20% BSF and 30% BSF were compared. No significant differences were found in height of mucosal folds and mucosal folds area between treatments. Significant differences were found in thickness of muscular layer between treatments. The results suggest that BSF larvae meal did not negatively affect intestine morphology in African catfish fingerlings, at least for the 91 day feeding period.

This study showed that BSF larvae meal is a viable protein source in the diet of African catfish. Furthermore, this investigation suggests that if unprocessed BSF larvae meal should be used for catfish production, the inclusion level should not exceed 10%. Moreover, this study recommends the defatting of BSF larvae to enable high inclusion levels in the diet of African catfish.

OPSOMMING

Vis as voedsel is ‘n goeie bron van kwaliteit proteïene en ander essensiële voedingstowwe benodig in menslike diëte. Die gesondheidsvoordele van vis eet word wyd verkondig. Vis voorsien die wêreld populasie met 17% van hul dierlike proteïen inname. Nieteenstaande, visvoorsiening vanaf vangste word beskou as stagnant of afnemend en word gesien dat dit nie sal toeneem in die toekoms nie. Akwakultuur word wyd beaam dat dit die potensiaal het om die gaping te vul in aanvraag vir vis vir die mensdom. Een van die vernaamste struikelblokke is die afhanklikheid van akwakultuur op nie-volhoubare proteïenbronne vir akwavoere.

Die soektog na alternatiewe en volhoubare proteïenbronne het insekte as ‘n aantreklike opsie vir visdiëte geplaas, want vis vreet natuurlik insekte. Insekte het ‘n aanvaarbare aminosuurprofiel en is ryk in vette, minerale en vitamiene. Hierdie studie het die effek van verskillende insluitingsvlakke van venstervlieg (VV) *Hermetia illucens* larwe-meel in die diet van die Afrika baber (*Clarias gariepinus*) vingerlinge ondersoek. Die produksie-parameters en voerverbruik van die vis wat die kontrole-diët en die VVM-supplement diëte ontvang het, vergelyk. Verder, is die invloed van die verskillende diëte op waterkwaliteitsparameters, dermhistologie en liggaamskomposisie van die eksperimentele vis ondersoek.

Vier eksperimentele diëte is geformuleer, bevattende 0% (kontrole), 10%, 20% of 30% VV larwe-meel. ‘n Totaal van 720 vingerlinge (5-6 g) was ewekansig geallokeer vir vier behandelings met ses herhalings per behandeling. Die proef is gestaak na ‘n 91 dae voedingsperiode. Proksimale analises is uitgevoer op die eksperimentele diëte op ‘n droë materiaal basis. Alhoewel die eksperimentele diëte voorberei is om dieselfde iso-stikstofagtig en iso-kalories te wees, het die proksimale analise aangedui dat die ruproteïen-inhoud gewissel het van 41.50% - 44.24% en ruvette van 11.16% - 16.49%. Die bruto-energie het gewissel van 19.10 – 20.06MJ/kg.

Vir die statistiese analises is die beduidenheidsvlak van p-waarde 0.05 gebruik. Behandelingseffekte met p-waardes kleiner as 0.05 is as beduidend aanvaar. Alternatiewelik, is behandelingseffekte met p-waardes groter as 0.05 as nie-beduidend aanvaar. Indien die ANOVA-toets minder as 0.05 was, is die Bonferroni *post hoc* toets gebruik vir die kleinstekwadrate gemiddels vir meertallige vergelykings.

Die resultate het getoon dat die waterkwaliteitsparameters gewissel het binne die aanvaarbare gebied vir Afrika babers en dit het nie die groei van die vis belemmer tussen die behandelings nie. Die groei van die vis wat die kontrole-diët en 10% VV meel ontvang het, het nie beduidend verskil nie ($P>0.05$); alhoewel, beide het beduidend verskil ($P < 0.05$) vir die vis wat VV 30% larwe-meel diët ontvang het. Die vis gevoer met VV 10% en VV 20% larwe-meel het nie beduidend van mekaar verskil nie. Geen beduidende verskille is waargeneem in voerinname tussen die behandelings in terme van gemiddelde daaglikse toevoeging, voeromsettingsverhouding, proteïeneffektiwiteitsverhouding en spesifieke groeitempo nie.

Geen verskille ($P > 0.05$) is waargeneem in die uitslagpersentasie tussen behandelings nie. Die proksimale samestelling vir die babers het geen beduidende verskille getoon nie ($P > 0.05$) vir persentasie vog, proteïene, vette en as tussen die behandelings. Die studie dui daarop dat die VV larwe-meel nie die proksimale

liggaamskomposisie van die Afrika baber beïnvloed het nie by enige van die verskillende insluitingsvlakke in vergelyking met die kontrole-diët.

Dermhistologie van die vis gevoer met die kontrole-diët, 10% VV, 20%VV en 30% VV is vergelyk. Geen beduidende verskille is waargeneem in terme van die hoogte en area van die mukosale voue tussen die behandelings nie. Beduidende verskille is wel waargeneem in die dikte van die spierlaag tussen die behandelings. Die resultate toon dat VV larwe-meel geen negatiewe effek op die intestine morfologie van die Afrika baber vingerlinge gehad het nie; ten minste vir die 91 dae voedingsperiode.

Die studie het aangedui dat VV larwe-meel ‘n haalbare proteïenbron in die dieët van die Afrika baber kan wees. Verder, toon die ondersoek dat indien die ongeprosesseerde VV larwe-meel vir die produksie van babers gebruik word, die insluitingsvlakke nie meer as 10% moet wees nie. Die studie stel voor dat die ontvetting van VV larwe moet plaasvind om hoër insluitingsvlakke in die dieët van Afrika babers te bewerkstellig.

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NOTES

This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BSF	Black soldier fly
FAO	Food and Agriculture Organisation
g	Grams
GLM	General Linear Model
H&E	Haematoxylin and eosin
HUFA	Highly unsaturated fatty acids
kg	Kilogram
L	Litres
mg	Milligram
MUFA	Mono-unsaturated fatty acids
PUFA	Poly-unsaturated fatty acids
µm	Micrometre

TABLE OF CONTENTS

1 Chapter One 1

1.1 Introduction 1

1.2 References 3

2 Literature Review 5

2.1 Alternative Sources of Protein in aquafeed 5

2.1.1 Nutritional Compositions of the Black Soldier Fly 7

2.1.2 The uses of Black Soldier Fly meal in aquaculture 10

2.2 Attribute of the African Catfish 11

2.3 Nutrient Requirements of the African Catfish 11

2.3.1 Protein and Amino Acids 11

2.3.2 Lipids and Fatty Acids 12

2.3.3 Carbohydrates 13

2.3.4 Minerals 13

2.3.5 Vitamins 14

2.4 Water Quality Requirements of the African Catfish 15

2.4.1 Dissolved Oxygen 15

2.4.2 Temperature 15

2.4.3 Ammonia, Nitrate and Nitrite 16

2.4.4 pH 16

2.5 Conclusion 17

2.6 References 18

3 The effects of black soldier fly *(Hermetia illucens)* larvae meal on growth performance and feed utilisation of African catfish *(Clarias gariepinus)* fingerlings 25

3.1 Introduction 25

3.2 Materials and Methods 26

3.2.1 Experimental Systems 26

3.2.2 Experimental Feed 27

3.2.3 Experimental Procedure 27

3.2.4 Water quality Parameters 28

3.2.5 Sampling Procedure 28

3.2.6 Analytical Procedures 29
3.2.6.1 Moisture Determination 29
3.2.6.2 Crude Protein Determination 29
3.2.6.3 Crude Fat Determination 29
3.2.6.4 Crude Fibre Determination 30
3.2.6.5 Ash Determination 30
3.2.6.6 Gross Energy Determination 30
3.2.6.7 Amino Acid Determination 30
3.3 Statistical Analysis 31
3.4 Results and discussions 31
3.5 Conclusion 36
3.6 References 37
4 The influences of black soldier fly *(Hermetia illucens)* larvae meal on the body composition of African catfish (*Clarias gariepinus*) fingerlings 41
4.1 Introduction 41
4.2 Materials and Methods 43
4.2.1 Experimental System 43
4.2.2 Experimental Feed 43
4.2.3 Experimental Procedures 43
4.3 Proximate Analysis 43
4.3.1 Moisture determination 43
4.3.2 Protein determination 43
4.3.3 Fat Content Determination 43
4.3.4 Ash determination 44
4.4 Statistical Analysis 44
4.5 Results and Discussion 44
4.6 Conclusion 46
4.7 References 47
5 The effects of different inclusion levels of black soldier fly *(Hermetia illucens)* larvae meal on the intestine of African catfish *(Clarias gariepinus)* fingerlings 50
5.1 Introduction 50

5.2 Materials and Methods 51
5.2.1 Histomorphological Procedure 51
5.3 Statistical Analysis 51
5.4 Results and discussion 52
5.5 Conclusion 53
5.6 References 54
6 General Conclusion 57

1 Chapter One

1.1 Introduction

According to the Food & Agriculture Organisation (FAO, 2014) fish and fishery products play a vital role in food security and meeting the nutritional needs of the human population in developing and developed countries. Fish is a good source of high value protein as well as an essential source of micronutrients including vitamins, minerals and polyunsaturated omega-3 fatty acids (FAO, 2012). The annual average global per capita consumption of fish increased from 9.9 kg to 17 kg between the years 1960 and 2000. It further expanded to 18.9 kg in 2010 (FAO, 2014). In the same year fish provided the global population with 16.7 percent of their animal protein intake and 6.5 percent of all the protein consumed (FAO, 2014). The world population is forecast to reach 9 billion by the year 2050 (Msangi *et al.,* 2013) while fish production from capture fisheries is believed to be stagnant or declining (Rana *et al.,* 2009). Therefore, aquaculture is widely acknowledged as a way to meet higher future food requirements for the human population (Tidwell, 2012).

Indeed aquaculture has become the fastest expanding food production sector (FAO, 2006). According to Delgado *et al.* (2003) the contribution of aquaculture to global food fish production rose from 7 percent in 1973 to 12 percent in 1985 and by 1997 it increased to more than 30 percent. Further, it rose from 34.5 percent in 2006 to 36.9 percent in 2008 (FAO, 2010). On the other hand, global fish production from aquaculture has increased from 32.4 million tonnes in 2000 to 66.6 million tonnes in 2012 (FAO, 2014). However, the implementation of aquaculture is limited by its heavy reliance on fishmeal and fish oil for aquafeed production (Klinkhardt, 2007).

Fishmeal is derived from capture fisheries which is a finite resource (Delgado *et al.,* 2003). It is manufactured by cooking, pressing, drying and milling of wild caught fish (Delgado *et al.,* 2003). Fishmeal has unique nutritional properties: it is highly palatable and its amino acid profile is ideal for most aquatic species. However, certain types of fishmeal contain gizzerosine (Okazaki *et al.,* 1983) which was reported to cause gizzard erosion in mono-gastric animals especially poultry (Masumura *et al.,* 1985). The toxicity of gizzerosine to fish is species-specific among other factors (Watanabe *et al.,* 1987; Dong *et al.,* 1994; Reyes-Sosa & Castellanos-Mollina, 1985). Nevertheless, the quality of fishmeal is related to the source of fish or fishery by-products used as well as on the processing method applied (Heuzé *et al.,* 2015).

Fishmeal production fluctuates annually which is largely affected by the El Niño phenomenon (FAO, 2012). Fishmeal production dropped from 30.2 million tonnes (MT) in 1994 to 15.0 MT in 2010 (FAO, 2012). In 2011 its production increased to 19.4 MT and dropped again to 16.3 MT in 2012 (FAO, 2014). The reducing supply and high demand for fishmeal from various animal husbandry sectors have escalated fishmeal prices until in 2013 it reached US $1,919 per tonne (FAO, 2014). There is currently ongoing research in an attempt to reduce dependence on fishmeal in fish diets. Therefore, it is justifiable to investigate insect based proteins to augment current fish feeds.

Insects have great potential to be used as feed for fish, since fish naturally consume insects (Rumpold & Schlüter, 2013). Henry *et al. (*2015) indicated that most insects/larvae have a well-balanced amino acid profile,

are rich in fats, minerals and vitamins. Additionally, insects can be reared on most organic waste streams and can transform the waste into a high quality feed stuff while reducing its inherent pollution potential (Barroso *et al.,* 2014). Moreover, insect rearing produce less greenhouse gases compared to livestock (Oonincx *et al.,* 2010).

This study aimed to establish the suitability and nutritional capacity of black soldier fly (BSF) (*Hermetia illucens*) larvae meal as a means to supplement fishmeal in fish feed. The production parameters and feed utilisation of African catfish (*Clarias gariepinus*) fingerlings fed on BSF larvae meal were compared. Furthermore, the influence of these different diets on water quality parameters, gut histology and body composition of the experimental fish were also investigated.

1.2 References

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

Delgado, C.L., Wada, N., Rosegrant, M.W., Meijer, S. & Ahmed, M., 2003. *Fish to 2020*: Supply and demand in changing global markets, The World Fish Center Technical Report 62.

Dong, M., Fairgrieve, W.T., Myers, M.S. & Hardy, R.W., 1994. Gastric abnormalities in rainbow trout *(Oncorhynchus mykiss)* fed amine-supplemented diets or chicken gizzard-erosion-positive fishmeal. *Aquaculture,* 127: 219-232.

FAO, 2014. The State of World Fisheries and Aquaculture. *FAO, Rome. pp 223.*

FAO, 2012. The State of World Fisheries and Aquaculture. *FAO, Rome. pp 209 .*

FAO, 2010. The State of World Fisheries and Aquaculture. *FAO, Rome. pp 197 .*

FAO, 2006. The State of World Fisheries and Aquaculture. *FAO fisheries technical paper, 500, pp.147*

Henry, M., Gasco, L., Piccolo, G. & Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: Past and future. *Anim. Feed. Sci.Tech*, 203: 1–22.

Heuzé, V., Tran, G. & Kaushik, S., 2015. Fish meal. Feedipedia, FAO. http://www.feedipedia.org.node/208.

Klinkhardt, M., 2007. The blue revolution- feed alternative for aquaculture. Global Trade Conference on Aquaculture.261-267. Qingdao, China, 29-31 May 2007.

Masumura, T., Sugahara, M., Noguchi, T., Mori, K. & Naito, H., 1985. The effects of gizzerosine, a recently discovered compound in overheated fishmeal, on the gastric acid secretion in chicken. *Poult. Sci.*, 64: 356-361.

Msangi, S., Kobayashi, M. & Batka, M., 2013. Fish to 2030: Prospects for Fisheries and Aquaculture. *World Bank Report*.

Okazaki, T., Noguchi, T., Igarashi, K., Sakagami, Y., Seto, H., Mori, K., Naito, H., Masumura, T. & Sugahara, M., 1983. Gizzerosine, a new toxic substance in fish meal, causes severe gizzard erosion in chicks. *Agric. Biol. Chem*., 47 (12): 2949-2952.

Oonincx, D.G.A.B., Van Itterbeeck, J., Heetkamp, M.J.W., Van den Brand, H., Van Loon, J.J.A. & Van Huis, A., 2010. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*, 5(12): E14445

Rana, K.J., Siriwardena, S. & Hasan, M.R., 2009. Impact of rising feed ingredient prices on aquafeeds and aquaculture production. *FAO Fisheries and Aquaculture Technical Paper*, 541: pp.63.

Reyes-Sosa, C.F. & Castellanos-Molina, R., 1995. Nutritional evaluation of gizzard erosion positive brown fishmeal in starter diet for Nile tilapia (*Oreochromis niloticus). Aquaculture 138: 323-329.*

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

Tidwell, J.H., 2012. Aquaculture Production Systems, John Wiley & Sons, Inc. pp 4-14.

Watanabe, T., Takeuchi, T., Satoh, S., Toyama, K. & Okuzumi, M., 1987. Effects of dietary histidine or histamine on growth and development of stomach erosion in rainbow trout. *Bull. Japan. Soc. Sci*, 53: 1207-1214.

Chapter 2

2 Literature Review

In fish culture, feeding constitutes more than 50% of the operating costs, with protein being the most expensive dietary source (El-Sayed, 1999). The majority of aquafeed formulations are based mainly on fishmeal as the dietary protein source. The availability of fishmeal in the future to sustain the rapid expansion of aquaculture and aquafeed production is questionable (Gatlin *et al.,* 2007). Tacon (1995) emphasised the importance of developing cost effective and more sustainable protein sources to sustain intensive farming systems. Insects are considered as a potential and high value sustainable protein source in animal feed. This chapter will discuss alternative protein sources used in aquafeed, the nutritional profile of black soldier fly (BSF), as well as the nutrient requirements and water quality parameters required for optimal growth of the African catfish.

2.1 Alternative Sources of Protein in aquafeed

The norms of studies that investigated alternative protein sources in aqua feed in the last three decades focuses on partial or complete replacement of fishmeal. However, in this study BSF larvae meal was used at various inclusion levels to supplement or complement conventional protein sources in the experimental diets.

Webster *et al.* (2005) indicated that the inclusion of alternative protein sources to reduce or eliminate dietary fishmeal in aquafeed should not compromise the growth and health of the cultured aquaculture fish species. Considerable studies have been carried out to find less expensive and relatively abundant nutrient rich sources for partial or complete substitution of the current expensive fishmeal protein source (El-Sayed, 1999; Odesanya *et al.,* 2011). These studies have evaluated alternative protein sources of either plant or animal origin. Plant protein sources include soybean meal (Fagbenro & Davis, 2001), cottonseed meal (El-Sayed, 1990) and ground nut/sunflower oil cake meal (Kestemont *et al.,* 2007). An aquatic plant such as duckweed (Mbagwu *et al.,* 1990) has also been evaluated. Animal protein sources include poultry by-products meal, and meat and bone meal (El-Sayed, 1998; Abdel-Warith *et al.,* 2001; Goda *et al.,* 2007). Moreover, fishery by-products were also evaluated (Fagbenro & Jauncey, 1994). Some of these ingredients are good protein sources available at a low price, however, certain factors have restricted their inclusion in fish feed.

