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Proximate composition and *in vitro* protein digestibility of extruded aquafeeds containing *Acheta domesticus* and *Hermetia illucens* fractions

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RESEARCH ARTICLE

Abstract

Major protein sources for feed manufacture have become costly, and this has led to increased cost of products such as aquafeeds. This has dampening effect on fish production especially in developing countries, and has necessitated a search for alternative protein sources in processed feeds. The effects of extrusion on aquafeed blends containing fractions of adult cricket (*Acheta domesticus*) meal (ACM) or black soldier fly (*Hermetia illucens*) pre-pupae meal (BSFM) on proximate composition and *in vitro* protein digestibility were investigated. Extrusion resulted in higher contents of protein and nitrogen free extract, and lower contents of ether extract and crude fibre as compared to the non-extruded feed. These observations were mainly associated with denaturation of proteins leading to increase in solubility, solubilisation of fibre due to the shearing forces at high temperature resulting in higher nitrogen free extract, and formation of starch-lipid complexes leading to lower ether extract. Our findings indicate that ACM or BSFM can be used to substitute fresh water shrimp meal up to 75 g/100 g level and still achieve highly nutritious processed fish feed.

Keywords: aquafeed, processing, edible insects, extrusion, nutritional value

1. Introduction

The lack of adequate protein is a major factor contributing to poor nutrition in many developing countries (FAO, 2015). To reduce the gap between protein demand and the supply, greater use of plant protein was suggested (Vrabec *et al.*, 2015). Plant protein sources are cheap and may have bioactive compounds that can help promote good health (Friedman, 1996). However, they also have limitations that include anti-nutritional factors, amino acid imbalances, and high contents of fibre and non-starch polysaccharides depending on the specific source (Kumar *et al.*, 2012). Thus animal protein sources are regarded superior. Fish has significantly higher protein quality than common livestock; a portion of 150 g of fish can provide about 50-60% of an adult's daily protein requirements (FAO, 2016; Neira *et al.*, 2009). In developing countries, fish consumption tends to be based on locally and seasonally available products largely from capture fishery. Because of the unsustainable nature of capture fishery, aquaculture has become increasingly important. Recently, Magnusson and Bergman (2014) reported that about 50% of the fish consumed by humans globally was contributed by aquaculture, and the FAO (2016) estimated that developing countries contributed 80% of world's aquaculture. For this reason, there is growing appreciation of the role that aquaculture can play in improving nutrition in these countries. However, the lack of access to affordable high-quality feeds threatens the participation of farmers in profitable aquaculture (Munguti *et al.*, 2014). Availability of ready-to-use or manufactured aquafeed is not widespread (Ngugi *et al.*, 2007), and the access is hindered by the high price (Njagi *et al.*, 2013) precipitated by the cost of protein sources (Davis *et al.*, 2009). In East Africa, commonly used protein sources in aquaculture feeds include silver cyprinid (*Rastrineobola argentea* Pellegrin, 1904) and fresh water shrimp (*Caradina nilotica* Roux, 1833), that also have competing uses (Munguti *et al.*, 2014). Therefore, there is need to explore other alternative and affordable sources of protein for use in aquafeeds.

In recent years, insects have gained attention as alternative protein sources. The nutritional profiles of the numerous edible insect species have been documented (Rumpold and Schlüter, 2013), and use of insects to feed fish recently reviewed (Tran *et al.*, 2015). Satisfactory growth performance was reported when various unprocessed insects such as black soldier fly larvae and dried termites were fed to fish such as the Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) and African catfish (*Clarius gariepinus* Burchell, 1822), respectively (Hem *et al.*, 2008; Sogbesan and Ugwumba, 2008). For other insects, processing proved to be important. Some of the approaches that have been applied to process and incorporate edible insects into feeds include drying followed by pulverisation into a meal which is compounded with other ingredients (Mutungi *et al.*, in press). The compounded mash is then formed into pellets using mechanical pressing tools or simple extrusion (e.g. Fasakin *et al.*, 2003).

