EFFECTS OF EXTRUSION PROCESSING ON THE PHYSICO-CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF FISH FEEDS CONTAINING *Acheta domesticus AND Hermetia illucens* MEALS

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A Thesis Submitted to Graduate School in Partial Fulfillment of the Requirements for Masters of Science in Food Science of Egerton University

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not, wholly or in part, been presented for the award of a degree in any other university.

Signature…………………… Date…......................

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KM16/14331/15

RECOMMENDATION

This thesis is the candidate's original work and has been prepared with our guidance and assistance. It has been submitted with our approval as the official university supervisors.

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DEDICATION

This Thesis is dedicated to my parents; Mr. and Mrs Irungu and my grandparents; Mr. and Mrs. Ndegwa.

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ABSTRACT

Fish farming in Kenya is faced with the challenge of high cost of feeds because of the high cost of fish meal, an important protein ingredient incorporated into feeds. Thus, there is the urgent need for cheaper alternative protein sources that have similar or higher protein quality to partially or completely substitute fish meal in feeds. The effects of substituting fresh water shrimps meal (FWSM) with black soldier fly larvae meal (*Hermetia illucens* – BSFM) or adult cricket meal (*Acheta domesticus* – ACM) on physico-chemical and nutritional properties of hot-extruded fish feed pellets were investigated, prior to optimizing processing conditions of the extruder. The FWSM in a basic 30% protein fish feed formulation was substituted at 0% (control), 25%, 50% and 75% and moisture content of the formulation adjusted to 10%, 20% or 30%. Black soldier fly larvae meal was significantly ($P < 0.05$) higher than ACM and the FWSM on dry matter, fibre content and ether extract. Adult cricket meal was significantly ($P < 0.05$) higher than FWSM and BSFM on crude protein and *in vitro* protein digestibility.

Optimization process gave optimum combination of extruder temperature, die diameter and feed pre-conditioning time of 120°C, 2 mm and 100 s respectively. The addition of both insect-based meals did not show significant effect on the physico-chemical properties of the extrudates ($P < 0.05$). The level of substitution, however, significantly influenced floatability ($P < 0.001$), bulk density ($P < 0.001$) and water stability ($P < 0.01$). Moisture content of formulation also significantly affected ($P < 0.05$) all the studied physico-chemical properties apart from durability index ($P < 0.05$) and water absorption index ($P < 0.05$). Level of FWSM substitution did not significantly ($P < 0.05$) influence dry matter, crude protein and crude fibre content of formulations. The level of feed moisture content influenced ($P < 0.05$) dry matter, ash content, crude protein, ether extract and carbohydrate content but did not affect ($P < 0.05$) fibre content and *in vitro* protein digestibility. Increasing the level of FWSM substitution from 0% to 75% led to a significant ($P < 0.05$) increase in the levels of phosphorus and potassium while significantly ($P < 0.05$) reduced calcium and sodium levels. Extruded pellets containing 75% BSFM or 75% ACM had desirable physico-chemical and nutritional properties when feed moisture content was increased to 30%. The findings from this study inform fish feed manufacturers and fish farmers that they can substitute freshwater shrimps with either crickets or black soldier fly larvae meals in fish feed to achieve better physico-chemical and nutritional properties.

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ACRONYMS AND ABBREVIATIONS

ACM	Adult cricket meal
AOAC	Association of Official Analytical Chemists
BD	Bulk density
BSFM	Black Soldier Fly Larvae Meal
ER	Expansion ratio
FAO	Food and Agriculture Organization of the United Nations
FDKS	Final Draft Kenya Standard
FWSM	Fresh water shrimp meal
IVPD	*in vitro* protein digestibility
KALRO	Kenya Agricultural and Livestock Research Organization
LA	Lectin activity
L/D	Length to diameter ratio
NSI	Nitrogen solubility index
PDI	Pellet durability index
RPM	Revolutions per minute
SSE	Single screw extruder
TDS	Total dissolved solids
TIA	Trypsin inhibitor activity
TSE	Twin screw extruder
TSS	Total suspended solids
WAI	Water absorption index
WSI	Water solubility index
WS	Water stability

CHAPTER 1: INTRODUCTION

1.1: Background information

Food insecurity is a threat to many nations especially the developing countries. In fact, 10.9% of world population (795 million people) is undernourished (FAO, 2015). This situation is worse in the sub-Saharan Africa. In East Africa, about 31.5% of the population (124 million people) is undernourished. According to FAO (2015), East Africa is second to Central Africa, which has 41.3% of its population being undernourished, in terms of regions that are hardly hit by food insecurity. *Van* Huis *et al*. (2013) noted that food insecurity is caused by among other factors the rapidly growing population coupled by the depletion of resources. However, poor nutrition also contributes to food insecurity (Durst *et al*., 2010). The major factor of poor nutrition is actually lack of adequate protein (FAO, 2015). Depletion of resources and rapid urbanization contribute to high prices of the available meat as a source of protein. In addition, livestock, pigs and poultry, the main sources of protein meat worldwide, depends on resources such as availability of feeds. However, Anderson and Gabrielsson (2012) affirm that the cost of feeds for livestock and poultry is relatively high which make them inaccessible by majority of the farmers. This is negatively affecting food security in developing regions which in effect leads to continued poverty.

Protein from fish is significantly bio-available than it is in livestock (Neira *et al*., 2009). However, the reliability of fish protein is hampered by the high cost of fish feeds (Njagi *et al*., 2013). One can therefore agree that there is a need for an alternative source of protein that is cheap and readily available. According to *van* Huis (2013), some of the potential alternative sources of protein include cultured meat, seaweed, vegetables, fungi, and mini-livestock. Edible insects such as field crickets (*Gryllus pennyslvaniccus*), house crickets (*Acheta domesticus*) and black soldier fly (*Hermetia illucens*) are examples of mini-livestock that could be useful alternative sources of protein (FAO, 2015). They have the potential to provide protein directly to human beings or indirectly through improvement of animal protein that is used for human food. Traditionally, insects have been consumed as food or delicacies in many communities such as in Thailand and Uganda. In Kenya, the Luhya community is associated with consumption of such insects as termites (Kinyuru *et al*., 2010). The practice of eating insects (entomophagy) dates back to thousands of years. However, in modern times, consumption of insects has drastically declined in many societies (Durst *et al*., 2010) as the practice is viewed as being primitive.

Insects can be fed to fish to increase their protein level. This is due to the fact that insects are natural feed sources for fish. In Asia and the Pacific, grasshoppers, crickets, cockroaches, termites, lice, stink bugs, cicadas, aphids, scale insects, psyllids, beetles, caterpillars, flies, fleas, bees, wasps and ants have all been used as complementary feed sources for fish (Ravindran & Blair, 1993). The amino acids derived from most insects' protein are superior to those from plant supplements in poultry feed formulations (Ravindran & Blair, 1993; Bukkens, 2005). In addition, various insect species have a higher proportion of protein content compared to conventional fish and soybean meals (Bukkens, 2005). Since protein is regarded as the most expensive ingredient in the diets of fish, feeding fish with insects appears to be an economically viable option. Furthermore, insects can be used to convert different types of organic waste materials into animal biomass rich in proteins which can later be used in animal nutrition, additionally providing environmentally friendly organic waste recycling opportunities (Ramos-Elorduy & Blair, 2005). Additionally, chitin, a polysaccharide found in the exoskeleton of insects is reported to have an enhancing effect on the functioning of the immune system of different organisms (*van* Huis *et al.*, 2013).

Nevertheless, insects as sources of fish feed remain underappreciated in most parts of the world including sub-Saharan Africa. While few reported cases are available from Angola, Benin, Burkina Faso, Cameroon, Nigeria, Democratic Republic of Congo and Togo, on the use of termites, house flies and cockroaches as fish feed (Ekoue & Hadzi, 2000; Téguia *et al.*, 2002; Munyuli Bin Mushambanyi & Balezi, 2002), very limited research has been conducted in East Africa to exploit insects as an alternative protein source in feeds. In Uganda, for example, only 5% of farmers use termites for feeding fish – either collecting the termites directly or purchasing them from local harvesters (Rutaisire, 2007). Given insects' natural role as feed for a number of livestock species and fish, it is worth reconsidering their role as feed for fish and to understand their contribution to the improvement of feed production. However, a key element to consider for the successful introduction of insects into the feed chain is that multiple quality aspects that relate to nutrition and safety could be influenced by processing, packaging, handling, as well as storage methods. Insects may harbor a number of hazards of microbial, parasitical, chemical and even allergenic nature. Consequently, it is necessary that effective decontamination procedures are developed and employed in order to ensure food and feed safety.

1.2: Statement of the problem

Poor nutrition contributes to food insecurity due to lack of adequate protein, a major factor of poor nutrition. Fish is particularly a superior protein source because, unlike poultry and beef, it is not prone to antibiotics that can accelerate drug-resistance development in human pathogenic bacterial strains (*van* Huis *et al.,* 2013). For this reason, aquaculture has become a fast-growing food production sub-sector to increase the supply of fish available for human consumption. About 80% of the world's aquaculture is practiced in developing countries mainly by small-scale farmers (FAO, 2016). Consequently, there is growing appreciation of the role small-scale aquaculture can play in rural household food, nutrition and income security in these countries. However, this protein source is scarce, expensive and proving to be accessible only to a small fraction of human population because of the high cost of fish feeds. Lack of access to affordable high-quality feeds limits the participation of many smallholder fish farmers, and threatens aquaculture profitability and sustainability (Munguti *et al*., 2014). Therefore, there is the need for alternative sources of protein ingredient in feeds that are cheap and readily available.

1.3: General objective

To contribute to food security in Kenya by incorporating edible insects as alternative sources of affordable high quality ingredients into fish feed through extrusion processing.

1.4: Specific objectives

1. To optimize extrusion cooking conditions for the manufacture of insects-based feed extrudates with respect to feed conditioning time, extruder die diameter and extrusion temperature.
2. To determine the effects of extrusion variables on the physico-chemical properties of the extruded products containing edible insects' meals.
3. To determine proximate composition, *in vitro* protein digestibility and minerals content of the extruded products as influenced by edible insects' meals.

1.5: Hypotheses

1. H_1: Feed conditioning time, extruder die diameter and extrusion temperature do not affect the extrusion of feed ingredient blends.
2. H_2: Extrusion variables do not affect the physico-chemical properties of the extruded products containing edible insects' meal.
3. H_3: Edible insects' meals do not affect the proximate composition, *in vitro* protein digestibility and minerals content of the extruded products.

1.6: Justification

This study is justified on the need to provide sources of proteins that are cheap and readily available. The success of this project is based on the fact that insects such as *Acheta domesticus* and *Hermetia illucens* are readily available and thus the composites formulated with these insects will be cheap. Extrusion process has been in use for many years in the production of pellets for use as feeds. Optimization of parameters in extrusion process can allow for the incorporation of insects as protein substitutes. Once incorporated as protein source into other feed raw materials sourced locally, the extruded products have the potential of providing same or higher nutritional value as conventional fish feeds but at a low cost, thus help to improve aquaculture productivity as a source of high quality protein for better nutrition and food security.

1.7: Outputs

1. A Master's Thesis
2. Findings presented in two international conferences:
    - ✓ 11TH Egerton University International Conference and Innovation Week. Theme: Knowledge and Innovation for Social and Economic Development, held at FEDCOS Complex, Njoro Campus, Egerton University - Kenya on 29th to 31st March, 2017. This paper won the best paper presentation award under the subtheme: Food Security.
    - ✓ 1st All Africa Postharvest Congress and Exhibition. Theme: Reducing food losses and waste; Sustainable solutions for Africa, held at Safari Park Hotel, Nairobi, Kenya on 28th – 31st March, 2017.
3. Two publications in peer-reviewed journals.
4. Three submitted manuscripts for publications in peer-reviewed journals.

CHAPTER 2: LITERATURE REVIEW

2.1: Insects as source of food and feed

Insects have both nutritional and economic value in many cultures across the world and thus a wide range of insect species are collected in the wild and eaten or used to feed animals. DeFoliart (1999) reported that some 209 insect species were being consumed either as delicacies or as components of the daily diets. However, Kelemu *et al.* (2015) reports over 470 species that are being consumed by mankind in Africa alone. In the western part of Kenya, black ants, lake flies, termites and grasshoppers have traditionally been consumed as meals (Ayieko *et al.*, 2010). As animal feed, insects have been used to feed fish, poultry and pigs. Dried termites, desert locust and migratory locusts have been shown to satisfactorily feed catfish fingerlings, African catfish and Nile tilapia respectively (Sogbesan and Ugwumba, 2008; Balogun, 2011; Emehinaiye, 2012). Termites have also been fed to guinea fowls and chicken in Togo (Farina *et al.*, 1991), Burkina Faso (Kenis *et al.*, 2014), Uganda (Rutaisire, 2007) and Democratic Republic of Congo (Munyuli Bin Mushambanyi and Balezi, 2002). In Nigeria, Black soldier flies were included in broiler rations without adversely affecting feed intake, weight gain or growth rate (Ijaiya and Eko, 2009). Adeniji (2008) demonstrated that maggot meal could be fed to weaned piglets without having any adverse effects.

2.2: Traditional processing of insects as food or feed

Traditionally, insects have been processed using different methods. After harvest, Mopane caterpillar is normally sun-dried with the aim of achieving higher shelf-life. In China, silkworm pupa, the most widely consumed insect, is normally fried in oil, boiled using water or in some cases milled into powder (Belluco *et al*., 2013). Other traditional processing methods of edible insects include, roasting, smoking, boiling as well as removal of unpalatable parts (Rumpold and Schluter, 2013). Sun-drying is cheap and easy to carry out. However, it is dependent on weather conditions and therefore is hampered by such climatic conditions as wet weather, strong winds as well as mist and fogs. Majority of these methods have far reaching negative impacts. Drying and toasting of termites and grasshoppers have been attributed to significant decrease in contents of individual vitamins including Pyridoxine, Folic acid, Ascorbic acid, Niacin, Riboflavin, Retinol, and α-Tocopherol depending on the insect species (Kinyuru *et al*., 2010). Additionally, the *in vitro* digestibility of proteins in tree locust has been shown to decline upon toasting and boiling probably because of the interaction of the protein with tannin and phytate present in the insect.

According to Afiukwa and Okereke (2013), removal of wings as one of the unpalatable insects' parts reduces the amount of iron that can be obtained from *Trinervitermes germinatus* (termite) - an edible insect from Nigeria. Thus, although these methods have been used to process edible insects in the past, they present a situation where their reliability is questioned (Chakravorty *et al.*, 2014).

2.3: Extrusion processing

Extrusion is the process in which the material (feed mixture) is pushed through a barrel by means of screws of different configurations and pressed through the die at the end of barrel (Levic and Sredanovic, 2010). High temperature short time (HTST) is the principle that governs the operation of an extruder. As a process, extrusion combines various operations such as cooking, mixing, shearing, kneading, shaping and forming (Aruna, 2005). According to Rokey *et al.* (2010), extrusion was first used to produce dry expanded pet foods in the 1950s. The method has so far being widely accepted to produce many tonnes of animal food throughout the world. It is an efficient method of producing pet and aqua feed (Moscicki, 2011). In the food industry, extrusion is used in cereal as well as in protein processing (Aruna, 2005). Examples of cereal based products that are produced through extrusion include weaning foods, pasta products, puffed breakfast cereals, expanded snack foods, crisp bread, pre-cooked composite flours and modified starches. The protein-based products that are made using extrusion process include caseinates, processed cheese, sausage products, surimi, texturized vegetable protein, pet foods and animal feeds. The benefits of extrusion process include the ability to gelatinize starch, destroying anti-nutritional factors, increasing the solubility of dietary fibres, reduction of lipid oxidation, retention of natural colors and flavors in final product as well as the denaturation of the undesirable enzymes (Nikmaram *et al*., 2015). The amounts of carbohydrate, protein, fibre and fat used in preparation of fish pellets plays a key role in the functional properties of the final product. Starch is the primary carbohydrate that is found in fish feed and can have levels varying from 5% to 60% (Rokey *et al*., 2010). The expansion and binding properties of the final fish feed is dependent on the type and amount of starch in the product. It is the amylose part of the starch that has higher binding properties than the amylopectin part of the starch (Aruna, 2005). Therefore, starches with higher amylose such as tuber starches (potato and tapioca) are preferred for use as binders as they improve the cohesion of the ingredients. Pre-cooked starches are also good binders though they are highly expensive (Rokey *et al*., 2010).

2.3.1: Classification of extruders

Extruders are classified in terms of operations as well as based on the type of construction. Classification based on operation results into hot and cold extruders (Aruna, 2005). In hot extrusion, the food or feed material is cooked at temperatures above 100^{o}C. This method is also known as extrusion cooking and is used to produce snack foods and ready-to-eat puffed foods. In cold extrusion, the temperature of the material being extruded remains at ambient level. This is the method used to produce pet foods as well as fish pastes. Based on their construction method there is the single screw as well as double (twin) screw extruders (Riaz, 2010; Aruna, 2005).

2.3.2: Single screw extruders (SSE)

These are extruders that have a single compressive screw with decreasing channel depth turning at high speeds to increase shear and mechanical energy input for heating (Riaz, 2010). The screw is normally contained in a barrel and has three process sections: feeding section, compression/transition section and metering section (Bouvier, 2010). The feeding section has deep channel (large screw pitch) to facilitate high transport capacity of raw materials. The transition section has a channel of decreasing pitch to facilitate compression and densification of the materials. In addition, inter-particular friction coupled with conductive heat transfer melts the material changing its state from solid particulate to viscous fluid state. Metering section has small channel depth to facilitate strong shearing of the molten dough. This is the section, also, where buildup of pressure needed to convey the feed through the die opening occurs as a result of die restrictions (Bouvier, 2010). A pre-conditioner is sometimes used to pre-heat ingredients through the use of a steam (Akdogan, 1999). Single screw extruders have been further categorized into cold forming (pasta type) extruder, high-pressure forming extruder, low shear cooking extruder, collet extruder and high shear cooking extruder (Bouvier, 2010).

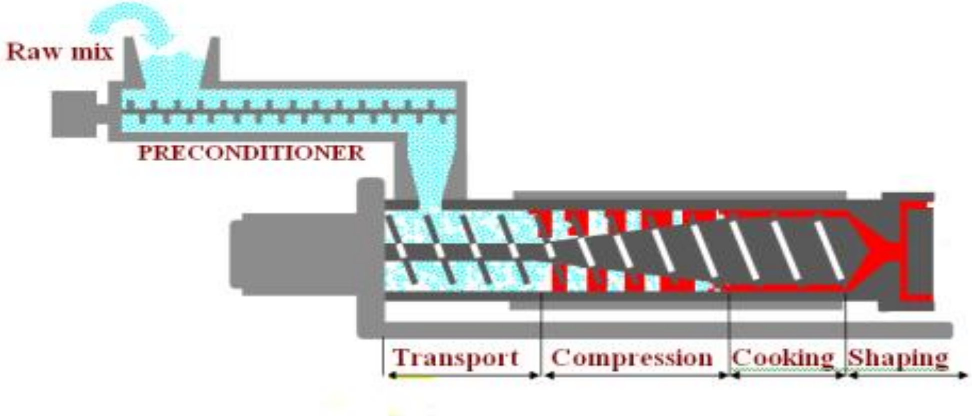

Figure 2.3-1: A single screw extruder (Source; Bouvier, 2010)

2.3.4: Twin screw extruder (TSE)

A twin screw extruder (TSE) has two parallel screws encased in a barrel with a figure 8 cross section (Martin, 2013). Twin screw extruders produce a more uniform product than a single screw extruder does (Riaz, 2010). However, they are more expensive than the single screw extruder (SSE). Categories of twin screw extruders include the non-intermeshed co-rotating, non-intermeshed counter rotating, intermeshed co-rotating and intermeshed counter rotating. It is the intermeshed co-rotating twin screw that has gained more acceptances in the food and feed industries

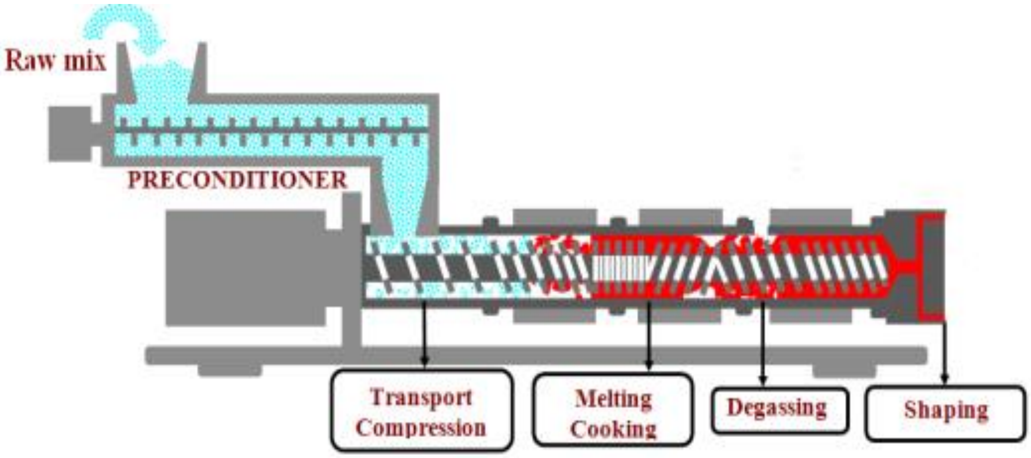

Figure 2.3-2: A twin screw extruder (Source; Bouvier, 2010)

A twin screw extruder has two shafts that are splined and that hold modular screw elements with a constant channel depth (Martin, 2013). The outside diameter (OD) and internal diameter (ID) are important dimensions that are used to characterize TSE. In a TSE, the ID and OD remain constant throughout the screws. The flight depth as well as the free volume in the process section of a TSE is determined by the OD/ID ratio. The ratio of the length to diameter (L/D) of the barrel determines the length of the barrel process. A higher L/D ratio will give a higher barrel length process. Dispersive and distributive mixing are enhanced by a TSE thus a homogeneous mixture is obtained using this equipment (O'Keefe & Newman, 2011). In dispersive mixing, the rotating screws subject liquid droplets (agglomerates) that are held together by interfacial tension, to mechanical strength in order to achieve size reduction. Repeated rearrangement of the minor components achieves distributive mixing and thus a homogeneous mixture. Figure 2.3-3 below shows the dispersive and distributive mechanisms of a twin screw extruder.

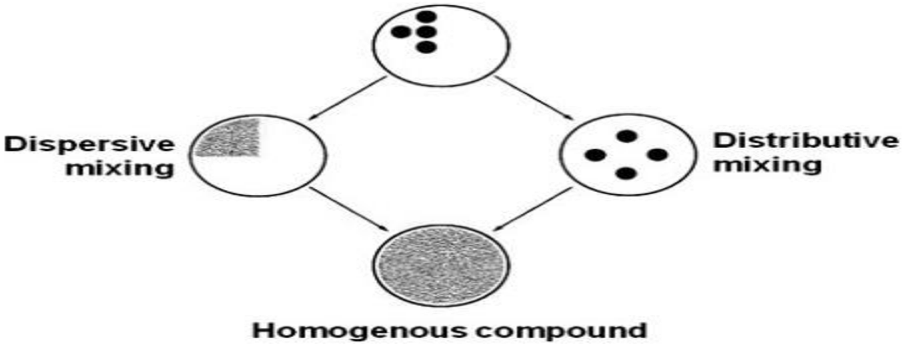

Figure 2.3-3: Dispersive and distributive mixing of a TSE (Source: Martin 2013)

A TSE has reverse pitches for shearing as well as positive pitches for venting. Intense mixing is obtained by use of mixing disks. Screws in a twin screw extruder interpenetrate each other thus creating a positive movement of the material. The parameters that are controlled in a TSE include the screw speed, process temperatures, feed rate, vacuum level and exit die diameter (Singh & Muthukumarappan, 2014).

2.3.5: Comparison between TSE and SSE

Unlike the single screw extruder, a twin screw extruder achieves a high level of micro-mixing and thus the equipment provides effective physico-chemical reactions. In addition, a TSE does not result in stagnant zones like those found in SSE (Martin, 2013). In a TSE, it is possible to achieve the desired processing conditions such as the degree of mixing, heat transfer, distribution of residence time, shear time, temperature of extrusion and the screw barrel assembly (O'Keefe & Newman, 2011). The throughput of a TSE remains as desired even when screws wear out. This is different from the SSE whose throughput is affected by wearing of the screw (Martin, 2013). Table 2.3-1 gives the comparison between single screw extruder and the twin screw extruder.

Table 2.3-1: Comparison of twin screw and single screw processing

Characteristics	**Twin Screw Extrusion**	**Single Screw Extrusion**
Initial Investment Cost	Higher investment cost (TSE:SSE = 1.4:2)	Smaller investment cost
Process Operation		
Wear cost	Slightly higher wear cost (TSE:SSE = 1.1:1.5)	Slightly lower wear cost
Energy	Large range of mechanical energy input	Low mechanical energy input
Process Flexibility		
Process Sections	Multiple	Single
Operating points	Multiple	Single
Recipe flexibility	Large range of raw materials and recipes	Tight specifications of raw materials and recipes
Process Performance		
Throughput consistency	Very low sensitivity to recipe composition and wear	Very sensitive to recipe composition and wear
Mixing degree	Good	Poor
Mechanical energy cost	From low to high	Low
Corrective heat transfer	Good	Poor
Residence time distribution	Relatively low dispersion	Relatively high dispersion
Shear rate and strain	Uniform	Non uniform
Melt characteristics		
Cooking extent	Uniform and complete	Non-Uniform, may be incomplete
Melt temperature	Uniform	Non uniform
Melt Composition	Homogenous	Heterogeneous
Physico-chemical binding	Good	Incomplete

Source: Bouvier (2010).