The inclusion of these ingredients in fish diets has been limited due to the fact that they frequently contain at least one or more undesirable characteristics (Hardy, 1996). Generally plant protein sources such as soybean meal, rapeseed meal, cottonseed, sunflower seed, lupin seed and pea seed are deficient in one or more essential amino acids that are required for fish. Soya protein products for example are limiting in lysine and methionine. The supplementation of amino acids are required in feed that is solely prepared from plants protein sources. Some plant feedstuffs contain anti-nutritional toxins and have issues of palatability (Tacon, 1995). Fishery by-products such as fish silage contains high levels of free amino acids due to protein degradation (Hertrampf & Piedad-Pascual, 2000). Terrestrial animals' by-products such as meat meal, blood meal, meat and bone meal, hydrolysed feather meal, poultry by-product and liver meal are also deficient in some amino

acids and sometimes also have mineral imbalances (Tacon, 1995; Hertrampf & Piedad-Pascual, 2000). On the other hand, the inclusion of single cell protein, legumes and other protein concentrates in fish diet has been limited to their availability, palatability and high cost (Tacon, 1995).

Currently, insects, especially fly larvae, have emerged as a new protein source for animal feed. Fly larvae meals have potential as a protein source in fish diets. Larvae meals have been reported to be a possible alternative because of their good nutritional value and are cheap to produce compared to other protein sources (Ogunji *et al.,* 2006; Aniebo *et al.,* 2009). Larvae meals are not normally directly eaten by man (Teotia & Miller, 1974). Insect larvae can be grown on several waste products such as animal manure and food wastes, whereby, a nutrient rich source is produced and at the same time those organic wastes are reduced and transformed (Aniebo *et al.,* 2009). Black soldier fly (BSF) has an advantage over other flies, as adult BSF is not considered as a pest species and as such a disease vector because it does not eat in its mature phase and therefore do not enter into human dwellings in search of food.

Black Soldier Fly (*Hermetia illucens*)

The black soldier fly (BSF), *Hermetia illucens* (L) (Diptera: Stratiomyidae) is described as a long wasp-like fly distributed throughout the tropical and warm temperate regions of the world (James, 1935; Mc Callan, 1974). Its larvae are reared on a wide variety of decomposing material including animal manures such as chicken manure (Sheppard *et al.,* 1994), swine manure (Newton *et al.,* 2005a; Newton *et al.,* 2005b; St-Hilaire *et al.,* 2007a), cow manure (St-Hilaire *et al.,* 2007b), dairy cow manure plus fish offal (Sealey *et al.,* 2011) and human excreta (Banks *et al.,* 2014). Agricultural waste such as coffee pulp (Larde, 1990) and palm kernel (Hem *et al.,* 2008), as well as household waste have also been used as nutrient sources (Diener *et al.,* 2011). Black soldier fly larvae was found to have successfully reduced these decomposing materials and converted them into a useful feedstuff (Sheppard *et al.,* 1994).

A reduction of over 50% in the manure of laying hens (Sheppard *et al.,* 1994) and swine manure (Newton *et al.,* 2005a; Newton *et al.,* 2005b) has been reported. Similarly, Diener *et al.* (2011) reported 66% to 79% of household wastes were reduced by BSF larvae. Banks *et al.* (2014) found a reduction between 25.2% and 54.6% in faeces volumes when BSF larvae were utilised in a trial to manage fresh human waste. Additionally, BSF larvae can also eliminate harmful microorganisms found in the organic wastes. *Salmonella* species were removed by BSF larvae reared on human faeces (Lalander *et al.,* 2013). Erickson *et al.* (2004) reported the reduction of *Escherichia coli* 0157:H7 by BSF larvae in chicken manure.

The larvae of BSF metamorphosise to pre-pupae, which is the sixth instar before pupation and metamorphosis into an adult fly. The pre-pupae have been the most desired stage used by previous researchers who had evaluated BSF in different animal feeds. This is because BSF pre-pupa are in a non-feeding migratory stage and are capable of self-harvesting from the feeding substrate in search for a suitable pupation site (Sheppard *et al.,* 1994).

In the present study we used BSF larvae because the pre-pupae has more chitin content which is indigestible to many fish species (Rust, 2002). Black soldier fly larvae have a dull whitish colour and are insatiable feeders which can only be harvested manually. There is a paucity of information with regards to the biology of the adult

black soldier fly. Adult BSF is a non pest fly, it does not enter human residence as a disease spreading organism because the adult fly lives on the fat reserves stored during its larval stage (Newton *et al.,* 2005b).

2.1.1 Nutritional Compositions of the Black Soldier Fly

Although substantial research has been conducted on the use of BSF pre-pupae in animals feeds (Newton *et al.,* 2005ab; St-Hilaire *et al.,* 2007a; Sealey *et al.,* 2011) there is little information on the nutritional composition of BSF larvae and its use as animal feed. From the literature different nutritional values have been reported for insects especially fly larvae. These were due to drying methods and age (Aniebo & Owen, 2010), method of processing (Fasakin *et al.,* 2003), variation in species and time of harvesting (Atteh & Ologbenla, 1993). Several studies (Newton *et al.,* 1977; Newton *et al,* 2005a; St-Hilaire *et al.*, 2007a) have shown that BSF larvae or pre-pupae are high quality feedstuff. Table 2.1 presents the approximate composition of BSF larvae or pre-pupae. Black soldier fly pre-pupae reared on cow and swine manure have similar protein content (42% and 43%, respectively). Regardless of the feeding substrate, the protein content of BSF larvae or pre-pupae (Table 2.1) can meet the protein requirements of the African catfish.

Table 2.1 Proximate compositions of BSF fly larvae and pre-pupae

Analysis	**BSF Larvae (Reared on cow manure)**	**BSF pre-pupae Reared on swine manure)**
Crude Protein (%)	42.1	43.6
Fats (%)	34.8	33.1
Crude Fibre (%)	7.0	-
Ash (%)	14.6	15.5

1 Newton *et al.* (1977)

2 St-Hilaire *et al.* (2007)

Black soldier fly larvae meals have the potential to be utilised as feed ingredients in fish diets due to their balanced amino acid profile. The essential amino acids of BSF larvae reared on cow manure (Newton *et al.,* 1977) and swine manure (Newton *et al.,* 2005b) are shown in Table 2.2. Not all the specific amino acid requirements for African catfish have been identified. In most cases the amino acid levels determined for channel catfish (Ictalurus punctatus) are used in African catfish formulations. Table 2.2 compares the amino acid profile of BSF larvae to the amino acid requirements of African catfish. Based on the essential amino acid requirements for African catfish reported by Pantazis, (2005), BSF larvae are deficient in methionine, threonine and tryptophan. However, when BSF larvae meal is combined with other ingredients; a balance can be achieved for these amino acids. Newton *et al.* (2005b) suggested that the levels of amino acids would increase by 40% compared to the values shown in Table 2.2 when BSF larvae are rendered to produced oil and protein meal. According to Newton *et al. (*2005), the removal of the cuticle would improve the amino acid profile in BSF pre-pupae.

Table 2.2 Comparison of amino acid of BSF larvae and amino acid requirements of African catfish expressed as % of lysine.

Amino Acids	BSF Larvae (Reared on cow manure)	BSF Larvae (Reared on swine manure)	African catfish[3] (Amino acid requirement)
Lysine	100	100	100
Arginine	66	80	ND[4]
Histidine	57	43	31
Isoleucine	58	68	35
Leucine	104	118	108
Methionine	26	38	71
Phenylalanine	65	67	102
Threonine	16	64	45
Tryptophan	6	27	58
Valine	101	101	46

1 Newton *et al.* (1977)

2 Newton *et al.* (2005b)

3 Amino acid requirement determined by Pantazis, (2005)

4 ND= not determined

The mineral compositions of fly larvae meals are influenced by the type of processing methods (Fasakin *et al.,* 2003) and variation in the diets used (Newton *et al,* 2005a). Newton *et al.* (2005a) found phosphorus content was higher in BSF pre-pupae grown on poultry manure compared to swine manure. Whereas calcium content was high in BSF pre-pupae reared on swine manure (Table 2.3). Black soldier fly larvae are rich in macro-minerals especially calcium and phosphorus that exceed the requirement of African catfish. Newton *et al.* (1977) reported high calcium content (5.0%) and phosphorus (1.5%) for BSF larvae grown on beef cattle manure and urine slurry. Newton *et al.* (2005a) also found calcium and phosphorus content increased with the increasing levels of BSF pre-pupae meal in the diet of channel catfish. They found calcium levels increased from 0.52 to 1.6 and phosphorus from 0.60 to 0.80 which corresponds to inclusion levels of BSF pre-pupae meal from 7.5% to 30% respectively.

Table 2.3 Minerals composition of black soldier fly pre-pupae reared on swine and poultry manure *Newton et al. (2005b).*

Minerals	**BSF (pre-pupae)** **Reared on swine manure**	**BSF (pre-pupae)** **Reared on poultry manure**
Calcium (%)	5.36	5.0
Phosphorous (%)	0.88	1.51
Potassium (%)	1.16	0.69
Magnesium (%)	0.44	0.39
Manganese (ppm)	348	246
Iron (ppm)	776	1370
Zinc (ppm)	271	108
Sodium (ppm)	1260	1325
Copper (ppm)	26	6

Black soldier fly larvae have a high lipid content compared to the lipid requirement of African catfish (Table 2.1). The fatty acids of BSF larvae correspond to the fatty acid composition of their feeding substrate. St-Hilaire *et al.* (2007a) found BSF pre-pupae grown on swine manure contain saturated fatty acids (SFAs) such as lauric, miristic, palmitic and stearic, Monounsaturated fatty acid (MUFAs) including palmitoleic and oleic, Polyunsaturated fatty acid (PUFAs), such as linoleic, α-linoleic as well as negligible amount of highly unsaturated fatty acids (HUFAs) (Table 2.4). Additionally, the lipid and fatty acid content of BSF larvae can be manipulated cheaply to enhance the level of omega-3 fatty acids by feeding them fish offal (St-Hilaire *et al.*, 2007b). St-Hilaire *et al.* (2007b) found that BSF larvae fed cow manure mixed with fish offal had approximately 43% more lipids and a 3% increase in omega-3 fatty acids compared to pre-pupae fed cow manure without fish offal. Sealey *et al.* (2011) attributed the better growth of rainbow trout fed on a BSF pre-pupae enriched diet (reared on cow manure mixed with rainbow trout offal) and normal BSF pre-pupae meal (reared on cow manure only) in the diet of rainbow trout to the high lauric acid content in the enriched BSF meal (Table 2.4). The quantitative essential fatty acids requirements for African catfish have not yet been determined. However, BSF larvae contain linoleic and linolenic fatty acids which were reported to be required by freshwater fish (NRC, 1993).

Table 2.4 Fatty acids composition of black soldier fly pre-pupae (St-Hilaire *et al.,* 2007a*;* Sealey *et al.,* 2011*)*

Fatty Acids	BSF pre-pupae[1]	NBSF pre-pupae[2]	EBSF pre-pupae[3]
Lauric acid (12:0)	49.3	23.6	37.1
Myristic acid (14:0)	6.8	5.1	6.3
Palmitic acid (16:0)	10.5	19.8	17.3
Palmitoleic acid (16:1n7)	3.5	6.3	7.6
Stearic acid (18:0)	2.8	6.5	2.0
Oleic acid (18:1n9)	11.8	22.7	18.8
Linoleic acid (18:2n6)	3.7	6.8	5.9
α-Linolenic acid (18:3n3)	0.1	0.0	0.5
Stearidonic acid (18:4n3)	0.0	0.0	0.5
Eicosapentanoic acid (20:5n3)	0.0	0.1	3.5
Docosapentaenoic acid (22:5n3)	0.0	0.0	0.35
Docosahexaenoic acid (22:6n3)	0.0	0.0	1.7

1 Reared on swine manure

2 Normal black soldier fly pre-pupae reared on cow manure

3 Enriched black soldier fly pre-pupae reared on cow plus fish offal

2.1.2 The uses of Black Soldier Fly meal in aquaculture

The BSF larvae or pre-pupae meal has been successfully utilised in the diets of a number of aquaculture species. These include channel catfish *(Ictalurus punctatus)*, blue tilapia *(Oreochromis aureus)* (Bondari & Sheppard, 1987), rainbow trout *(Oncorhynchus mykiss)* (St-Hilaire *et al.,* 2007a; Sealey *et al.,* 2011), turbot *(Psetta maxima)* (Kroeckel *et al.,* 2012) and Atlantic salmon *(Salmo salar)* (Lock *et al.,* 2014). The findings of these feeding trials were that BSF larvae or pre-pupae meal was able to partially replace fishmeal in diets of these fish species. To our knowledge BSF larvae or pre-pupae meal has not been evaluated in the diet of the African catfish. This fish species was selected in this study because it has a high growth rate, ability to feed on a wide range of organisms including insects. It can tolerate low levels of dissolved oxygen and is highly demanded as food fish in many African countries. Although African catfish has been reported to tolerate estuarine conditions, it is considered to be a freshwater fish species which is accessible in landlocked African countries.

2.2 Attribute of the African Catfish

The African catfish (*Clarias gariepinus*) belong to the family Claridae. It is widely distributed in the African continent from the southern Natal and Orange River in the South through Central, West and North Africa. It has been reported to occur in the Middle East and Eastern Europe (Hecht *et al.,* 1988). The African catfish can tolerate wide environmental conditions. The ability to accept a variety of feedstuff, withstand poor water quality and maintain a high growth rate has made the African catfish one of the most suitable fish for aquaculture (Huisman & Richter, 1987; Graaf & Janssen, 1996; Pillay & Kutty, 2005). Moreover, African catfish has a high resistance to handling and stress (Graaf & Janssen, 1996); it can be cultured at high stocking density as well as maintain an all year round production (if the water temperature is high enough) and it has high consumer demand (Huisman & Richter, 1987).

A uniform pattern has not emerged with regards to the natural dietary composition of the African catfish. It is regarded as opportunistic omnivore or predator (Graaf & Janssen, 1996). The dietary composition for this fish species ranged from phytoplankton to fish (Hecht *et al.,* 1988). African catfish have been reported to feed on aquatic and terrestrial insects, crabs, shrimp, snails, plankton, dead animals, fish, amphibians, reptiles, seeds and fruits (Hecht *et al.,* 1988; Graaf & Janssen, 1996). Under intensive culture condition the larvae and fry of African catfish require live starter food such as *Artemia nauplii*, small daphnia, moina or rotifer until weaning. Thereafter, the fry/fingerlings are fed on a formulated feed.

2.3 Nutrient Requirements of the African Catfish

Fish require nutrients such as protein, lipids, carbohydrates, minerals, and vitamins for growth, reproduction and to perform other physiological functions (Lovell, 1991). To date, not all the specific dietary requirements of the African catfish for essential amino acids, essential fatty acids, vitamins and mineral have been determined

2.3.1 Protein and Amino Acids

African catfish requires essential amino acids and nonspecific nitrogen for growth. Protein is the most expensive component in most fish feeds. Fish require higher dietary protein levels compared to farmed terrestrial animals (El-Sayed, 2006). Young fish require higher protein levels, which decrease as the fish size increases. According to Davis *et al.* (2009), the optimum dietary protein levels depend on the fish growth rate, feed intake, amount of non-protein energy in the diet, protein quality, presence of natural food and management practices. According to Viveen *et al.* (1985), the optimal dietary crude protein requirement for catfish is 30-35%. Hecht *et al.* (1988) suggested the dietary crude protein requirement for African catfish ranging from 40% to 50% on a dry weight basis. A wide range of protein levels can be used to produce fish; however, to achieve the best economic return, a balanced protein to energy ratio is required in the diet (Davis *et al.,* 2009). Using iso-nitrogenous diets a crude protein of 350 g/kg diet and different energy levels in juvenile African catfish diets, the best growth was obtained with diets consisting of 12.73 MJ/Kg diet (Yilmaz *et al.,* 2006).

Amino acids are the basic units of protein. All fish require the same ten essential amino acids: arginine, lysine, histidine, methionine, leucine, isoleucine, phenylalanine, valine, threonine, and tryptophan. Fish cannot synthesize these essential amino acids and therefore, it has to be provided in their diets. The total sulphur containing amino acids requirement for catfish can be met by methionine alone or by a methionine and cystine mixture. Cystine can be synthesized by fish from dietary methionine and not *vice versa* (Wilson, 2003). Similarly, tyrosine can be synthesized from dietary phenylalanine (aromatic amino acid) (Houlihan *et al.,* 2001). A fish diet that lacks some of the essential amino acids, results in slow growth and decrease in feeding efficiency (Guillaume, 1999). The quantitative essential amino acids for several aquaculture species have been determined (Wilson, 2003). However, only few have been determined for African catfish (Wilson, 2003) therefore, when formulating diets for African catfish, the essential amino acids values for Channel catfish have been used instead (Fagbenro & Nwanna, 1999). The dietary essential amino acids reported for African catfish were arginine 45 g/kg protein (Fagbenro *et al.,* 1999a), lysine 57 g/kg protein (Fagbenro *et al.,* 1998), methionine 32 g/kg protein (Fagbenro *et al.,* 1999b) and tryptophan 11 g/kg protein (Fagbenro & Nwanna, 1999).