Extrusion is a more robust aquafeed processing technique. The benefits include improvement of feed nutritional value (Sørensen, 2007) through destruction of anti-nutritional factors and undesirable enzymes (Filipovic *et al.*, 2010; Nikmaram *et al.*, 2015), thus improving the digestibility of feed (McDonald *et al.*, 2010; Moscicki, 2011). Other advantages are increased solubility of dietary fibres (Nikmaram *et al.*, 2015) as well as the destruction pathogenic microbes (Levic and Sredanovic, 2010). Furthermore, hot extrusion makes it possible to produce water-stable floating aquafeeds for top feeders thereby allowing better feed utilisation and less pollution in fish ponds. Feed composition influences the choice of the extruder variables (Alam *et al.*, 2016) which may in turn influence the nutritional quality of the extruded product. The optimum extrusion conditions for production of expanded aquafeeds from insect meal containing blends using low-cost single screw extruder were investigated and the findings are reported elsewhere. The aim of this study was to investigate the effects of extrusion of fish feed blends containing cricket (*Acheta domesticus* L.) and black soldier fly (*Hermetia illucens* L.) pre-pupae meal on the proximate composition. A further aim was to study the effect of the extrusion process on *in-vitro* protein digestibility.

2. Materials and methods

Research sites, source of ingredients, experimental design, feed formulation and extrusion process were as outlined in Irungu *et al.* (2018). Briefly, extruded pellets were produced from iso-proteineous (26.45±0.76 protein) composite blends containing maize germ, sunflower cake, wheat pollard, cassava flour and dried fresh-water shrimp meal (FWSM) substituted with adult cricket meal (ACM) or black soldier fly pre-pupae meal (BSFM) at different levels (0, 25, 50 and 75%). Thus the experimental blends were: the control (0%) which did not contain insect meal; BSFM25 and ACM25 in which BSFM or ACM substituted 25% of the protein supplied by FWSM in control; BSFM50 and ACM50 in which BSFM or ACM substituted 50% of the protein supplied by FWSM in control; and BSFM75 and ACM75 in which BSFM substituted 75% of the protein supplied by FWSM in control, respectively. Extrusion was performed at different moisture contents of the composite blends (10, 20 and 30 g/100 g on wet weight basis) using a low-cost single-screw extruder (DOLLY, Unitech, New Delhi, India; screw length to diameter ratio of 9.1) operating at fixed conditions of screw speed (200 rpm), feeding rate (1 kg/min), feed pre-conditioning time (2 min with steam at an inlet pressure of 4 bars), extrusion temperature (120 °C), and die diameter (2 mm).

Determination of dry matter

Dry matter content was determined gravimetrically according to AOAC 930.15 (AOAC, 2000). Approximately 2 g of ground sample was weighed and placed into a pre weighed dish (W_1). The weight of the dish together with the sample was recorded (W_2). The dish was placed into a drying oven (Memmert, Schwabach, Germany) at 105 °C for 3 h, then cooled in a desiccator and reweighed (W_3). Dry matter (%) was determined as:

$$100 \times \frac{(W_3 - W_1)}{(W_2 - W_1)}$$

Determination of crude protein

Crude protein was determined according to AOAC method 984.13 (AOAC, 2000). 2 g of ground sample were mixed with 20 ml of concentrated sulphuric acid in a digestion tube. Kjeldahl tablets (catalyst) were added to the mixture in the tube and the sample digested in a Gerhardt Kjeldatherm digester (model KB40; Gerhardt GMBH & CO. Kg, Königswinter, Germany) for 1 h at 420 °C. Distilled water was added to the digest to make 80 ml volume. Exactly 50 ml of Sodium hydroxide solution was added to the mixture and this was followed by distillation of the ammonia into concentrated boric acid using a 2200 Kjeltec™ auto distillation unit (Foss Analytical, Höganäs, Sweden). Titration was done using hydrochloric acid (0.1

mol/l) after adding a few droplets of indicator solution. Nitrogen content (g/100 g) was obtained using the formula:

$$N(g/100\ g) = \frac{(V_s - V_b) \times M_{(HCl)} \times 1 \times 14.007}{W \times 10}$$

Where, V_s is volume of HCl (ml) needed to titrate sample; V_b is volume of HCl (ml) needed to titrate the blank test; $M_{(HCl)}$ is the molarity of hydrochloric acid; the numeral one (1) is the acid factor; 14.007 is the molecular weight of nitrogen; W is the weight of the sample (g) and 10 is the conversion factor from mg/g to g/100 g. Crude protein content was obtained by multiplying the nitrogen content by 6.25.