2.3.6: Effects of extrusion on extruded food/feed products

Extrusion results into a product with improved nutritional value and improved organoleptic properties (Alam *et al*., 2016). In addition, the microbial count in the extruded composite has been found to be low as shown in Table 2.3-2. The food/feed product obtained through hot extrusion can thus be said to be microbiologically safe (Slavko *et al*., 2010).

Table 2.3-2: Microbiological analysis of extruded mixture of "wild forage fish" and soya grits

Micro-organisms	Amount in sample (in grams)	No. in extruded dry matter
Salmonella spp.	50	0
Coagulase-positive staphylococci	50	0
Sulphite reducing clostridia	1	0
Proteus species	50	0
Escherichia coli	50	0
Total Yeast	1	0

Source: Slavko *et al*. (2010).

Unlike cooking and roasting, extrusion is an effective method of inactivating trypsin inhibitor activity (TIA), lectin activity (LA) and urease activity. (Levic and Sredanovic, 2010; Slavko *et al*., 2010; Sorensen, 2009) as shown by Tables 2.3-3 and 2.3-4.

Table 2.3-3: The influence of various heat treatments on inactivation of TIA and LA

	Inactivation	
Heat Treatment	**TIA [%]**	**LA[%]**
Cooking (100^{0}C; > 15MIN	65-97	90-100
Cooking under Pressure (121^{0}C; > 15Min)	85-100	99-100
Roasting (different temperatures and time)	54-82	85-99
Extrusion (145^{0}C; > 16s	78-98	93-98

Source: Levic and Sredanovic (2010).

Table 2.3-4: Quality indicators relevant to assessing adequacy of applied heat treatment

Quality Parameter	Soybeans	Extruded full fat soya grits
Trypsin Inhibitor (mg/g)	61.66	3.27
Urease Activity (mgN/g/min at 30^0C)	10.95	0.26
Nitrogen solubility index NSI (%)	65.82	25.64

Source: Levic and Sredanovic (2010).

Extrusion has very minimal influence on the nutrient content of food and feed (Slavko *et al*., 2010). However, this process often leads to loss of vitamins particularly water soluble and heat sensitive vitamins. Vitamin C is the most affected vitamin where after extrusion, only about 20% of the initial vitamin is retained (Sorensen *et al*., 2010: Moscicki, 2011). The B-complex vitamins also experience considerable loss and especially vitamin B1, B2 and Niacin (Moscicki, 2011). Vitamins A, D and E are also lost during extrusion though in minimal amounts. Table 2.3-5 shows the effect of extrusion on vitamins.

Table 2.3-5: Effects of the extruding process on vitamin activity

Vitamins	Remaining Activity (%)
Vitamin A	80
Vitamin D3	75
Vitamin E	80
Vitamin K3	20
Vitamin B1	90
Vitamin B2	>95
Vitamin B6	>95
Vitamin B12	>95
Biotin	>95
Folic Acid	>95
Nicotine Acid	>95
Pantothenic acid	>95
Vitamin C crystal	25
Vitamin C polyphosphate	-

Source: Levic and Sredanovic (2010).

It is estimated that 15-20% of the vitamins in the raw materials are lost during extrusion. To improve content of vitamins and other heat labile nutrients, coating of extrudates with films containing these nutrients may be done in some instances. In addition, extrusion has been shown to cause maillard reactions on the final product (Nikmaram *et al*., 2015). As a result, extreme extrusion cooking conditions may lead to loss of the nutritional value of proteins.

CHAPTER 3: MATERIALS AND METHODS

3.1: Research site

Extrusion trials were performed using a small-scale fish feed extruder at a local cottage fish feed processing facility owned by a group of small-scale fish farmers in Vihiga County, Kenya. This cottage facility formulates and processes the feed using locally obtained ingredients that include rice bran, wheat pollard, wheat bran, maize germ, maize bran, cotton seed cake, soybean, sunflower cake, fresh water shrimps and cassava for its group members who farm Tilapia, *Oreochromis niloticus* (L.) and Catfish, *Clarias gariepinus* (Burchell). Laboratory analyses were carried out at the Department of Dairy and Food Science and Technology, Egerton University, Kenya, and Kenya Agricultural and Livestock Research Organization KALRO Njoro and Naivasha centers.

3.2: Materials

Sunflower cake, maize germ, wheat pollard, dried fresh water shrimps and dried cassava chunks were purchased from a local vendor in Luanda town, Vihiga County. Blanched and sundried black soldier fly larvae (*Hermetia illucens* [L.]) were obtained from Sanergy Limited, a commercial insect rearing farm based in Nairobi, Kenya, while blanched and sundried adult house crickets (*Acheta domesticus* [L.]) were purchased from farmers in Homa Bay County, Kenya. Each ingredient was separately ground into fine meal using a hammer mill (Model 4, ARTHUR H. THOMAS, Philadelphia, PA, USA) and passed through a 1.0 mm aperture sieve.

3.3: Formulation of fish meal substituted blends

Based on the proximate composition of ingredients, the quantities required to formulate 5 kg of iso-proteinous blends (30.00±0.75 protein) were calculated using Microsoft Excel® function on Windows 2007. The amount of water needed to achieve desired moisture content was calculated, weighed, and added to the other ingredients in 20 L plastic bucket. These were mixed manually by hands for 2 min, then transferred to a multi-vane paddle mixer (Model: MX – 25, Unitech, New Delhi, India) and mixed further for 2 min at moderate speed. Compositions of the various blends are given in Table 3.3-1.

Table 3.3-1: Inclusion levels of insects' meals in fish meal substitution

Ingredient	Control	Inclusion level of insects (%)					
		BSFM25	BSFM50	BSFM75	ACM25	ACM50	ACM75
Maize germ	19	18.5	18.1	17.6	19.2	19.4	19.6
Sunflower cake	19	18.5	18.1	17.6	19.2	19.4	19.6
Cassava flour	5	4.9	4.8	4.6	5	5.1	5.2
Wheat pollard	28.5	27.8	27.1	26.5	28.8	29.1	29.4
FSWM	28.5	20.9	13.6	6.6	21.6	14.5	7.3
BSFM	-	9.5	18.4	27.1	-	-	-
ACM	-	-	-	-	6.3	12.5	19.1

FWSM: fresh water shrimp meal; BSFM: black soldier fly meal; ACM: adult cricket meal.
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

3.4: Extrusion process

A low-cost single screw extruder (Model: DOLLY, Unitech, New Delhi, India), with a screw length to diameter ratio of 9.1 and operating at a speed of 200 rpm was used. A schematic representation is depicted in Figure 3.4-1. The extruder was equipped with a pre-conditioning chamber where the incoming feed was first preheated with steam at an inlet pressure of 4 bars. The blended ingredients were introduced into the steam conditioning chamber through a manual hopper at a feed rate of 1kg/min.

For the first objective, extrusion process was first optimized on the basis of three factors: residence time in the preconditioning chamber, extruder temperature, and die diameter using response surface methodology. Residence time in the conditioning chamber was varied to have 50 s, 100 s and 150 s of conditioning, after which the mash was channeled into the extrusion barrel that housed a single screw with a barrel length and diameter of 55 cm and 6 cm, respectively. The extruder was set to operate at temperatures of 80, 100 or 120°C, which was measured at the exit end of the barrel. The extrudates exited through a die of 2, 3 or 4mm in diameter. Speed of the screw was maintained at 200 rpm. Each extruded sample was collected into separate 20 L bucket and solar dried to constant weight. About 500 g of the dried pellets were sampled in duplicate and packed into zip-lock bags for analysis.

For the second and third objectives, on the effects of extrusion variables on the physico-chemical and nutritional characteristics of insect's based products, the optimal extrusion conditions determined in objective 1 were used. The feed was conditioned by passing through the conditioning chamber for 100 s after which the mixture was channeled into the extrusion jacket. The extruder was set to operate at 120°C. The extrudates exited through a die with a diameter of 2 mm. Each extruded batch was collected into separate 20 L bucket and then dried in a solar tent for about 4 h to constant weight. About 500 g of the dried pellets were taken in duplicate and packed into zip-lock bags for further analysis.

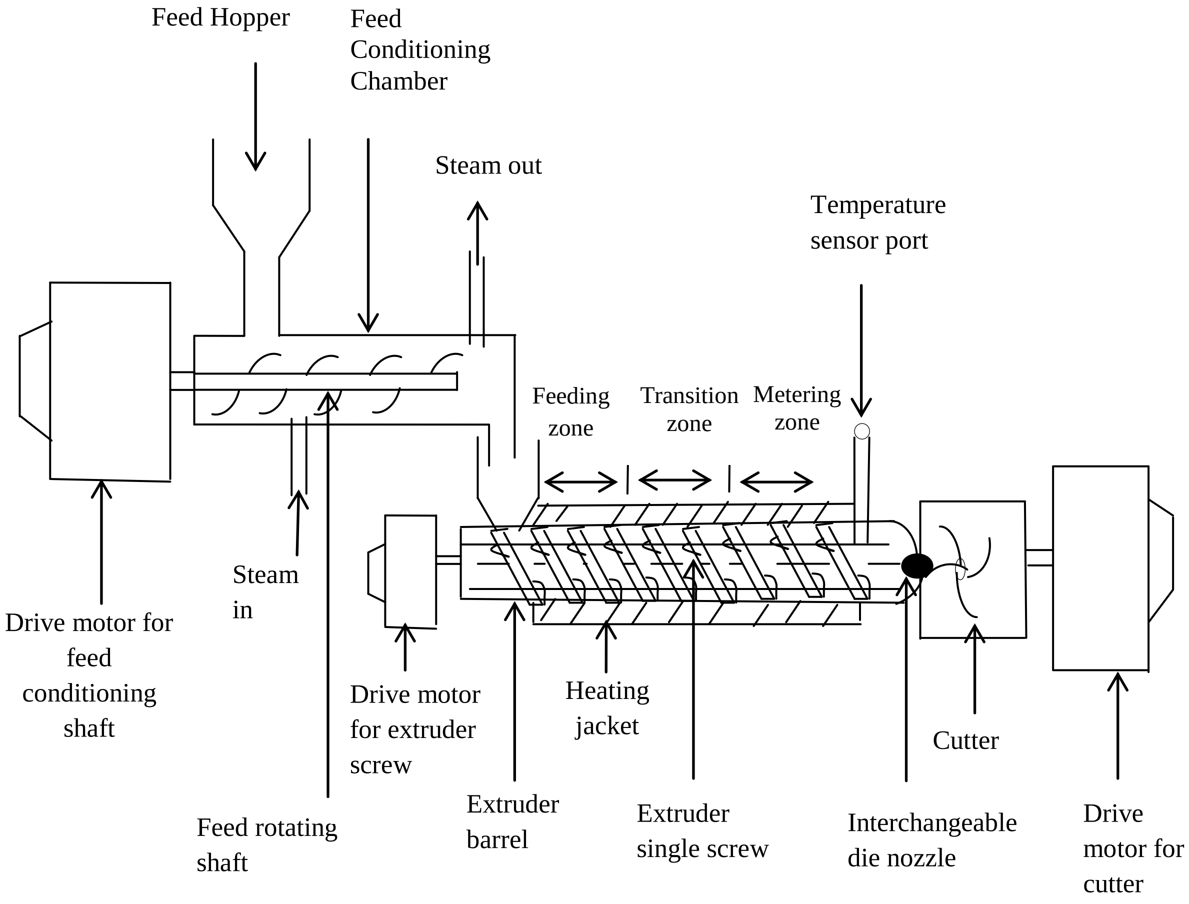

Figure 3.4-1: Schematic representation of a single screw extruder (Model: DOLLY, Unitech, New Delhi, India).

3.5: Optimization of extrusion parameters

A surface response methodology using Box-Behnken design with three independent variables: Extrusion temperature (Factor A), die diameter (Factor B), and feed pre-conditioning time (factor C) was used. The dependent variables were floatability, durability index, expansion ratio, water absorption index, water solubility index, water stability, bulk density and *in vitro* protein digestibility of extruded pellets. The design was generated using Minitab 14.12.0 (State College PA, USA) and comprised 30 trials. Experimental arrangement of Box-Behnken design and the treatment combinations is presented in Table 3.5-1. Sunflower, maize germ, wheat pollard, fresh water shrimps (fishmeal) and cassava were ground into fine flour using a laboratory mill (Model 4, ARTHUR H. THOMAS, Philadelphia, PA, USA) and sieved through a 1.0 mm aperture sieve. The quantities of the ingredients required to formulate feed mash containing 27% (db) protein were proportionately weighed using electronic digital weighing scale (Model No: 7765, Ashton Meyers, China) into 20 L buckets. The ingredients were then mixed manually by hand for 1 min after which the amount of water needed to raise the moisture content of the mash to 20g/100g was added and further mixing done for 1 min. This moistened mash was then transferred to a multi-vane paddle mixer (Model: MX – 25, Unitech, New Delhi, India) for further mixing for 2 min. The blended ingredients were introduced into the steam conditioning chamber (with a steam inlet set at 4 bars) through a manual hopper at a feed rate of 60kg/h and conditioning time was varied to have 50 s, 100 s or 150 s after which the mash was channeled into the extrusion barrel. The extrusion temperature was set at 80, 100 or 120°C, as measured at the die end of the extruder barrel. Extrudates exited through a die of 2, 3 or 4 mm diameter. Each extruded sample was collected into separate 20 L bucket and then dried in a solar tent to constant weight. Data on floatability, pellet durability, expansion ratio, water absorption index, water solubility index, water stability, bulk density and *in vitro* protein digestibility were analyzed using multiple regression analysis using Minitab 14.12.0 (State College PA, USA). Each response variable was fitted to a second order model expressed with the coded variables (A, B, C) with the following equation;

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \mathcal{E}$$

Where Y is the estimated response, β_0 is the constant term, β_i represents the linear terms, β_{ii} represents the quadratic terms for a single variable, β_{ij} represents the interaction terms (i = 1–3 and $j = 1–3$), and $\mathcal{E}$ represents the random error. Response surface plots and contour plots were developed as a function of two independent variables while keeping the other independent variable at optimal value.

Table 3.5-1: Experimental arrangement of Box-Behnken design and the treatment combinations

Run	Coded variables			Actual variables		
	A	B	C	A	B	C
1	-1	0	-1	80	3	50
2	1	0	-1	120	3	50
3	1	0	1	120	3	150
4	0	0	0	100	3	100
5	1	1	0	120	4	100
6	-1	1	0	80	4	100
7	0	-1	-1	100	2	50
8	-1	-1	0	80	2	100
9	-1	-1	0	80	2	100
10	1	-1	0	120	2	100
11	0	0	0	100	3	100
12	0	1	1	100	4	150
13	0	1	-1	100	4	50
14	1	0	1	120	3	150
15	0	0	0	100	3	100
16	0	0	0	100	3	100
17	0	-1	-1	100	2	50
18	0	0	0	100	3	100
19	1	1	0	120	4	100
20	0	1	1	100	4	150
21	-1	0	-1	80	3	50
22	1	0	-1	120	3	50
23	-1	0	1	80	3	150
24	1	-1	0	120	2	100
25	-1	0	1	80	3	150
26	0	-1	1	100	2	150
27	-1	0	+1	80	3	150
28	+1	0	+1	120	3	150
29	0	-1	+1	100	2	150
30	0	-1	+1	100	2	150

A: Temperature (°C); B: Die diameter (mm); C: Feed conditioning time (s).

3.6: Determination of physico-chemical properties of pellets

3.6.1: Floatability

For each sample, 10 randomly selected pellets were put into 250 mL beakers containing 200 mL of distilled water at room temperature. This was done in 3 replicates and average number of pellets that were found floating after 20 min was recorded. Floatability was calculated as the number of floating pellets after 20 min divided by the total number of pellets introduced in the water multiplied by 100 (Umar *et al.*,2013; Khater *et al*., 2014).

3.6.2: Expansion ratio (ER), surface area (SA) and volume (V)

Expansion ratio, surface area and volume were determined as outlined by Khater *et al*. (2014). For each sample, the diameter (D) and length *(l)* of 10 randomly selected pellets were measured using a digital vernier caliper (SR NO: 09070705763, MARS, USA) and their average value recorded. Expansion rate was calculated using the expression:

$$ER(\%) = \left[\left(D - D_i\right) / D_i\right] \times 100$$

[Equation 3-1]

where (D_i) is the die diameter.

The surface area (*SA*) and volume (*V*) of extrudates were estimated using the expressions:

$$SA = \pi D\left(l + D/2\right)$$

[Equation 3-2]

$$V = \pi D^2 l/4$$

[Equation 3-3]

3.6.3: Bulk density (BD)

Extruded pellets were milled using a laboratory-scale grinder and passed through a 1 mm aperture sieve. A 50 mL graduated measuring cylinder was tared and gently filled with 50 g of the powder. The bottom of the cylinder was repeatedly tapped gently until there was no further reduction in sample volume. Bulk density was calculated as weight of the sample divided by the respective volume (g/L).

3.6.4: Pellet durability index

Pellet durability index was determined as outlined by Umar *et al.* (2013). About 15 g of each sample was sieved on a 2.36 mm sieve in triplicate. The pellets that were retained on the sieve were weighed (W_i) and placed in a flask mounted on a Lab-line shaker (SR NO: 0486-0002, Lab-

Line Instruments, Inc., USA), which was then shaken for 20 minutes at 260 rpm. The pellets were then sieved and re-weighed (W_r) and the pellet durability index (*PDI*) calculated using the expression:

$$PDI(\%) = (W_r / W_i) \times 100$$ [Equation 3-4]

3.6.5: Water absorption index (WAI) and water solubility index (WSI)

Water absorption index (WAI) and WSI were determined as described by Rosentrater *et al.* (2009). About 0.625 g of each sample (W_i) was ground and suspended in 8 mL distilled water in a tared 12 mL centrifuge tube. The contents were shaken vigorously for 3 min and then centrifuged (using a bench-top centrifuge, Model: DSC – 200T, Digisystem Laboratory Instruments Inc., Taiwan) at 2500 rpm for 10 min. The supernatant was decanted and transferred into a tared aluminum dish and placed in a hot air oven (SR NO: WOF 500 80502004, DAIHAN SCIENTIFIC CO, Ltd., Korea) maintained at 135°C for 2 h. The dish and its contents was cooled in a desiccator and re-weighed on a sensitive weighing scale (Model: DJ-1505-S, SHINKO DENSHI CO Ltd, Japan), and the difference in weight (W_s) obtained. The mass of the gel remaining in the centrifuge tube (W_g) was obtained as well. The WAI and WSI were calculated using the expressions:

$$WAI(\%) = (W_g / W_i) \times 100$$ [Equation 3-5]

$$WSI(\%) = (W_S / W_i) \times 100$$ [Equation 3-6]

3.6.6: Water stability (WS)

The procedure described by Umar *et al*. (2013) was used. About 4 g of each sample was weighed and put on a 0.5 mm wire mesh screen in 3 replicates. The screen with the sample was immersed into a 250 mL beaker containing 200 mL distilled water for 20 min. The pellets retained on the wire mesh were then dried in hot-air oven (105°C) for 24 h. The percent ratio of weight of pellets retained on the wire mesh to the initial weight gave the WS.

3.6.7: Sinking behavior

The procedure outlined by Umar *et al.* (2013) and Rosentrater *et al*. (2009) was used with modification. Ten pellets from each sample were put into a 250 mL measuring cylinder containing 200 mL distilled water, in 3 replicates. The number of pellets sinking at onset (time 0), 2, 4, 8, 24 and 48 h were counted. A graph of the percent number of sinking pellets against time was plotted.

3.6.8: Total suspended solids (TSS) and total dissolved solids (TDS) in water

These were determined according to AOAC (1998). For each measurement, 3 replicates were performed. About 5 g of sample were weighed and introduced into a 250 mL measuring cylinder containing 200 mL distilled water. Aliquots (5 mL) of the supernatant were drawn before introducing the pellets (time 0), and after 4, 8, 24 and 48 h, and filtered through a pre-weighed Whatmann® filter paper No. 2 (110 mm diameter, Whatmann International Ltd., Maidstone, England). The residue was dried at 105°C for 1 h. The filtrate was poured into a pre-weighed aluminium dish and evaporated at 180°C for 2 h. Both the dried residue and filtrate were cooled in a desiccator and then weighed. Total suspended solids (TSS) was the percent difference in dry weight of the filter paper while TDS was the percent difference in dry weight of the aluminium dish.

3.6.9: Leaching activity

For each measurement, 3 replicates were performed. About 5 g of sample were weighed and introduced into a 250 mL measuring cylinder containing 200 mL distilled water. Aliquots (5 mL) of the supernatant were drawn after 4, 8, 24 and 48 h and sodium azide added at a rate of 0.02% into the tubes containing the aliquots. Mineral analyses were then determined as outlined in 3.9.

3.7: Proximate Analysis

3.7.1: Determination of dry matter

Determination of dry matter was done according to AOAC 930.15 (2000). Approximately 2g of ground sample was weighed and placed into a pre weighed dish. The weight of the dish together with the sample was recorded. The dish was placed into a drying oven (S/N: 100-11-00-22; Memmert; Schwabach; Germany) at 105°C for 3h. The dish was then cooled in a desiccator and reweighed. Dry matter was determined using the following formula:

$$Dry \text{ matter } (\%) = (W3 - W1) / (W2 - W1) \times 100 \qquad \text{Equation 3-7]}$$

Where

W_1 is the weight of the empty dish (g)

W_2 is the weight of dish and sample (g)

W_3 is the weight of dish and sample after drying (g)

3.7.2: Ash content determination

This was determined according to AOAC 942.05 (2000). Two (2) g of dry sample were weighed into a crucible that was previously calcined and weighted and then heated in a muffle furnace (Model: MR170; S/N: 6800616; Hereaus, GMBH, Hanau, Germany) at 550°C for 12 hours. The crucible with the ashed sample was then cooled in a desiccator and reweighed again. Weight of ash was calculated as below:

$$Ash\ content\ (\%)\ =\ [weight\ of\ crucible\ +\ ash\ -\ weight\ of\ crucible]\ /\ (weight\ of\ original\ sample) \times 100$$

[Equation 3-8]

3.7.3: Crude protein determination

Crude protein was determined according to AOAC 984.13 (2000). Two (2) g of sample were mixed with 20 mL of concentrated sulphuric acid in a digestion tube. Kjeldahl tablets were added to the mixture in the tube and then digested using a Gerhardt Kieldathem digester (Model: KB40; Gerhardt GMBH & CO. Kg; Germany) for 1 h at 420°C. Distilled water was added to the digest to 80 mL volume mark. Exactly 50 mL of sodium hydroxide solution was added to the mixture and this was followed by distillation using a Kjeltec auto distillation unit (Model: 11014901; S/N: 91708870; Foss Analytical, Sweden) to distill ammonium into concentrated boric acid. Titration was done using hydrochloric acid after adding a few droplets of indicator solution. The nitrogen content was obtained using the formula below;

$$Nitrogen\ (\%)\ =\ [(Vs - Vb) \times M(HCl) \times 1 \times 14.007)] / (W \times 10) \qquad \text{[Equation 3-9]}$$

Where;

V_s is the mL HCl needed to titrate sample

V_b is the mL HCl needed for the blank test

$M_{(HCl)}$ is the molarity of HCl

1 is the acid factor

14.007 is the molecular weight of N

W is the weight of the sample (g)

10 is the conversion from mg/g to %

Protein content was then calculated as follows;

$$Crude\ protein\ (\%) = \%N \times 6.25 \qquad \text{(Equation 3-10)}$$

where 6.25 is the conversion factor

3.7.4: Fat content determination

Determination of crude fat was done according to AOAC 920.39 (2000). Five (5) g of the sample were weighed into the extraction thimble and covered with a fat-free wad of cotton wool. The thimble was then fitted to a clean round bottom flask that had been cleaned, dried and weighed. Exactly 25 mL of petroleum ether was added into the extraction flask. The Electro-thermal Soxlet Apparatus (Model: EME 6250/CF; Cole Parmer; England) was set to extract the sample for 6 h. All the solvent was evaporated, flask dried in a desiccator and then reweighed. Fat content was determined as follows;

$$Crude\ fat\ (\%) = (W3 - W2)/W1 \times 100 \qquad \text{[Equation 3-11]}$$

Where;

W_1 is the initial sample weight in grams,

W_2 is the tare weight of flask in grams

W_3 is the weight of flask and fat residue in grams

3.7.5: Crude fibre determination

Determination of crude fibre was done according to ISO 6865 (2000). Two (2) g of sample was weighed and digested in a Fibertec digester (S/N; 91710358; FOSS; Sweden) using sulphuric acid for 30 minutes and then digested using potassium hydroxide for 30 minutes. Each digest was followed by filtration through a crucible. The residue was washed 5 times with 10 mL of hot distilled water. The crucible and its contents were then dried in an oven at 105°C for 4 h, cooled in a desiccator and then reweighed. The dried crucible and residue were then incinerated using a muffle furnace at 550°C for 2 h, cooled in a desiccator and re-weighed again. The following equation was used to calculate crude fibre content:

$$Crude\ fibre\ = (W2 - W3)/W1 \times 100 \qquad \text{[Equation 3-12]}$$

Where;

W_1 is the weight of sample (g),

W_2 is the weight of crucible and residue after drying (g)

W_3 is the weight of crucible and residue after incineration.