Table 2.5 Amino acids requirements of juvenile Channel Catfish adapted from (NRC, 1993)

Amino acids	**Ideal amino acid profile expressed as a % of lysine**	**Requirement as % of dry diet**
Lysine	100	1.2
Arginine	84	1.0
Histidine	29	0.4
Isoleucine	51	0.6
Leucine	69	0.8
Methionine	45	0.6
Phenylalanine	98	1.2
Threonine	39	0.5
Tryptophan	10	0.12
Valine	59	0.71

2.3.2 Lipids and Fatty Acids

Dietary lipids not only supply the fish with energy and essential fatty acids, it also facilitates in the intestinal absorption of vitamins and carotenoid pigments for normal growth and reproduction (NRC, 1993; De Silva & Trevor, 1995; Guillaume *et al.,* 1999; Lim & Webster, 2001). Other benefits of lipids in fish feeds are that it enhances feed palatability and helps to reduce dust while stabilising the pellets during manufacture, transportation and storage (De Silva & Trevor, 1995). Dietary lipids supplied in fish diets must be sufficient to meet the energy requirements of the fish in order to save or spare protein from being used as an energy source. Lipid high diets result in reduced feed intake in fish which in turn decreased their growth. It also results

in increased fat deposition which affects the quality of fish product (Li *et al.,* 2004). African catfish require 8 to 12% of lipid in their diets for fingerlings and grow out (Uys, 1989).

Alternatively, different diets ranging from semi purified diets (Henken *et al.,* 1986) to fishmeal and soybean meal based diets (Ali & Jauncey, 2005) were used to determine protein to energy ratio in African catfish. Ali & Jauncey (2005) reported the best growth performance was obtained with diet containing 430 g/kg protein and 20.5 mg protein/KJ as protein to energy ratio. Henken *et al.* (1986) established the requirements of the African catfish for crude protein and metabolizable energy were comparable to other omnivorous fish species. Salhi *et al.* (2004) obtained the optimal protein content of 37% and digestible protein/energy (DP)/DE ratio of 23.6 mg/KJ for black catfish *(Rhandia quelen)* fry.

Fish, as well as other vertebrates cannot synthesise the polyunsaturated fatty acids linolenic (n-3) and linoleic (n-6) acids, therefore these fatty acid must be provided in the diet (NRC, 1993). Freshwater fish have a dietary requirement for linolenic (n-3) and linoleic (n-6) fatty acids alone or both (NRC, 1993). Hoffman & Prinsloo, (1995) fed juvenile African catfish diets containing different sources of lipids such as sunflower oil (high in oleic: linoleic fatty acids and C18:2 omega-6 fatty acids), cod liver oil (high in 20 and 22 C omega-3 fatty acids) and tallow (contain saturated and monounsaturated fatty acids) which was a mixture of cattle, pig and sheep fat. They obtained lower growth performance with cod liver oil as a lipid source. Ng *et al.* (2003) found similar results with cod liver oil and suggested a balance ratio between n-3 and n-6 fatty acids for optimum growth of African catfish. On the other hand, channel catfish have been reported to perform better with diets containing fish oils compared to vegetable oils (Stickney & Andrews, 1972).

2.3.3 Carbohydrates

Carbohydrates are cheap energy sources that can be used in the diets of aquaculture species (Milikin, 1982). Freshwater and warm water fish including catfish appear to be able to use much higher levels of dietary carbohydrates compared to cold water or marine fish (Li *et al.,* 2004). The maximal inclusion level of dietary carbohydrates in fish diets, without reducing growth depends on the feeding habits, whether the fish is herbivorous, omnivorous or carnivorous (Milikin, 1982). No requirement for dietary carbohydrates has been established in fish, although it was found that growth was retarded in certain fish species fed diets without carbohydrates (Wilson, 1994). Ali & Jauncey, (2004) found that, the optimal dietary carbohydrate to lipid ratio ranging from 1.7 to 3.4 in the diet of African catfish resulted in good growth performance.

2.3.4 Minerals

Since fishes have the ability to obtain some minerals from their surrounding water environment and diets, it is not easy to study their minerals requirements (Guillaume *et al.,* 1999). Minerals are categorised into macro minerals needed in large amounts and micro minerals needed in minute amounts (Webster, 2002). Minerals also play a vital role in structural components of the skeletal system, components of organic compounds, enzyme system activators, as well as maintaining acid base and osmotic balances (Webster & Lim, 2002). Minerals such as calcium, magnesium, sodium, potassium, iron, zinc, copper and selenium can be obtained by fish from their surrounding water (NRC, 1993). On the other hand dietary phosphorus should be supplied in the fish diet due to poor phosphorus in feedstuffs of plant sources and low dissolved phosphorus in natural

water. Phosphorus in catfish diets are supplied in the form of dicalcium and deflourinated phosphates. Trace minerals are supplied in premix form to meet or exceed catfish dietary requirements (Robinson & Li, 2002).

2.3.5 Vitamins

Vitamins are needed in small quantities in fish diets. It assists in maintenance of normal metabolic and physiological function (Pillay & Kutty, 2005). Vitamins are categorised into two groups: fat soluble vitamins A, D, E, K and the water soluble vitamins, the B group, vitamin C and some specific cofactors (Lucas & Southgate, 2003). Fish require all the 15 vitamins (Table 2.6) but only some of these vitamins are dietary essential for certain fish species. The vitamin requirements of a fish depends on fish species, size, growth rate, nutrient interrelationship as well as environment and metabolic function of the fish (Lovell, 1989). Fish that partially feed on natural aquatic organisms do not require some vitamins to be provided in their diets. Fish diets high in vitamins can cause avitaminosis; especially fat soluble vitamins which are difficult to excrete by the animal (Lucas & Southgate, 2003). On the other hand, a diet low in vitamin causes poor growth, fish become susceptible to infection and other nutritional diseases (De Silva & Trevor, 1995). Adewolu & Aro, (2009) used seven iso-nitrogenous diets supplemented with different levels of vitamin C (ascorbic acid). Clinical symptoms of ascorbic acid deficiency were observed after 12 weeks in fish fed an ascorbic acid free diet. The best growth performance and feed utilisation was observed in fish that received 1500 mg/kg ascorbic acid. However, Adewolu & Aro, (2009) recommended a minimum of 50 mg/kg of ascorbic acid is required in the diet of African catfish.

Table 2.6 Vitamin requirements of Channel Catfish *(Ictalurus punctatus)* adapted from (NRC, 1993)

Vitamin	**Unit**	**Requirement (units/kg diet)**
Vitamin A	*IU*	1000 – 2000
Vitamin D	*IU*	500
Vitamin E	*Mg*	25
Vitamin K		R[1]
Thiamin (vitamin B_1)	*mg*	1
Riboflavin (vitamin B_2)	*mg*	9
Vitamin B6	*mg*	3
Pantothenic acid	*mg*	10
Niacin	*mg*	14
Biotin		R
Vitamin B12		R
Folate	*mg*	1.5
Choline	*mg*	400
Myoinositol		NR[2]
Ascorbic acid (vitamin C)	*mg*	60

1 R= Required

2 ND= No requirement determined

2.4 Water Quality Requirements of the African Catfish

Fish carry out all their bodily activities in water which includes breathing, feeding, growing, excreting wastes, maintaining a salt balance and reproduction (Swann, 1997). The measurement of water quality in aquaculture is therefore imperative to assess whether the quality of water is good enough to support aquatic life. The most important parameters to measure are dissolved oxygen, water temperature, ammonia, nitrite and total suspended solids (Zweig *et al.,* 1999; Timmons & Ebeling, 2010).

2.4.1 Dissolved Oxygen

Dissolved oxygen is one of the most critical water quality parameters for aquaculture (Nathalie *et al.,* 2010; Timmons & Ebeling, 2010). Low dissolved oxygen concentration reduces fish appetite, induces slow growth and fish become more susceptible to infections (Boyd, 2012). Growth is affected when dissolved oxygen is lower than 3 to 4 mg/L for warm water fish (Boyd, 2012). To avoid fish stress the levels of dissolved oxygen should be kept above 4 mg/L (Parker, 2012). A minimum of 5 mg/L dissolved oxygen concentration is required for optimum growth, by warm water fish (Masser *et al.,* 1999). Fish grow very fast, convert feed efficiently and are healthiest at dissolved oxygen concentrations above 5 mg/L (Timmons & Ebeling, 2010; Boyd, 2012). Oxygen consumption depends on fish size, feeding rate, activity level, species, temperature and salinity. Small fish have high metabolic rates, therefore, they consume more oxygen than large fish (Boyd, 2012). The African catfish has been known to survive poorly oxygenated water by their ability to breathe atmospheric air, making it an attractive fish for rural aquaculture (Pillay & Kutty, 2005). Additionally, African catfish can survive for considerable periods out of water if they stay moist (Hecht *et al.,* 1988). As such they can be cultured intensively without the needing of aeration or high water exchange rates (Hecht *et al.,* 1988).

2.4.2 Temperature

The majority of fish are poikilothermic ectotherm, which means that their environmental temperature has a pronounced effect on their physiology and behaviour (Nathalie *et al.,* 2010). The respiratory rate of fish, feeding efficiency, growth and reproduction are directly influenced by temperature (Timmons & Ebeling, 2010). Based on their temperature tolerance, fish are categorised as cold water, cool water or warm water species (Timmons & Ebeling, 2010); the African catfish is a warm water species. According to Timmons & Ebeling (2010) each fish species has an optimum temperature in which performance is maximised as well as minimum and maximum tolerance limit beyond which they cannot survive (Timmons & Ebeling 2010). The African catfish has an optimal temperature which varies from 20 °C to 30 °C with 25 °C and 27 °C as the most favourable for adults and juveniles respectively (Viveen *et al.,* 1985). On the other hand, Isyagi *et al.* (2009) reported the ideal temperature range for pond production of African catfish was 26 °C to 32 °C. Below 15 °C, growth ceases and death occurs at extreme cold temperatures (Isyagi *et al.,* 2009). Between 15 °C and 26 °C growth rate and feed intake were reduced and fish undergo stress (Isyagi *et al.,* 2009). Parker (2012) reported that water temperature affects oxygen solubility in water and the rate of un-ionized ammonia. Water temperature has also

been reported to affect other water quality parameters such as ammonia either directly or indirectly (Boyd, 2012).

2.4.3 Ammonia, Nitrate and Nitrite

In water ammonia is generated as a by-product of protein metabolism by aquatic animals (Stickney, 2005; Parker, 2012). It occurs in two forms as ionized (nontoxic) and un-ionized (toxic) (Boyd, 2012; Parker, 2012). The sum of these two forms is called total ammonia (Stickney, 2005). At high pH most of the ammonia is in the un-ionized form which is toxic to fish (Boyd, 2012; Parker, 2012). Ammonia tolerance by aquatic organisms differs among species, their physical condition and environmental factors (Boyd, 2012). Warm water fish tolerate ammonia toxicity more than cold water fish, likewise freshwater fish tolerate ammonia better compared to saltwater fish (Timmons & Ebeling, 2010). Isyagi *et al.* (2009) suggested the range of 0.3 to 2 mg/l of toxic form of ammonia is acceptable or optimal for African catfish production.

Nitrite is the ionised form of nitrous acids and is the intermediate product in the conversion of ammonia to nitrate (Timmons & Ebeling, 2010). Nitrate is generated through the nitrification process (Timmons & Ebeling, 2010). Although nitrites in an aquaculture system are easily transformed to nitrates, it can be problematic especially if the medium required to convert nitrite to nitrate are insufficient (Stickney, 2005; Timmons & Ebeling, 2010). The diffusion of nitrite into blood haemoglobin of fish results in the oxidation of ferrous to ferric iron (Stickney, 2005; Timmons & Ebeling, 2010). These nitrite and haemoglobin combinations then form methaemoglobin which does not combine with oxygen. Methaemoglobin gives fish blood a brown colour and hence nitrite poisoning is known as brown colour disease (Stickney, 2005; Timmons & Ebeling, 2010; Boyd, 2012; Parker, 2012).

Nitrate is the end product of nitrification. It is unlikely that nitrate levels will reach toxic concentrations to fish especially in aquaculture systems with regular water exchange (Pillay & Kutty, 2005; Timmons & Ebeling, 2010).

2.4.4 pH

The water pH is its hydrogen ion concentration. It expresses how acidic or basic the water is. The log pH scale ranges from 0 to 14 (Timmons & Ebeling, 2010). A pH value of 7 is considered neutral. Values above 7 are considered alkaline whereas values below 7 are acidic (Timmons & Ebeling, 2010). The optimal pH for the growth and health of freshwater aquatic animals ranges from 6.5 to 9.0 (Timmons & Ebeling, 2010; Boyd, 2012). Acid and alkaline death points are at pH 4 and pH 11 respectively (Boyd, 2012). Lower pH levels affect aquatic animals in maintaining their internal ion balance (Boyd, 2012). Lower pH also affects respiratory efficiency, cause excessive mucus production and gill damage (Boyd, 2012). The optimal pH range for African catfish is similar to the pH range determined for freshwater fish species (Isyagi *et al.,* 2009).

2.5 Conclusion

The black soldier fly is a good candidate for bioconversion of food wastes and animal wastes while producing animal feedstuff of high quality which can be incorporated into fish diets. The BSF has a good amino acid profile that is comparable to the requirements of most fish. There are numerous benefits of incorporating insect meal in aquaculture diets; the problem of low fishmeal availability and high cost can be alleviated. Also, the production of insect biomass as animal feedstuff through bioconversion of organic wastes reduces pollution. Additionally, insect rearing is more environmentally friendly than livestock because it produces less greenhouse gases, consumes less water and requires less land. The general conclusion for several studies that utilised BSF larvae or pre-pupae meal to replace conventional protein sources were that this fly species can partially substitute high quality fishmeal without negative effects on growth.

2.6 References

Abdel-Warith, A.A., Russell, P.M. & Davies, S.J., 2001. Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish *Clarias gariepinus* (Burchell 1822). *Aquac Res.* 32: 296–305.

Adewolu, M. A. & Aro, O.O., 2009. Growth, feed utilization and haematology of (*Clarias gariepinus* Burchell, 1822) fingerlings fed diets containing different levels of vitamin C. *Am. J. Applied. Sci. 6(9): 1675-1681.*

Ali, M. Z. & Jauncey, K., 2005. Approaches to optimizing dietary protein to energy ratio for African catfish (*Clarias gariepinus* Burchell, 1822). *Aquac. Nutr*. 11: 95-101.

Ali, M.Z. & Jauncey, K., 2004. Optimal dietary carbohydrate to lipid ratio in African catfish (*Clarias gariepinus* Burchell 1822). *Aquac. Int*, 12(2): 169–180.

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

Aniebo, A.O. & Owen, O.J., 2010. Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica* Linnaeus) meal (HFLM). *Pakistan. J. Nutr. 9(5): 485-487.*

Atteh, J.O. & Ologbenla, F.D., 1993. Replacement of fishmeal with maggots in broiler diets: effects on performance and nutrient retention*. Nig. J. Anim. Prod*. 20:44-49.

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.

Bondari, K., Coastal, D.C.S. & Experiment, P., 1987. Soldier fly , Hermetia illucens L ., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia *Oreochromis aureus* (Steindachner). *Aquaculture and fisheries management*. 18: 209–220.

Boyd, C., 2012. Water quality. In: Aquaculture: farming of aquatic animals and plants. 2nd edition. Eds. Lucas, J.S. & Southgate, P.C. John Wiley & Sons.

Davis, D., Nguyen, T. & Li, M., 2009. Advances in aquaculture nutrition: catfish, tilapia and carp nutrition. *New technologies in aquaculture: improving production efficiency, quality and environmental management* (2009): 440-458.

De Silva, S.S., & Anderson, T.A., 1995. Fish nutrition in aquaculture. Springer Science & Business Media. pp 320.

Diener, S., Nandayure, M., Solano, S., Gutierrez, F.R., Zurbrugg, C. & Tocker, K., 2011. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valor*, 2(4): 357–363.

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

El-Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, Oreochromis spp. *Aquaculture*, 179(1-4): 149–168.

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., 1990. Long-term evaluation of cotton seedmeal as a protein source for Nile tilapia *Oreochromis niloticus*. Aquaculture 84: 315-320.

Erickson, M.C., Islam, M., Sheppard, C., Liao, J. & Doyle, M.P., 2004. Reduction of *Eschericha coli* 0157:H7 and *Salmonella enterica* serovar Enteritidis in chicken manure by larvae of black soldier fly. *J. Food Protection*. 67(4): 685-690.

Fagbenro, O. & Davies, S., 2001. Use of soybean flour (dehulled solvent-extracted soybean) as fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed. *J. App. Ichthyol*. 17: 64-69.

Fagbenro, O.A. & Nwanna, L.C., 1999. Dietary tryptophan requirement of the African catfish *Clarias gariepinus. J. Appl. Aquac.9(1): 65-72.*

Fagbenro, O.A., Balogun, A.M., Fasakin, E.A.,1999b. Dietary Methionine Requirement of the African Catfish, *Clarias gariepinus*. *J.Appl.Aquac*, 37–41.

Fagbenro, O.A., Nwanna, L.C. & Adebayo, O.T., 1999a. Dietary arginine requirement of the african catfish, *Clarias gariepinus*. *J.Appl.Aquac.9(1): 59-64.*

Fagbenro, O.A., Balogun, A.M. & Fasakin, E.A., 1998b., Dietary Lysine Requirement of the African Catfish, *Clarias gariepinus*. *J.Appl.Aquac.8(2): 71-77*

Fagbenro, O.A., & Jauncey, K., 1994. Chemical and nutritional quality of dried fermented fish silage and their nutritive value for Tilapia (*Orechromis niloticus). Anim. Feed Sci. Technol*. 45 (2): 167-176.