Determination of fat content (ether extract)

Ether extract was determined according to AOAC method 920.39 (AOAC, 2000). About 5 g of the sample was weighed (W_1) 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 (W_2). Exactly 25 ml of petroleum ether was added into the extraction flask. The electro-thermal soxlet apparatus (model EME 6250/CF; Cole Parmer, Saint Neots, UK) was set to extract the sample for 6 h, after which the solvent was evaporated, flask dried in a desiccator and reweighed (W_3). Crude fat content (%) was calculated as:

$$100 \times \frac{(W_3 - W_2)}{W_1}$$

Determination crude fibre

The ISO 6865 method was used (ISO, 2000). 2 g of sample were weighed (W_1) and digested in a Fibertec digester (Foss Analytical) using sulphuric acid for 30 min and then digested using potassium hydroxide for 30 min. 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 (W_2). The dried crucible and residue were then incinerated in a muffle furnace at 550 °C for 2 h, cooled in a desiccator and reweighed (W_3). Crude fibre content (%) was obtained as:

$$100 \times \frac{(W_2 - W_3)}{W_1}$$

Determination of ash content

The AOAC 942.05 method (AOAC, 2000) was used. About 2 g of ground sample was weighed (W_1) into a crucible that was previously calcined and weighed (W_2), and then heated in a muffle furnace (model MR170, S/N: 6800616; Hereaus GMBH, Hanau, Germany) at 550 °C for 12 h. The crucible with the ashed sample was then cooled in a desiccator and reweighed (W_3). Ash content was calculated using the expression:

$$Ash(\%) = 100 \times \frac{(W_3 - W_1)}{W_2}$$

Determination of nitrogen free extract content

Carbohydrate content was calculated as weight by difference between 100 and summation of the other proximate parameters and given as nitrogen free extract (NFE, g/100 g) using the equation:

$$NFE = 100 - (P + F + A + C)$$

Where, P is the crude protein content (g/100 g), F is ether extract content (g/100 g), A is the ash content (g/100 g) and C is the crude fibre content (g/100 g).

Determination of *in vitro* protein digestibility

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 containing 0.02 g/100 g sodium azide and 1.5 mg pepsin from porcine gastric mucosa (Sigma P7000-25G, activity ≥250 units/mg solid; Sigma-Aldrich, St. Louis, MO, USA) was added, and the tube incubated in a shaking water bath (WSB-30; Witeg Labortechnink GmbH, Wertheim, Germany) maintained at 15 °C for 3 h. The sample was then centrifuged at 4,000×*g* for 20 min 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 Gerhardt Kjeldatherm digester (KB40; Gerhardt GMBH & Co. Kg) and Kjeltec auto distillation unit (model 11014901, S/N: 91708870; Foss Analytical). Nitrogen in the supernatant was expressed as a percentage of the total nitrogen in the sample and reported as *in vitro* protein digestibility (IVPD).

Statistical analysis

General linear model procedure of the Statistical Analysis System (SAS) software version 9.1.3 (SAS Institute Inc., Cary, NC, USA) was used for analysis of variance (ANOVA). Means were separated using Tukey's HSD test at $P<0.05$ level of significance.

3. Results

Proximate composition of ingredients and the formulated blends

Proximate composition of ingredients is reported in Irungu *et al.* (2018). All the ingredients of plant origin (sunflower cake, wheat pollard, maize germ and cassava) had significantly higher (P<0.05) contents of NFE but lower contents of crude protein and ash compared to FWSM, BSFM and ACM. In addition, with the exception of sunflower cake, the ether extract contents were lower. There was no significant (P>0.05) difference in the crude fibre contents of BSFM and ACM, but these insect meals had significantly higher (P<0.05) amounts, of crude fibre and crude fat than FWSM. The ACM contained 1.5 times more protein than BSFM and 1.15 times more protein than FWSM. The blends containing the insect meal had higher contents of fat and fibre than the control. Blends containing BSFM particularly had significantly higher (P<0.05) fat content. The control formulation had higher ash content than the insect meal-containing blends. NFE contents of the blends were fairly equal.