3.7.6: Carbohydrate content determination

Carbohydrate content was calculated as weight by difference between 100 and summation of other proximate parameters and given as Nitrogen Free Extract % carbohydrate.

$$NFE = 100 - (P + F + A + C) \qquad \text{[Equation 3-13]}$$

Where;

P is the crude protein content,

F is the crude fat content,

A is the ash content, and

C is the crude fibre content.

3.8: Determination of *in vitro* protein digestibility (IVPD)

Procedures outlined by Saunders *et al*. (1973) and Babiker *et al*. (1995) were used with modifications. About 0.2 g of the sample was weighed into a 50 mL centrifuge tube. Exactly 15 mL of 0.1 mol/L hydrochloric acid solution (pH = 1.0) containing 0.02% sodium azide and 1.5 mg pepsin from porcine gastric mucosa (Sigma P7000-25G; activity ≥250 units/mg solid, SIGMA-ALDRICH CO., USA) was added, and the tube incubated in a shaking water bath (Model; WSB-30, Witeg Labortechnink GmbH, Wertheim; Korea) maintained at 15°C for 3 h. The sample was then centrifuged at 4000 x *g* for 20 minutes at room temperature and the supernatant was decanted. Nitrogen content of the supernatant was determined by the Kjeldahl method according to the AOAC Method 984.13, using a UDK 20S digester (SN: 106242; VELP SCIENTIFICA; Europe) and UDK 127 distillation unit (SN: 130489, VELP SCIENTIFICA; Europe). Nitrogen in the supernatant was expressed as a percentage of the total nitrogen in the sample and reported as *in vitro* protein digestibility.

3.9: Minerals analyses

All samples (fresh water shrimp meal, adult cricket meal, black soldier fly larvae meal and extruded samples) were each separately milled using an oscillating mill (Model: MM400; S/N: 129251116; Retsch, Germany) and weighed using an electronic weighing balance (Model: ABS 220-4; S/N: WB1210455; KERN & SOHN GmbH, Germany). Mineral contents were then determined by atomic absorption spectrometry in an atomic absorption spectrophotometer (Model: AA-6300; S/N: A305243009165A; Shimadzu, Japan) after digestion in sulphuric acid and selenium powder. Calcium, potassium, magnesium, sodium, copper, zinc, manganese and iron were measured at wavelengths of 422.7, 766.5, 285.2, 589.0, 324.8, 213.9, 279.5 and 248.3 nm, respectively. Phosphorus was determined using a UV-Visible spectrophotometer (Model; UV-1700; S/N: A11024302429LP; Shimadzu, Japan) at 880 nm.

3.10: Statistical analysis

A 2 x 4 x 3 factorial arrangement in a completely randomized design was used for the second and third objectives. The factors investigated were; insect type (2 levels; black soldier fly larvae and adult cricket), level of freshwater shrimp meal (FWSM) protein substitution with black soldier fly larvae meal (BSFM) or adult cricket meal (ACM) (4 levels: 0%, 25%, 50% and 75%), and feed moisture content (3 levels: 10%, 20% and 30% on wet weight basis). The experiment was replicated four times.

Statistical model:

$$Yijk = \mu + \alpha i + \beta j + \gamma k + \alpha\beta ij + \beta\gamma jk + \alpha\gamma ik + \alpha\beta\gamma ijk + \varepsilon ijk$$

Where: Y_{ijk} is the response from i^{th} level of type of insect, j^{th} level of freshwater shrimp meal protein substitution and k^{th} level of feed moisture content; μ is the overall mean responses, α_i is the effect of i^{th} level of type of insect; β_j is the effect due to the j^{th} level of freshwater shrimp meal protein substitution; γ_k is the effect due to the k^{th} level of feed moisture content; $\alpha\beta_{ij}$ is the interaction between the i^{th} level of type of insect and j^{th} level of freshwater shrimp meal protein substitution, $\beta\gamma_{jk}$ is the interaction between the *jth* level of freshwater shrimp meal protein substitution and the k^{th} level of feed moisture content; $\alpha\gamma_{ik}$ is the interaction between the i^{th} level of type of insect and k^{th} level of feed moisture content; $\alpha\beta\gamma_{ijk}$ is the interaction between the i^{th} level of type of insect, j^{th} level of freshwater shrimp meal protein substitution and k^{th} level of feed moisture content; and e_{ijk} is the random error component.

Analysis of variance was performed using General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS) software version 9.1.3 (SAS Institute Inc., USA). Means were separated using Tukey's HSD (Honestly Significant Difference) test at 95% confidence level.

CHAPTER 4: RESULTS AND DISCUSSION

4.1: Optimization of extrusion conditions for the manufacture of floating fish feeds with respect to feed conditioning time, extruder die diameter and extrusion temperature

Properties of extrudates processed under treatment combinations of the various runs are shown in Table 4.1-1. Analyses of variance for the responses are given in Tables 4.1-2 and 4.1-3. Regression models fitted for each variable were highly significant *($P<0.001$)* with high values of R^2. Surface plots for each response are shown in Figures 4.1-1 to 4.1-4. The predictive models after excluding the insignificant terms for each response are shown in Table 4.1-4. Figure 4.1-5 shows the optimized treatment combinations for desirable physico-chemical properties. These were extrusion temperature of 120°C, die diameter of 2 mm and feed pre-conditioning time of 100 seconds.

Expansion ratio

Expansion ratio is a measure of how extrudates puffs at the die exit. It is influenced by such factors as extrusion temperature, feed rate, die dimension, steam conditioning, moisture content and composition of the ingredients used. Figures 4.1-1(a) and 4.1-2(a) give the surface plots for expansion ratio against temperature, die diameter and feed conditioning time. The study revealed that increasing temperature resulted into higher pellet expansion wherein low temperatures of 80°C yielded minimal expansion. The expansion rate decreased with an increase in die diameter (Figure 4.1-1[a]). According to Rosentrater *et al*. (2009), expansion of extruded fish feeds start to occur at around 100°C. Similarly, Figure 4.1-2(a) shows that an increase in the number of feed conditioning time resulted into higher rate of expansion. There is a high positive correlation between floatability and expansion rate (Table 4.1-5); as expansion rate increased so did floatability and vice versa.

Table 4.1-1: Properties of extrudates processed under treatment combinations of the various runs

Run	Response variables							
	Y_1 (%)	Y_2 (%)	Y_3 (g/L)	Y_4 (%)	Y_5 (-)	Y_6 (%)	Y_7 (%)	Y_8 (%)
1	25.48	27.50	489.38	99.76	3.88	11.62	75.11	65.22
2	37.38	87.50	494.94	99.68	4.06	11.92	82.52	65.77
3	44.85	85.00	430.40	99.71	4.22	7.71	81.33	65.55
4	45.32	80.00	761.64	99.73	4.20	8.75	75.99	63.57
5	22.43	22.50	409.32	99.77	3.86	11.95	82.07	60.93
6	14.25	10.00	500.03	99.79	3.98	8.03	81.56	61.82
7	45.63	100.00	431.92	99.88	4.00	10.31	83.62	73.28
8	38.41	80.00	470.98	99.87	3.80	13.29	82.03	63.89
9	41.20	65.00	477.54	99.86	3.79	13.22	79.87	63.99
10	54.57	97.50	499.96	99.86	4.11	9.07	86.75	64.22
11	39.32	77.50	643.11	99.69	4.19	9.26	74.72	62.17
12	22.84	57.50	413.29	99.79	4.15	6.68	84.28	66.97
13	18.50	10.00	451.05	99.88	3.91	7.65	80.45	64.93
14	47.83	85.00	464.00	99.73	4.16	8.91	82.93	65.56
15	37.06	85.00	634.82	99.73	4.17	7.89	76.61	61.34
16	36.33	79.50	676.16	99.71	4.19	8.79	78.09	64.46
17	51.22	100.00	461.99	99.87	3.98	9.50	82.31	70.26
18	40.60	97.50	712.48	99.73	4.17	8.95	75.73	63.43
19	22.43	22.50	409.32	99.77	3.86	11.95	82.07	60.94
20	20.02	52.50	426.11	99.79	4.13	7.59	84.31	68.18
21	26.37	27.50	474.23	99.72	3.91	11.98	76.51	65.29
22	37.20	100.00	491.00	99.69	4.07	11.29	84.69	65.58
23	24.09	75.00	655.98	99.70	4.07	11.09	83.83	58.62
24	52.50	100.00	477.33	99.86	4.14	7.52	85.78	63.04
25	21.20	80.00	599.69	99.69	4.02	11.15	84.18	57.89
26	51.82	100.00	477.89	99.96	4.06	6.66	86.09	57.67
27	51.18	97.50	486.30	99.93	4.06	6.91	85.24	57.98
28	13.45	5.00	499.06	99.80	3.94	7.74	83.33	62.25
29	43.23	77.50	687.68	99.73	4.13	8.95	77.80	63.28
30	23.31	35.00	424.41	99.85	3.87	8.07	80.00	64.49

Y_1:Expansion ration, Y_2: Floatability; Y_3: Bulk density; Y_4: Pellet durability index, Y_5: Water absorption index; Y_6: Water solubility index; Y_7: Water stability; $Y_{8:}$ *In-vitro* protein digestibility.

The increase in expansion as the temperature is increased could be attributed to the fact that higher temperature increases the dough melt temperature which reduces melt viscosity of the dough and as thus results into the material being expanded more longitudinally while reducing sectional expansion (Singh *et al*., 2014). Reduction in melt viscosity results into growth of large bubbles which upon extrusion expands more (Majumdar and Singh, 2014). In addition, increasing barrel temperature results into development of increased die pressures (Meng *et al*., 2010) which then leads to higher extrudates expansion. Moreover, higher temperatures unfolded amylose and amylopectin in the formulated mash leading to more cohesive and expanded pellets (Rokey *et al.,* 2010). These findings are in agreement with several studies that reported an increase in expansion rate with an increase in temperature of the barrel (Badrie and Mellowes, 1991; Jozinović *et al*., 2013; Tumuluru, 2013; Peluola-Adeyemi *et al*., 2014). However, Rosentrater *et al*. (2009) reported a decrease in expansion with the increase in processing temperature. High amount of feed conditioning time also reduced the melt viscosity of the dough resulting into higher longitudinal expansion. Increased feed conditioning time could also have resulted into higher rate of starch gelatinization (Adeparusi and Fameruwa, 2011) which influences expansion. These findings are in agreement with Rokey *et al.* (2010) who stated that increasing the amount of steam in the pre-conditioner increases the amount of feed expansion. The decrease in expansion as the size of the die was increased is due to the fact that increasing the size of the die diameter reduced the pressure that was developed inside the extrusion barrel. A small die on the other hand resulted into a lot of pressures being accumulated in the barrel and upon exit of the material through the die, the product expanded more before equalizing with the atmospheric pressure. However, Akdogan (1999) relates this to melt viscosity, which increases as the size of the die decreases. These findings are in agreement with Singh and Muthukumarappan (2014) who noted that expansion rate increased as the size of the die diameter reduced. The relationship between expansion rate and floatability has been reported by Umar *et al*. (2013) who identified a positive relationship between expansion and floating of feeds.

Table 4.1-2: Analysis of variance for floatability, expansion ratio, bulk density and durability index

Source of variation	Df	Expansion ratio		Floatability		Bulk density		Durability index	
		Ms	*F* value	Ms	*F* value	Ms	*F* value	Ms	*F* value
Regression	9	497.72	66.48***	3121.39	50.68***	30896.9	33.49***	0.02	111.64***
Linear	3	76.80	10.26***	987.70	16.04***	60990.2	66.11***	0.03	143.08***
Square	3	85.34	11.40***	1295.67	21.04***	76786	83.23***	0.05	257.68***
Interaction	3	30.84	4.12*	786.20	12.77**	8426.3	9.13**	0.00	26.04***
Lack of fit	3	13.96	2.20^{ns}	129.69	2.262^{ns}	1332.5	1.57^{ns}	0.00	0.02^{ns}
Pure error	17	6.34		49.57		850.3		0.00	
Total	29								
R^2			0.97		0.96		0.94		0.98
R^2-Adj			0.94		0.94		0.91		0.97
S			2.74		7.85		30.37		0.01

ms: mean square; s: standard error of the regression; *** significant at $P<0.001$; ** significant at $P<0.01$; *significant at $P<0.05$; ^{ns} not significant at $P<0.05$

Floatability

Floatability is a measure of feed buoyancy and is affected by several factors including ingredients used in formulation, processing conditions and post-processing handling practices. Figures 4.1-1 (b) and 4.1-2 (c) shows the response surface plots for floatability as influenced by temperature, die diameter and feed conditioning time. Increasing extrusion temperature from 80°C to 120°C increased floatability (Figure 4.1-1[b]). This was also true when feed conditioning time was varied where floatability increased with increasing feed conditioning time (Figure 4.1-2[c]). Figures 4.1-1 (b) and 4.1-2 (c) show that floatability of extrudates increased with a decrease in the size of the die where a larger die diameter resulted into pellets with low floatability. From this study, it can be said that maximum floatability can be achieved where temperature, die diameter and feed conditioning time are 120°C, 2mm and 150 s respectively. These findings correspond to Muyang Group (2014) who argued that floating aqua feeds are best produced with temperatures and conditioning time of between 120°C – 130°C and 90-120 seconds respectively.

Gelatinized starch improves binding of feed ingredients and thus a stable product that can float easily. The increase in floatability can be due to increased gelatinization as the time of feed conditioning as well as temperature increased. Adeparusi and Famurewa (2011) also found a positive correlation between floatability and temperature as well as the time of feed conditioning. In addition, Foley and Rosentrater (2013) reported that floatability of extrudates increased as the extruding temperature increased. However, Saalah *et al*. (2010) found a contrary trend where temperature changes did not affect floatability of the fish feed. Dies with low diameter increases the amount of pressures developed within the extrusion barrel where after extrusion, a greater expansion is experienced by the feed. On the other hand, large dies create more room for the material to expand sectional and not longitudinally (Vijayagopal, 2004). This then explains why floatability was high for the small die and low with large dies.

Bulk density

According to Meng *et al*. (2010), extruded products are desired to have low bulk densities. However, there are various data on the bulk density for floating fish feeds. Vijayagopal (2004) states that feed with bulk density of 550g/L will float on water. On the other hand, Muyang Group (2014) states that bulk densities of below 480g/L are desired for floating feeds. This study found similar findings where majority of floating feeds had bulk densities of between 400g/L and 500g/L (Table 4.1-1).

The effects of temperature, die diameter and feed conditioning time on bulk density are shown in Figures 4.1-1 (c) and 4.1-2 (b). It was observed that increasing barrel temperature decreased bulk density (Figure 4.1-1[c]). However, increasing the number of steam conditioning cycles (Figure 4.1-2[b]) resulted in increase in extrudates' bulk densities. From Figure 4.1-1(b) increasing the size of die diameter gave a curvilinear effect on bulk density, where low bulk densities were recorded for 2mm and 4mm dies at a temperature of 120°C. Figures 4.1-1 and 4.1-2 show an inverse relationship between expansion rate and bulk density with respect to temperature. As temperature was increased from 80°C to 120°C there was an increase in expansion rate which corresponded to a decrease in bulk density as expected.

The decrease in bulk density with the increase in temperature can be attributed to the decreased melt viscosity that encourages bubble growth that is accompanied by more expansion of the product but with reduced bulk density. Several studies have also observed a decrease in bulk density as temperature was increased (Giri and Bandyopadhyay, 2000; Meng *et al*., 2010; Singh and Muthukumarappan, 2015). On the contrary, Singh *et al*. (2014) reported an increase in bulk density as temperature increased. The increase in bulk density with the rise in the time of feed conditioning could be attributed to the increase in shear stress which resulted in lower expansion of the extrudates and thus low bulk density. The initial increase in bulk density when the size of the die was increased from 2mm to 3mm is due to the reduction in die pressure as the die size increases. Singh and Muthukumarappan (2014) reported a decrease in bulk density as the size of the die reduced. In addition, Rokey *et al*. (2010) state that bulk density of extruded feeds reduces as the open area of the die reduces. The decrease in bulk density as the size of the die further increases from 3mm to 4mm could be due to reduced shear stress with the 4mm die. Similar to these findings, Majumdar and Singh (2014) also reported an inverse relationship between expansion rate and bulk density

Pellet durability index (PDI)

Pellet durability index is a parameter of how strong the pellets can withstand mechanical handling during transportation, storage and subsequent use. Figures 4.1-1(d) and 4.1-2(d) shows surface plots for pellet durability index against temperature, die diameter and feed conditioning time. In this study, temperature was found to affect PDI in a curvilinear pattern. High PDI was observed with the temperature of 100°C, while temperatures of 80°C and 120°C resulted into low PDIs. Feed conditioning time influenced PDI in that an increase in the time of feed conditioning reduced PDI (Figure 4.1-2[d]). It was observed that lowest PDI was obtained with the 3mm die. On the other hand, extrudates produced by the 2mm die were found to have high PDI. The 4mm die produced extrudates with relatively high PDI. However, all pellets obtained in this study had PDIs of over 99% and thus they can withstand much of mechanical handling. This are encouraging results as feeds with high PDI are highly desired by both aqua feed manufacturers and fish farmers because they can keep for long periods without losing their physical qualities (Ayadi *et al*., 2011).

It has been reported that starches have a peak gelatinization temperature of around 110°C (BeMiller and Whistler, 2009). This then explains why in this study, maximum PDI was observed at the extrusion temperature of 100°C, which can be said to be optimum for PDI. At 80°C, the temperatures were not optimal for the gelatinization of starch, which determines PDI (Chevanan *et al*., 2009). At 120°C, the temperatures could be said to be too high as to cause fragility of the pellets. The decrease in PDI with increased time of feed conditioning could be as a result of increased melt temperature that was accompanied by increased high amount of steam obtained in 100 and 150 s. It can also be as a result of low friction created by increased amount of steam (Sitaula, 2012).

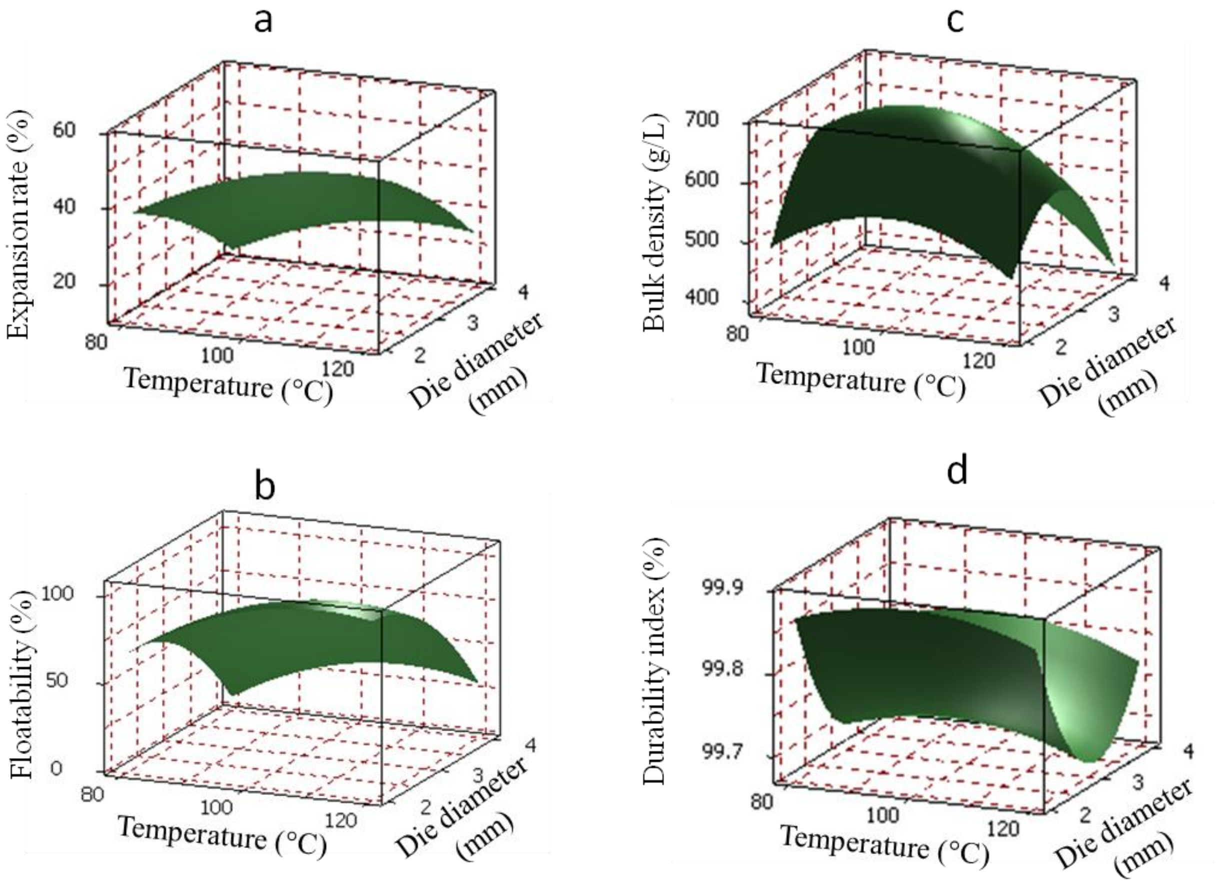

Figure 4.1-1: Surface plots for the effects of temperature and die diameter at constant steam conditioning time of 100 s on expansion rate (a), floatability (b), bulk density (c) and durability index (d).

These findings are in agreement with Sitaula (2012) who reported a decrease in PDI with the inclusion of steam conditioning. The low PDI associated with the 3mm die could be attributed to the fact that small dies increase shear stress of the dough which upon extrusion results into a fragile product (Akdogan, 1999). However, the 2mm die had a conical pre-nozzle shape rather than a cylindrical shape found in the 3mm and 4mm dies. As a result, a lot of pressures were developed in the 2mm die where upon extrusion rapid cooling was experienced and thus extrudates with high PDI. For the 4mm die, less shear stress was experienced by the dough resulting into strong and durable extrudates.

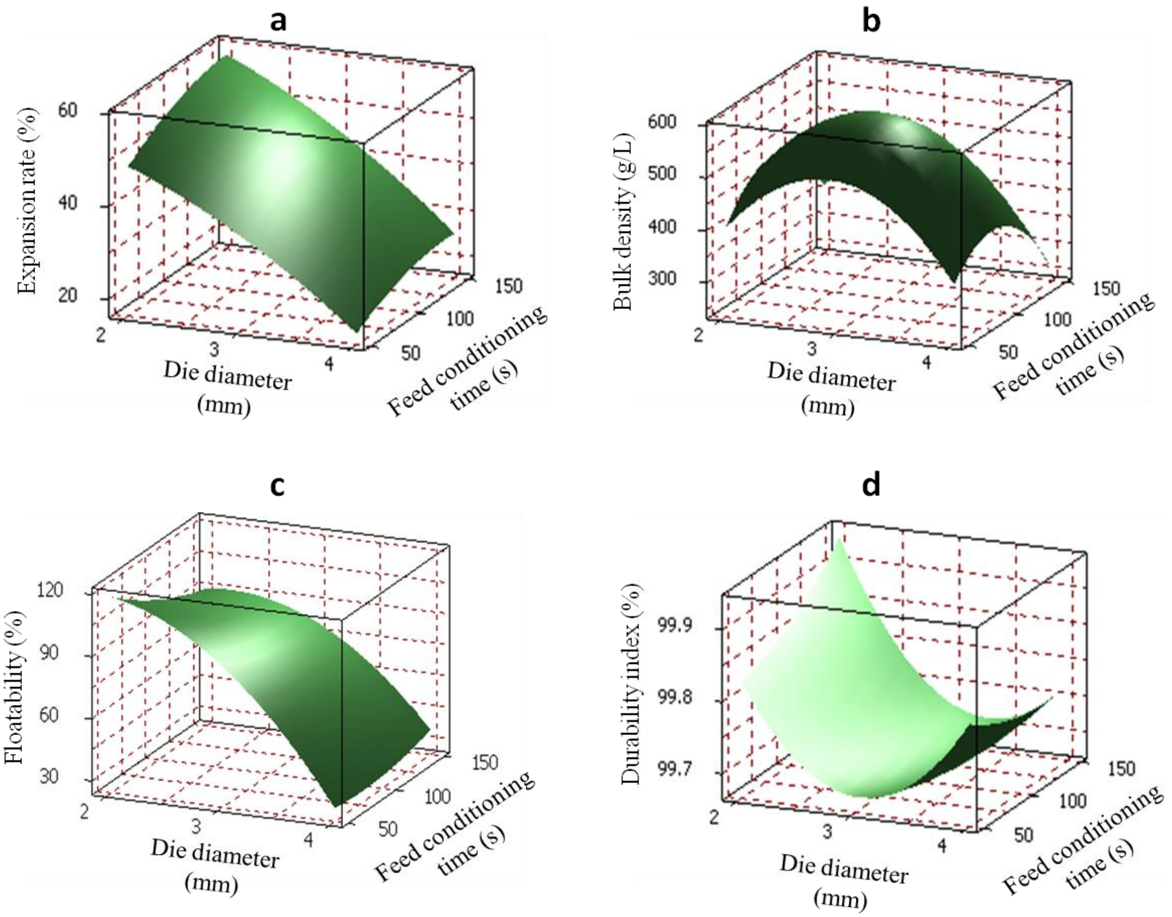

Figure 4.1-2: Surface plots for the effects of die diameter and feed conditioning time at constant temperature of 120°C on expansion rate (a), bulk density (b), floatability (c) and durability index (d).