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.

Gatlin, D.M., Barrows, F., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone D., Wilson, R. &

Wurtele, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquac. Res*, 38(6): 551–579.

Goda, M., El-Haroun, E.R. & Kabir Chowdhury, M. A., 2007. Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish Clarias gariepinus (Burchell, 1822) reared in concrete tanks. *Aquac. Res,* 38(3): 279–287.

Graaf, G. D. & Janssen, H., 1996. Artificial reproduction and pond rearing of the Arican catfish *(Clarias gariepinus)* in sub-Saharan Africa. *FAO fisheries technical Paper* 362**.**

Guillaume, J.,1999. Nutrition and feeding of fish and crustaceans. Springer Science & Business Media.

Hardy, R.W., 1996. Alternate protein sources for salmon and trout diets. *Anim. Feed. Sci. Technol*, 59 (1-3): 71-80.

Hecht, T., Uys, W. & Britz, P., 1988. Culture of sharptooth catfish, *Clarias gariepinus* in Southern Africa. Foundation for Research Development: CSIR.

Hem, S., Toure, S., Sagbla, C. & Legendre, M., 2008. Bioconversion of palm kernel meal for aquaculture : Experiences from the forest region (Republic of Guinea). *Afr. J. Biotechnol.*7(8): 1192–1198.

Henken, A.M., Machiels, M.A.M., Deckker, W. & Hogendoorn., 1986. The effect of dietary protein and energy content on growth rate and feed utilization of the african catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture,* 58: 55-74.

Hertrampf, J.W. & Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds, Springer Science & Business Media.

Hoffman, L. & Prinsloo, J., 1995. The influence of different dietary lipids on the growth and body composition of the African sharptooth catfish. *S. Afr. J. Sci.* 91.6: 315-320.

Houlihan, D., Boujard, T. & Jobling, M., 2001. Food intake in fish. Oxford, UK: Blackwell Science Ltd.

Huisman, E. & Richter, C., 1987. Reproduction, growth, health control and aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture*.

Isyagi, N.A., Veverica, K.L., Asiimwe, R. & Daniels, W.H., 2009. Manual for the commercial pond production of the African catfish in Uganda. *Kampala*.

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

Kestemont, P., Wathelet, B. & Nyina-wamwiza, L., 2007. Potential of local agricultural by-product for the rearing of African catfish (*Clarias gariepinus)* in Rwanda: effects on growth, feed utilization and body composition. *Aquaculture*: 38: 206-214.

Kroeckel, S., Harjes, A.G.E. Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C., 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly *(Hermetia illucens)* as fish meal substitute — Growth performance and chitin degradation in juvenile turbot *(Psetta maxima)*. *Aquaculture*, 364: 345–352.

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. *The Science of the total environment*, 458: 312–318.

Larde, G., 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Biol. Wastes*, 33(4): 307–310.

Li, M.H., Robinson, E.H. & Manning, B.B., 2004. Nutrition. In: Biology and culture of channel catfish. Eds. Tucker, C.S. & Hargreaves, J.A. Elsevier. pp 279-323.

Lim, C. & Webster, C.D., 2001. Nutrient Requirements and Feeding of Finfish for Aquaculture. CABI. pp 448.

Lim, C., Webster, C.D. & Brady, Y.J., 2001. Nutrition and Fish Health. Taylor & Francis. pp 365.

Lock, E., Arsiwalla, T. & Waagbø, R., 2014. Insect meal: A promising source of nutrients in the diet of Atlantic salmon *(Salmo salar)*. *Abstract Book Conference Insects to Feed The World. The Netherlands, 14-17 May 2014.*

Lovell, R.T., 1991. Nutrition of aquaculture species. *J. Anim. Sci. 69: 4193-4200.*

Lovell, R.T., 1989. Nutrition and Feeding of Fish. Springer Science & Business Media, USA. pp 267.

Lucas, J. & Southgate, P. C., 2012. Aquaculture: Farming aquatic animals and plants. 2nd edition. John Wiley & Sons. pp 648.

Lucas, J.S. & Southgate, P.C., 2003. Aquaculture: Farming of aquatic animals and plants.

Masser, M.P., Rakocy, J. & Losordo, T.M., 1999. Recirculating aquaculture tank production systems: Management of recirculating systems. *SRAC publication* no. 452:12.

Mbagwu, I.G., Okoye, F.C., Adinji, H.A., 1990. Studies on use of duckweed (*Lemna paucicostata* Hegelm) as fish food. *Tech. Rep.Ser. Natl.Freshwater fish. Resh.Nigeria, Bussa, No. 22: pp 25*.

McCallan, E., 1974. *Hermetia illucens* (L.) (Dipt. Stratiomyidae), a cosmopolitan American species long established in Australia and New Zealand. *Entomol. Mo. Magazine*, 109: 232-4.

Milikin, M.R., 1982. Qualitative and quantitative nutrient requirements of fishes- A review. *Fishery bulletin*, 80(4).

Nathalie, L.F., Jobling, M., Carter, C. & Pierre, B., 2010. Finfish aquaculture diversification. CAB International, Wallinford, UK.

Newton, L. Sheppard, C. Watson, D. Burtle, G. & Dove, R., 2005b. Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. *Report for Mike Williams. Director of the animal and poultry waste management centre, North Carolina State University, Raleigh, NC.*

Newton, G.L., Sheppard, D., Watson, W., Burtle, G.J., Dove, R.C., Tomberlin, J.K. & Thelen, E.E., 2005a. The Black Soldier Fly, Hermetia Illucens, as a Manure Management / Resource Recovery Tool. In *Symposium on the State of the Science of Animal Manure and Waste Management*. pp. 0–5.

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

Ng, W., Lim, P. & Boey, P., 2003. Dietary lipid and palm oil source affects growth , fatty acid composition and muscle a -tocopherol concentration of African catfish *(Clarias gariepinus)*. *Aquaculture.* 215(1): 229–243.

NRC, 1993. Nutrient Requirements of Fish, National Academies Press.

Odesanya, B.O., Ajayi, S.O., Agbaogun, B.K.O. & Okuneye, B., 2011. Comparative evaluation of nutritive value of maggots*. Int. J. Sci. Eng. Res*, 2(11): 1–5.

Ogunji, J.O., Kloas, W., Wirth, M., Schulz, C. & Rennert, B., 2006. Housefly maggot meal (magmeal): An emerging sudstitute of fishmeal in Tilapia diets. Conference on International Agricultural Research for Development, Stuttgart-Hohenheim, October 11-13, 2006.

Pantazis, P.A., 1999. Nutritional studies in African catfish, *Clarias gariepinus* (Burchell, 1822). Ph.D. Thesis, University of Sterling.

Parker, R., 2012. Aquaculture science. Third edition. Cengage Learning. Clifton Park, NY, USA. pp 348-381.

Pillay, T.V.R. & Kutty, M.N., 2005. Aquaculture: Principles and Practices. *Blackwell Publishing Ltd.*

Robinson, E.H. & Li, M.H., 2002. Channel catfish. In: Nutrient requirements and feeding of finfish for aquaculture. Eds. Webster, C.D. & Lim, C. CAB International, Wallinford, UK.

Robinson, E.H., Wilson, R.P. & Poe, W.E., 1980. Total aromatic amino acid requirement, phenylalanine requirement and tyrosine replacement value for fingerling channel catfish. *J. Nutr,* 110(9): 1805–12.

Rust, M. B. 2002. Nutrional physiology. In: Fish nutrition, 3rd ed. J. E. Halver & R. W. Hardy, (eds). Academic Press, New York, USA. pp 368–446.

Salhi, M., Bessonart, M., Chediak, G., Bellagamba, M., & Carnevia, D., 2004. Growth, feed utilization and body composition of black catfish, *Rhamdia quelen*, fry fed diets containing different protein and energy levels. *Aquaculture* 231: 435-444.

Sealey, W.M., Gaylord, T.G., Barrows, F.T., Tomberlin, J.K., McGuire, M.A., Ross, C. & St-Hilaire, S., 2011. Sensory analysis of Rainbow trout, *Oncorhynchus mykiss*, fed enriched Black Soldier fly prepupae, *Hermetia illucens*. *J. World Aquac. Soc,* 42(1): 34–45.

Sheppard, C.D., Newton, L.G., Thomson, S.A. & Savage, S., 1994. A value added manure management system using the black soldier fly. *Bioresour. Technol*. 50(3): 275–279.

Silva, S.S. de & Anderson, T.A., 1995. Fish Nutrition in Aquaculture. *Springer Science & Business Media.*

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C., & Irving, S., 2007a. Fish offal recycling by the Black Soldier fly produces a foodstuff high in omega-3 fatty Acids. *J. World Aquac. Soc*, 38(2): 309–313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., & Sealey, W., 2007b. Fly prepupae as a feedstuff for Rainbow trout, *Oncorhynchus mykiss. J. World Aquac. Soc*, 38(1): 59–67.

Stickney, R., 2005. Aquaculture: An introductory text. CAB International. London.

Stickney, R.R. & Andrews, J.W., 1972. Effects of dietary lipids on growth, food conversion, lipid and fatty acids compositionof channel catfish. *J. Nutr., 102: 249-258.*

Swann, L., 1997. A fish farmer's guide to understand water quality. *Aquaculture extension. Fact sheet AS-503.*

Tacon, A., 1995. Feed ingredients for carnivorous fish species: alternatives to fishmeal and other fishery resources. In: Sustainable fish farming. Eds. Reinertsen, H. & Haaland, H. CRC Press.

Teotia, J.S. & Miller, B. F., 1974. Nutritive content of housefly pupae manure residue. *Brit. Poult. Sci.* 15: 177-82.

Timmons, M. & Ebeling, J., 2010. Recirculating Aquaculture Cayuga Aquaculture Ventures. *Ithaca, NY, USA*.

Uys W. 1989. Aspects of the nutritional physiology and dietary requircmcnts of juvenile and adult sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). PhD Thesis, Rhodes University, South Africa, pp 190. (cited in Van Weerd, 1995).

Van Weerd, J. H. (1995). Nutrition and growth in Clarias species-a review. *Aquat. Living Resour*, *8*(04): 395-401.

Viveen, W.J.A.R., Richter, C.J.J., van Oort, P.G.W.J., Janssen, J.A.L. & Huisman, E.A., 1985. Practical manual for the culture of the African catfish *(Clarias gariepinus)*. *Joint Publication of DGIS, Dept. of Fish Culture and Fisheries and Research Group for Comparative Endocrinology, Wageningen, pp 94.*

Webster, C.D., & Lim, C., 2002. Nutrient requirements and feeding of finfish for aquaculture. CABI Publishing. UK.

Webster, C.D., Thompson, K.R., Muzunic, L.A. & Engler, L.S., 2005. Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish *(Cherax quadricarinatus). Aquaculture.244: 241-249.*

Wilson, R.P., 2003. Amino acid requirements of finfish and crustaceans. In: DMello, J.P.F. (ed). Amino acids in animal nutrition, 2nd ed. CAD International, London, pp 427-447.

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

Yilmaz, E., Akyurt, I. & Mutlu, E., 2006. Effects of energetic diets on growth, blood chemistry, and liver pathology of african catfish, *Clarias gariepinus* (Burchell 1822). *Isr.J.Aquac.*58(3): 191-197.

Zweig, R.D., Morton, J.D. & Stewart, M.M., 1999. Source water quality for aquaculture: A guide for assessment. *The International Bank for Reconstraction and Development/The Work Bank. Washington,D.C.*

Chapter 3

3 The effects of black soldier fly *(Hermetia illucens)* larvae meal on growth performance and feed utilisation of African catfish *(Clarias gariepinus)* fingerlings

Abstract

A feeding trial to investigate different inclusion levels of black soldier fly (BSF) larvae meal in African catfish diets was carried out in a water recirculation system. Four experimental diets were formulated containing 0% (control), 10%, 20% or 30% BSF larvae meal. A total of 720 fingerlings (5 – 6 g) were randomly allocated to treatments with six replicates per treatment. Fish were feed to satiation twice a day (10:00 and 16:00) and six times a week, except on the sampling days. The trial was terminated after a 91 day feeding period. There were no significant differences in feed utilisation in terms of feed conversion ratio, protein efficiency ratio, average daily gain and specific growth rate between treatments. However, a reduction in growth was observed in fish fed 20% and 30% BSF larvae meal diet. Although the experimental diets were prepared to be similar (iso-nitrogenous and iso-calorific), the proximate analysis showed that protein content ranged from 41.50 to 44.24%. The fat content (11.16 – 16.49%) increased with the increasing level of BSF larvae meal. It is believed that the reduced growth is attributed to this high fat content. It is concluded that, should full fat BSF larvae meal be used for catfish production, the inclusion level should not exceed 10%. This study recommends the defatting of BSF larvae to enable higher inclusion levels in the diet of African catfish.

Key words: Black soldier fly, growth performance, feed utilisation, African catfish

3.1 Introduction

Traditionally, fishmeal has been the main dietary protein source in aquafeeds (El-Sayed, 1999; Gatlin *et al.,* 2007). Fishmeal contains a high quality protein, is an excellent source of essential amino acids and fatty acids as well as digestible energy, minerals and vitamins (El-Sayed, 1999; Gatlin *et al.,* 2007). Due to the balanced nutritional profile of fishmeal, it is no surprise that fishmeal is the most expensive protein source in animal feeds (Tacon, 1993). Fishmeal supply decreased due to climatic phenomena (El Niño events) and exploitation of fish stocks used for fishmeal (Naylor *et al.,* 2009). The high demand for fishmeal in aquaculture and other animal husbandry has greatly increased its cost (Olsen & Hasan, 2012). This is of particular concern to developing countries which rely on fishmeal as a major protein source for aqua feed (El-Sayed, 1998; El-Sayed, 1999). The search for alternative and sustainable protein sources in animal feeds has been on-going (Sealey *et al.,* 2011). Factors such as protein content, amino acids profile, digestibility, palatability and anti-nutritional factors must all be considered when evaluating an alternative protein source (Hardy, 2010). However, a possible option is supplementation using other less expensive protein sources.

The norm of the studies conducted with plants' or animals' protein usually focuses on dietary replacement, but this study sought to supplement fishmeal or conventional protein sources. There is meagre scientific information available on dietary supplementation studies in aquaculture, particularly as pertaining to the African catfish. Hoffman *et al.* (1997) determined that African catfish successfully utilised soybean, tomato or yeast

meal as alternative protein sources. Hoffman *et al.* (1997) used iso-nitrogenous diets and observed that none of the diets yielded the growth equivalent to a fishmeal control diet. In a substitution of dietary protein, Fagbenro & Davies, (2001) demonstrated that soybean flour (dehulled, solvent extracted soybean) can replace 50% of fishmeal protein without additional amino acids supplementation in the diet of African catfish. With regards to supplementation of methionine, soybean flour can replace up to 75% of fishmeal. Goda *et al.* (2007) used iso-nitrogenous diets to evaluate poultry by-product meal, meat and bone meal and soybean meal as partial or total replacement of fishmeal in the diet of African catfish. They found that there were no significant differences in growth between poultry by-product meal and soybean meal diets with that of the control diet at 100% inclusion level, whilst meat and bone meal diet can only replace up to 75% of fishmeal protein in the control diet (Goda *et al.*, 2007).

In contrast to Goda *et al.* (2007), Abdel-Warith *et al.* (2001) established that poultry by-product meal can successfully replace up to 40% of fishmeal protein in the diet of African catfish without affecting growth. The diets were iso-nitrogenous and iso-caloric. However, the growth was affected when the inclusion level of poultry by-product meal was increased ranging from 60% to 100% (Abdel-Warith *et al.,* 2001). Hlophe & Moyo (2014) evaluated kikuyu grass and moringa leaves in the diet of African catfish. Better growth performance and feed utilization were observed in fish fed kikuyu meal. Hlophe & Moyo (2014) concluded that in iso-nitrogenous and iso-caloric diets, kikuyu meal can replace 25% of the fishmeal component in the diet of African catfish. Feather meal has also been evaluated in the diet of African catfish and it can successfully replace 20% of fishmeal protein in the diet of African catfish fry (Chor *et al.,* 2013).

There is paucity in the research regarding the use of the black soldier fly (BSF) larvae as potential feed for fish. The extant of the studies conducted with BSF have focused on the potential of this fly species as a waste management agent (Larde, 1990; Sheppard *et al.,* 1994; St-Hilaire *et al.,* 2007b; Hem *et al.,* 2008; Diener *et al.,* 2011; Lalander *et al.,* 2013; Banks *et al.,* 2014; Cičková *et al.,* 2014). Different growth stages of BSF have been successfully fed to several aquaculture species: St-Hilaire *et al.* (2007a) and Sealey *et al.* (2011) fed BSF pre-pupae meal to rainbow trout *(Oncorhynchus mykiss)*. Chopped larvae or dried larvae mixed with pre-pupae meal were fed successfully to channel catfish and blue tilapia (Bondari & Sheppard, 1981; Bondari & Sheppard, 1987).

No study has been published on the BSF larvae as a protein source in the diet of African catfish. Therefore the aims of this study were to compare growth performance and feed utilisation of African catfish fingerlings fed diets supplemented with BSF larvae meal.