Effect of the type of insect meal, level of substitution and feed moisture content on proximate composition of extruded pellets

Crude protein contents of extruded pellets are presented in Figure 1. Interaction effects of insect meal and feed moisture content at extrusion, as well as level of substitution and moisture content at extrusion were significant (P=0.0005 and P=0.0102, respectively). At 10 g/100 g moisture content, the pellets extruded from BSFM25 and ACM25 had significantly (P<0.05) higher levels of crude protein than those extruded from the other formulations. At 20 g/100 g moisture content, pellets extruded from ACM25 and ACM75 had significantly (P<0.05) higher crude protein contents than the pellets extruded from the other formulations, whereas at 30 g/100 g moisture ACM50- and ACM75-based pellets had significantly (P<0.05) higher crude protein contents. The variation in protein contents of pellets extruded from ACM50 and ACM75 followed the same trend as the control, in which increase in feed moisture content seemed to increase crude protein content. There was a tendency of the crude protein content of pellets extruded from BSFM formulations to decrease with increasing feed moisture content. However, on average, compared to the non-extruded feed, the protein content of pellets were higher by 6.6, 8.1 and 9.6% when extruded at feed moisture contents of 10, 20, and 30 g/100 g, respectively.

Contents of ether extract are as shown in Figure 2. Interaction effect of insect meal type, level of substitution and moisture content was significant (P=0.0257). As expected, the BSFM75 and BSFM50 formulations had significantly (P<0.05) higher amounts of ether extracts than all the other formulations across the three feed moisture contents. At 30 g/100 g feed moisture, the

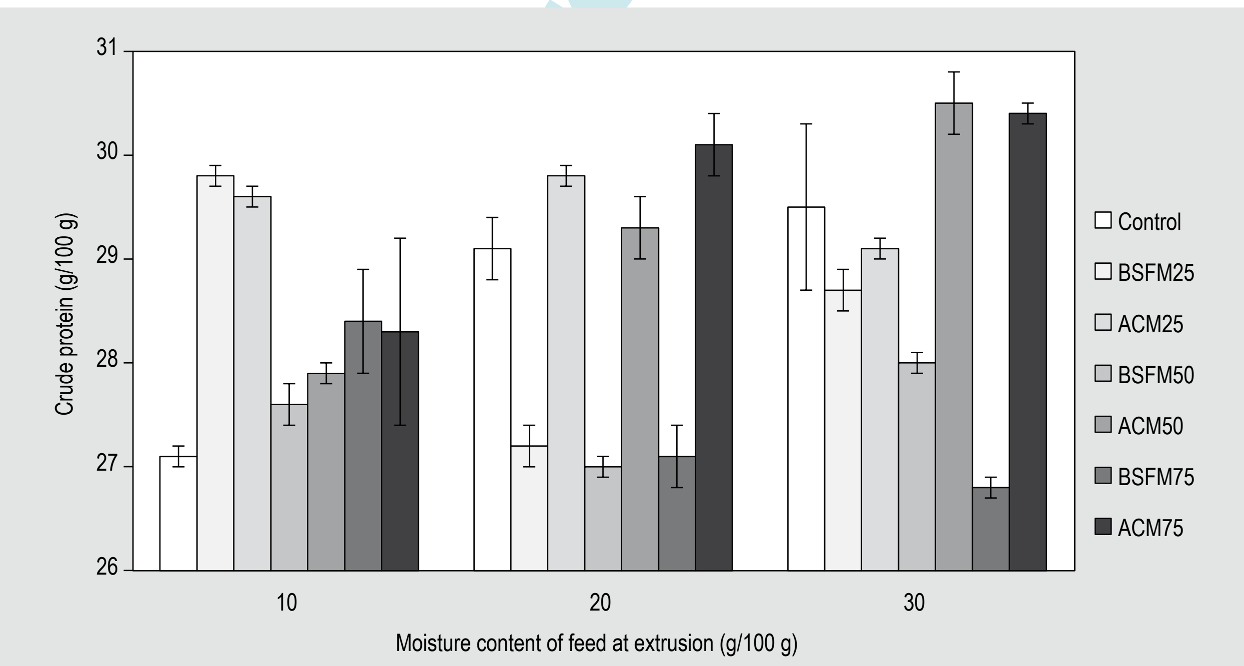

Figure 1. Crude protein contents (g/100 g dry weight basis) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: does not contain insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.