The high levels of PDI obtained in this study could be attributed to the fact that some of the ingredients that were used such as wheat pollard and maize germ normally have carbohydrate levels of over 50% dwb. As such, these ingredients provided high amount of resistant starch, which according to BeMiller and Whistler (2009) promotes better functional properties of extrudates. In addition, cassava, an ingredient that was used in this study was added at 5% and thus the high durable products. According to Tumuluru *et al*. (2016), binders added at about 4% inclusion results into PDI of over 98%. These results correspond with those found by Fallahi *et al*. (2012) who also found PDIs of over 99% in their study.

Water absorption index (WAI) and water solubility index (WSI)

Water absorption index (WAI) is a measure of starch gelatinization as it measures the amount that starch polymers occupy after swelling in water. On the other hand, WSI is a measure of starch degradation as it measures the amount of soluble starch components (Gui *et al*., 2012). Figures 4.1-3 and 4.1-4 give the surface plots for WAI and WSI against temperature, die diameter and feed conditioning time. Water absorption index (WAI) increased with an increase in temperature as well as with the increase in the amount of feed conditioning time. On the other hand, WSI reduced with increasing temperature as well as increasing feed conditioning time. From both Figures, WAI decreased as the size of the die diameter increased while WSI increased as the size of the die increased. These findings reveal that WAI and WSI are inversely related to each other where an increase in WAI results into a decrease in WSI and vice versa at 0.01 level of significance (Table 4.1-5).

Table 4.1-3: Analysis of variance for water absorption index, water solubility index, water stability and in-vitro protein digestibility.

Source of variation	df	Water absorption		Water solubility		Water stability		Protein digestibility	
		Ms	F value	Ms	F value	Ms	F value	Ms	F value
Regression	9	0.05	80.70***	12.18	46.04***	38.0	39.29***	32.84***	12.95
Linear	3	0.09	136.49***	19.11	72.27***	17.13	17.70***	57.79***	22.79
Square	3	0.07	102.13***	13.53	51.15***	63.92	66.06***	12.2**	4.82
Interaction	3	0.04	55.40***	15.67	59.25***	21.29	22.00***	55.09***	21.72
Lack of fit	3	0.00	2.41^{ns}	0.37	1.52^{ns}	0.24	0.22^{ns}	12.68**	17
Pure error	17	0.00		0.25		1.09		0.75	
Total	29								
R^2			0.97		0.95		0.95		0.85
R^2-Adj			0.96		0.93		0.92		0.79
S			0.03		0.51		0.98		1.59

ms: mean square; s: standard error of the regression; *** significant at P<0.001; ** significant at P<0.01; *significant at P<0.05; ^{ns} not significant at P<0.05.

Increasing temperature and feed conditioning time increased gelatinization of starch and thus high WAI with low WSI. These findings are in agreement with many researchers who reported an increase in WAI with increase in barrel temperature as WSI decreased (Chevanan *et al.*, 2007; Rosentrater *et al.*, 2009; Gui *et al.*, 2012; Singh and Muthukumarappan 2014:) but contradict other researchers (Badrie and Mellowes, 1991; Peluola-Adeyemi *et al.*, 2014). Low WAI associated with large die diameter could be due to the fact that pressure accumulation within the barrel decreased with large die sizes (Chiang and Johnson 1977), and as a result, the amount of starch gelatinization reduced. The increase in WSI as the size of die diameter increases translates that as the degree of starch gelatinization decreases, the degree of starch degradation increases. The inverse relationship between WAI and WSI has also been reported by Fallahi *et al.* (2012).

Water Stability

According to Ayadi *et al*. (2011) high values of water stability indicates that the extruded pellets will resist dissolution and thus low loss of nutrients when placed in water. Figures 4.1-3 (c) and 4.1-4 (c) show the effect of temperature, die diameter and feed conditioning time on water stability. It was observed that as the temperature increased from 80°C to 120°C, there was a corresponding increase in water stability of the feeds (Figure 4.1-3[c]). Figures 4.1-3(c) and 4.1-4(c) show a curvilinear relationship between water stability and the size of the die; as the size of the die increases from 2mm to 3mm, water stability reduces but from 3mm to 4mm, water stability increases slightly. From Figure 4.1-4(c), increasing the time of feed conditioning resulted into a decrease in water stability when feeds were extruded at the same temperature of 120°C. From Table 4.1-1, it can be seen that high levels of water stability were obtained in this study. In fact, only 9 out of the 30 runs gave water stability values of between 74% and 79%. The rest (21 runs) gave water stability of over 80%.

Increasing temperature to optimum levels of 120°C (Vijayagopal, 2004) results into higher starch gelatinization coupled with the formation of a strong starch - protein matrix that is water stable (Tumuluru, 2013). These findings correspond with Bandyopadhyay and Rout (2001) who found an increase in water stability with increasing barrel temperature. The decrease in water stability as the size of the die increases from 2mm to 3mm can be attributed to the increase in melt viscosity with the increase in die diameter (Akdogan, 1999). On the other hand, increase in water stability as die diameter increases from 3mm to 4mm can be attributed to the ability of the large die to promote both longitudinal and sectional expansion of the pellet to some extent. As a result, the extruded feeds become somewhat more compact and thus the slight increase in water stability. Increasing the number of steam conditioning cycles resulted into an increase in temperature of the feed material which when combined with the barrel temperature of 120°C damaged the gluten protein of the ingredients (Vijayagopal, 2004). As a result, their binding properties were reduced with increase in time of steam feed conditioning and thus the reduction in water stability. These results are in agreement with those by Sitaula (2012) who reported a decrease in stability of extrudates with the inclusion of steam conditioning. The high values of water stability reported in this study translates that products obtained will least contribute to water pollution.

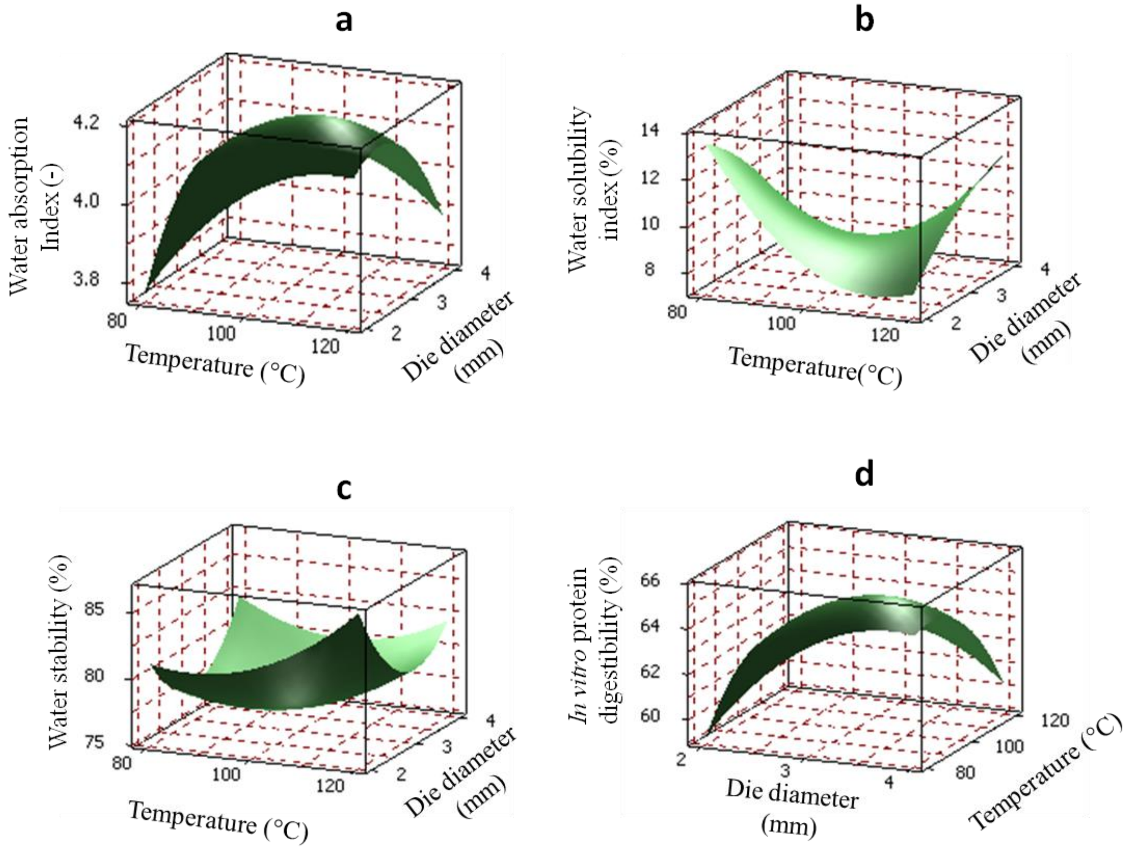

Figure 4.1-3: Surface plots for the effects of temperature and die diameter at constant steam conditioning time of 100 s on water absorption index (a), water solubility (b), water stability (c) and in vitro protein digestibility (d).

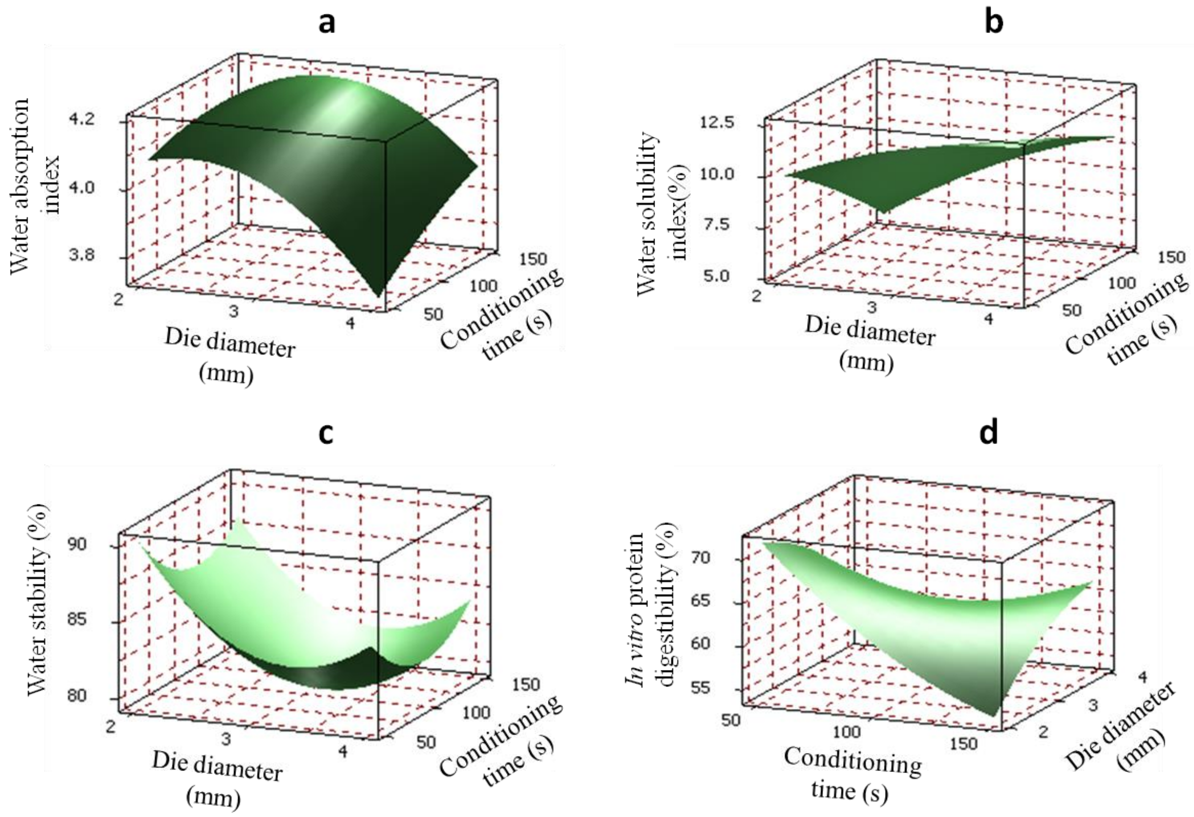

Figure 4.1-4: Surface plots for the effects of die diameter and feed conditioning time (s) at constant temperature of 120°C on water absorption index (a), water solubility (b), water stability (c) and *in vitro* protein digestibility (d).

In vitro protein digestibility (IVPD)

Extrusion improves *in-vitro* protein digestibility of feeds in comparison to feeds produced through pelletization (Fenerci and Sener, 2005). Figures 4.1-3 (d) and 4.1-4 (d) show surface plots for the effects of die diameter, temperature and feed conditioning time on *in vitro* protein digestibility. Increasing die diameter from 2mm to 4mm resulted in a linear increase in IVPD (Figure 4.1-3[d]). On the other hand, increasing temperature from 80°C to about 100°C resulted in an increase in IVPD, but further increase in temperature from 100°C to 120°C resulted in a decrease in IVPD (Figure 4.1-3[d]). Increasing the feed conditioning time (Figure 4.1-4[d]) from 50 s to 150 s decreased IVPD linearly. Most values for IVPD were between 60% and 70%. These results agree with those obtained by Fenerci and Sener (2005) which also ranged between 60% and 69%.

The initial increase in IVPD with increasing extrusion temperature could be attributed to protein denaturation which exposes more polypeptide bonds to enzymatic activity (Opstvedt *et al.*, 2003) and degradation of enzyme-specific inhibitors. On the other hand, the decrease in IVPD as temperature was increased from 100°C to 120°C could be due to the formation of protein-polyphenol complexes with high temperatures that resists pepsin hydrolysis. The high values of IVPD obtained in this study could be due to the fact that extrusion unfolds and exposes hydrophobic groups of protein increasing the number of susceptible sites to enzymatic activity (Onyango, 2005).

Multivariate regression analysis

Optimum conditions for processing of floating feeds

The optimal conditions for processing good quality floating fish pellets were established using the graphical method of response surface methodology with the aim of obtaining extrudates with most desirable properties. The main criteria involved maximizing water stability and floatability, minimizing water solubility index and targeting *in-vitro* protein digestibility of about 70%.

The final optimized plots for the response optimization of extrusion conditions (temperature, die diameter and feed conditioning time) in making fish feeds are shown in Figure 4.1-5. The optimized responses for *in vitro* protein digestibility, water stability water solubility index and floatability gave higher desirability values of 0.50168, 0.96898, 0.79359 and 1.0000 respectively. The optimum extrusion conditions of temperature, die diameter and feed conditioning were identified as 120°C, 2 mm and 100 seconds respectively. The optimum values had a composite desirability of 0.8227 which is acceptable though it implies that other than the study parameters, there are other factors such as feed composition and rate of feeding (not within the scope of this study) that also affect the physico-chemical properties of extruded fish feeds.

Table 4.1-4: Explanatory equations for various pellet properties after excluding the insignificant terms

Model Equation	R^2 (%)	R^2-Adj (%)	S
$Y_1 = 549.7 + 9.98A + 55.1B + 1.144C - 0.039A^2 - 17.46B^2 - 0.015AC + 0.169BC$	95.3	93.8	7.93
$Y_2 = 39.05 + 7.171A - 14.331B - 5.05A^2 - 2.54B^2 + 3.08AC$	95.3	94.3	3.02
$Y_3 = 686.0 - 30.66A - 76.1A^2 - 141.9B^2 - 97.4C^2 - 26.2AB - 48.0AC$	90.8	88.4	3.42
$Y_4 = +99.72 - 0.041B - 0.02813A^2 + 0.131B^2 + 0.01813C^2 + 0.0200AC - 0.03625BC$	97.4	96.7	0.01
$Y_5 = -1.54438 + 0.0723A + 1.23875B + 0.00044C - 0.00026A^2 - 0.13438B^2 - 0.00001C^2 - 0.00538AB + 0.00090BC$	97.3	96.2	0.03
$Y_6 = +95.00 - 1.399A - 9.40B + 0.0099C + 0.0055A^2 - 0.585B^2 + 0.11281AB - 0.0007AC + 0.0120BC$	95.0	93.1	0.52
$Y_7 = +128.2 - 0.613A - 18.766B + 0.065C + 0.006A^2 + 4.171B^2 + 0.001C^2 - 0.071AB - 0.002AC$	94.4	92.2	0.98
$Y_8 = +62.809 - 2.275C + 1.768C^2 + 1.720AC + 4.203BC$	78.3	74.8	1.73

Y_1: Floatability, Y_2: Expansion ratio; Y_3: Bulk density; Y_4: Pellet durability index, Y_5: Water absorption index; Y_6: Water solubility index; Y_7: Water stability; Y_8: *In-vitro* protein digestibility R^2: Co-efficient of determination, R^2-Adj: Adjusted co-efficient of determination, s: Standard error of the regression.

Table 4.1-5: Pearson's correlation coefficients of response variables (n=30)

	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
Y_1	0.849^{**}	0.311	0.034	0.567^{**}	-0.085	0.219	0.086
Y_2		0.168	0.271	0.413^{*}	-0.122	0.117	0.127
Y_3			-0.479^{**}	0.511^{**}	-0.052	-0.535^{**}	-0.313
Y_4				-0.383^{*}	-0.278	0.448^{*}	0.043
Y_5					-0.581^{**}	-0.040	0.017
Y_6						-0.169	0.054
Y_7							-0.062

Y_1: Floatability, Y_2: Expansion ratio; Y_3: Bulk density; Y_4: Pellet durability index, Y_5: Water absorption index; Y_6: Water solubility index; Y_7: Water stability; Y_8: *In-vitro* protein digestibility

**Correlation is significant at the 0.01 level (2-tailed);

*Correlation is significant at the 0.05 level (2-tailed).

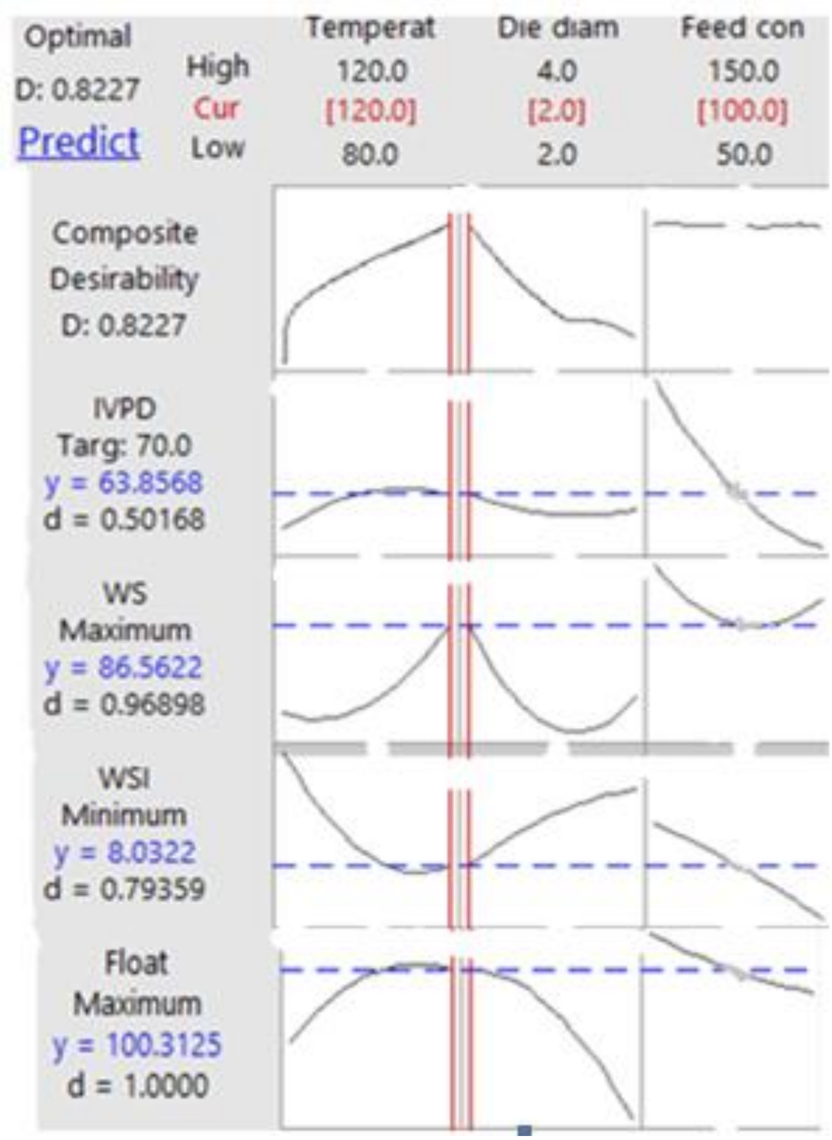

Figure 4.1-5: Optimization plot for in vitro protein digestibility (IVPD), water stability (WS), water solubility index (WSI) and floatability with respect to temperature (ºC), die diameter (mm) and feed conditioning time (s)

4.2: Effect of insect type, level of inclusion and feed moisture content on the physico-chemical properties of fish feed extrudates containing edible insect meal.

Floatability, expansion ratio, surface area, volume and bulk density of pellets

Insect type did not significantly influence (P = 1.0000) pellet floatability (Table 4.2-1). The effect of interaction between level of substitution and moisture content of formulation was however significant (P = 0.0198). In all formulations, pellet floatability increased with increasing moisture content (Table 4.2-2), but seemingly decreased with increasing level of insect meal substitution when moisture content of formulation was maintained at 10 and 20%. All blends with 30% moisture content produced floating pellets with over 93% floatability and the values were not significantly different ($P = 0.05$).

Expansion ratio, surface area and volume of pellets were not influenced by insect type ($P = 0.2800$: expansion ratio; $P = 0.6050$: surface area; $P = 0.5728$: volume). Level of FWSM substitution also did not influence these parameters ($P = 0.0658$: 25% substitution; $P = 0.1805$; 50% substitution; $P = 0.1645$: 75% substitution). The effect of moisture content was, however, significant ($P = 0.0389$: expansion; $P=0.0440$: surface area; $P = 0.0371$: volume). Blends containing 30% feed moisture content produced pellets with higher expansion ratio, surface area and volume compared to those containing 10 and 20% feed moisture content (Table 4.2-2). Also, at 10 and 20% feed moisture contents, pellets processed from 50 and 75% ACM containing blends had higher expansion than the respective BSFM blends.

Bulk density of extruded pellets was not influenced by insect type, but by the level of inclusion ($P < 0.001$), and moisture content ($P<0.001$). The high value of R^2 (87.90%; Table 4.2-1), however, shows that the three factors and their interactions, sufficiently accounted for bulk density of extrudates. The BSFM and ACM containing blends had higher bulk densities than the control formulation at 10 and 20% feed moisture contents, and increasing the level of insect meal inclusion from 25% to 75% gave a curvilinear relation with bulk density (Table 4.2-2). An increase in feed moisture content significantly ($P < 0.05$) decreased bulk density. Moreover, at 30% moisture, lower bulk densities were attained in pellets extruded from the BSFM containing blends (BMSF50, BMSF75) as compared to the control and the ACM containing ones.

Table 4.2-1: Mean squares for the effects of insect type, level of fresh water shrimp meal substitution, feed moisture content and their interactions on physico-chemical properties of extruded fish feeds

Source of variation	df	F	ER	SA	V	BD	DI	WAI	WSI	WS
Insect type	1	0.00^{ns}	41.24^{ns}	300.81^{ns}	189.57^{ns}	4530.13^{ns}	0.11^{ns}	0.06^{ns}	11.24^{ns}	7.59^{ns}
LS	3	3201.74**	92.46^{ns}	1933.21^{ns}	1074.71^{ns}	44716.59***	0.07^{ns}	0.03^{ns}	6.71^{ns}	17.76**
MC	2	22474.35***	126.08*	3753.04*	2198.23*	282984.17***	0.26^{ns}	0.04^{ns}	13.47*	48.07***
Insect type x LS	3	551.74^{ns}	5.54^{ns}	2014.58^{ns}	1058.46^{ns}	1795.16^{ns}	0.34^{ns}	0.09^{ns}	4.43^{ns}	22.98***
Insect type x MC	2	37.89^{ns}	8.24^{ns}	269.82^{ns}	114.14^{ns}	2443.92^{ns}	0.03^{ns}	0.01^{ns}	2.18^{ns}	43.09***
LS x MC	6	1084.94*	21.22^{ns}	385.55^{ns}	245.64^{ns}	10700.08^{ns}	0.37*	0.04^{ns}	5.79^{ns}	31.02***
Insect type x LS x MC	6	808.89^{ns}	63.69^{ns}	934.91^{ns}	489.58^{ns}	2348.22^{ns}	0.12^{ns}	0.02^{ns}	3.34^{ns}	6.59*
Error		343.23	33.80	1094.73	579.85	4552.123	0.15	0.03	3.46	2.75
R^2 (%)		89.15	57.85	51.69	52.89	87.89	57.25	50.93	61.19	89.06
CV		28.97	10.24	28.15	31.85	11.71	0.39	4.61	16.05	1.99

***significant at $P < 0.001$; ** significant at $P < 0.01$; * Significant at $P < 0.05$; ^{ns} not significant at $P < 0.05$; R^2: coefficient of determination; CV: coefficient of variation; LS: level of fresh water shrimp meal substitution; MC: moisture content; df: degrees of freedom; F: floatability; ER: expansion ratio; SA: surface area; V: volume; BD: bulk density; DI: durability index; WAI: water absorption index; WSI: water solubility index; WS: water stability.