3.2 Materials and Methods

3.2.1 Experimental Systems

The experiment was conducted at the Aquaculture section at the Welgevallen Experimental Farm, University of Stellenbosch. A water recirculation system housed in a greenhouse was used for the experiment. The greenhouse consisted of 88 plastic tanks (120 L), each tank has an adjustable inflow valve and receive a maximum flow rate of 150 ml per second which replaces the tank volume in 14 minutes. Aeration to the tanks

was provided by a blower and air stones. The sump has a water capacity of 3500 L. The sump consists of UV lights, four biofilters (3 m×1.5 m×1.5 m) and element type heaters (2×4 kw) regulated by a digital control box. One water pump (450 w) provides water flow over the biofilters and four pumps (450 w) provide water flow to the tanks. The protocol of this experiment was approved by the Animal Ethics Committee of the Stellenbosch University.

3.2.2 Experimental Feed

Four diets were formulated according to dietary requirements of catfish (NRC, 1993), containing 41% protein and 8% fat. The experimental diets were supplemented with black soldier fly (BSF) larvae meal at inclusion levels of 0% in the control diet, 10% in diet two, 20% in diet three and 30% in diet four, respectively. The diets were prepared as sinking pellets. The feed were pelletized with a 3 mm die. The pellets were dried and stored at room temperature in polythene bags until used.

Table 3.1 Ingredients of control diet and experimental diets

Ingredients (%)	**Control (0%)**	**BSF (10%)**	**BSF (20%)**	**BSF (30%)**
BSF larvae		10.00	20.00	30.00
Yellow maize fine	8.03	11.70	9.38	11.55
Soybean full fat meal	34.92	20.51	19.65	0.26
Soybean meal 50	28.91	31.40	24.66	31.99
Fishmeal 70	20.02	20.64	20.56	20.42
Vitamin mineral premix	0.15	0.15	0.15	0.15
Limestone	3.61	5.60	5.60	5.63
Salt	0.01	-	-	-
Monocalcium phosphate	4.35	-	-	-
Total	100.00	100.00	100.00	100.00

3.2.3 Experimental Procedure

Fertilized African catfish eggs were obtained from the Aquaculture Innovations Company in Grahamstown, Eastern Cape, and were reared to experimental size of 6 g at Welgevallen Experimental Farm. A total of 720 catfish fingerlings were recorded at the start of the experiment and were stocked randomly into the experimental tanks. A total of 24 tanks were used for four dietary treatments, six replicates per treatment with 30 fish per tank. The fingerlings were hand fed to satiation twice a day at 10:00 h and 16:00 h, six days a week excluding the sampling/weighing day. The feed administered was recorded daily. At the end of the experiment, growth performance and feed utilisation were expressed as feed conversion ratio (FCR) (Equation 1), specific

growth rate (SGR) (Equation 2), protein efficiency ratio (PER) (Equation 3), feed intake (FI) (Equation 4) and average daily gain (ADG).

Equation 1 $$\text{FCR: } = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Equation 2 $$\text{SGR: } = \frac{(\text{Ln Wf} - \text{Ln Wi}) \times 100}{\text{Time}}$$

Where:

Wf= Final weight (g)
Wi= Initial weight (g)

Equation 3 $$\text{PER: } = \frac{\text{Weight gain(g)}}{\text{Protein intake (g)}}$$

Equation 4 $$\text{FI: } = \frac{Total\ feed\ consuned}{(Initial + final\ number\ of\ animal)/2}$$

Average daily gain (ADG): 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 average daily gain.

3.2.4 Water quality Parameters

Water temperature, dissolved oxygen and pH were monitored daily. Water temperature and dissolved oxygen was measured using a portable temperature meter and an Oxyguard dissolved oxygen meter, respectively. Water pH was measured using a YS160 pH meter. Ammonia, nitrate, nitrite, phosphorous and total suspended solids were measured bi-weekly using a HACH DR/850 colorimeter.

3.2.5 Sampling Procedure

Individual fish measurements from each tank (20 fish) were recorded weekly throughout the 13 weeks experimental period. On the sampling day, fish were anaesthetized in clove oil to minimize handling stress. Thereafter the fish were dried on a moist towel and total and standard lengths were measured in millimetres (mm) using a fish measuring board. Fish weight was recorded in grams (g) using a digital weighing scale (UWE, HGS-1500) to nearest 0.01 g. The tanks were cleaned weekly on the sampling day and the water replaced.

3.2.6 Analytical Procedures

Proximate analysis for the experimental feeds were carried out at the Department of Animal Sciences, Stellenbosch University, excluding amino acids where only the hydrolysis was conducted at the department where after the samples were sent to the Central Analytical Facility at University of Stellenbosch for amino acids analysis.

Duplicate measurements were obtained for each feed sample analysed for moisture, ash, crude protein, crude fibre, crude fat, gross energy and amino acids.

3.2.6.1 Moisture Determination

Moisture content was determined by drying 2 g of sample in an oven at 105°C for 24 hours according to (AOAC) Association of Official Analytical Chemist International (2002), Official Method 934.01. Moisture content was calculated using Equation 5.

Equation 5 $\%\ \text{Moisture} = \frac{(A+B)-C}{B} \times \frac{100}{1}$

(% DM= 100 - % Moisture).

Where:

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

3.2.6.2 Crude Protein Determination

The crude protein was determined by measuring the total nitrogen content of the feed using the Dumas method with a LECO FP 528 (LECO FP 528, USA) according to AOAC (2002), Official Method 4.2.07. A foil cup with 0.1 g of sample was folded and put into the LECO FP 528 sample tray. Total nitrogen content was determined and then the value 6.25 was used as conversion factor of total nitrogen to protein. Protein content was calculated using Equation 6.

Equation 6 Crude protein (%) = Nitrogen (%) × 6.25

3.2.6.3 Crude Fat Determination

Crude fat was determined according to AOAC (2000), Official method 954.02. A volume of 2 ml of ethanol and 10 ml of HCl was added into 2 g of sample in a test tube and boiled for 30 min. Thereafter the test tube sample was cooled to room temperature and poured into a separating funnel. Then 25 ml of diethyl ether and 25 ml of petroleum ether was added to the test tube and the test tube was shaken for 1 min. The upper portion of the solvent was poured into a fat cup. Again 15 ml of diethyl ether and 25 ml of petroleum ether was added to the test tube and the test tube was shaken for 1 min. The upper portion of the solvent was poured into a fat cup

and placed on a sand bath at 30°C for 45 min until all solvent had evaporated. Crude fat content was calculated using Equation 7.

Equation 7 Fat%= $((Mass\ of\ fat\ cup + fat))/(Mass\ of\ sample) \times 100/1$

3.2.6.4 Crude Fibre Determination

Crude fibre was determined by the filter bag technique with an ANKOM fibre analyser (ANKOM 220, USA). A mass of 0.1 g of sample was sealed in an ANKOM filter bag F57 and soaked in petroleum ether for 10 min to extract the fat. Thereafter it was air-dried at ambient temperature and then placed in a fibre analyser vessel. A quantity of 0.255 N H_2SO_4 was added and the samples were left to extract for 40 min. Afterwards the samples were rinsed twice with hot water. Then 0.313 N NaOH was added and the samples were left to extract for 40 min. The samples were again rinsed thrice with hot water and soaked in acetone for 5 min. The samples were dried in an oven at 105°C and ashed in a furnace for 2 hours at 550°C. Crude fibre content was calculated using Equation 8.

Equation 8 % Crude fibre = $100 \times \frac{W3-(W1\times C1))}{W2}$

Where:

W_1= Bag tare weight
W_2= Sample weight
W_3= Weight of organic matter (loss of weight on ignition of bag and fibre)
C_1= Ash corrected blank bag factor (loss of weight of weight on ignition of blank bag)

3.2.6.5 Ash Determination

The moist free samples after moisture determination were used to determine ash content by placing it in a furnace at 500°C for 6 hours according to AOAC (2002), Official Method 942.05. Percentage ash content was calculated using Equation 9.

Equation 9 % Organic material = 100 - % ash

3.2.6.6 Gross Energy Determination

Gross energy was determined by using a CP 00 bomb calorimeter (IKA C200, Germany). A mass of 0.3 g of sample was converted into a pill and placed in a metal crucible. The pill in the metal crucible was then placed in a decomposition vessel and filled with oxygen for approximately 30 sec. The decomposition vessel was placed into the bomb calorimeter, filled with 2 litres of tap water. The device was then triggered for combustion and gross energy was measured in MJ/kg.

3.2.6.7 Amino Acid Determination

The hydrolysis of amino acids was determined according to AOAC (2003), Official method 994.12. A quantity of 0.1 g of sample was added into a hydrolysis tube. Then 6 ml of 6N hydrochloric acid and 15% phenol solution

was added into the sample. The samples were kept alternating between the vacuum for 20 seconds and the nitrogen for 20 seconds. This process was repeated twice for the vacuum and nitrogen alteration, but another cycle of the vacuum was added. Thereafter, the hydrolysis tubes were sealed. The sealed hydrolysis tubes were left to hydrolyse in an oven for 24 hours at 110°C. After hydrolysis the samples were left to cool to room temperature. After cooling, 2 ml was poured into eppendorf eppis and stored at -20°C until amino acid determination. The hydrolysed samples were subjected to the Water AccQ Tag Ultra Derivitisation kit for amino acids detection.

3.3 Statistical Analysis

Data for the first 20 days of the trial was not used in the analyses, due to logistical problems with the environmental conditions. The results of the analyses of the data recorded from day 21 to 91 are therefore discussed.

Statistical analyses were carried out using PROC General Linear Model (GLM) procedure in SAS software (2009). The growth performance and feed utilisation data were subjected to one way analysis of variance (ANOVA) to determine if there were significant differences between treatments. The level of significant used was at p-value 0.05. Treatment effect with p-value less than 0.05 were considered significant. Alternatively, treatment effect with p-value more than 0.05 were not significantly different. If the ANOVA test was found to be less than 0.05, then a Bonferroni *post hoc* test for least square means was used for multiple comparisons. The results are shown as means of six replicate tanks per treatment.

3.4 Results and discussions

Proximate analysis of control diet and experimental diets are illustrated in Table 3.2.

Table 3.2 Proximate analysis of control diet and experimental diets

Proximate Analysis (%)	**BSF (0%)**	**BSF (10%)**	**BSF (20%)**	**BSF (30%)**
Moisture	5.76	5.52	5.30	4.93
Crude Protein	44.24	43.88	41.50	41.50
Crude Fat	11.16	11.48	13.39	16.49
Fibre	2.93	3.28	3.97	3.76
Ash	7.56	7.73	8.95	9.91
Gross energy MJ/Kg	20.06	19.94	19.10	20.00

Although the experimental diets were formulated to be iso-nitrogenous and iso-caloric, differences were observed in proximate analysis of the diets with respect to crude protein and crude fat. The crude protein content ranged from 41.50% - 44.24% and crude fat ranged from 11.16% - 16.49%. The gross energy of the diets was similar and it ranged from 19.10 – 20.06 MJ/kg. It was noted that crude fat content increased with the increasing inclusion level of BSF larvae meal, similar problems were reported by Newton *et al.* (2005a).

The amino acid analyses of the experimental diets are portrayed in Table 3.3 and Table 3.4.

Table 3.3 Essential amino acid compositions of control diet and experimental diets (Amino acid expressed in g/100g)

Essential amino Acids	Control (0%)	BSF (10%)	BSF (20%)	BSF (30%)
Arginine	2.49	2.65	2.01	1.81
Histidine	0.38	0.33	0.24	0.14
Isoleucine	1.64	1.83	1.54	1.49
Leucine	2.76	3.16	2.55	2.42
Lysine	2.28	2.67	2.16	2.08
Methionine	0.55	0.70	0.57	0.55
Phenylalanine	1.86	2.04	1.62	1.49
Threonine	1.46	1.69	1.38	1.29
Valine	1.82	2.14	1.83	1.80
Tryptophan*	-	-	-	-

*Tryptophan not identified

Table 3.4 Non-essential amino acid compositions of control diet and experimental diets (Amino acid expressed in g/100g)

Non-essential amino acids	Control (0%)	BSF (10%)	BSF (20%)	BSF (30%)
Alanine	1.70	2.10	1.88	1.88
Asp	3.68	4.33	3.52	3.34
Cysteine	0.13	0.14	0.09	0.08
Glutamine	5.71	6.49	5.22	4.86
Glycine	1.77	2.07	1.96	1.78
Proline	1.73	2.02	1.77	1.72
Serine	1.66	1.82	1.35	1.23
Tyrosine	1.39	1.59	1.37	1.35

Water quality parameters are shown in Table 3.5.

Table 3.5 Water quality parameters

Parameters	**Control (0%)**	**BSF (10%)**	**BSF (30%)**	**BSF (30%)**
Temperature (°C)	25.66	24.87	25.71	25.8
Dissolve oxygen (mg/L)	7.27	7.13	7.16	7.08
pH	6.40	6.37	6.3	6.33
Ionized ammonia (mg/L)	4.6	4.26	3.18	3.25
Nitrite (mg/L)	1.34	1.36	1.30	1.36
Nitrate (mg/L)	3.44	2.66	2.95	2.71
Phosphate (mg/L)	7.79	7.84	8.16	8.98
Total suspended solids (mg/L)	70.75	56.75	69.87	87.75

Water quality parameters fluctuated within the acceptable range for African catfish (Table 3.4). Water temperature ranged from 24.8 to 25.8°C. Dissolved oxygen ranged from 7.08 – 7.27 mg/l. Water pH was slightly lower and ranged from 6.30 – 6.40. Additionally, ammonia, nitrite, nitrate, phosphate and total suspended solids ranged from 3.2 – 4.6mg/L, 1.3 -1.4mg/L, 2.7 – 3.4mg/L, 7.8 9.0mg/L and 56.8 – 87.8 mg/L, respectively during the trial. Similar values were reported to be acceptable for African catfish growth (Haylor, 1989; Pistelok & Pruszynski, 1999). In this study the water quality parameters reported would not compromise the growth of fish between treatments.

The growth performance and feed utilization are shown in Table 3.5. There were no differences ($P > 0.05$) in the mean final weights of fish that received the control diet and fish that were supplemented with 10% BSF larvae meal. Both were different ($P < 0.05$) from fish that were supplemented with 30% BSF larvae meal. The fish fed diets supplemented with 10% and 20% BSF larvae meal were not significantly different from each other. The fish that received 20% and 30% BSF larvae meal supplemented diets were also not significantly different from each other. There were no differences ($P > 0.05$) in feed conversion ratio, protein efficiency ratio, average daily gain and specific growth rate between treatments.

Table 3.6 Growth performance and feed utilisation of African catfish fingerlings fed experimental diets.[1]

Parameter	Control (0%)	BSF (10%)	BSF (20%)	BSF (30%)	SEM[2]
Initial weight (g)	6.32	6.06	5.53	5.45	0.17
Final weight (g)	237.76[a]	233.67[ab]	208.13[bc]	202.17[c]	6.50
Feed intake (g)	142.36[a]	143.28[a]	135.89[ab]	128.60[b]	2.99
ADG[3]	3.19	3.19	3.14	3.13	0.03
SGR[4]	2.90	2.82	2.90	2.89	0.06
FCR[5]	1.63	1.59	1.49	1.53	0.04
PER[6]	3.67	3.63	3.59	3.69	0.11

1The values are means of six replicate tanks per treatment. Means in the same row with common superscript were not significantly different (P > 0.05).
2SEM= Standard error of the means
3ADG= Average daily gain
4SGR= Specific growth rate
5 FCR= Feed conversion ratio
6PER= Protein efficiency ratio

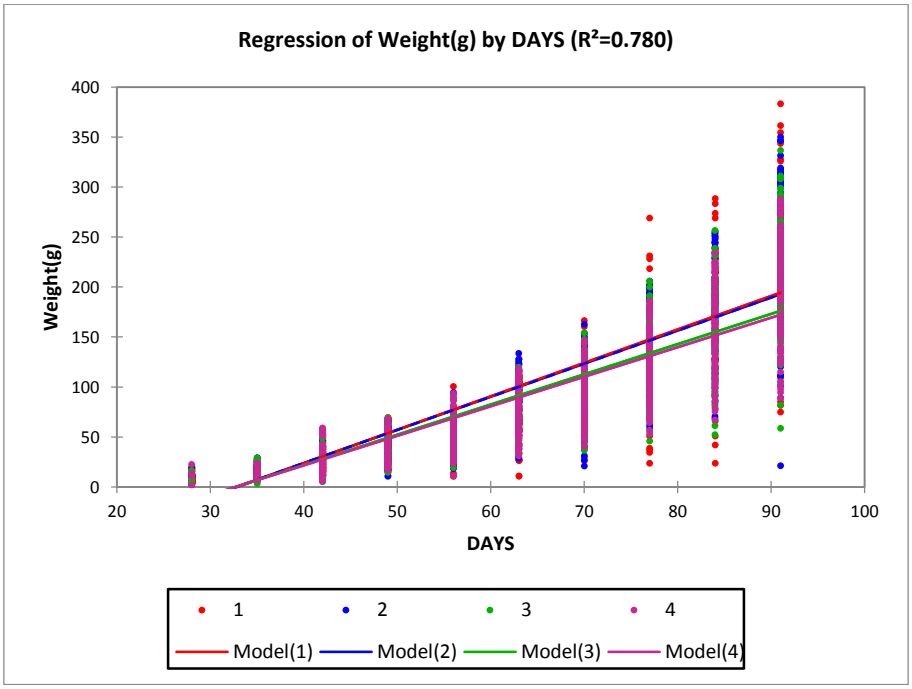

Figure 3.1 Comparison of regression of weight by days

Black soldier fly larvae or pre-pupae meal has not been previously evaluated in the diet of African catfish. The results of this study demonstrate that BSF larvae meal are a suitable protein source for African catfish and it could be supplemented up to 30% without adverse effects on feed utilisation.