Extrusion of feed blends offers opportunity for efficient utilization of the feed in fish ponds. Floatability of extrudate is an important parameter that determines whether an aqua feed is to be utilized by bottom, mid-level or top feeders that feed on sinking, slow sinking and floating feeds, respectively. It is related to structure formation, which is a function of composition of the feed material. Starch, and occasionally some proteins, play important role in structure formation during extrusion. According to Ayadi *et al*. (2011) and Vijayagopal (2004) starch plays role in expansion of pellets which in turn affects the floatability. Depending on moisture content and temperature, starch granules are melted or gelatinized to form an amorphous continuous phase that traps the gases released at the extruder die enabling formation of expanded structures. In the present case, starch in various blends was supplied mainly by wheat pollard, maize germ, sunflower cake and cassava, and these were not varied. The substitution of fishmeal with BSFM or ACM, however, altered the composition of fat, and fibre. Contents of crude fibre and crude fat were particularly higher in BSFM and ACM than in FWSM. Increasing fat content may have been responsible for a decrease in extrudate expansion and therefore floatability particularly for the BSFM containing blends. High fat content of feed causes reduced mechanical energy input during extrusion, which leads to an undercooked dough with low amount of dispersed starch and a decreased overall die expansion of extrudate (Ilo *et al.,* 2000). Other ingredients such as fibre, depending on their polymeric nature, affect moisture binding of the feed or modify the behavior or viscosity of the melt as it flows and exits through the die. The increase in floatability as feed moisture content was increased from 10 to 30% could be attributed to increased starch gelatinization and a reduced melt viscosity that facilitated growth and maintenance of gas bubble growth (Singh *et al.,* 2014). These findings are in agreement with the findings of Umar *et al*. (2013) who reported that moisture content influenced floating characteristics of extruded fish feeds. Feed moisture content of 30% offers the possibility to produce floating pellets at different levels of BSFM or ACM inclusion as may be desired.

In addition to floatability, expansion defines the brittleness of pellets (Rosentrater *et al*., 2009) and also affects the digestibility of feeds (Fallahi *et al*., 2012); higher expansion ratios improve feed digestibility. Surface area and volume, which are related to expansion, give an indication of how the material will pack during storage and transportation (Khater *et al.,* 2014). As the plasticized dough exits the die under pressure, the extrudate swells, moisture flushes out, and cooling and relaxation of the extruded material occur. These events, affect the actual size and shape of extrudate. Expansion ratio, surface area and volume increased with moisture content because the water promoted starch gelatinization leading to higher expansion at the exit of the die (Umar *et al*., 2013). However, the finding somehow contradicts the findings of Rosentrater *et al*. (2009) and Bandyopadhyay and Rout (2001) who found that an increase in moisture content decreased the expansion ratio of extruded aqua feeds, but agrees well with the findings by Tumuluru (2013) that medium moisture content of 30 – 40% increased die expansion. Fallahi *et al.* (2012) has also observed that moisture content, feed composition and the flow rate of the material through the extrusion barrel influences expansion rate. Although the effect of insect meal substitution on expansion ratio was not significant, a peculiar observation was that increasing the level of insect meal tended to decrease expansion of extrudates at each moisture content level, an observation that could be attributed to increase in lipids content in the formulated blends (Vijayagopal, 2004).

Bulk density measures the extent of puffing of extrudates, and thus is related to expansion of the final products (Singh *et al*., 2014), but unlike volume and surface area, takes into consideration the pores and voids that were developed during extrusion process and therefore informs better on how the packaging material and storage facilities could be designed (Fallahi *et al*., 2012; Ayadi *et al*., 2011; Chevanan *et al*., 2007). The decrease in bulk density with increase in moisture content is explained by the fact that moisture content favors expansion. Several studies reported similar findings (Singh and Muthukumarappan, 2014; Chevanan *et al*., 2007; Singh and Muthukumarappan, 2015). The inverse relationship between floatability and bulk density has also been reported (Adeparusi and Famurewa, 2011). Vijayagopal (2004) gives bulk densities for different categories of expanded aqua feeds: floating pellets < 550g/L, sinking pellets 550g/L – 650g/L, fast sinking pellets > 650g/L. Thus, from this study, BSFM and ACM can be used to produce pellets for all feeders by varying insect meal inclusion and feed moisture content. For example, at 10% moisture content, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75 are all suited for bottom feeders such as Catfish (*Clarias gariepinus*); at 20% moisture content, BSFM25, BSFM50, and ACM25 are well suited for top feeders, ACM50 and ACM75 for mid-level feeders, while BSFM75 is suited for bottom feeders; at 30% moisture content, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75 can all be used for top feeders such as Tilapia (*Oreochromis niloticus*).

Table 4.2-2: Effect of insect type, level of fresh water shrimp meal substitution and feed moisture content (MC) on floatability (F), expansion ratio (ER), surface area (SA), volume (V), and bulk density (BD) of pellets.

Property	Formulation							
	MC (%)	Control	BSFM25	BSFM50	BSFM75	ACM25	ACM50	ACM75
F (%)	10	68.8±6.3^{a}	0.0±0.0^{e}	20.3±1.3^{c}	7.5±0.5^{d}	46.3±6.3^{b}	25.0±5.0^{c}	0.0±0.0^{e}
	20	98.8±1.6^{a}	95.0±4.0^{ab}	71.3 ± 6.3^{c}	12.5±2.5^{e}	95.0±0.0^{b}	42.5±4.5^{d}	51.3±6.3^{d}
	30	100.0±0.0^{a}	100.0±0.0^{a}	98.8±1.3^{a}	98.8±1.3^{a}	93.8±6.3^{a}	93.8±6.4^{a}	96.3±3.8^{a}
ER (%)	10	54.5±1.8^{b}	61.7±4.8^{a}	48.4±2.6^{c}	49.4±3.5^{bc}	51.2±1.5^{bc}	53.5±1.2^{b}	57.7±3.2^{ab}
	20	59.5±3.6^{ab}	58.6±6.1^{ab}	54.9±1.2^{b}	52.5±3.6^{b}	67.8±5.0^{a}	56.4±3.3^{b}	54.0±2.3^{b}
	30	52.6±7.8^{a}	60.7±2.8^{a}	61.7±4.5^{a}	55.7±1.5^{a}	62.4±6.1^{a}	59.5±1.2^{a}	54.5±3.7^{a}
SA (mm^{2})	10	88.5±7.0^{b}	115.4±8.5^{a}	94.2±10.4^{b}	73.3±8.7^{b}	85.3±6.3^{b}	122.3±7.6^{a}	121.6±14.2^{a}
	20	118.0±7.2^{c}	136.7±7.8^{b}	120.3±5.0^{c}	122.8±0.3^{c}	111.8±11.2^{c}	167.8±16.9^{a}	91.9±7.6^{d}
	30	116.9±11.3^{b}	149.9±14.6^{a}	151.1±13.0^{a}	93.3±8.7^{c}	112.7±5.8^{bc}	154.6±8.7^{a}	140.7±16.3^{ab}
V (mm^{3})	10	53.9±3.2^{b}	75.8±9.3^{a}	56.9±6.6^{b}	42.5±5.1^{b}	50.9±4.1^{b}	77.5±8.5^{a}	78.4±8.4^{a}
	20	76.7±8.2^{c}	89.8±3.0^{b}	76.7±3.5^{c}	77.6±0.8^{c}	75.3±7.9^{c}	111.9±5.3^{a}	55.9±5.3^{d}
	30	72.9±7.9^{b}	100.4±8.3^{a}	102.5±9.4^{a}	57.8±7.4^{b}	73.2±6.8^{b}	103.5±7.9^{a}	91.9±4.7^{a}
BD (g/L)	10	570.5±49.2^{d}	720.4±51.5^{bc}	719.3±44.1^{c}	861.3±14.3^{a}	771.9±97.3^{ab}	713.8±54.7^{c}	822.9±24.8^{a}
	20	466.3±3.9^{c}	502.5±40.2^{b}	517.1±39.2^{b}	676.8±34.3^{a}	545.2±78.4^{b}	564.2±71.5^{ab}	597.7±58.5^{ab}
	30	453.8±5.2^{b}	456.7±13.7^{b}	427.7±14.4^{e}	428.1±17.7^{e}	513.4±47.9^{a}	457.5±18.5^{b}	520.7±32.0^{a}

FWSM: fresh water shrimp meal; BSFM: black soldier fly meal; ACM: adult cricket meal; BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control. For each property, means followed by same superscript letters along the same row are not significantly different at $P < 0.05$.

Table 4.2-3: Effect of insect species, level of fresh water shrimp substitution, and feed moisture content (MC) on durability index (DI), water absorption index (WAI), water solubility index (WSI), and water stability (WS) of pellets

Property	MC (%)	Formulation						
		Control	BSFM25	BSFM50	BSFM75	ACM25	ACM50	ACM75
DI (%)	10	99.8±0.2^{a}	99.4±0.5^{a}	99.7±0.0^{a}	99.1±0.0^{a}	99.9±0.0^{a}	99.5±0.2^{a}	98.9±0.1^{a}
	20	98.8±1.1^{a}	99.7±0.1^{a}	99.9±0.0^{a}	99.6±0.1^{a}	99.9±0.1^{a}	99.3±0.0^{a}	99.9±0.0^{a}
	30	99.7±0.2^{a}	99.6±0.2^{a}	99.9±0.1^{a}	99.9±0.1^{a}	99.8±0.1^{a}	99.6±0.2^{a}	99.8±0.1^{a}
WAI (%)	10	4.1±0.1^{a}	4.2±0.1^{a}	3.8±0.1^{b}	3.8±0.1^{b}	4.1±0.1^{a}	4.1±0.1^{a}	4.2±0.1^{a}
	20	4.1±0.1^{a}	4.0±0.0^{a}	3.9±0.2^{a}	4.1±0.1^{a}	3.9±0.1^{a}	4.2±0.0^{a}	4.2±0.1^{a}
	30	4.1±0.2^{a}	3.9±0.2^{a}	3.8±0.0^{a}	3.9±0.0^{a}	3.9±0.2^{a}	4.1±0.2^{a}	3.9±0.3^{a}
WSI (%)	10	12.8±2.0^{a}	10.2±0.7^{a}	10.5±0.7^{a}	10.3±0.0^{a}	11.2±0.5^{a}	13.4±2.6^{a}	9.5±0.8^{a}
	20	12.3±2.6^{b}	15.1±0.4^{a}	10.4±0.4^{b}	9.9±0.6^{b}	11.7±0.3^{b}	9.1±0.3^{b}	10.1±0.2^{b}
	30	14.1±1.8^{a}	13.6±0.4^{a}	14.0±3.8^{a}	11.7±0.2^{b}	11.0±0.7^{b}	13.6±0.3^{a}	11.3±0.0^{b}
WS (%)	10	85.8±0.4^{a}	78.4±3.5^{bc}	77.6±0.0^{c}	74.1±1.0^{c}	86.1±1.3^{a}	80.1±0.6^{b}	79.6±0.3^{b}
	20	84.7±1.1^{bc}	83.4±0.0^{c}	87.0±0.7^{a}	80.3±0.4^{d}	86.3±2.7^{ab}	81.3±0.1^{b}	85.6±0.4^{b}
	30	83.7±0.6^{b}	83.2±0.0^{b}	87.1±0.3^{a}	87.2±0.1^{a}	83.7±1.7^{b}	83.0±1.2^{b}	83.5±1.3^{b}

FWSM: fresh water shrimp meal; BSFM: black soldier fly meal; ACM: adult cricket meal; BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control. For each property, means followed by same letter superscript letters along the same row are not significantly different at $P < 0.05$.

Durability index, water absorption index, water solubility index and water stability

Insect type, level of fish meal substitution and moisture content of formulation, and their interactions did not influence pellet durability ($P<0.05$; Table 4.2-1). Processed pellets were generally highly durable under the test conditions applied for the present study. Similarly, WAI was not influenced by either of the investigated factor or their interaction effects. However, WSI was significantly influenced ($P < 0.05$) by feed moisture content (Table 4.2-1); WSI increased with increasing moisture content (Table 4.2-3). Water stability of pellets was significantly influenced ($P<0.05$) by the interaction effect of insect type, level of insect meal inclusion and moisture content (Table 4.2-1), and the high value of R^2 (89.062%) and low value of CV (1.996) show that this interaction sufficiently explained the parameter.

Durability index measures whether extrudates can withstand mechanical handling (Singh and Muthukumarappan, 2015), and is dependent on factors such as the degree of heat treatment during extrusion, and the extent of starch transformation and its interaction with other macromolecules (Chevanan *et al.*, 2007; Chevanan *et al*., 2009; Rosentrater *et al*., 2009). The durability indices (>99%) indicate that the pellets produced can withstand major handling impacts such as loading and unloading during transportation. Fallahi *et al*. (2012) also obtained > 99% pellet durability index on extruded aqua feeds. The linear relationship between expansion ratio and durability index can be attributed to the fact that both properties are influenced by pressures developed within the barrel. Other studies also reported a linear relationship between expansion ratio and durability index (Khater *et al*., 2014; Kraugerud *et al*., 2011). The finding that feed moisture content did not influence durability index agrees with the findings of Singh and Muthukumarappan (2015), but negates the findings of Chevanan *et al*. (2007) and Foley and Rosentrater (2013) who reported that moisture content had a significant effect on durability of extruded aqua feeds containing distillers dried grains.

Water absorption and water solubility indices give the relationship that the pellets will have with water when introduced into fish ponds (Rosentrater *et al*., 2009), and give an indication of the changes in ingredient content of the pellets in water (Fellows 2000). Like these findings, Rosentrater *et al*. (2009) reported a curvilinear relationship between the inclusion levels of distillers dried grains and WAI and WSI of the extruded aqua feeds. Similarly, Fellows (2000) reported an inverse relationship between WSI and WAI. The increase in WSI with an increase in moisture content can be attributed to starch gelatinization that increased with increase in moisture content of up to 30% (Jackson *et al.*, 1990). It has also been reported that depolymerisation of macromolecules during extrusion produces simpler molecules that are more water soluble (BeMiller and Whistler, 2009; Peluola-Adeyemi *et al*., 2014). Several other studies have reported an increase in WAI and a decrease in WSI with an increase in moisture content (Badrie and Mellowes, 1991; Singh and Muthukumarappan, 2014). With respect to starch, WAI and WSI are also dependent on amylose and amylopectin (Moscicki *et al*., 2012) and their resulting ratios in the extruded product. An increase in water solubility index, as was the case in this study, is desirable in that it increases nutrient digestibility.

Water stability indicates how the product will withstand water dissolution (Ayadi *et al*., 2011). Starch plays an important role in water stability (Vijayagopal, 2004), where its dextrinization results in the products being able to absorb water fairly well. Similar findings were reported by Umar *et al*. (2013) and Foley and Rosentrater (2013) who found an increase in water stability with an increase in moisture content. Bandyopadhyay and Rout (2001) found an inverse relationship between moisture content and water stability. Tumuluru (2013) also reported that moisture content significantly affected water stability of extruded fish feeds. Vijayagopal (2004) observed that feed mixture needed to be wetted to moisture content of about 25% for maximum water stability. The slightly higher water stability for BSFM75 and ACM75 pellets extruded at 20 and 30% moisture contents indicate that these products will resist nutrient leaching in water and thus allow better availability of nutrients for fish. In addition, minimal pollution of water would be experienced as a result of their use. For instance, FAO (2010) cites a water stability of 84% for extruded feeds intended for Catfish, which are bottom feeders.

Sinking velocity, total suspended solids and total dissolved solids in water

Sinking velocity, TSS and TDS were determined on pellets processed from the control formulation and insect meal containing blends at 75% substitution (BSFM75 and ACM75) and 30% feed moisture content because the three exhibited better properties in comparison to other treatments. All the three differently processed pellets exhibited floatability > 95% in the first 8 h (Figure 4.2-1[A]). About 50% and 60% of pellets extruded from BSFM75 and control blends respectively, remained floating after 24 h compared to 72% of the ACM75 pellets. This difference was significant ($P < 0.05$). At 48 h, 90, 95 and 100% of the control, BSFM75 and ACM75 pellets respectively, did not float.

Figure 4.2-1[B] shows that waters introduced with BSFM75 or ACM75 pellets did not differ in TDS levels. Moreover TDS of water introduced with these pellets was significantly lower ($P < 0.05$) than that of water introduced with the control pellets. Figure 4.2-1[C] shows the TSS concentration overtime. At onset, water introduced with the control and ACM75 pellets had significantly higher ($P<0.05$) TSS concentration than that introduced with BSFM75 pellets. However, at 8 h TSS concentrations of water introduced with the three types of pellets were not significantly different ($P<0.05$). There was a sharp decrease in TSS concentration between 8 - 24 h and a slight increase from 24 h onwards in the waters introduced with BSFM75 and ACM75 pellets. A peculiar observation is that water introduced with the BSFM75 pellets had higher TSS than that introduced with the control or ACM75 pellets from 8 to 48 h.

Sinking velocity gives an indication of how long the pellets would remain floating on water. The first 8 h after feed has been introduced into the pond is the time within which fish (top feeders such as Tilapia) would be expected to have fed on the pellets. Expansion of extrudates influences sinking velocity (Chevanan *et al*., 2007). The control, BSFM75 and ACM75 pellets had similar expansion ratios when extruded at 30% moisture content. The three products thus had similar sinking characteristics within the first 8 h. Pellet sinking behavior also coincides with changes in pond water quality. Suspended as well as the dissolved solids affect turbidity of water and thus light penetration (Bhavimani and Puttaiah, 2014). Within 0 - 8 h, majority of the pellets would still be floating hence the high concentration of suspended solids. The drop in TSS between 8 - 24 h is explained by the fact that, about half of the pellets are no longer floating while a slight increase in TSS between 24 -48 hours is because non-floating pellets begin to disintegrate. The high level of TSS in BSFM75 from 8 - 48h is probably due to high fat content of BSFM making the pellets less intact. The TDS profiles imply that although all the extrudates continued to lose small amounts of soluble nutrients into the water, BSFM75 and ACM75 pellets were more water stable than the control.

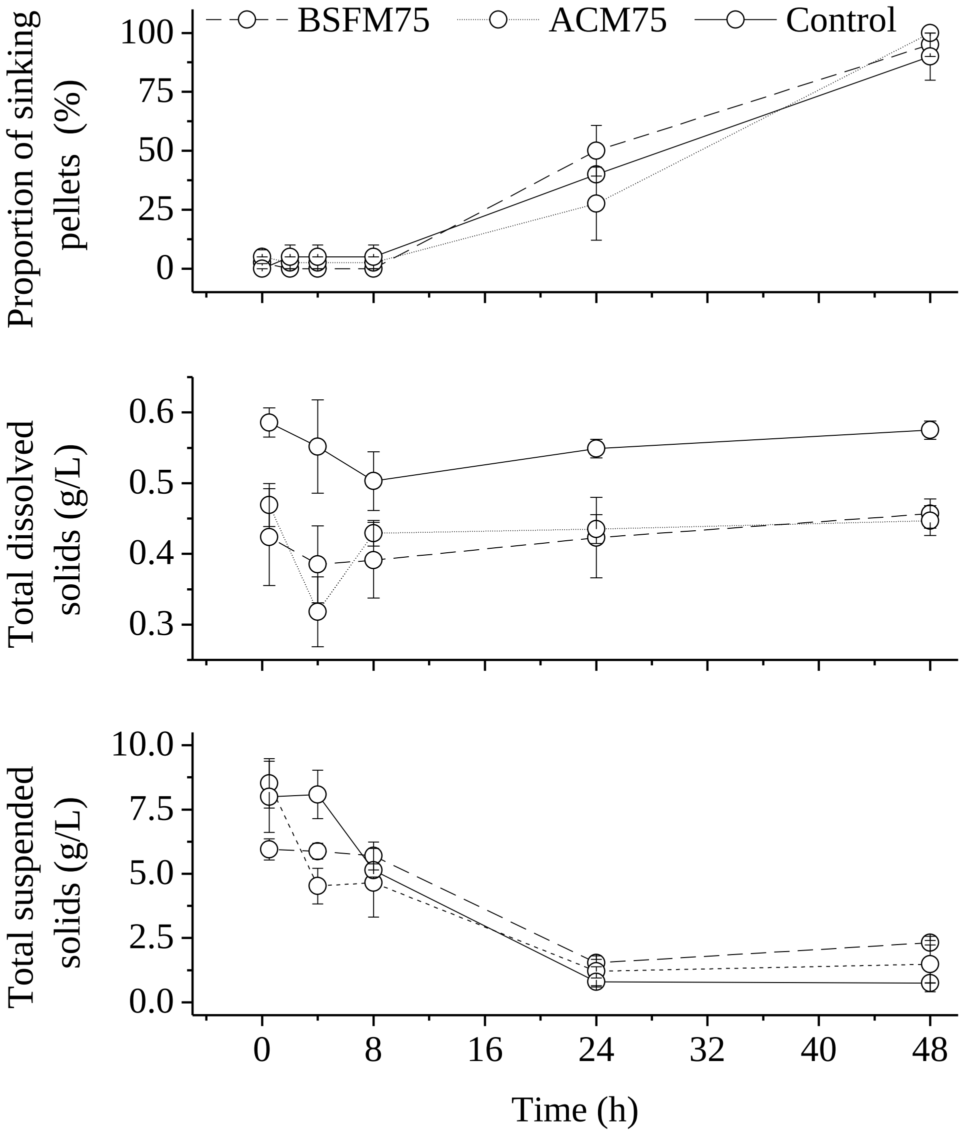

Figure 4.2-1: Graphs of sinking profile of pellets [A], total dissolved solids [B] and total suspended solids [C] of water introduced with pellets extruded from the control blend, black soldier fly meal containing blend [75% substitution; BSFM75] and adult cricket meal containing blend [75% substitution; ACM75] adjusted to 30% feed moisture content.

4.3: Proximate composition and in vitro protein digestibility of extruded fish feeds containing black soldier fly larvae (*Hermetia illucens*) and cricket (*Acheta domesticus*) flours.

Proximate composition of raw ingredients

Proximate composition of ingredients is as shown in Table 4.3-1 Cassava had highest values of dry matter and carbohydrate content that were significantly higher ($P < 0.05$) than all other ingredients with FWSM and ACM giving the least ($P < 0.05$) of the dry matter and carbohydrate contents respectively. Generally, ingredients of plant origin (sunflower cake, wheat pollard, maize germ and cassava) contained significantly ($P < 0.05$) higher values of carbohydrate content than animal based ingredients (FWSM, BSFM and ACM). On the other hand, ingredients of animal origin contained higher values of crude fat than all plant-based ingredients apart from sunflower cake. In addition, animal-based ingredients had significant ($P < 0.05$) higher values of crude ash and protein than ingredients of plant origin. There was no significant ($P < 0.05$) difference in dry matter and crude fibre contents of BSFM and ACM, although the two showed significant ($P < 0.05$) higher amounts of dry matter, crude fibre and crude fat than FWSM. Crude protein was highest ($P < 0.05$) in ACM than all other ingredients. This was followed by FWSM and BSFM respectively.

Table 4.3-1: Proximate composition of raw ingredients

Ingredient	Dry matter	Crude ash	Crude protein	Crude fibre	Ether extract	Carbohydrate
Sunflower cake	94.18±0.01	4.78±0.97	20.60±0.67	31.89±0.74	21.29±0.51	21.44±1.31
Wheat pollard	91.64±0.03	3.44±0.03	16.01±0.33	11.49±1.34	8.50±0.49	60.56±0.43
Maize germ	94.56±0.06	5.89±0.14	13.81±0.16	16.17±1.20	9.87±0.05	54.26±0.09
FWSM	90.77±0.02	22.34±2.02	53.98±1.52	4.18±0.23	10.53±1.44	11.97±1.26
Cassava	98.20±0.08	2.34±0.02	1.96±1.12	1.90±0.03	0.26±0.00	93.54±0.25
BSFM	94.25±0.07	10.8±0.03	41.77±0.65	8.81±0.35	24.95±0.35	13.67±0.31
ACM	94.02±0.16	8.36±0.14	62.35±1.03	8.62±0.08	13.34±0.19	7.30±1.10

FWSM: fresh water shrimp meal; BSFM: black soldier fly meal; ACM: adult cricket meal.
Values are presented on dry matter basis

The high values of carbohydrate that were recorded in ingredients of plant origin is due to the fact that cell wall of plants is mainly composed of carbohydrate while that of animals is composed of proteins and lipids. In addition, plants store their energy in form of carbohydrates such as fructans and starch (McDonald *et al*., 2010) with animals storing their energy in form of lipids. This also explains why BSFM, ACM and FWSM had significant high values of crude fat than most of plant ingredients. The fact that ingredients of animal origin gave significant higher amounts of crude ash and crude protein translate that these sources, such as BSFM and ACM are good sources of minerals as well as protein than plant based ingredients. In addition, both BSFM and ACM are good sources of crude fat given that the two recorded higher crude fat values than FWSM.

Effects of insect species, level of fish meal substitution and level of feed moisture content on proximate composition of extrudates

The effects of main factors (insect species, level of fish meal substitution and feed moisture content) on nutritional composition of extruded feeds are given in Table 4.3-2. There was significant difference ($P < 0.05$) among the insect species on all the study parameters. Black soldier fly larvae meal was significantly ($P < 0.05$) higher than ACM and the control formulations on dry matter, fibre content and ether extract. Adult cricket meal was significantly ($P < 0.05$) higher than the control and BSFM on crude protein and *in vitro* protein digestibility. The control formulation was only higher ($P < 0.05$) than both BSFM and ACM on ash content. There was no significant ($P < 0.05$) difference among the level of fish meal substitution on dry matter, crude protein and crude fibre (Table 4.3-2). However, the 25% level of substitution was higher ($P < 0.05$) than both 50% and 75% substitution levels on ash content and *in vitro* protein digestibility. Ether extract was higher ($P < 0.05$) on the 75% level of substitution than both the 25% and 50% levels of fish meal substitution. However, the 75% level of fish meal substitution was lower ($P < 0.05$) than both 25% and 50% levels of substitution on carbohydrate content. The level of moisture content influenced ($P < 0.05$) dry mater, ash content, crude protein, ether extract and carbohydrate content but did not affect ($P < 0.05$) fibre content and *in vitro* protein digestibility.

Table 4.3-2: Effect of insect species, level of fish meal substitution and level of feed moisture on proximate composition of extrudates

	DM	ASH	CP	CF	EE	CHO	IVPD
Insect Species							
BSFM	93.7±0.1^{a}	8.2±0.1^{b}	27.9±0.2^{b}	9.9±0.1^{a}	9.6±0.8^{a}	44.4±0.8^{b}	57.17±0.8^{b}
ACM	93.3±0.1^{b}	7.7±0.1^{c}	29.3±0.2^{a}	9.6±0.1^{b}	6.3±0.4^{b}	47.0±0.4^{a}	61.72±1.6^{a}
Level of fish meal substitution (%)							
0	93.4±0.2^{a}	8.5±0.0^{a}	28.5±0.5^{b}	9.1±0.1^{c}	4.8±0.2^{c}	49.2±0.5^{a}	55.82±0.7^{c}
25	93.5±0.1^{a}	8.5±0.1^{a}	29.0±0.3^{a}	9.9±0.1^{a}	6.8±0.5$_{c}$	45.8±0.7^{a}	62.74±2.2^{a}
50	93.5±0.1^{a}	7.5±0.1^{b}	28.4±0.4^{a}	9.9±0.1^{a}	8.8±0.8^{b}	45.4±0.5^{a}	59.73±2.4^{b}
75	93.6±0.2^{a}	7.3±0.2^{b}	28.5±0.4^{a}	10.2±0.2^{a}	11.4±1.0^{a}	42.6±0.9^{b}	59.48±1.3^{b}
Level of feed moisture content (%)							
10	93.3±0.1^{b}	8.1±0.1^{a}	28.2±0.3^{b}	9.6±0.2^{a}	8.8±0.8^{a}	45.3±1.0^{b}	Xx
20	93.9±0.1^{a}	7.9±0.2^{b}	28.6±0.4^{ab}	9.8±0.2^{a}	8.0±0.9^{b}	45.7±0.8^{ab}	59.35±1.31^{a}
30	93.3±0.1^{b}	7.8±0.2^{b}	29.0±0.4^{a}	9.8±0.1^{a}	7.1±0.9^{c}	46.2±0.7^{a}	59.54±1.42^{a}

BSFM: black soldier fly meal; ACM: adult cricket meal; DM: Dry matter; CP: Crude protein; CF: Crude fibre; EE: Ether extract; CHO: Carbohydrates; IVPD: *in vitro* protein digestibility. For each component, means followed by same superscript letters along the same column are not significantly different at $P < 0.05$.

The effect of insect species on proximate composition of extruded feeds is attributed to the differences between the two insects in regards to proximate composition. From Table 4.3-1, BSFM had significant higher amounts of dry matter and fibre content than ACM and fresh water shrimps, and this explains why BSFM based extrudates had higher amounts of dry matter and fibre content than FWSM and ACM. Similarly, Table 4.3-1 shows that ACM had higher amounts of crude protein than both the control (FWSM) and BSFM, and thus ACM gave products with significant higher crude protein than BSFM and the control. The high values in dry matter content of the extruded feeds indicate that the products had significant low moisture contents which resulted into extrudates with high stability. This is due to the fact that extrusion process cooks and dries the feed simultaneously (Onyango *et al.*, 2004) and this reduces the time and cost of post drying the extruded products. Among all the treatments, the control (0% fish meal substitution) was only higher than both BSFM and ACM on ash content. This again can be attributed to the fact that FWSM had far higher levels of ash content than BSFM and ACM (Table 4.3-1). This also explains why the 25% level of fish meal substitution had significant higher values of ash content than both 50% and 75% levels of substitution. The non-significant difference among the levels of fish meal substitution for crude protein is due to the fact that iso-proteineous formulations were formulated across all levels. The non-significance difference among the levels of fish meal substitution for most nutritional parameters such as dry matter, crude protein and fibre content indicate that either of the insects (BSFM and ACM) can be used to substitute fish meal at any of the three levels (25%, 50% and 75%) and still achieve nutritional quality as that of the control. Feed moisture content affected most of nutrients due to its ability to solubilize them and also due to its influence on extrusion variables such as melt temperature, melt viscosity and extrusion pressures.

Effect on crude ash

The effects of all the formulations on ash content of feeds extruded at 10%, 20% and 30% feed moisture contents are shown in Figure 4.3-1. At 25% level of fish meal substitution, BSFM, ACM and control formulations gave high values of ash content, though BSFM25 was higher than both the control and ACM25 in terms of crude ash content. However, it was observed that increasing the level of fish meal substitution with BSFM and ACM beyond 25% reduced the ash content of feeds at $P < 0.05$ level of significance. At 10% moisture content, ACM75 and ACM50 were significantly ($P < 0.05$) lower than all the other formulations. At 20% and 30% moisture contents, the control, BSFM25 and ACM25 gave higher values of crude ash than other formulations at $P < 0.05$ level of significance. At 20% and 30% moisture contents, BSFM25 had significantly ($P < 0.05$) higher amounts of ash content than all other formulations, while ACM75 had lower ($P < 0.05$) amounts of ash content than the other six formulations. Moisture content did not seem to affect ash content on feeds produced with formulations having low levels of fish meal substitution as well as the control formulation. However, ash content decreased with increasing level of moisture content for the BSFM50, BSFM75 and ACM75 formulations.

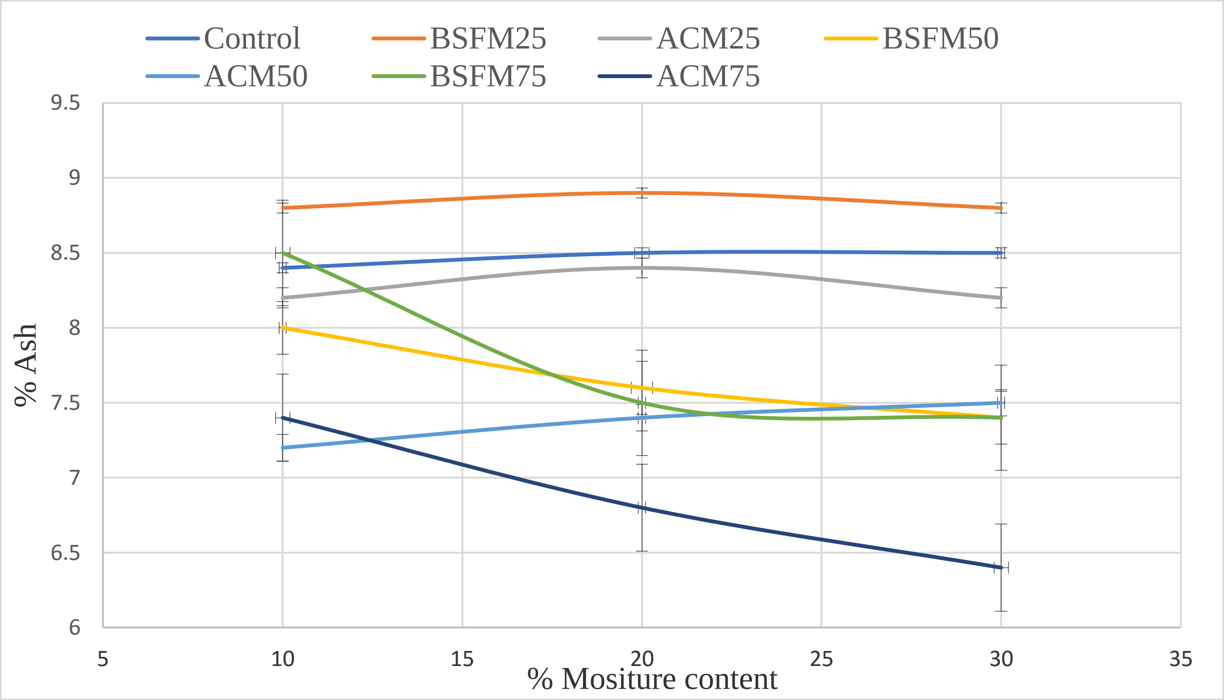

Figure 4.3-1: Ash content (%) as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents

Control: 0% insect substitution of FWSM;
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

Ash content gives an indication of the inorganic constituents in animal feeds. The decrease in ash content for the BSFM50, BSFM75 and ACM75 formulations as feed moisture content increased from 10% to 30% could be due to the leaching effect of minerals that are soluble in water. There are no set ash requirements for Tilapia and Catfish though a feed with considerably high ash content shows that the feed has relatively high amounts of minerals. However, some organic material may be present in ash and as thus this parameter do not give a good representative of the mineral composition in feeds (McDonald *et al.*, 2010), the need for further analysis to determine the mineral composition using the atomic absorption spectrometry.

Effect on crude protein

The effects of formulations under study and levels of feed moisture content on crude protein of the extrudates are presented in Figure 4.3-2. Substituting fish meal with ACM slightly increased the crude protein of the final feeds while substituting with BSFM reduced protein content of the final feeds. At 10% moisture content, BSFM25 and ACM25 had significant ($P < 0.05$) higher levels of crude protein than other formulations. The formulations ACM75 and ACM25 had crude protein levels that were significantly ($P < 0.05$) high than other formulations at 20% level of moisture content. However it is the ACM50 and ACM75 formulations that had significant ($P < 0.05$) higher crude protein than the control, BSFM25, ACM25, BSFM50 and BSFM75 formulations at 30% feed moisture content.

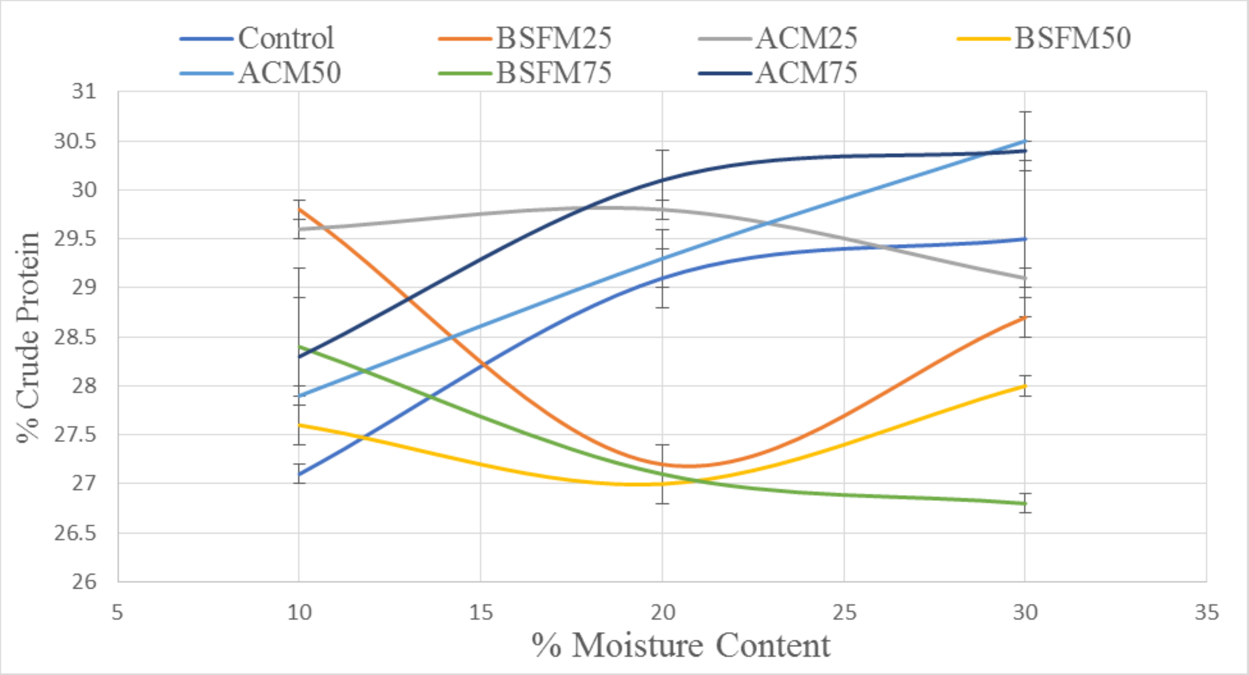

Figure 4.3-2: Crude protein (%) as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents
Control: 0% insect substitution of FWSM;
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

It is only the ACM50 and ACM75 that exhibited the same trend as the control where an increase in moisture content resulted in a corresponding increase in crude protein though in all, ACM50 and ACM75 had higher amounts of crude protein than the 0% fish meal substitution. Formulations BSFM25 and BSFM50 exhibited a peculiar relationship with feed moisture content where crude protein decreased with increasing level of moisture content up to 20% feed moisture content giving crude protein values that were not significant ($P < 0.05$) different from each other, probably due to uncontrollable changes in extruder conditions, but further increase in feed moisture content to 30% resulted in an increase in crude protein. For the BSFM75, there was a negative linear relationship between crude protein and feed moisture content where increasing feed moisture content led to a decrease in crude protein.

Proteins form the major organic constituent in tissue of fish and are thus the most expensive component in fish feeds as their supply are needed continuously throughout the life of fish for growth and maintenance (McDonald *et al*., 2010; Burnel and Allan, 2009; Robinson *et al*., 2006). Adult cricket has higher protein content than both FWSM and BSFM (Table 4.3-1; Barker *et al*., 1998; Finke 2002), while BSFM has low protein content than FWSM (Newton *et al*., 1977; St-Hilaire *et al*., 2007; Makkar *et al*., 2014). This is the reason as to why feeds substituted with ACM recorded high protein content than those substituted with BSFM. This also explains why the ACM based formulations had significant high amounts of protein throughout the three levels of feed moisture content than most of the test formulations. The increase in protein content for the ACM and control formulations as feed moisture content was increased linearly could be due to the ability of feed moisture to reduce absorption of thermal energy. The more a food material absorbs thermal energy during extrusion, the high amount of proteins that are lost (Omohimi *et al*., 2013) due to denaturation that may cause re-orientation of proetins to form insoluble structures (Onyango, 2005). At 10% and 20% feed moisture contents, , amino acids in BSFM formulations could have been involved in maillard reactions resulting into low crude protein content (Onyango *et al.*, 2004) as extrusion at low feed moisture content has been shown to facilitate maillard reactions (Guy, 2001). At 30% feed moisture content, feed moisture was enough to reduce mechanical dissipation and to solvate protein polymers allowing them to freely move and change from glassy to viscous elastic state making proteins more bio-available, the reason why crude protein for BSFM based formulations increased at 30% feed moisture content.

The minimum protein requirement for Tilapia and Catfish is 28-32% and 40-42% respectively (FDKS-2289-2:2010; FAO 2017) and from this study, both the ACM and the control formulations can satisfactory meet the minimum requirements for Tilapia. Quality of protein obtained from feed is expressed well in terms of digestible protein and the ability of that feed to supply the limiting amino acids (Burnel and Allan, 2009). In fish feeds, lysine and methionine are the limiting amino acids and their minimum requirements are 1.2 g/Kg and 0.5 g/Kg for Tilapia respectively (FDKS-2289-2:2010) and 4.49 g/Kg and 3.2 g/Kg for Catfish respectively (FAO 2017). Black soldier fly larvae and adult cricket have methionine levels of 9.04 g/Kg DM and 10.52 g/kg DM respectively (Vrabec *et al*., 2015). Adult cricket has lysine content of 11 g/Kg (Finke 2002) while BSFM has been shown to contain significant high amount of lysine (Dahiru *et al*., 2016) to an extent that its inclusion in feeds do not necessitate further fortification and this means that both BSFM and ACM are good protein substitutes to fish meal in the feeding of both Tilapia and Catfish. This study did not examine the changes in lysine and methionine that might occur as a result of extrusion. Future studies should target investigation such changes.

Effect on crude fiber

The effects of extrusion and test formulations on crude fibre content of fish feeds under various feed moisture contents are given in Figure 4.3-3. The control formulation (100% fishmeal) was significantly ($P < 0.05$) lower than all other formulations at 10% and 20% moisture contents. In addition, the control formulation still had the lowest crude fibre at 30% feed moisture content, though this was not significant ($P < 0.05$) different with ACM25, BSFM25 and BSFM50 formulations. Highest amount of crude fibre was recorded with BSFM75 at 20% moisture content. All formulations that had insects (BSFM and ACM) substituting fish meal showed significant ($P < 0.05$) higher amounts of crude fibre. Different formulations responded differently to the three levels of feed moisture content and thus feed moisture content did not have a significant ($P < 0.05$) influence on crude fibre.

Fibre supplies fish with no nutrients or energy but may play a vital role in regulating movement of bowel (Robinson *et al*., 2006). Both BSFM and ACM have high fibre contents than FWSM (Table 4.3-1) mainly due to the high presence of chitin, a non-starch polysaccharide that is found in insects such as BSFM and ACM than in FWSM (Tran *et al*., 2015), the reason why increasing the substitution level of FWSM with FWSM and ACM increased the crude fibre content of the feeds. According to Kumar *et al*. (2012) and McDonald *et al*. (2010) non-starch polysaccharide, especially those that forms gel acts as anti-nutritional elements in aquatic animals as they reduces the digestibility of nutrients by binding them and increasing the viscosity of the digested matter in intestines. Thus there is need to reduce the chitin content of both BSFM and ACM while substituting FWSM in fish feeds so that the fibre content, especially the non-starch polysaccharide may reduce significantly. Food and Agriculture Organization (2017) has put the maximum crude fibre requirement for Tilapia to be 8-10% and ACM25 formulation met this condition satisfactorily at all the three levels of feed moisture content. The formulations BSFM25 and BSFM50 also met this condition at 10% and 30% feed moisture contents with ACM75 formulation satisfying this condition at 10% and 20% feed moisture contents. It is only the BSFM75 that surpassed the maximum crude fibre requirement throughout the three feed moisture contents which could be due to the high crude fibre content in BSFM than in ACM and FWSM. According to Guy (2001) and Omohimi *et al*. (2013), extrusion has no significant effect on fibre content and this could explain why individual formulations responded differently to extrusion at different feed moisture contents. However, the changes in crude fibre with different feed moisture contents could be attributed to the structural changes such as shifts from insoluble to soluble fibres and formation of resistant starch that are not digestible at normal fibre analytical procedures.

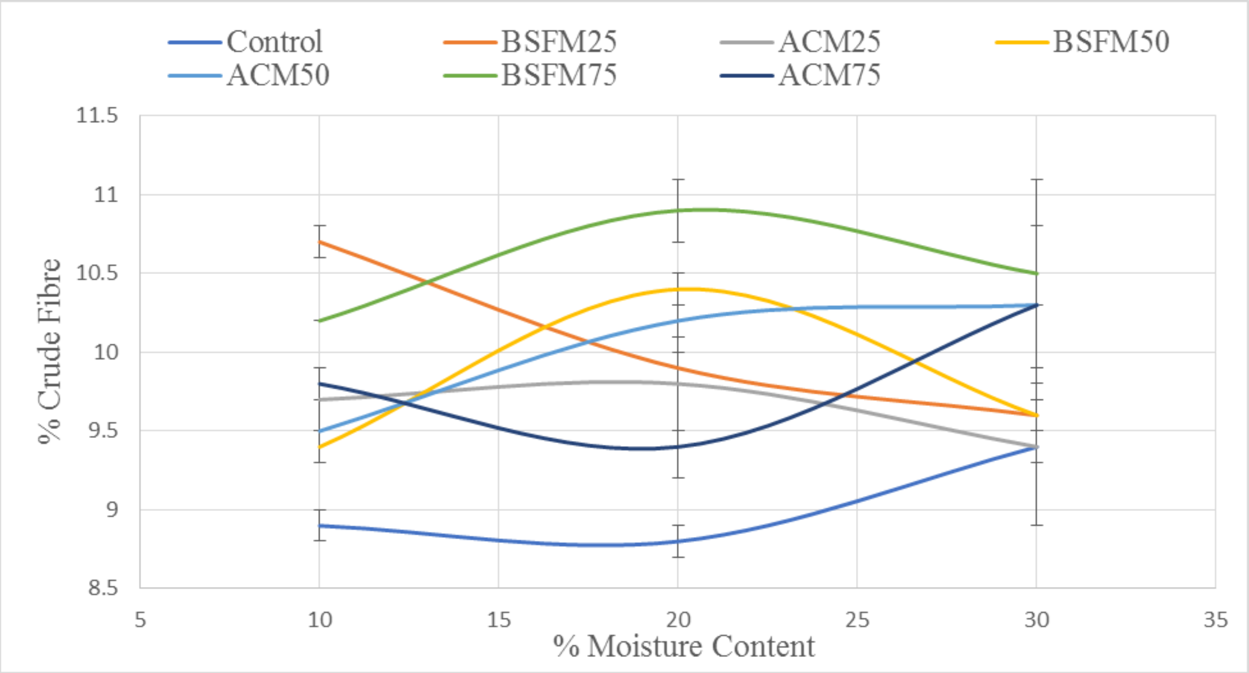

Figure 4.3-3: Fibre content (%) as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents

Control: 0% insect substitution of FWSM;

BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;

BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;

BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;

ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;

ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;

ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

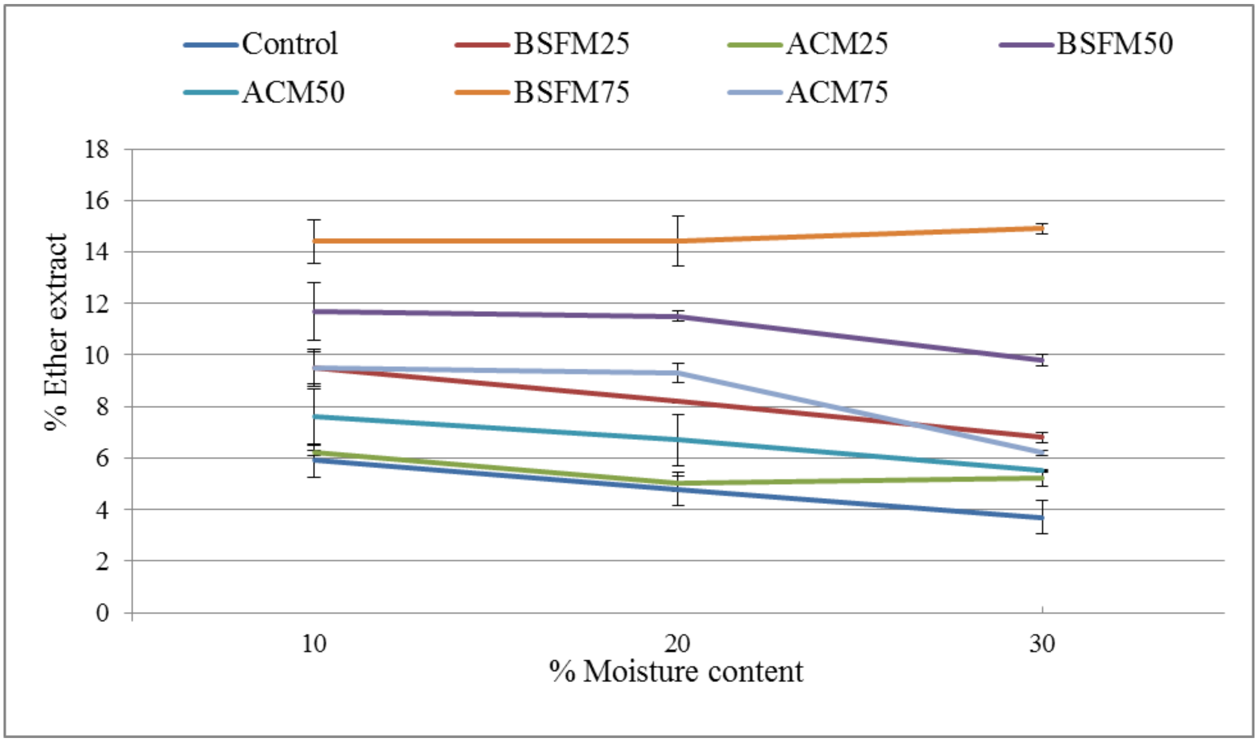

Figure 4.3-4: Ether extract (%) of extrudates as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents

Control 0% insect substitution of FWSM;
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

Effect on ether extract

The effects of different formulations and feed moisture content on ether extracts of pellets are as shown in Figure 4.3-4. The BSFM75 and BSFM50 formulations gave significant ($P < 0.05$) higher amounts of ether extracts than all other formulations across all the three feed moisture contents. At 30% feed moisture content, the control formulation showed the least ($P < 0.05$) amount of ether extract than all other formulations. In addition, the control formulation (100% fish meal) recorded significant ($P < 0.05$) lower levels of ether extracts than BSFM25, BSFM50, BSFM75, ACM50 and ACM75. Feed moisture was shown to have a significant ($P < 0.05$) effect on ether extract concentration where increasing the amount of feed moisture content from 10% to 30% reduced the amount of fat available in the feed. All the formulations except BSFM75 exhibited this phenomenon.