The possible reasons for the reduced growth may be due to high fat content in BSF 20% and BSF 30% supplemented diets that might have reduced feed consumption or diet digestibility. High fat content in the diet

inhibits the intake of nutrients required for growth (Ali & Jauncey, 2004) and could reduce digestibility (Watanabe, 1982). Similarly, Fasakin *et al.* (2003) reported a significant reduction in growth of African catfish fed full-fat maggot *(Musca domestica)* meal compared to fishmeal control diet. Fasakin *et al*. (2003) attributed the growth reduction to palatability, digestibility and amino acid content. Their diets were iso-nitrogenous and iso-caloric (Fasakin *et al.,* 2003).

Presently, there is not enough literature available on insects' meal supplementation in fish diets. Due to the lack of similar studies, it is difficult to compare the results of the present study with other studies. Most of the previous studies on BSF as a protein source in fish diets utilised BSF pre-pupae which is the last stage before pupation into the adult fly (Sheppard *et al.,* 1994) to replace fishmeal at various inclusion levels (Newton *et al.,* 2005; St-Hilaire *et al.,* 2007; Sealey *et al.*, 2011). However, in the present study, BSF larvae meal was used to supplement fishmeal or conventional protein sources. The variations in results of the above studies compared to the present study were due to protein replacement versus supplementation. Also the BSF life stage used pre-pupae versus larvae, the trials duration and fish species used were also all different; all these factors would cause differences and variation between studies.

The acceptable growth of the African catfish in this study indicated that the amino acid profile of the test diets had met the dietary requirements of the fish. Several researchers have demonstrated that fly larvae meals have an ideal amino acid profile that is comparable to conventional protein sources such as soybean or fishmeal (Akpodiete *et al.,* 1997; Newton *et al.*, 2005b). Bondari & Sheppard, (1987) indicated that insects in various growing phase can be suitable alternative sources of protein. A review by Rumpold & Schlüter, (2013) indicated that insects have a great potential to be incorporated in fish feeds as insects fall within the nutrient range of fish in their natural habitat.

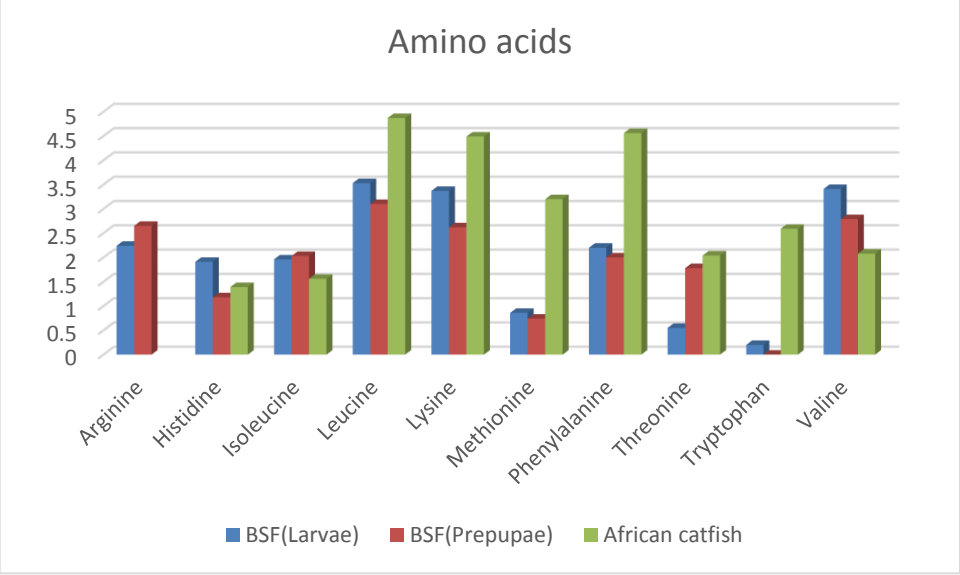

Figure 3.2 Comparison of the essential amino acids composition of BSF larvae and pre-pupae meal to amino acids requirements of the African catfish (adapted from Newton *et al.,* 1977; Newton *et al.,* 2005b; Pantazis, 2005).

The feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and average daily gain (ADG) did not differ ($P > 0.05$) between the experimental diets and the control. This indicates that BSF larvae meal supplemented in the diets were well utilised and supported the growth of the African catfish in this study. The lack of information about BSF larvae supplementation in fish feeds does not allow us to say much at this point.

Considering the lack of previous research and the resultant lack of comparability, except in the broadest sense or for general nutritional knowledge, different insect species have been fed experimentally to various fish species. Nandeesha *et al*. (1990) studied the effects of silkworm pupae on the growth of common carp *(Cyprinus carpio)* and found superior growth of diet containing 30% silkworm without fishmeal over the fishmeal control diet. Nevertheless, statistically no significant differences were noted between the dietary treatments. Jabir *et al.* (2012) reported super worm meal (iso-nitrogenous diets) can replace 25% of fishmeal protein in the diet of Nile tilapia. However, growth was reduced at inclusion level of super worm meal above 50%. Similarly, when 25% of variegated grasshopper was used to replace fishmeal in the diet of African catfish, this resulted in comparable growth performance with the control diet (Alegbeleye *et al.,* 2012). The diets were iso-nitrogenous and iso-caloric (Alegbeleye *et al.,* 2012). However, growth was negatively influenced at over 50% inclusion level of variegated grasshopper meal. Furthermore, growth declined significantly at 100% inclusion level (Alegbeleye *et al.,* 2012).

3.5 Conclusion

The results of this study effectively indicate that BSF larvae are a viable protein source for African catfish. Despite this fact, BSF larvae meal supplemented up to 30% in the diet of African catfish did not compromise feed utilisation in terms of feed conversion ratio, protein efficiency, specific growth rate and average daily gain. However, from the above discussion, the reduced growth of fish that were supplemented with 20% and 30% BSF larvae meal diets were believed to be due to high fat content in the unprocessed BSF larvae. Furthermore, this study recommends subsequent studies to evaluate defatted BSF larvae to enable high inclusion levels in the diet of African catfish.

3.6 References

Abdel-Warith, A.A., Russell, P.M. & Davies, S.J., 2001. Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish *Clarias gariepinus* (Burchell 1822). *Aquac. Res*., 32: 296–305.

Akpodiete, O.J., Ologhobo, A. D. & Oluyemi, J.A., 1997. Production and nutritive value of Housefly maggot meal on three substrates of poultry faeces. *J. Appl. AniM. Res*, 12(1): 101–106.

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.

Ali, M.Z. & Jauncey, K., 2004. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). *Aquac. Int*, 12(2): 169–180.

AOAC, 2003. Association of Official Analytical Chemists. Official methods of analysis (17th edition), Arlington,Virginia, USA.

AOAC, 2002. Association of Official Analytical Chemists. Official methods of analysis (17th edition), Official Method 934.01, 942.05, 4.2.07, 920.39. Arlington,Virginia, USA.

AOAC, 2000. Association of Official Analytical Chemists. Official methods of analysis (16th edition), Official Method 954.02. Arlington,Virginia, USA.

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.

Bondari, K., Coastal, D.C.S. & Experiment, P., 1987. Soldier fly *Hermetia illucens* L larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia *Oreochromis aureus* (Steindachner). *Aquaculture and fisheries management*. 18: 209–220.

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

Chor, W.K., Lim, L.S. & Shapawi, R., 2013. Evaluation of feather meal as a dietary protein source for African catfish fry, *Clarias gariepinus*. *J. Fish. Aquat. Sci*. 8(6): 697-705.

Cičková, H., Newton, L.G., Lacy, R.C. & Kozanek, M., 2014. The use of fly larvae for organic waste treatment. *Waste management (New York, N.Y.)*. In press

De Silva, S.S., & Anderson, T.A., 1995. Fish nutrition in aquaculture. Chapman and Hall, London.

Diener, S., Nandayure, M., Solano, S., Gutierrez, F.R., Zurbrugg, C. & Tocker, K., 2011. Biological treatment of municipal organic waste using black soldier fly larvae. *Waste Biomass Valor*, 2(4): 357–363.

El-Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis spp*. *Aquaculture*, 179(1-4): 149–168.

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.

Fagbenro, O. & Davies, S., 2001. Use of soybean flour (dehulled solvent-extracted soybean) as fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed. *J. Appl. Ichthyol*.17(2): 64-69.

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.

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

Goda, A.M., El-Haroun, E.R. & Kabir Chowdhury, M. A., 2007. Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquac. Res*. 38(3): 279–287.

Hardy, R., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquac. Res.41(5): 770-776.*

Haylor, G.S., 1989. The case for the African catfish, *Clarias gariepinus*: a comparison of the relative merits of Tilapine fishes, especially *Oreochromis niloticus* (L.) and *C. gariepinus* Burchell for African aquaculture. *Aquaculture and fisheries management:* 20: 279-285.

Hem, S., Toure, S., Sagbla, C. & Legendre, M., 2008. Bioconversion of palm kernel meal for aquaculture : Experiences from the forest region (Republic of Guinea). *Afr. J. Biotechnol.*7(8): 1192–1198.

Hlophe, S.N, & Moyo, N.A.G., 2014. Replacing fishmeal with Kikuyu grass and Moringa leaves: Effects on growth, protein digestibility, histological and haematological parameters in Clarias. *Turk. J. Fish. Aquat. Sci.*14(2): pp.22.

Hoffman, L., Prinsloo, J. & Rukan, G., 1997. Partial replacement of fish meal with either soybean meal, brewers yeast or tomato meal in the diets of African sharptooth catfish *(Clarias gariepinus)*. *Water SA*.23(2): 181-186.

Jabir, M.A.R., Jabir, S.A.R. & Vikineswary, S., 2014. Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. *Afri. J. Biotechnol*, 11(24): 6592–6598.

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. total. environ*, 458-460: 312–8.

Larde, G., 1990. Recycling of coffee pulp by *Hermetia illucens* (Diptera: Stratiomyidae) larvae. *Biol. Wastes*, 33(4): 307–310.

Nandeesha, M.C., Srikantha, G.K., Keshavanatha, P., Varghesea, T.J., Basavarajaa, N. & Dasa, S.K.,1990. Effects of non-defatted silkworm-pupae in diets on the growth of common carp, *Cyprinus carpio*. *Biol. Wastes*, 33(1): 17–23.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrel, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K. & Nicholas, P.D., 2009. Feeding aquaculture in an era of finite resources. *Proc. Natl. Acad.Sci. of the USA*, 106(36): 15103–15110.

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

Newton, G.L., Sheppard, D., Watson, W., Burtle, G.J., Dove, R.C., Tomberlin, J.K. & Thelen, E.E., 2005a. The Black Soldier Fly, (*Hermetia Illucens)* as a Manure Management / Resource Recovery Tool. In *Symposium on the State of the Science of Animal Manure and Waste Management*. pp. 0–5.

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

NRC, 1993. Nutrient Requirements of Fish, National Academies Press.

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

Olsen, R.L. & Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends. Food. Sci. Tech*, 27(2): 120–128.

Pistelok, F. & Pruszynski, T., 1999. Biological and economical evaluation of African and European catfish rearing in water recirculation systems. *Arch. Ryb. Pol. Vol*.7. Fasc2: 343-352

Rumpold, B. A. & Schlüter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. *Innov. Food. Sci. Emerg.* 17: 1–11.

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 (*Hermetia illucens)* prepupae,. *J. World Aquacult. Soc*. 42(1): 34–45.

SAS, 2009. Version 9.3. SAS Institute Inc., Cary, North Carolina, USA.

Sheppard, C.D., Newton, L.G., Thomson, S.A & Savage, S., 1994. A value added manure management system using the black soldier fly. *Bioresour. Technol*, 50(3): 275–279.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C., & Irving, S., 2007a. Fish offal recycling by the Black Soldier fly produces a foodstuff high in omega-3 fatty Acids. *J. World Aquacult. Soc*. 38(2): 309–313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., & Sealey, W., 2007b. Fly prepupae as a feedstuff for Rainbow trout *(Oncorhynchus mykiss)*. *J. World Aquacult. Soc*. 38(1): 59–67.

Tacon, A., 1993. Feed ingredients for warmwater fish fish meal and other processed feedstuffs.

Watanabe, T., 1982. Lipid nutrition in fish. *Comp. Biochem. Physiol*. 73B: 3-15.

Chapter 4

4 The influences of black soldier fly *(Hermetia illucens)* larvae meal on the body composition of African catfish (*Clarias gariepinus*) fingerlings

Abstract

The study investigated the influences of different inclusion levels of black soldier fly (BSF) larvae meal on the body composition and dressing percentage of African catfish fingerlings. Four experimental diets were formulated containing 0% (control), 10%, 20% or 30% BSF larvae meal. A total of 720 fingerlings (5 – 6 g) were randomly allocated to treatments with six replicates per treatment within a water recirculating system. Fish were fed to satiation (10:00h and 16:00h) daily except on the sampling day for 91 days experimental period. At the end of the trial six fish from each treatment were selected randomly, euthanized, eviscerated and filleted. Proximate analysis tested on the fillets showed no significant differences in percentage moisture (76.56-74.45±0.70), protein (16.88-15.69±0.31), lipids (8.43-5.24±0.86), and ash (1.19-.113±0.02) between treatments. No significant difference was found in dressing percentage between treatments. This study shows that BSF larvae meal can be incorporated into the diet of African catfish without affecting proximate body composition.

Key words: Body composition, dressing percentage, black soldier fly, proximate analysis, African catfish

4.1 Introduction

Fish as food are highly nutritious, tasty, and easily digested (FAO, 2015). Fish are a source of quality protein, vitamins and minerals (Murray & Burt, 2001). The amino acid profile in fish protein is similar to that of milk, eggs and beef, and has a very high biological value (Huss, 1995; Murray & Burt, 2001). Moreover, fish are regarded as the best food into body tissue converters compared to all other animals (Lovell, 1989). Fish muscles contain more edible lean tissue to that of beef, pork or poultry (Lovell, 1989). Regular consumption of fish as a source of omega-3 fatty acids may reduce cardiovascular diseases (Kris-Etherton *et al.,* 2002). Steyn *et al.* (1995) reported improvement in nutritional status (heights, weights and head circumference) of underweight rural preschool children supplemented with 300g of African catfish per child per week for a period of 12 months. As the human population is increasing globally, the demand for fish and fish products is likely to increase substantially, assuming the current world per capita consumption of 19 kg/year remains constant (FAO, 2014). Fish provide the global population with 17% of their animal protein intake (FAO, 2014). Fish also play a vital role in food security, poverty alleviation and general well-being especially in places where protein sources from livestock is relatively scarce (Tidwell, 2012; FAO, 2014).

Proximate body composition of fish involves the measurement of moisture, protein, lipid and ash content (Shearer, 1994). According to Love (1970), the principal constituent of fish is water ranging from 66% to 81%, protein 16% to 21%, lipid 0.2% to 25%, minerals 1.2% to 1.5% and carbohydrates less than 0.5%. Information about the proximate composition of fish and factors affecting it, may assist in the evaluation of the health

condition of fish, the determination of food conversion efficiency and may enable the prediction of carcass composition (Shearer, 1994). Knowledge of the body composition of fish is essential and valuable especially to the fish processors, nutritionists, those who prepare fish, food scientists, fishery biologists as well as the consumers (Hoffman *et al.,* 1992; Murray & Burt, 2001). More importantly, the measurement of some proximate profiles of fish is often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Waterman, 2000).

Under culture conditions, the chemical composition of fish is influenced by feed composition, environment and fish size as well as genetic traits. However, feed composition is the factor having a major effect on fish chemical composition (Huss, 1995). According to Shearer (1994), the body composition of fish appeared to be largely affected by endogenous and exogenous factors that operate simultaneously. Endogenous factors are genetically controlled and are associated with fish size and life cycle. Exogenous factors include numerous environmental factors and dietary origins. The chemical composition of fish differs substantially from one fish to another of the same species as well as within an individual fish (Murray & Burt, 2001). Hoffman *et al.* (1994) found variations in proximate composition along the fillet of African catfish. Moisture and protein content decreased from head to tail, whereas fat content showed the reverse. Ash content was uniform (Hoffman *et al.,* 1994). Dorsa-ventrally, no differences occurred in moisture, protein and fat contents (Hoffman *et al.,* 1994). Moreover, the variation in proximate composition along the fish fillet is associated with the ratios of light and dark muscles (Hoffman *et al.*, 1994). Dark muscle is low in moisture, protein and ash, and high in lipid content compared to light muscle (Hoffman *et al.,* 1995).

Freshwater fish have been reported as a good source of animal protein, essential polyunsaturated fatty acids, and contain considerable quantities of minerals and vitamins (Steffens, 2006). African catfish is nutritionally important; it contains all the essential amino acids and is particularly high in lysine (Hoffman *et al.,* 1992). Hoffman *et al.* (1993) identified essential polyunsaturated fatty acids of both series n-3 and n-6 in African catfish. Hoffman *et al.* (1993) found 1.2% eicosapentaenoic acid, trace concentrations of docosapentaenoic and 3% decosahexaenoic acids. Percentages of saturated, monounsaturated and polyunsaturated fatty acids were 36.6%, 37.6% and 19.6%, respectively (Hoffman *et al.*, 1993). This nutritional profile makes African catfish a suitable fish species for the development of the aquaculture industry in the African continent. Additionally, African catfish have several other desirable characteristics. It has a high growth rate, tolerates high stocking density under culture conditions and is preferred by consumers in several African countries (Huisman & Richter, 1987). It is also a species that is an opportunistic omnivore and is known to eat various animals and insects. It has also been shown that this species is a suitable utiliser of agricultural waste convertors such as black soldier fly (BSF) larvae into fish protein (Chapter 3). However, the question remains on what the effect(s) of this insect dietary source would be on the quality/composition of the catfish flesh?

Therefor the objectives of this study were to investigate the proximate body composition and dressing percentage of African catfish fed diets supplemented with BSF larvae meal.

4.2 Materials and Methods

4.2.1 Experimental System

The experiment was conducted in a water recirculation system as described in section 3.2.1 in Chapter 3.

4.2.2 Experimental Feed

Details on the experimental feed and the inclusion levels of black soldier fly (BSF) larvae meal were described in section 3.3.2 in Chapter 3.

4.2.3 Experimental Procedures

Details on the dietary treatments, number of African catfish fingerlings stocked and the feeding were described in section 3.2.3 in Chapter 3. At the end of the 91 days feeding trail, the fish were fasted for 24 hours to empty the digestive tract. A total of 24 fish representing six fish from each treatment were randomly selected and euthanized. The fish were dressed (removal of head, viscera and tail) and filleted individually. Live weight was recorded before the fish were euthanized and dressed (body) weight, head weight and visceral fat weight were recorded after slaughter. The fillets from each fish were vacuumed and sealed separately in vacuum plastic bags and kept frozen for further analysis. The dress out yield and waste yield were computed as weight of dressed fish relative to live weight according to Clement & Lovell (1994).

4.3 Proximate Analysis

All the proximate body composition analysis for the African catfish fillet was carried out at the Department of Animal Sciences, Stellenbosch University. Prior to proximate analysis, each fish fillets sample was thawed and homogenised. The mixer bowl was cleansed between each sample. Duplicate measurements were done for each homogenised sample analysed for body moisture, ash, protein, and lipid content.

4.3.1 Moisture determination

Moisture content was determined according to Association of Official Analytical Chemist International (AOAC, 2002), AOAC Official Method 934.01following the procedure described in section 3.2.6.1 in Chapter 3.

4.3.2 Protein determination

The protein content was determined by measuring the total nitrogen content of the sample using the Dumas method with a LECO FP 528 according to AOAC (2002), AOAC Official Method 992.15. A foil cup with 0.1g of the sample was folded and put into the LECO FP 528 sample tray. Then total nitrogen content was determined.

4.3.3 Fat Content Determination

Fat content was determined by chloroform/methanol (2:1) method according to Lee *et al.* (1996). A volume of 50ml chloroform/methanol solution was poured into a beaker containing 5 g homogenized fish fillet sample and thoroughly mixed/homogenised. The solution was filtered through filter paper (Whatman no.1) into a separation funnel where after 0.5% NaCl was added and the separation funnels were shaken 4 times and were allowed to stand for 30 minutes. The bottom layer of liquid from the separation funnel was collected in a 100

ml Erlenmeyer flask. Then, using a 5 ml pipette, 5 ml collected liquid was poured into pre-weighed glass fat beakers. The beakers were placed onto a sand bath at 30˚C for 45 minutes, thereafter cooled in a desiccator for 30 minutes and weighed accurately. The residues (fat free) of samples on the filter papers were dried in an oven for crude protein analysis.

4.3.4 Ash determination

Ash content was determined according to AOAC (2002), AOAC Official Method 942.05 as described in section 3.2.6.5 in Chapter 3.

4.4 Statistical Analysis

Statistical analyses were carried out using PROC General Linear Model (GLM) procedure in SAS software (2009). Experimental data were subjected to one way analysis of variance (ANOVA) to determine if there were significant differences between treatments. The level of significant used was at p-value 0.05. If the ANOVA test was found less than 0.05, then a Bonferroni *post hoc* test for least square means was used for multiple comparisons.

4.5 Results and Discussion

The dressing percentage and waste yield (head, viscera, and visceral fat) are presented in Table 4.1. No differences (P > 0.05) were found in dressing percentages and waste yield between treatments. The dressing percentage of fish ranged from 65.32% - 68.41% (Table 4.1). Waste yield of the fish heads ranged from 24.14% - 27.26%, viscera ranged from 4.99% - 5.09% and visceral fat ranged from 0.58% - 1.20% (Table 4.1).

Table 4.1 Mean dressing percentage (%) of African catfish fingerlings fed experimental diets.[1]

Parameter	Control (0%)	BSF[2] (10%)	BSF (20%)	BSF (30%)	SEM[3]	P > F
Body weight	307.8	300.1	268.2	259.8	10.68	
Dressing Percentage (%)	68.41	67.40	65.32	65.56	0.94	0.09
Head (%)	24.14	24.84	27.26	26.70	1.06	0.16
Viscera (%)	4.99	5.06	5.09	5.08	0.31	0.99
Visceral Fat (%)	1.19	1.20	0.58	1.11	0.17	0.06

1Values are means of six fish per treatment.

2BSFL: Black soldier fly larvae

3SEM: Standard error of the means

The results of the final body composition are illustrated in Table 4.2. One way analysis of variance (ANOVA) showed no differences (P > 0.05) in body composition of African catfish between treatments in terms of moisture, protein, lipid and ash (Table 4.2). Moisture content ranged from 74.45% - 76.56%, crude protein

content ranged from 91.14% - 92.86%, crude lipid levels ranged from 5.24% - 8.43% and ash ranged from 1.13% - 1.19%.

Table 4.2 Mean final proximate body composition (%) of African catfish fingerlings experimental diets.[1]

Parameter	Control (0%)	BSF[2] (10%)	BSF (20%)	BSF (30%)	SEM[3]	P > F
Moisture (%)	76.6	75.52	74.45	74.77	0.70	0.19
Protein (% as is)	16.88	16.79	15.69	16.40	0.31	0.05
Lipid (%)	5.24	6.37	8.43	7.26	0.86	0.10
Ash (%)	1.15	1.14	1.13	1.19	0.02	0.38

1Values are means of six fish per treatment.

2BSFL: Black soldier fly larvae

3SEM: Standard error of the means

The findings of this study show that final body (fillet) composition of African catfish was not affected by the dietary treatments. The dressing percentage ranged from 65.3% to 68.4% which did not differ ($P > 0.05$) between treatments. The values of dressing percentages obtained in this study were in the range of percentage dressed mass of African catfish reported by Hoffman & Prinsloo, (1990). Hoffman & Prinsloo, (1990) compared the dressing percentage of the red/golden and normal coloured strains of African catfish. In this study, it was also noted that excess lipids in BSF20% and BSF30% did not reduce the fillet dressing percentage between treatments.

No clear trend has been observed in the proximate body (fillets) composition of African catfish, as the level of BSF larvae meal was increased. The high body protein content reported for all treatments in this study are in agreement with the report of Steffens (2006); that protein forms the main component in the dry matter of fish muscle or flesh. The relationship between percentage body moisture and body lipid found in this study is in agreement with Kang *et al.* (2011) on Asian red tailed catfish *(Hemibagrus wyckioides)* and Hoffman, (1995) on African catfish. Kang *et al.* (2011) and Hoffman, (1995) found an inverse relationship between body lipid and moisture content.

No literature was found on the effects of BSF larvae meal on the proximate body composition of African catfish. There is also a paucity of information on the body (fillet) composition of African catfish. Several studies have reported the proximate carcass or whole-body composition of the African catfish (Abdel-Warith *et al.,* 2001; Fagbenro & Davies, 2001; Ng *et al.*, 2001; Goda *et al.,* 2007; Alegbeleye *et al.*, 2012; Chor *et al.*, 2013) which cannot be compared to fillet composition in the present study.

Information on the effects of BSF larvae meal supplementation on body composition of fish is limited. However, the effects of BSF pre-pupae meal as a partial replacement for fishmeal on carcass composition of rainbow trout has been investigated (St-Hilaire *et al.* 2007; Sealey *et al.* 2011). As such, it is very difficult to compare these studies with the current study. The reason were differences in BSF life stages used as well as the nature of the trials, supplementation versus percentage replacement of protein sources.

The body moisture content ranged from 74.45% to 76.56%. The results clearly indicate no significant difference in moisture content between treatments. The moisture content found in this study is within the suitable range 65% to 80% for freshwater fish, as moisture content is an important measure of fish flesh quality (Steffens, 2006). For comparative purpose, Hoffman *et al.* (1993) reported similar moisture content in African catfish fed commercial diet. Additionally, the body protein content ranged from 15.69% to 16.88%. The fillet protein content in the present study was consistent with the findings of Hoffman *et al.* (1993) on African catfish fed a commercial diet. This study show African catfish fed diets supplemented with BSF larvae meal is a good source of protein. According to Murray & Burt, (2001) the protein content of fish muscle ranges between 15% and 20%.

The body lipid content ranged from 5.24% to 8.43% (Table 4.1). The results clearly show no significant difference in body lipid content between treatments. Hoffman *et al.* (1993) obtained similar fillet lipid content compared to the control diet but lower lipid content compared to other experimental diets in this study. It was reported that body lipid levels depend upon the dietary lipid levels in the feed (Houlihan *et al.,* 2001; Pillay & Kutty, 2005). These excess lipids are then deposited into the body and may affect fish flesh quality in terms of fillet texture, dressing losses during processing as well as the nutritional value of the fillet (Houlihan *et al.,* 2001). According to Haard, (1992) the fillet lipid content of the African catfish reported in this study would place this fish species into average fat fish category.

The body ash content ranged from 1.13% to 1.19%. The results reveal no significant difference in body ash content between treatments. Hoffman *et al.* (1993) found that African catfish fed commercial diet had lower fillet ash content. The ash content of the fillets in this study indicates the presence of different minerals which constitute 1 – 2% of the edible portion in fish (Murray & Burt, 2001). The fillet ash content of African catfish reported in this study was within the acceptable range of the edible portion in fish according to Murray & Burt, (2001).

4.6 Conclusion

From the above discussion, it is evident that the fillet which is the chief food source was not altered at the studied inclusion levels of BSF larvae meal. This result indicates that BSF larvae meal could supplement conventional protein in the diet of African catfish. No treatment differences were found in terms of dressing percentage. Finally, the present study showed no significant differences in proximate body composition between dietary treatments when BSF larvae meal was included up to 30% in the diet of African catfish fingerlings. It may then conclusively be stated that BSF larvae meal can be incorporated into the diet of African catfish without negatively affecting body proximate chemical composition, however it would be interesting to see whether the inclusion of BSF larval meal will influence the sensory properties of African catfish fillets.

4.7 References

Abdel-Warith, A.A., Russell, P.M. & Davies, S.J., 2001. Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish *Clarias gariepinus* (Burchell 1822). *Aquac. Res*, 32: 296–305.

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.

AOAC (2002). Association of Official Analytical Chemists. Official methods of analysis (17th edition), Arlington,Virginia, USA.

Chor, W.K., Lim, L.S. & Shapawi, R., 2013. Evaluation of feather meal as a dietary protein Source for African catfish fry, *Clarias gariepinus*. *J. Fish. Aquat. Sci*.8(6): 697-705.

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

Fagbenro, O. & Davies, S., 2001. Use of soybean flour (dehulled solvent-extracted soybean) as fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed. *J. Appl. Ichthyol*.17(2): 64-69.

FAO, 2015. Fisheries and Aquaculture topics. Nutritional elements of fish. Topics Fach Sheet. Rome. Updated 27 May 2005. Accessed 3 February 2015.

FAO, 2014. State of World Fisheries and Aquaculture. Rome. pp 223.

Goda, A.M., El-Haroun, E.R. & Chowdhury, K. 2007. Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquac. Res*, 38(3): 279–287.

Haard, N.F. 1992. Control of chemical composition and food quality attributes of culture fish. *Food. res. Int, 25: 289-307.*

Hoffman, L.C., 1995. Factors affecting the meat quality parameters of *Clarias gariepinus* (Burchell). Unpubl. PhD. thesis. University of the North, Sovenga.

Hoffman, L., Prinsloo. J., Casey, N. & Theron. J., 1994. The anatomical heterogeneity in the proximate composition, amino acid, fatty acid and mineral concentrations of muscle of the African sharptooth catfish. *S. Afr. J. Food. Sci. Nutr*. 6:30-35.

Hoffman, L., Casey, N. & Prinsloo, J., 1993. A further investigation into the fatty acid composition of the lipids of the African catfish (*Clarias gariepinus*). *S. Afr. J. Food. Sci. Nutr*. 5(2): 41-42.

Hoffman, L., Casey, N. & Prinsloo, J., 1992. Fatty acid, amino acid and mineral contents of African sharptooth catfish (*Clarias gariepinus*) fillets. *S. Afr. J. Food. Sci. Nutr*. 4(2): 36-40.

Hoffman, L. & Prinsloo, J., 1990. A comparison of the dress-out percentage of the red and normal coloured strains of the African sharptooth catfish, *Clarias gariepinus* (Burchell). *S. Afr. J. Food. Sci. Nutr. 2(2):35-38.*

Hoffman, L. & Prinsloo, J., 1995. The intrinsic variation in the chemical composition of the African sharptooth catfish, Clarias gariepinus (Burchell). I. Lipid, amino acid and mineral composition of dark and light muscle. *S. Afr. J. Food. Sci. Nutr.7:13-17.*

Houlihan, D., Boujard, T. & Jobling, M., 2001. Food intake in fish. Oxford, UK: Blackwell Science Ltd.

Huisman, E. & Richter, C., 1987. Reproduction, growth, health control and aquacultural potential of the African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture*.63 (1): 1-14.

Huss, H.H., 1995. Quality and quality changes in fresh fish. *FAO fisheries technical paper* 348. FAO. Rome.

Kang. B., Deng. J., Zhang. Xi., Baoliang. Bi. & Lingfu. K., 2011. Dietary protein requirement of juvenile Asian red-tailed catfish *Hemibagrus wyckioides*. *Anim. Feed. Sci. Technol.* 170.3 (2011): 231-238.

Kris-Etherton. P.M. Harris. W.S. & Appel. L.J., 2002. Fish Consumption, fish oil, omega-3 fatty Acids, and cardiovascular disease. *Circulation* 106 (21): 2747-2757.

Lavell, T., 1989. Nutrition and Feeding of Fish, Boston, MA: Springer US.

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): 487–92.

Love, R.M., 1970. The chemical biology of fishes: with a key to the chemical literature, Academic Press Inc., London: New York.

Murray, J. & Burt, J.R., 2001. The Composition of Fish. Ministry of Technology, Torry Research Station, Torry Research Note No. 38

Ng, W., Liew, F-L., Ang. L-P. & Wong. K-W., 2001. Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquac.Res,32:273-280.*

NRC, 1993. Nutrient Requirements of Fish, National Academies Press.

Ojgunji. J., Kloas. W., Wirth. M., Schulz. C. & Rennert. B., 2008. Housefly maggot (Magmeal) as a protein source for *Oreochromis niloticus* (Linn.). *Asian. Fish. Sci.* 21.3 (2008): 319-331.

Pillay, T.V.R. & Kutty, M.N., 2005. Aquaculture: Principles and Practices. No. Ed. 2. *Blackwell Publishing Ltd.*

Sealey, W.M. Tomberlin. J.K. McGuire, M.A. Ross, C. & ST-Hilaire, S., 2011. Sensory analysis of Rainbow trout, *Oncorhynchus mykiss*, fed enriched Black soldier fly prepupae, *Hermetia illucens*. *J.World Aquacul. Soc*. 42(1): 34–45.

SAS, 2009. Version 9.3. SAS Institute Inc., Cary, North Carolina, USA.

Shearer, K., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture*.119:63-88.

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

Steyn, N.P., Nel, J.H., Prinsloo, J.F., Tichelaar, H.Y., Dhansay, M.A., Theron, J., Hoffman, L.C. & Benade, A.J.S., 1995. Nutritional status of underweight rural preschool children consuming a diet rich in *Clarias gariepinus. S. Afr. J. Food. Sci. Nutr*. 7(4): 158-162.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., & Sealey, W., 2007. Fly prepupae as a feedstuff for Rainbow trout, *Oncorhynchus mykiss*. *J. World. Aquacult. Soc*. 38(1): 59–67.

Tidwell, J.H., 2012. Aquaculture Production Systems, John Wiley & Sons.

Waterman, J., 2000. Composition and quality of fish. *Torry Research Station, Edinburgh*.

Chapter 5

5 The effects of different inclusion levels of black soldier fly *(Hermetia illucens)* larvae meal on the intestine of African catfish *(Clarias gariepinus)* fingerlings

Abstract

Intestinal morphohistological effects of feeding black soldier fly (BSF) larvae meal to African catfish fingerlings were investigated. Four experimental diets were formulated containing either 0% (control), 10%, 20% or 30% BSF larvae meal. A total of 720 fingerlings (5 – 6 g) were randomly allocated to treatments with six replicates per treatment. Fish were fed to satiation twice daily at 10:00 and 16:00, six times a week except on the sampling days. After 91 days, six fish from each treatment were euthanized and dissected. Samples from the intestine were preserved for histological examination. No significant differences were found in height of mucosal folds and mucosal folds area between treatments. Significant differences were found in the measurements of thickness of muscular layer between treatments. The results suggest that BSF larvae meal can be incorporated up to 30% without negatively affecting intestine morphology in African catfish fingerlings, at least for a 91 days feeding period.

Keywords: intestinal morphology, black soldier fly larvae, fishmeal, African catfish

5.1 Introduction

The intestine plays an important role in the digestion and absorption of nutrients in all animal species including fish (Caballero *et al.,* 2003; Li *et al.,* 2004; Wilson & Castro 2010; Zhu *et al.,* 2012). Moreover, the intestine plays a vital role in immunity, water and electrolyte balance and regulation of digestion and metabolism (Buddington, 1996). The intestinal morphology of the fish is influenced by the type of feed administered and fish species used (Raskovic *et al.,* 2011). Thus, histological examination of the fish intestine is considered a good sign of the nutritional status of fish (Caballero *et al.,* 2003).