Lipids act as storage and sources of energy, as carriers for fat-soluble nutrients and as components of biological membranes (McDonald *et al*., 2010; Burnel and Allan, 2009). Fat, a constituent of lipids not only supplies energy to the animal but also acts as a thermal insulator. Black soldier fly larvae has been shown to contain significant amounts of fat than both ACM and FWSM (Table 4.3-1; Newton *et al*., 1977; Finke, 2002; Makkar *et al*., 2014) and thus the reason why BSFM based formulations had significant high values of fat than both the control and ACM formulations. The increase in amount of fat content as BSFM inclusion levels is increased has also been reported by Dahiru *et al*. (2016). The decrease in amount of crude fat as feed moisture content increased from 10% to 30% could be due to the formation of starch-lipid complexes, as a result of extrusion at high temperature with increased feed moisture content, that resisted lipid extraction (Omohimi *et al*., 2013;Onyango *et al*., 2004; Guy, 2001). The minimum requirements for crude fat for Tilapia and Catfish are 10% and 10-12% crude fat respectively (FDKS-2289-2:2010; FAO, 2017). Thus, from this study, it is only BSFM75 that can satisfactory meet these requirements with other formulations necessitating spraying of extrudates with supplementary oils. Given the facts that fats contributes to palatability of fish feeds (Burnel and Allan, 2009) and that BSFM and ACM formulations, such as BSFM75, BSFM50, ACM75 and ACM50 gave significant higher fat contents than the control formulation, BSFM and ACM formulations would probably be more palatable than the control formulation

Effect on carbohydrate content

The carbohydrate content as influenced by different test formulations that were extruded with different feed moisture content are as shown in Figure 4.3-5. Substituting FWSM with ACM slightly reduced the available carbohydrates in the final product but substituting FWSM with BSFM greatly reduced the carbohydrate content in the feeds. The reduction was significant ($P < 0.05$) for BSFM throughout the 4 levels of substitution but for ACM, it was significant ($P < 0.05$) only for the 75% level of substitution. Feed moisture content had a significant ($P < 0.05$) influence on carbohydrate content of extrudates in that; for most formulations (ACM25, BSFM25, BSFM50, BSFM75 and ACM75) carbohydrate content increased as the level of feed moisture content increased. However, carbohydrate content decreased for the control and ACM50 formulations as feed moisture content was increased from 10% to 20%. This trend continued for ACM50 where further increase in feed moisture content to 30% further reduced the carbohydrate content.

Carbohydrates are chemical compounds that contain the elements carbon, hydrogen and oxygen (McDonald *et al*., 2010) and they are primary sources of energy for living organisms. Carbohydrates dietary requirement in fish have not been demonstrated but if they are not supplied in the formulation, fish will catabolize proteins and fats for energy and thus these nutrients will not be able to perform their primary functions. Even though, FAO (2017) has recommended a maximum carbohydrate content of 40% for Tilapia which could be due to the consideration of energy to protein ratio balance. As thus BSFM75 formulation is well suited to meet this condition**.** Between 10% and 20% feed moisture content, ACM and control formulations unlike BSFM formulations were involved in dissipation of mechanical energy that resulted into unavailability of carbohydrates. Black soldier fly has lot of fats that acted as lubricants reducing the frictional and mechanical energy and thus their increasing carbohydrates between 10% and 20% feed moisture content due to intense shearing. At 30% feed moisture content, the moisture resulted into swelling of starches that facilitated their availability to shearing forces and thus the increased carbohydrate content of all formulations at 30% feed moisture content.

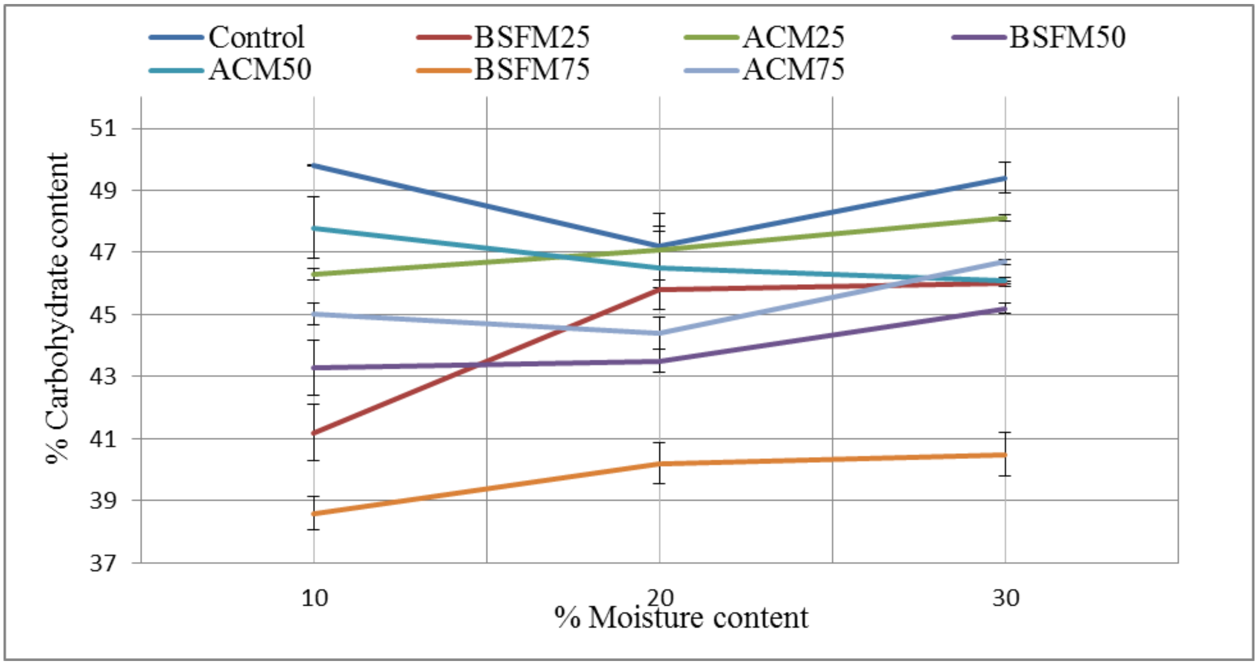

Figure 4.3-5: Carbohydrate content (%) of extrudates as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents

Control: 0% insect substitution of FWSM
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.

Effect on in vitro protein digestibility

The effects of different formulations on *in vitro* protein digestibility (IVPD) of extruded pellets are shown in Figure 4.3-6. At 20% feed moisture content, all ACM formulations had significant ($P < 0.05$) higher digestibility than BSFM and control formulations. However, ACM extrudates exhibited decreasing IVPD while BSFM showed increasing IVPD as feed moisture content was increased from 20% to 30%. Extrudates of the control formulation (0% level insect meal) also showed reducing IVPD with increasing level of feed moisture content.

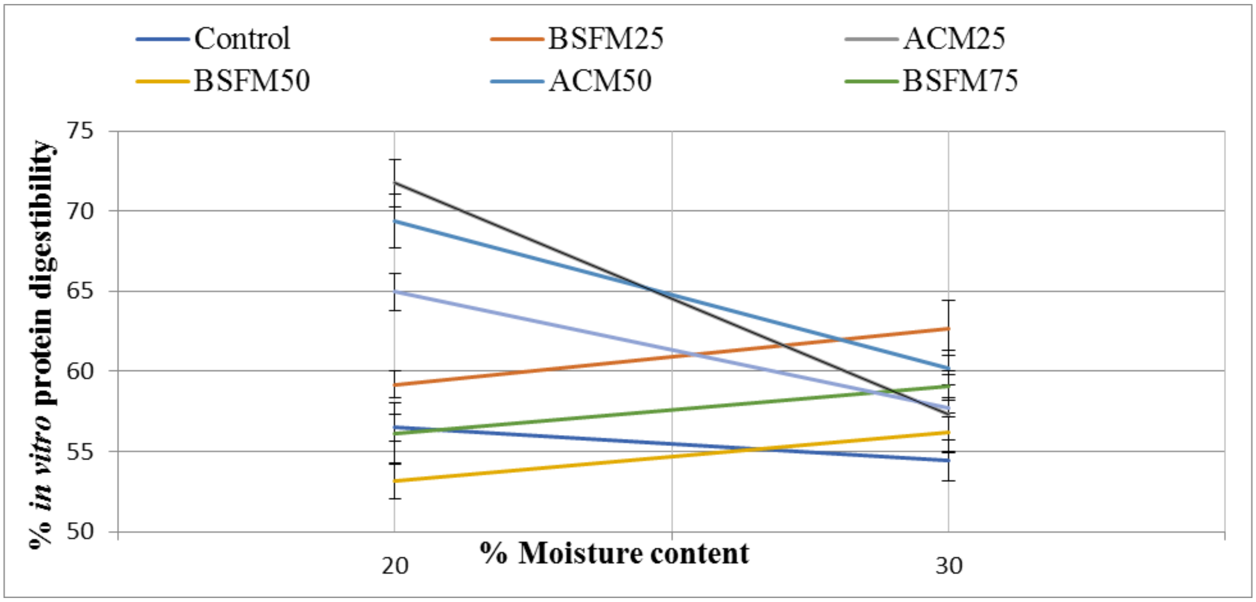

Figure 4.3-6: *In vitro* protein digestibility (%) of extrudates as influenced by different formulations (Control, BSFM25, BSFM50, BSFM75, ACM25, ACM50 and ACM75) under various feed moisture contents

Control: 0% insect substitution of FWSM
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control

Anti-nutritional factors such as protease and saponins, found in ingredients such as sunflower cake bind to proteins and amino acids reducing their digestibility (Kumar et al., 2012; McDonald et al., 2010). However, extrusion process reduces these anti-nutritional factors (Levic and Sredanovic, 2010; Filipovic et al., 2010; Sorensen, 2009) which in turn increases the digestibility of protein. This explains the high values of protein digestibility that were reported in this study. Nutrient excesses or deficiencies affects protein digestibility. For example, fat inhibits the activity of the enzyme pepsin to chemically digest the protein in food and since BSFM has high fat content than ACM (Table 4.3-1), the *in vitro* protein digestibility was low in BSFM based formulations than in ACM based formulations. The decrease in protein digestibility for ACM and control formulations as feed moisture content was increased from 20% to 30% could be attributed to the ability of high moisture to promote formation of protein-polyphenol complexes under high extrusion temperatures (Onyango *et al.*, 2004) that resist digestibility by hindering access sites to enzyme activity given that these formulations were rich in protein content.

4.4: Minerals content of extruded fish feeds containing cricket (*Acheta domesticus*) and black soldier fly larvae (*Hermetia illucens*) fractions

Minerals results for FWSM, ACM and BSFM are given in Table 4.4-1. Phosphorus and potassium were significantly ($P < 0.05$) higher in BSFM than in FWSM, though the contents of the two elements did not differ significantly ($P < 0.05$) in BSFM and ACM. Calcium was significantly ($P < 0.05$) higher in FWSM than in ACM and BSFM. Magnesium was significantly ($P < 0.05$) higher in BSFM than in ACM and FWSM. Sodium, copper and zinc were higher ($P < 0.05$) in both BSFM and ACM than FWSM. Adult cricket meal showed significant ($P < 0.05$) higher amounts of manganese than FWSM and BSFM. Iron was higher ($P < 0.05$) in ACM and FWSM than in BSFM.

The fact that ACM and BSFM were higher in majority of minerals than FWSM indicates that the two insects are better sources of minerals than FWSM and the two are good substitutes of FWSM in fish feeds.

Phosphorus and Calcium

The level of phosphorus in control formulations was significantly ($P < 0.05$) low than all the insect based formulations (Table 4.4-2). The level of phosphorus increased with increasing levels of insect substitution. However, for each level of insect substitution, BSFM based formulations recorded high values of phosphorus than ACM formulations. Moisture content of feed prior to extrusion did not have a significant ($P < 0.05$) effect on phosphorus. Table 4.4-2 shows the effects of different study formulations with different feed moisture contents on calcium. It was observed that the control formulation extruded with feed moisture of 20% retained the highest amounts of Calcium. This level however did not show significant ($P < 0.05$) difference with BSFM25 and ACM formulations that were produced at 20% feed moisture content. The increase in level of insect substitution led to a corresponding decrease in calcium levels. However, BSFM based formulations had higher calcium levels than the corresponding ACM formulations. Moisture content was also found to have a significant ($P < 0.05$) influence on calcium where for each formulation; calcium was high for the extrudates that had 20% feed moisture content than those that had a feed moisture content of 30%.

Phosphorus and calcium are important minerals required by fish for healthy bone formation as well as in enhancing growth performance of fish (FAO, 2016; Braga *et al*., 2016). The two have an antagonistic effect where an inadequate supply of one element affects the availability of the other due to the formation of Calcium-Phosphorus complex (Chavez-Sanchez *et al*., 2000), the reason why the two have to be discussed together. The high values of phosphorus recorded in BSFM based formulations can be attributed to the fact that BSFM had the highest amounts of phosphorus in comparison to ACM and FWSM (Table 4.4-1). Newton *et al*. (1977) and Barker *et al*. (1998) have also shown that BSFM has higher calcium and phosphorus than ACM. Likewise, the decrease in calcium amounts as the level of substitution increased is attributed to the high values of calcium that are found in FWSM in comparison to BSFM and ACM (Table 4.4-1). The low levels of calcium as the feed moisture content was increased to 30% could be due to the formation of solid bridge complexes (Tumuluru *et al*., 2011) that hindered the availability of the element. It could also be due to the fact that calcium is highly soluble in water (Nielsen, 2010) and thus a lot of it could have been solubilized in water which upon extrusion was evaporated. On the other hand, phosphorus has poor solubility in water and this could explain why feed moisture content did not have an influence on the amount of available phosphorus. Unlike calcium, phosphorus must be supplied by the feed because fish cannot get it readily from the surrounding water environment (Tessenderlo Group, 2005; Tang *et al*., 2012).

As such BSFM and ACM based formulations are good sources of phosphorus as both resulted in higher levels of phosphorus than the control (fresh water shrimps based) formulation. However, none of the study formulations resulted into minimum requirements for phosphorus for Tilapia (*Oreochromis Niloticus* [L]) and Catfish (*Clarias gariepinus* [Burchell]) which is 5000 ppm and 4500 ppm respectively (FDKS-2289:2010; FAO, 2017), and thus there is need to supplement the feeds with an inorganic source such as Dicalcium Phosphate so as to improve feed utilization and growth performance. Since calcium and phosphorus have an antagonist effect (Hassan *et al*., 2013; FAO, 2016), the Ca/P ratio of these formulations on growth and development of fish need to be studied.

Potassium

The effects of treatment combinations on potassium are as shown in Table 4.4-2. It was observed that the level of potassium increased with increasing level of fish meal substitution for each insect based formulation. This increase was significant (P < 0.05) apart from the ACM25 formulation that did not show significant (P < 0.05) difference with the control formulation. Another peculiar observation was that for each formulation, moisture content showed a significant (P <0.05) influence on Potassium where increasing the amount of feed moisture content from 20% to 30% resulted in corresponding increase in the level of potassium.

Potassium is essential in the growth and development of fish as it regulates acid base balance, intracellular osmotic pressure and is also required for protein and glycogen synthesis (FAO, 2017; N.R.C., 1993). The increase in potassium as level of substitution increased is due to the high potassium levels that are found in both ACM and BSFM in comparison to FWSM (Table 2). The fact that moisture content influenced potassium levels shows that extrusion has an influence on this element, which could be due to chemical alteration (Singh *et al*., 2007) and destruction of polyphenols (Alonso *et al*., 2001) through extrusion at high feed moisture content, that allows phosphorus to become bio-available. Razzak *et al*. (2012) also found an increase in potassium amounts of maize extrudates as moisture content was increased. The minimum potassium requirements for Tilapia and Catfish are 2100-3300 ppm and 2600 ppm respectively, and thus all the study formulations meet these requirements.

Table 4.4-1: Mineral composition of fresh water shrimps meal (FWSM), adult cricket meal (ACM) and black soldier fly meal larvae (BSFM)

Ingredient	P (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
FWSM	4385.8±960.6^{b}	3932.4±95.8^{a}	10065.2±962.9^{b}	2191.5±35.7^{b}	21246.1±23.5^{b}	1512.5±23.5^{b}	454.9±14.7^{b}	1932.1±95.6^{b}	922.2±25.6^{a}
ACM	6542.5±1246.7^{ab}	1600.7±170.7^{b}	11981.6±957.6^{ab}	1467.7±26.8^{c}	22633.6±97.4^{a}	1628.8±30.6^{a}	566.4±23.6^{a}	3126.1±96.7^{a}	931.9±24.8^{a}
BSFM	8579.7±900.5^{a}	1971.4±212.4^{b}	13853.1±978.7^{a}	3542.2±12.5^{a}	22546.9±92.4^{a}	1609.4±36.7^{a}	576.5±14.7^{a}	1762.2±86.7^{b}	845.1±23.8^{b}

FWSM: fresh water shrimp meal; BSFM: black soldier fly meal; ACM: adult cricket meal.

Table 4.4-2: Macro minerals content of extruded fish feed pellets containing varying levels of black soldier fly larvae and cricket meal as fish meal substitute.

Formulation	MC (%)	P (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)
Control	20	625.9±19.9^{e}	15063.8±300.6^{a}	6220.8±0.7^{e}	3287.6±4.5^{e}	1268.3±9.2^{a}
	30	649.4±13.5^{e}	14476.2±114.6^{ab}	6229.4±2.4^{e}	3318.9±5.8^{de}	1157.8±31.5^{bc}
BSFM25	20	712.6±26.6^{d}	14696.9±420.8^{ab}	6335.4±0.59^{d}	3436.1±31.4^{abcde}	1223.9±7.0^{ab}
	30	735.9±39.8^{d}	10752.5±37.8^{cd}	6341.8±3.8^{c}	3519.1±4.4^{ab}	1107.6±19.7^{c}
BSFM50	20	810.6±10.1^{b}	13077.1±1412.7^{abc}	6359.5±8.55^{b}	3451.9±13.4^{abcd}	991.9±44.4^{d}
	30	789.3±6.7^{c}	10386.2±370^{cd}	6370.5±10.4^{b}	3467.6±10^{abcd}	804.7±7.4^{ef}
BSFM75	20	804.2±14.9^{b}	9646.2±1053.9^{cde}	6362.5±2.3^{b}	3491.6±60.9^{abc}	492.1±16.4^{g}
	30	840.9±4.9^{a}	8855.8±218.6^{de}	6385.9±5.3^{a}	3597.5±49.1^{a}	442.9±5.3^{g}
ACM25	20	718.2±2.3^{d}	14613.2±392.4^{ab}	6226.3±3.6^{e}	3450.3±42.4^{abcd}	1145.7±18.4^{bc}
	30	715.9±23.3^{d}	12824.8±409.3^{bc}	6243.1±6.7^{e}	3383.8±14.2^{bcde}	1049.6±10.1^{d}
ACM50	20	749.4±6.5^{cd}	8911.8±1519.3^{de}	6359.7±9.2^{b}	2956.6±32.6^{f}	812.4±33.6^{ef}
	30	769.9±30.7^{bc}	4326.1±39.2^{f}	6383.5±8.4^{a}	2837.6±38.4^{f}	717.9±11.6^{f}
ACM75	20	782.1±12.7^{bc}	6439.6±392.4^{ef}	6385.3±0.6^{a}	3334.8±12.02^{cde}	878.2±4.8^{e}
	30	767.8±21.6^{bc}	4074.9±357.7^{f}	6389.5±3.3^{a}	2934.4±17.8^{f}	766.09±18.47^{f}

MC: Moisture content of feed prior to extrusion:
Control: 0% insect substitution of FWSM
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.
Extrusion was done at a barrel temperature of 120ºC and feed exited through a 2 mm die:
For each component, means followed by same superscript letters along the same column are not significantly different at $P < 0.05$.

Magnesium

The effects of the treatment combinations on magnesium are as shown in Table 4.4-2. Increasing the level of fish meal substitution with BSFM increased the content of magnesium. On the contrary, increasing the level of fish meal substitution with ACM led to a decreasing amount of magnesium. In addition, increasing the level of feed moisture content resulted into higher amounts of magnesium for the control and BSFM formulations but resulted in lower levels of magnesium for the ACM formulations.

Magnesium is required by fish as it is an essential cartilage and bone component and it also stimulates nerve and muscle irritability (Jahnen-Dechent and Ketteler, 2012; FAO, 2017). The decrease in amount of magnesium as FWSM was substituted with BSFM is due to the high magnesium content that is found in BSFM than it is found in FWSM (Table 4.4-1). Likewise, ACM has lower magnesium content than FWSM (Table 4.4-1) the reason why formulations substituted with ACM had lower magnesium content than the control formulation. Adult Cricket meal has higher protein content than both FWSM and BSFM (Makkar *et al*., 2014). This could explain why increasing level of FWSM substitution with ACM led to decreasing amount of magnesium where extrusion under high feed moisture content facilitated softening of proteins, that acts as natural binders (Tumuluru, 2014) and thus binding elements such as magnesium and rendering them unavailable. Fibre in ACM could also have been more susceptible to restructuring due to extrusion process to an extent of promoting chelating effects that could have rendered magnesium un-extractable (Cheftel, 1986). According to FAO (2017), the minimum magnesium requirements for Tilapia and Catfish are 600-800 ppm and 400 ppm respectively, and all the study formulations exceeded these levels. Thus both BSFM and ACM can be used to substitute FWSM in fish feed and satisfy magnesium requirements for both Tilapia and Catfish, given that fish does not meet all its magnesium requirements from surrounding water and that feeds must supply the demand (FAO, 2017).

Sodium

The effects of the treatment combinations on sodium are given in Table 4.4-2. Increasing the level of fish meal substitution with insect meal beyond 25% resulted in lower levels of sodium where BSFM50, BSFM75, ACM50 and ACM75 contained significantly ($P < 0.05$) lower amounts of sodium than the control formulation. Moisture content gave a negative correlation with the final amount of sodium where increasing the amount of feed moisture resulted in a significant ($P < 0.05$) decrease in the amount of sodium for all the formulations apart from BSFM75 whose reduction was not significant ($P < 0.09$).

Fish needs sodium for maintenance of acid base balance and for regulation of osmotic pressure (FAO, 2017; Webster and Lim, 2002). Extrusion at high temperature and high moisture content may have led to the formation of phenolic complexes that changed the chemical structure of the compounds and thus rendering sodium un-extractable (Alonso *et al*., 2001). In addition, high feed moisture content may have lowered the glass transition temperature leading to formation of solid-bridges (Tumuluru, 2014) that resulted into dense products that made sodium un-available. More studies need to be done to explain why sodium contents reduced as the level of fish meal substitution increased, yet both BSFM and ACM had higher sodium contents that FSWM. There is little information on the minimum sodium requirements for both Tilapia and Catfish and thus it is impossible to conclude whether the study formulations (especially insect based formulations) can successfully substitute fish meal and meet the sodium demand for these fishes.

Copper

The content of copper in the control formulation did not differ significantly ($P < 0.05$) from the content in BSFM75, ACM25 and ACM50 formulations that were extruded with 30% feed moisture content (Table 4.4-3). The ACM75 formulation extruded at both 20% and 30% feed moisture gave highest values of copper that significantly ($P < 0.05$) differed with all other formulations. Moisture content showed significant ($P < 0.05$) influence on the level of copper in that, for each formulation copper levels increased with increasing moisture content.

Copper is directly involved in enzymes activity such as dopamine hydroxylase and cytochrome oxidase (Watanabe *et al*., 1997) and also acts as catalysts for some enzymes (Camire *et al*., 1990). The higher values of copper in ACM75 formulation than BSFM and control formulations is due to the higher copper amounts that were found in ACM in comparison to BSFM and FWSM (Table 4.4-3). The increase in copper as feed moisture content increased could be due to the ability of water to increase the contact area of feed particles through *van der wal* forces (Tumuluru, 2014) and thus increasing the extractability of copper. In addition, water used in extrusion could have had traces of copper which led to more amount of the element as moisture content was increased to 30%. All the study formulations met the minimum requirements for copper in Tilapia (6 ppm) as outlined by KEBS standards (FDKS-2289:2010) as well as the minimum requirements for Catfish (5 ppm) as specified by FAO (2017) and thus ACM75 formulation can be used to substitute FWSM and still obtain the same or higher values of copper.

Zinc

The effects of treatment combinations on the Zinc are shown in Table 4.4-3. The control formulation that had 20% feed moisture content did not have significant ($P < 0.06$) difference with BSFM25, BSFM50 and ACM50 formulations that were also extruded at 20% feed moisture content. Similarly, the control formulation that had 30% feed moisture content did not show significant ($P < 0.05$) difference with BSFM50 and ACM75 formulations that were also extruded at 30% feed moisture content, while ACM75 formulation with 20% feed moisture content gave the highest level of zinc that had significant ($P < 0.05$) difference with all other formulations. Moisture content of feed did not influence the levels of zinc across all formulations.

Zinc in fish is involved in many metabolic pathways, serves as a specific cofactor for various enzymes and has also been shown to assist in healing of wounds (Watanabe *et al*., 1997: FAO, 2017). The KEBS minimum requirements for Tilapia ranges between 50 and 100 ppm depending on the stage of fish that is being targeted (FDKS-2289:2010), while the minimum requirements for Catfish is 20 ppm (FAO, 2017). The facts that all the study formulations were between 100 ppm and 200ppm and that the control formulation did not differ with BSFM50 and ACM75 demonstrate that BSFM and ACM can be used to substitute FWSM up to 50% and 75% level of substitution respectively in regards to Zinc. It can be said that extrusion process has little effect on zinc composition as moisture content did not show significance influence on its content. Other studies have also documented the non-significance influence of extrusion on zinc and its bio-availability (Guy, 2001: Kang and Chenoweth, 2000)

Manganese

As Table 4.4-3 shows, that there were no definite trends in manganese contents. However, ACM75 formulations gave high values of manganese that did not differ significantly ($P < 0.05$) with BSFM50 formulations as well as the BSFM75 formulation that was extruded at 20% feed moisture content.

Manganese is required in fish for normal brain functioning as well as for the metabolism of carbohydrates and lipids (Watanabe *et al*., 1997).