Plant protein sources especially soybean have been widely explored as a fish protein source as it is inexpensive and easy to obtain (Gatlin *et al.,* 2007). However, the use of plant protein in animal feed has been limited due to protein quality and amino acids profile, palatability, phosphorus bioavailability, digestibility and presence of anti-nutritional factors (Becker *et al.*, 2001b; Gatlin *et al.,* 2007; FAO, 2012). Moreover, plant protein sources have been demonstrated to induce morphological changes in fish gastrointestinal tracts especially in carnivorous fish (Baeverfjord & Krogdahl 1996; Krogdahl *et al.,* 2003). These changes include shortening of mucosal folds, widening of lamina propria of mucosal folds, enteritis, and infiltration of inflammatory cells in the lamina propria as well as loss of supranuclear vacuolisation of the absorptive cells in the intestinal epithelium (Baeverfjord & Krogdahl, 1996; Krogdahl *et al.,* 2003).

Not much attention has been given to the utilisation of insects as a source of protein in aqua feeds. However, recently, published reviews have addressed the nutritive values of various insects as a potential source of

protein that will open new perspectives in animal feeding (Van Huis, 2013; Rumpold & Schluter, 2013; Makker *et al.*, 2014; Manzano-Agugliaro *et al.,* 2014; Makker *et al.,* 2015). There is little scientific information on the use of BSF larvae meal as a protein source in fish feed. Current knowledge on the effects of BSF larvae meal on gut morphology of fish is very limited. However, investigations on intestinal health of fish have been widely performed with soybean meal on carnivorous fish (Van den Ingh *et al.,* 1991; Baeverfjord & Krogdahl 1996; Krogdahl *et al.,* 2003; Silva *et al.,* 2015), although not many studies have been carried out on omnivorous fish, in particular African catfish.

This study is novel in its approach on assessing the effects of BSF larvae meal supplementation on the intestine morphology of African catfish.

5.2 Materials and Methods

The experimental system, feed and experimental procedure were described in Chapter 3. Briefly, the study was conducted in a water recirculation system. Four dietary treatments with six replicates per treatment were used. African catfish fingerlings 5-6 g were fed to satiation twice daily different inclusion levels of black soldier fly (BSF) larvae meal for 91 days feeding.

5.2.1 Histomorphological Procedure

At the end of the feeding trial, the fish were not fed for 24 hours to empty the digestive tract. Six fish from each treatment were randomly selected euthanized and dissected. Intestinal samples were cut 2 cm away from stomach, rinsed with 9% saline solution and fixed in 10% buffered formalin solution. After fixation, the samples were subjected to routine histological techniques of trimming, dehydration in alcohol, clearing in xylene and embedding in paraffin wax. Five µm cross-sections were cut using a rotary microtome and stained with haematoxylin and eosin (H&E). Images were recorded using 4.0 magnification objective lens and a digital camera mounted on Olympus IX70 microscope. Morphometrical analyses were determined using analysis imager software. Ten measurements per individual fish were recorded for height of mucosal folds, area of mucosal folds and thickness of longitudinal muscle.

5.3 Statistical Analysis

Statistical analyses were carried out using PROC General Linear Model (GLM) procedure in SAS software (2009). Morphohistological data of the African catfish intestine were analysed using ANOVA procedure in SAS to determine if there were significant differences between treatments. The level of significance used was at p-value 0.05. Treatment effects were considered non-significant at p-value more than 0.05. If the ANOVA test was found less than 0.05, then a Bonferroni *post hoc* test for least square means was used for multiple comparisons.

5.4 Results and discussion

Morphohistological measurements of the African catfish intestine are displayed in Table 5.1.

Due to several artefacts in BSF 10% slides, it is considered an outlier. There were no significant differences ($P > 0.05$) in mucosal folds height and mucosal folds area between treatments up to 30% inclusion level of black soldier fly larvae meal. There was significant difference ($P < 0.05$) in longitudinal muscle thickness of muscularis layer.

Table 5.1 Intestinal morphology of African catfish fed experimental diets

Measurements	Control (0%)	BSF[2] (10%)	BSF (20%)	BSF (30%)	SEM[3]	P value
Height of mucosal folds (µm)	1234.3^{a}	985.0^{b}	1191.9^{a}	1272.0^{a}	35.144	<.0001
Perimeter of mucosal fold (µm)	4305.4^{b}	4007.2^{b}	4633.7^{ab}	5074.2^{a}	176.301	0.0002
Area of mucosal folds (µm)	330967^{ab}	300340^{b}	366100^{a}	355367^{ab}	16708.79	0.029
Thickness of longitudinal muscle	73.72^{a}	59.25^{b}	55.10^{bc}	48.60^{c}	1.899	<.0001

1Values are means of six fish per treatment. Values in the same row with the same superscript did not differ significantly ($P > 0.05$) from each other.

2BSFL: Black soldier fly larvae

3SEM: Standard error of the means

The results clearly show the inclusion levels of BSF larvae meal up to 30% did not induce severe morphological alteration in the intestine of African catfish. Longer mucosal folds found in fish that received 30% BSF larvae meal may indicate that BSF larvae meal promotes mucosal folds development to further enlarge surface area for nutrient absorption. The mucosal folds play a role by expanding the surface area and enhancing the absorptive capacity in fish (Wange & Cao, 2009; Zhu *et al.,* 2012). Positive correlations were found between height of mucosal folds and area of mucosal folds in all treatments. Adesulu & Mustapha, (2000) suggested that larvae meals have advantages to other ingredients in fish diet due to the easily digested nature of larvae meals.

A significant difference was observed in the longitudinal muscle thickness of the muscularis layer between treatments. There were no correlation between intestinal muscles thickness and the height or the area of mucosal folds in all treatments. The muscle layer of fish intestines have been reported to be responsible for the peristaltic movement of the digesta (Smith, 1980).

To date, studies showing the effects of BSF larvae meal on intestinal histology of African catfish are lacking. However, to our knowledge the only two studies available that investigated the digestive tract of African catfish were reported by Ikpegbu *et al*. (2013) and Hlophe & Moyo, (2014). Hlophe & Moyo, (2014) demonstrated that mucosal folds length in African catfish decreased significantly when fishmeal in the control diet was replaced by 75% moringa leaves meal and 100% kikuyu grass meal in an iso-nitrogenous and iso-caloric diets. Ikpegbu *et al.* (2013), focused on the study of the normal histological structure of the digestive tract of African catfish.

Lock *et al.* (2014) investigated the effects of BSF meal on the intestine of Atlantic salmon (*Salmo salar*). Due to the lack of similar studies in omnivorous fish, it is difficult to contrast our findings with that of existing studies.

Fish are classified according to their feeding habits as herbivore, omnivore or carnivore (Harpaz & Uni, 1999). The African catfish is classified as an opportunistic omnivore or predator (Hecht *et al.,* 1988; Graaf & Janssen, 1996). There is also a paucity of information about the influence of BSF larvae meal in the intestine of omnivore fish. On the other hand, regarding the effects of diets in the digestive tract of fish, soybean meal has been the focus of several studies that investigated the gut health of fish especially carnivorous fish. These studies demonstrated the severe negative effects induced by soybean meal inclusion on the intestinal tract (Baeverfjord & Krogdahl, 1996; Krogdahl *et al.,* 2003). Nevertheless, different fish species respond differently to anti-nutritional factors present in plant based diets. Evans *et al.* (2005) fed omnivore channel catfish iso-nitrogenous and iso-caloric diets of defatted, dehulled raw soybean meal as test diets and dehulled, solvent-extracted soybean meal as a control diet for ten weeks. They found no histological changes in fish intestines between treatments. However, a significant reduction in weight gain, feed intake and feed efficiency ratio were observed in fish fed non-heat treated raw soybean meal compared to the control diet (Evans *et al.,* 2005). When 20% of a fishmeal based control diet was replaced by soybean meal in the diet of omnivore common carp, an inflammation was observed in the distal intestine after one week. However, common carp was able to recover from the enteritis towards the end of the trial (Rombout *et al.,* 2008).

5.5 Conclusion

The findings of this study indicate no morphological differences in mucosal folds height and mucosal folds area between treatments. Black soldier fly larvae meal can be incorporated up to 30% without negatively affecting intestinal morphology in African catfish fingerlings, at least during 91 days feeding period. Further investigation is required for longer periods to validate this conclusion and to evaluate the impact of BSF larvae meal on possible histological alterations in other organs.

5.6 References

Adesulu, E.A. & Mustapha, A.K., 2000. Use of housefly maggots as a fishmeal replcaer in Tilapia culture: A recent vogue in Nigeria. 5th Inter. Symp. On Tilapia aquaculture (Fitzsimmons, K. & Filho, J.C. eds) 1:138. Rio De Janeiro, Brasil.

Baeverfjord, G. & Krogdahl, A., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L., distal intestine: a comparison with the intestines of fasted fish. *J. Fish. Dis.* 19(5): 375–387.

Becker, K., Hossain, M.A. & Focken, U., 2001b. Evaluation of an unconventional legume seed, Sesbania aculeata, as a dietary protein source for common carp, *Cyprinus carpio* L. *Aquaculture.* 198:129-140.

Becker, K., Francis, G. & Makker, H.P.S., 2001b. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*. 199: 197-227.

Buddington, R., 1996. The intestines of carnivorous fish: structure and functions and the relations with diet. *Acta physiol. Scand. Suppl*. *638*: 67-80

Caballero, M.J., Izquierdo, M.S., Kjorsvik, E., Montero, D., Socorro, J., Fernandez, A.J. & Rosenlund, G., 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. *Aquaculture*. *225*(1): 325–340.

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.

Evans, J.J., Pasnik, D.J., Peres, H., Lim, C. & Klesius, P.H., 2005. No apparent differences in intestinal histology of channel catfish (*Ictalurus punctatus*) fed heat-treated and non-heat-treated raw soybean meal. *Aquac. Nutr*, 11(2):123–129.

FAO (Food & Agriculture Organisation), 2012. *The State of World Fisheries and Aquaculture 2012*,

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

Graaf, G. de & Janssen, H., 1996. Artificial reproduction and pond rearing of the Arican catfish *Clarias gariepinus* in sub-Saharan Africa. *FAO fisheries technical Paper* 362 (1996).

Harpaz, S. & Uni, Z., 1999. Activity of intestinal mucosal brush border membrane enzymes in relation to the feeding habits of three aquaculture fish species. *Comp.Biochem.Physiol. Part a124*:155-160.

Hecht, T., Uys, W. & Britz, P.J., 1988. The culture of sharptooth catfish, *Clarias gariepinus* in Southern Africa. *South African National Scientific Programme Report no.153*.

Hlophe, S.N, & Moyo, N.A.G., 2014. Replacing Fishmeal with Kikuyu grass and Moringa leaves: Effects on growth, protein digestibility, histological and haematological parameters in Clarias. *Turk. J. Fish. Aquat. Sci.*14(2): pp.22.

Ikpegbu, E., Ezeasor, D.N., Nwogu, C., Nlebedum, U.C., Nnadozie, O., & Agbakwuru, I.O., 2013. The structure and functional significance of branching anastomosing mucosal folds in the proximal intestine of the domesticated African catfish (*Clarias gariepinus* Burchell 1822).*World. Appl. Sci. J.* 28 (11): 1815-1818.

Krogdahl, Å., Bakke-McKellep, A.M. & Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities and pancreatic response in Atlantic salmon (*Salmo solar* L.). *Aquac. Nutr*, 9(6): 361–371.

Li, M.H., Robinson, E.H. & Manning, B.B., 2004. Nutrition. In Biology and culture of channel catfish. Eds. Tucker, C.S., & Hargreaves, J.A. Vol 34. Elsevier.

Lock, E., Arsiwalla, T. & Waagbø, R., 2014. Insect meal: A promising source of nutrients in the diet of Atlantic salmon (Salmo salar). *Abstract Book Conference Insects to Feed The World. The Netherlands, 14-17 May 2014.*

Makker, H.P.S., Tran. G. & Heuze, V., 2015. Insects in fish diets. *Animal Frontiers*. 5(2): 37-44

Makkar, H.P.S., Tran, G., Heuze, V. & Ankers, P., 2014. State-of-the-art on use of insects as animal feed. *Anim. Feed. Sci. Technol*. 197: 1-33In press.

Manzano-Agugliaro, F., Sanchez-Muros, M.J., & Barroso, F.G., 2014. Insect meal as a renewable source of food for animal feeding: a review*. J. Cleaner. Production*.65:16-27.

NRC, 1993. Nutrient Requirements of Fish, National Academies Press.

Olsen, R.L. & Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends. Food. Sci. Tech*. 27(2): 120–128.

Rašković, B. & Stanković, M., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. *J. Agr. Sci, Belgrade* 56.1 (2011): 87-100.

Rombout, J.H.W.M., Uran, P.A., Goncalves, A.A., Taverne-Thiele, J.J., Schrama, J.W. & Verreth, J.A.J., 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish. Shellfish. immunol*. 25:751-760.

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

SAS, 2009. Version 9.3. SAS Institute Inc., Cary, North Carolina, USA.

Sealey, W.M. Gaylord, T.G. Barrows, F.T. Tomberlin, J.K. McGuire, M.A. Ross, C. & St-Hilaire, S., 2011. Sensory analysis of Rainbow trout, *Oncorhynchus mykiss*, fed enriched Black Soldier fly prepupae, *Hermetia illucens*. *J. World Aquacult. Soc*. 42(1):34–45.

Silva,P.F., McGurk, C., Knudsen, D.L., Adams, A., Thompson, K.D. & Bron, J.E. 2015. Histological evaluation of soybean-induced enteritis in Atlantiv salmon (*Salmo salar*): quantitative image analysis vs semi-quantitative visual scoring. *Aquaculture 445:42-56.*

Smith, L.S., 1980. Digestion in teleost fishes. In FAO, aquaculture development and coordination programme. *Lectures presented at the FAO/UNDP Training Course in fish feeding technology, ACDP/REP/80/11* (1980): 3-17

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W., & Sealey, W., 2007. Fly prepupae as a feedstuff for Rainbow trout, *Oncorhynchus mykiss*. *J. World Aquacult. Soc*. 38(1): 59–67.

Tacon, A., 1995. Feed ingredients for carnivorous fish species: alternatives to fishmeal and other fishery resources. *Sustainable fish farming*. 89-114.

Van den Ingh, T.S.G.A.M., Krogdhal, a., Olli, J.J., Hendriks, H.G.C.J.M. & Knoninkx, J.G.J.F., 1991. Effects of soybean containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture 94:297-305.*

Van Huis, A., 2011. Potential of Insects as Food and Feed in Assuring Food Security. *Annu. Rev. Entomol.* 563-583.

Wange, M.W., & Cao, X.J., 2009. Histology and mucin histochemistry of the digestive tract of yellow catfish (*Pelteobagrus fulvidraco*). *Anat. Histol. Embryol*. 38:254-261.

Wilson, J.M. & Castro, L.F.C., 2010. Morphological diversity of the gastrointestinal tract in fishes. *Fish. Physiol*. *30*: 1-55

Zhu, H., Liu, H., Yan, J., Wang, R. & Liu, L., 2012. Effect of yeast polysaccharide on some hematologic parameter and gut morphology in channel catfish (*Ictalurus punctatus*). *Fish. Physiol. Biochem*. 38(5):1441–1447.

Chapter 6

6 General Conclusion

Aquaculture needs to reduce reliance on fishmeal in order to grow sustainably. The findings of this study revealed the nutritional capacity of BSF larvae meal as a dietary protein source that could supplement fishmeal or conventional protein sources in the diet of African catfish.

In the present study, the data reported for production parameters when BSF larvae meal were incorporated up to 30% inclusion levels in the diets of African catfish fingerlings showed significant difference in the final weights between treatments. This study suggests that, should the unprocessed BSF larvae meal be incorporated in the diet of African catfish, the inclusion level should not exceed 10%. Moreover, this study demonstrated that BSF larvae meal were efficiently utilised and had supported the growth of the catfish.

The data reported on the effects of BSF larvae meal on the proximate body composition showed no significant difference between treatments. This investigation also shows neither dressing percentages nor proximate body compositions (in terms of percentage moisture, protein, lipid and ash) were negatively affected at all studied inclusion levels of BSF larvae meal diets. Similarly, no morphological changes were induced in mucosal fold height and mucosal fold area at all studied inclusion levels of BSF larvae meal diets.

Thus, the larvae meal of this non-pest fly species has proven to be a viable protein source in the diet of African catfish. As the cost of conventional protein sources such as fishmeal and soybean meal continues to increase, it will only be a matter of time before the economic advantages of producing BSF larvae meals as a cheap source of protein becomes apparent to many aquaculturists and feed manufacturers.

Suggestions for future research:

In this study the reduced growth at BSF20% and BSF30% inclusion levels was suggested to be caused by the high fat content in the unprocessed BSF larvae that may have affected nutrient digestibility. As a result, a viable option is the defatting of BSF larvae in subsequent investigations and the evaluation thereof to determine the optimum dietary inclusion levels as well as conducting a digestibility study in African catfish. Moreover, future studies with BSF larvae should also investigate haematological studies as well as liver histology in African catfish.

This investigation was conducted for 91 days. Further studies should consider long term feeding trials with fish approaching marketable size. Additionally, with regards to consumers' perception of fish as a healthy food, future studies with BSF larvae meal should evaluate organoleptic properties of fish flesh as well as consumers acceptance.