Table 4.4-3: Micro minerals content of extruded fish feed pellets containing varying levels of black soldier fly larvae and cricket meal as fish meal substitute.

Formulation	MC (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
Control	20	83.7±0.4^{b}	158.1±1.3^{efg}	14.9±0.1^{cd}	356.9±1.6^{cd}
	30	89.9±1.5^{b}	181.2±4.3^{b}	10.1±0.2^{ghi}	376.7±2.2^{cd}
BSFM25	20	12.5±1.2^{d}	159.5±1.6^{efg}	12.1±0.8^{efgh}	347.4±30.45^{d}
	30	16.8±2.7^{d}	145.8±0.5^{hi}	13.8±0.7^{cde}	355.5±11.2^{d}
BSFM50	20	19.4±0.1^{d}	168.3±1^{cde}	18.3±0.3^{a}	271.6±6.4^{e}
	30	52.8±0.5^{c}	177.4±1.2^{bc}	15.6±0.9^{bc}	309.7±15.3^{d}
BSFM75	20	44.8±0.0^{c}	171.4±2.8^{bcd}	15.6±0.3^{bc}	207.8±11.9^{f}
	30	73.1±5.8^{b}	161.9±3.2^{def}	12.4±0.5^{efg}	255.5±11.2^{e}
ACM25	20	50.9±8.8^{c}	143.1±2.7^{i}	9.9±0.3^{hi}	429.2±5.3^{b}
	30	75.1±3.4^{b}	155.3±0.9^{fgh}	12.8±0.2^{def}	469.6±5.9^{ab}
ACM50	20	43.5±4.2^{c}	153.5±1.5^{fghi}	10.6±0.0^{fghi}	467.2±3.2^{ab}
	30	75.7±0.0^{b}	148.7±0.8^{ghi}	12.9±0.0^{def}	492.2±6.4^{a}
ACM75	20	152.3±3.8^{a}	199.4±1.2^{a}	17.8±0.2^{ab}	502.4±1.7^{a}
	30	170.8±5.6^{a}	186.1±2.5^{b}	18.3±0.3^{a}	521.4±8.2^{a}

MC: Moisture content of feed prior to extrusion:
Control: 0% insect substitution of FWSM
BSFM25: black soldier fly meal substitutes 25% of the protein supplied by FWSM in control;
BSFM50: black soldier fly meal substitutes 50% of the protein supplied by FWSM in control;
BSFM75: black soldier fly meal substitutes 75% of the protein supplied by FWSM in control;
ACM25: adult cricket meal substitutes 25% of the protein supplied by FWSM in control;
ACM50: adult cricket meal substitutes 50% of the protein supplied by FWSM in control;
ACM75: adult cricket meal substitutes 75% of the protein supplied by FWSM in control.
Extrusion was done at a barrel temperature of 120ºC and feed exited through a 2 mm die:
For each component, means followed by same superscript letters along the same column are not significantly different at $P < 0.05$.

The minimum requirements for Manganese in feeds intended for Tilapia and Catfish are 12 ppm and 2.4 ppm respectively (FAO, 2017). Findings from this study indicate that BSFM50 and ACM75 are best suited for feeding tilapia as they contain more than 12 ppm of Manganese. However, all the formulations exceeded the minimum requirements for Manganese that is required by Catfish. Manganese is one of the elements that are least affected by extrusion process and its parameters (Razzaq *et al*., 2012; Singh *et al*., 2007), partly due to its electronic configuration that lenders it less susceptible to formation of chelates (Alonso *et al*., 2001). This explains why feed moisture content did not influence manganese content of the final products.

Iron

Effects of treatment effects on iron are also shown in Table 4.4-3. An increase in level of fish meal substitution with BSFM resulted in a decrease in final iron content, where BSFM75 formulations had significant ($P < 0.05$) lower levels of iron than the control. On the other hand substitution with ACM resulted in increased levels of iron as the level of substitution increased with all the ACM formulations giving significant ($P < 0.05$) higher levels of iron than the control and BSFM formulations. It was also noted that increasing feed moisture content gave a corresponding increase in the amount of iron for all the formulations, though this increase was only significant ($P < 0.05$) for BSFM50 and BSFM75 formulations.

Iron is necessary for haemoglobin and myoglobin pigments (FAO, 2017) and is actively involved in oxidation/reduction reactions (Watanabe *et al*., 1997). The decrease in iron content as the level of FWSM substitution with BSFM increased is attributed to the low iron content that is found in BSFM in comparison to FWSM (Table 4.4-3). Similarly, the corresponding increase in iron content as FWSM is substituted with ACM is due to the higher iron content in ACM than in FWSM (Table 4.4-3). Other researchers have also shown that ACM has higher iron content than BSFM (Makkar *et al*, 2014; Finke, 2002; Barker *et al*., 1998). It should be noted that all the formulations exceeded the minimum iron requirements for Tilapia and Catfish (60 and 30 ppm respectively) and thus any of the insect formulation can substitute FWSM in regards to iron content in fish feed (FAO, 2017; FDKS-2289-2:2010). Extrusion process influenced the amount of iron in the final products where for each formulation, the 30% feed moisture content resulted in feeds having higher iron content. This is attributed to the fact that moisture and high temperature of the extrusion (120°C) increased the abrasion of extruder barrel, screw and the die thereby increasing the final iron content. Similar phenomena have been reported (Razzaq *et al*., 2012; Guy, 2001; Alonso, 2001; Camire *et al*., 1990).

Mineral leaching activity of extrudates

Leaching activity was determined on pellets processed from the control formulation and insect meal containing blends at 75% substitution (BSFM75 and ACM75) and 30% moisture content because the three exhibited relatively better properties in comparison to other treatments. Figure 4.4-1 shows the leaching activity of minerals that were studied over a period of 48 hours. The control formulation led to high leaching of phosphorus, calcium, potassium, sodium and manganese where across all hours, the control showed significant ($P < 0.05$) higher leaching effect as compared to ACM75 and BSFM75 formulations. The leaching of magnesium and zinc were significantly ($P < 0.05$) higher with the ACM75 formulations than BSFM75 and control formulations throughout the 48 hours study period. At 4 hours, BSFM75 had significant ($P < 0.05$) lower leached copper levels than ACM75 and the control both of which were not significant at the time. However, at 48 hours, all the three formulations had no significant ($P < 0.05$) difference on the amount of leached copper. At 4, 8 and 24 hours, BSFM75 had significant ($P < 0.05$) higher amounts of leached iron levels than the control and ACM75 formulations. This scenario changed at 48 hours where BSFM75 showed significant ($P < 0.05$) low amounts of leached iron than both the control and ACM75 formulations both of which were not significant ($P < 0.05$). Another peculiar observation is that; while it is expected for the level of leached minerals to increase as time progresses, BSFM75 showed a decreasing level from 24 to 48 hours in regards to copper, zinc and iron elements. The control also showed the same behavior with zinc and copper while ACM75 only showed a decreased behavior with copper.

Leaching activity is determined by water stability of feeds where pellets with low water stability exhibits higher leaching effect (Saalah *et al*., 2010; Ayadi *et al*., 2011). At 30% moisture content, ACM75 and BSFM75 based pellets had higher water stability than fish feeds that had 100% FWSM. This explains why the control formulation had higher leaching effect for majority of minerals than ACM75 and BSFM75 formulations. These findings are in agreement with Haghbayan and Mehryan (2015) and Hardy *et al*. (2002) who reported that fish meal in fish feed, increased the nutrient load in waste water and thus contributes to higher leaching effect.

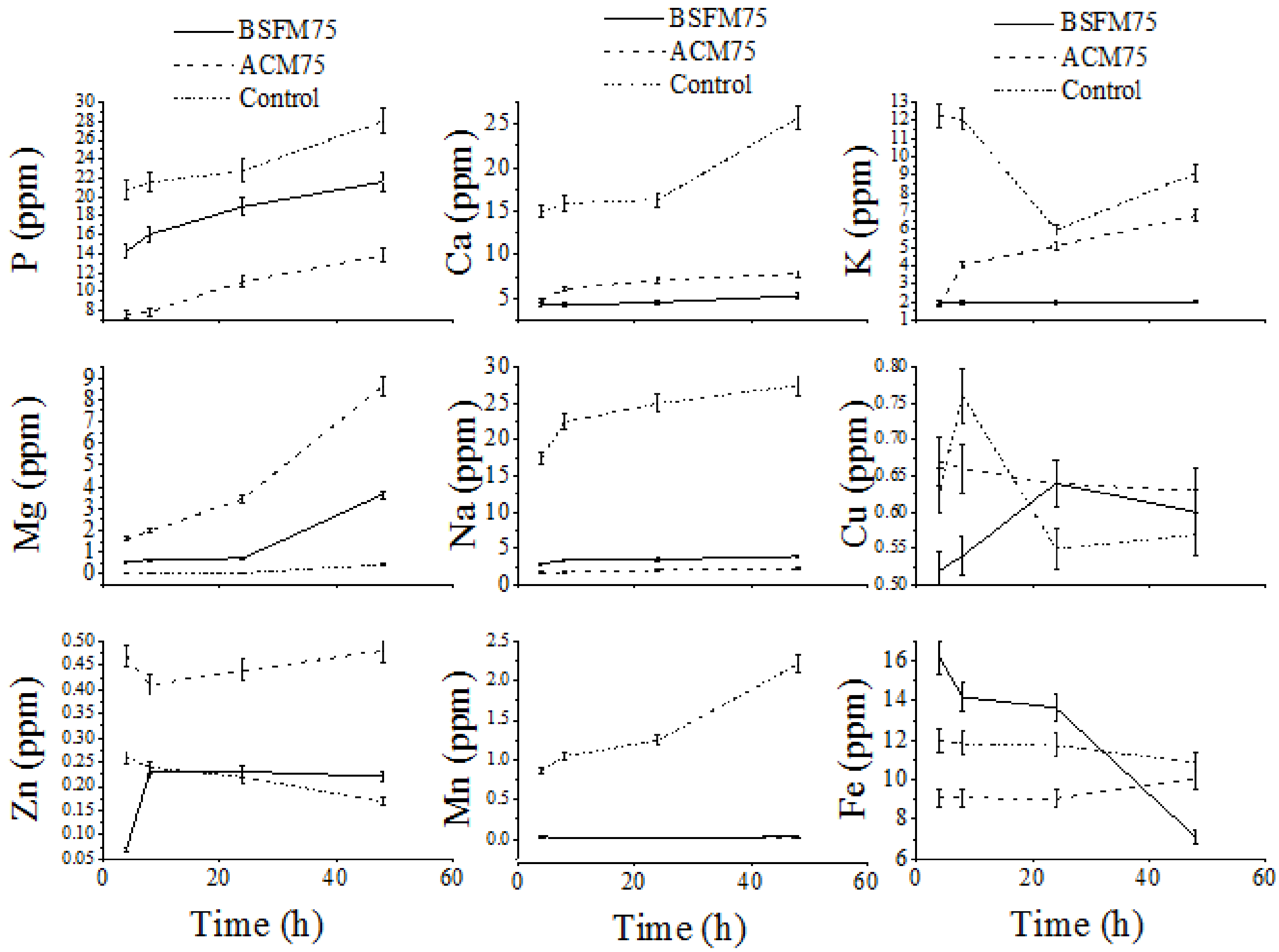

Figure 4.4-1: Leaching activity of minerals in water introduced with pellets extruded from the control blend (round dotted line), black soldier fly larvae meal containing blend [75% substitution; BSFM75] (solid line), and adult cricket meal containing blend [75% substitution; ACM75] (square-dotted line) adjusted to 30% feed moisture content.

Phosphorus and sodium leaching leads to eutrophication and algal blooms (Jia *et al.*, 2015; Xiang and Zhou, 2011; Saalah *et al.*, 2010) while lack of adequate potassium affects nerve and muscle formation in fish (Wurts, 1993) and thus there is need to supplement fish meal with either ACM75 or BSFM75 meals as the two leads to low leaching of phosphorus, sodium and potassium as compared to the control. In addition, fish do not get phosphorus readily from the water environment (Tang *et al.*, 2012) and thus feeds that have higher phosphorus leaching activity contributes to phosphorus deficiency in fish. Calcium and magnesium leaching do not pose a serious challenge as both contribute to water hardness which is important for the growth of fish. For example, relatively high amounts of free calcium ions in water reduce the loss of sodium and potassium salts from the body of fish (Wurts, 1993). However, high concentration of hard water may increase the susceptibility of fish to columnaris disease (Avant, 2015). The decreasing copper, zinc and iron leached levels in BSFM75 formulations and copper and zinc in ACM75 and control formulations respectively could be due to formation of complexes by the macro minerals rendering these micro elements unavailable with time.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1: Conclusions

1. Response surface methodology gave optimum treatment combinations of temperature, die diameter and feed conditioning time of 120°C, 2 mm and 100 sec respectively with a composite desirability of 0.8227.
2. Both black soldier fly larvae and adult cricket meals can be used to process extruded fish feeds with desirable physico-chemical characteristics. The substitution levels can be increased up to 75% but moisture content of the blends has to be increased progressively to about 30% so as to obtain good quality fish feed pellets with regards to floatability, durability index, bulk density as well as water stability.
3. Substituting fresh water shrimps with adult cricket led to a significant increase in protein content of the products while black soldier fly decreased the protein content. At all levels of fresh water shrimps substitution, ACM based formulations gave higher levels of *in vitro* protein digestibility than BSFM based formulations though both ACM and BSFM recorded higher *in vitro* protein digestibility than the control. Increasing the level of fresh water shrimps with either cricket or black soldier fly larvae led to increase in levels of phosphorus and potassium but reduced the calcium and sodium amounts in final extruded products.

5.2: Recommendations

1. The optimized extruder cooking conditions are applicable to fish feed processors who use a single screw extruder to make floating fish feeds. One limitation of extrusion is that it may lead to loss of vitamins particularly vitamin C. Consequently, coating of extruded feeds is recommended to improve on the vitamin content.
2. Further work should investigate nutrient availability and digestibility as well as performance of the pellets in feeding trials.
3. Leaching leads to degradation of the surrounding water environment through water pollution and this not only affects the growth and behavior of fish but also other aquatic life. Thus this study recommends ACM75 and BSFM75 formulations as they have less leaching activity than the control at 30% moisture content.

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APPENDICES

Appendix 1: Abstract of accepted paper for publication by the Journal of Insects as Food and Feed

Physico-chemical properties of extruded fish feed pellets containing black soldier fly (*Hermetia illucens*) larvae and adult cricket (*Acheta domesticus*) meals

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Running header: physico-chemical properties of extruded insect based fish feeds

Abstract

Fish farming in Kenya is faced with the challenge of high cost of feeds because of the high cost of fish meal, an important protein ingredient incorporated into feeds. Thus, there is urgent need for cheaper alternative protein sources that have similar or higher protein quality to partially or completely substitute fish meal in feeds. The effects of substituting freshwater shrimp meal (FWSM) with black soldier fly larvae meal (BSFM) or adult cricket meal (ACM) on physico-chemical properties of hot-extruded fish feed pellets were investigated. The FWSM protein in a 26g/100g protein fish feed formulation was substituted at 0%, 25%, 50% and 75%, and moisture content of the formulated blends adjusted to 10, 20 or 30g/100g prior to extrusion. Floatability, expansion rate, bulk density, durability index, water absorption index, water solubility index, and water stability of extruded pellets were determined. Sinking velocity and the total suspended and dissolved solids in water were determined for the optimal pellets. The addition of BSFM or ACM did not affect properties of the extrudates ($P<0.05$). The level of substitution, however, influenced pellet floatability ($P<0.001$), bulk density ($P<0.001$) and water stability ($P<0.01$). Moisture content of formulation influenced ($P<0.05$) all parameters except durability index ($P>0.05$) and water absorption index ($P>0.05$). Good quality fish feed pellets can be produced with 75% BSFM or 75% ACM at 30g/100g feed moisture.

Keywords: aqua feed, extrusion, edible insects

Appendix 2: Selected statistical outputs for objective 1

```
Welcome to Minitab, press F1 for help.
```

Box-Behnken Design

```
Factors:       3     Replicates:     2
Base runs:    15     Total runs:    30
Base blocks:   1     Total blocks:   1

Center points: 6


Design Table (randomized)

Run  Blk  A  B  C
  1    1  -  0  -
  2    1  +  0  -
  3    1  +  0  +
  4    1  0  0  0
  5    1  +  +  0
  6    1  -  +  0
  7    1  0  -  -
  8    1  -  -  0
  9    1  -  -  0
 10    1  +  -  0
 11    1  0  0  0
 12    1  0  +  +
 13    1  0  +  -
 14    1  +  0  +
 15    1  0  0  0
 16    1  0  0  0
 17    1  0  -  -
 18    1  0  0  0
 19    1  +  +  0
 20    1  0  +  +
 21    1  -  0  -
 22    1  +  0  -
 23    1  -  0  +
 24    1  +  -  0
 25    1  -  0  +
 26    1  0  -  +
 27    1  0  -  +
 28    1  -  +  0
 29    1  0  0  0
 30    1  0  +  -
Analysis of Variance for Floatability

Source          DF   Seq SS   Adj SS   Adj MS      F      P
Regression       9  28092.5  28092.5  3121.39  50.68  0.000
  Linear         3  21846.9   2963.1   987.70  16.04  0.000
  Square         3   3887.0   3887.0  1295.67  21.04  0.000
  Interaction    3   2358.6   2358.6   786.20  12.77  0.000
Residual Error  20   1231.8   1231.8    61.59
  Lack-of-Fit    3    389.1    389.1   129.69   2.62  0.085
  Pure Error    17    842.7    842.7    49.57
Total           29  29324.2

S = 7.848   R-Sq = 95.8%   R-Sq(adj) = 93.9%
```

```
Analysis of Variance for WSI

Source          DF   Seq SS   Adj SS   Adj MS      F      P
Regression       9  109.597  109.597  12.1774  46.04  0.000
  Linear         3   21.998   57.342  19.1138  72.27  0.000
  Square         3   40.587   40.587  13.5289  51.15  0.000
  Interaction    3   47.012   47.012  15.6708  59.25  0.000
Residual Error  20    5.290    5.290   0.2645
  Lack-of-Fit    3    1.117    1.117   0.3724   1.52  0.246
  Pure Error    17    4.172    4.172   0.2454
Total           29  114.886


S = 0.5143   R-Sq = 95.4%   R-Sq(adj) = 93.3%
```

Response Surface Regression: BD versus Temperature, Die diameter, Feed conditioning

```
Analysis of Variance

Source                                       DF  Adj SS  Adj MS  F-Value
Model                                         6  269267   44878    37.87
  Linear                                      1   15044   15044    12.69
    Temperature                               1   15044   15044    12.69
  Square                                      3  230358   76786    64.79
    Temperature*Temperature                   1   42770   42770    36.09
    Die diameter*Die diameter                 1  148769  148769   125.53
    Feed conditioning time*Feed conditioning time  1   70093   70093    59.15
  2-Way Interaction                           2   23865   11933    10.07
    Temperature*Die diameter                  1    5472    5472     4.62
    Temperature*Feed conditioning time        1   18394   18394    15.52
Error                                        23   27257    1185
  Lack-of-Fit                                 6   12802    2134     2.51
  Pure Error                                 17   14455     850
Total                                        29  296525

Source                                       P-Value
Model                                          0.000
  Linear                                       0.002
    Temperature                                0.002
  Square                                       0.000
    Temperature*Temperature                    0.000
    Die diameter*Die diameter                  0.000
    Feed conditioning time*Feed conditioning time  0.000
  2-Way Interaction                            0.001
    Temperature*Die diameter                   0.042
    Temperature*Feed conditioning time         0.001
Error
  Lack-of-Fit                                  0.063
  Pure Error
Total


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
34.4254  90.81%     88.41%      84.43%
```

```
Coded Coefficients

Term                                         Effect    Coef  SE Coef  T-Value
Constant                                              686.0     14.1    48.81
Temperature                                  -61.33  -30.66     8.61    -3.56
Temperature*Temperature                      -152.2   -76.1     12.7    -6.01
Die diameter*Die diameter                    -283.9  -141.9     12.7   -11.20
Feed conditioning time*Feed conditioning time  -194.9   -97.4     12.7    -7.69
Temperature*Die diameter                      -52.3   -26.2     12.2    -2.15
Temperature*Feed conditioning time            -95.9   -48.0     12.2    -3.94

Term                                         P-Value   VIF
Constant                                       0.000
Temperature                                    0.002  1.00
Temperature*Temperature                        0.000  1.01
Die diameter*Die diameter                      0.000  1.01
Feed conditioning time*Feed conditioning time    0.000  1.01
Temperature*Die diameter                       0.042  1.00
Temperature*Feed conditioning time             0.001  1.00


Regression Equation in Coded Units

BD = 686.0 - 30.66 Temperature - 76.1 Temperature*Temperature
     - 141.9 Die diameter*Die diameter
     - 97.4 Feed conditioning time*Feed conditioning time
     - 26.2 Temperature*Die diameter - 48.0 Temperature*Feed conditioning time
```

Appendix 3: Selected statistical outputs for objective 2

```
                                   The GLM Procedure

Dependent Variable: Float

                                                 Sum of
                      Source                      DF         Squares     Mean Square    F Value    Pr > F

                      Model                       23     67647.91667      2941.21377       8.57    <.0001

                      Error                       24      8237.50000       343.22917

                      Corrected Total             47     75885.41667


                                R-Square     Coeff Var      Root MSE    Float Mean

                                0.891448      28.96643      18.52645      63.95833


                      Source                      DF       Type I SS     Mean Square    F Value    Pr > F

                      Insect                       1         0.00000         0.00000       0.00    1.0000
                      level                        3      9605.20833      3201.73611       9.33    0.0003
                      Moisture                     2     44948.69792     22474.34896      65.48    <.0001
                      Insect*level                 3      1655.20833       551.73611       1.61    0.2138
                      Insect*Moisture              2        75.78125        37.89063       0.11    0.8959
                      level*Moisture               6      6509.63542      1084.93924       3.16    0.0198
                      Insect*level*Moistur         6      4853.38542       808.89757       2.36    0.0623


                      Source                      DF     Type III SS     Mean Square    F Value    Pr > F

                      Insect                       1         0.00000         0.00000       0.00    1.0000
                      level                        3      9605.20833      3201.73611       9.33    0.0003
                      Moisture                     2     44948.69792     22474.34896      65.48    <.0001
                      Insect*level                 3      1655.20833       551.73611       1.61    0.2138
                      Insect*Moisture              2        75.78125        37.89063       0.11    0.8959
                      level*Moisture               6      6509.63542      1084.93924       3.16    0.0198
                      Insect*level*Moistur         6      4853.38542       808.89757       2.36    0.0623
```

```
                                   The GLM Procedure

Dependent Variable: Stabilty

                                                       Sum of

                      Source                      DF          Squares     Mean Square    F Value    Pr > F


                      Model                       23      537.8252134      23.3837049       8.50    <.0001


                      Error                       24       66.0531141       2.7522131


                      Corrected Total             47      603.8783276



                           R-Square     Coeff Var      Root MSE    Stabilty Mean

                             0.890619      1.995940      1.658980         83.11770


                      Source                      DF       Type I SS     Mean Square    F Value    Pr > F
```

Appendix 4: Selected statistical outputs for objective: 3

```
                                                            The GLM Procedure

Dependent Variable: PROT

                                                      Sum of
                       Source                 DF         Squares     Mean Square    F Value    Pr > F

                       Model                  23     75.38629792      3.27766513       4.89    0.0001

                       Error                  24     16.09465000      0.67061042

                       Corrected Total        47     91.48094792


                                R-Square     Coeff Var      Root MSE     PROT Mean

                                0.824066      2.863586      0.818908      28.59729


                       Source                 DF       Type I SS     Mean Square    F Value    Pr > F

                       INSECT                  1     25.18651875     25.18651875      37.56    <.0001
                       LEVEL                   3      3.08592292      1.02864097       1.53    0.2313
                       MOIST                   2      5.65045417      2.82522708       4.21    0.0270
                       INSECT*LEVEL            3      3.19142292      1.06380764       1.59    0.2187
                       INSECT*MOIST            2     14.43263750      7.21631875      10.76    0.0005
                       LEVEL*MOIST             6     14.70194583      2.45032431       3.65    0.0102
                       INSECT*LEVEL*MOIST      6      9.13739583      1.52289931       2.27    0.0707


The SAS System

                                                           The GLM Procedure
                                                          Least Squares Means
                                                Adjustment for Multiple Comparisons: Tukey

                                                   Least Squares Means for Effect LEVEL
                                                 t for H0: LSMean(i)=LSMean(j) / Pr > |t|

                                                                        LSMEAN
                                                  LEVEL     PROT LSMEAN      Number

                                                  0         28.4866667          1
                                                  25        29.0291667          2
                                                  50        28.3783333          3
                                                  75        28.4950000          4


                                                   Least Squares Means for Effect LEVEL
                                                 t for H0: LSMean(i)=LSMean(j) / Pr > |t|

                                                       Dependent Variable: PROT

                                   i/j             1             2             3             4

                                      1                   -1.62271      0.324043      -0.02493
                                                            0.3854        0.9879        1.0000
                                      2      1.622707                    1.94675      1.597781
                                               0.3854                     0.2359        0.3987
                                      3      -0.32404      -1.94675                   -0.34897
                                               0.9879        0.2359                     0.9850
                                      4      0.024926      -1.59778      0.348969
                                               1.0000        0.3987        0.9850
```

Appendix 5: Assorted fish feeds with different formulations extruded at various feed moisture contents