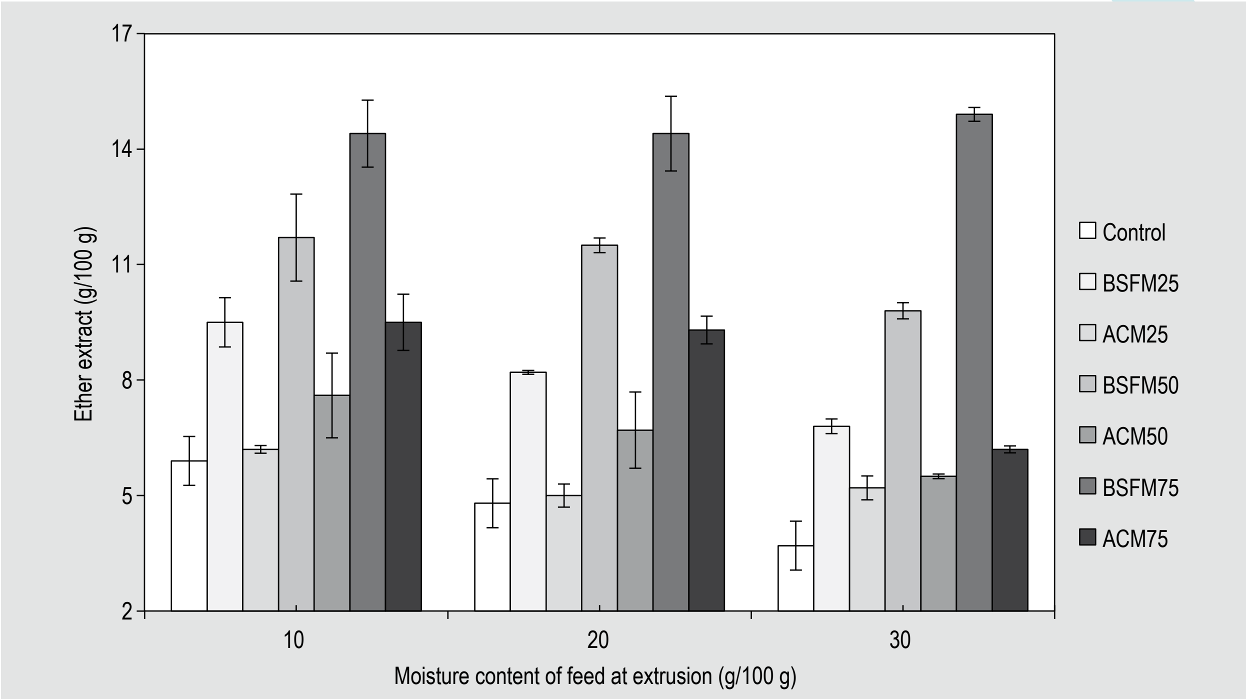

Figure 2. Ether extract contents (g/100 g dry weight basis) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: contains FWSM but no insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.

control formulation had the least content of ether extract that was significant with BSFM25 (P=0.0441), BSFM50 (P<0.0001) and BSFM75 (P<0.0001). In addition, the control formulation had significant lower levels of ether extract than BSFM25 (P=0.0047), BSFM50 (P<0.0001), BSFM75 (P<0.0001), ACM50 (P=00197) and ACM75 (P<0001). Generally, increasing feed moisture content decreased fat contents of the extruded pellets which was significant in majority of the formulations (P=0.0271 between 10 and 20 g/100 g; P<0.0001 between 10 and 30 g/100 g; and P=0.0028 between 20 and 30 g/100 g moisture contents). Ether extract decreased from an average of 12.6 g/100 g in the non-extruded feed to 8.8, 8.0 and 7.1 g/100 g in pellets extruded at 10, 20, 30 g/100 g feed moisture, respectively, representing 30.15-43.65% decline.

Crude fibre of the pellets extruded from the various feed blends is given in Figure 3. Interaction effect of type of insect meal, level of substitution and moisture content was significant (P=0.0304). Pellets extruded from the control blend had significantly (P<0.05) lower fibre content than pellets from all the other formulations when extruded at 10 and 20 g/100 g moisture, in line with compositions of the raw blends. In addition, the control formulation had the lowest crude fibre at 30 g/100 g feed moisture, although this was not significantly (P=1.0000) different from the fibre content of ACM25, BSFM25 and BSFM50 pellets. Highest content of crude fibre was determined in the pellets extruded from BSFM75 at 20 g/100 g moisture. Generally, the different formulations responded differently to extrusion at the three levels of feed moisture; the effect of feed moisture was not significant (P=0.1619). Nonetheless, crude fibre contents of extruded products were 23-36% lower than those of the raw blends.

Ash content of pellets are presented in Figure 4. As with ether extract and crude fibre, the interaction effect of insect meal type, level of substitution and moisture content was significant (P=0.0255). Higher ash contents were determined in the control, and in the blends substituted with 25 g/100 g of insect meal at the three extrusion moisture contents, which agreed with the compositions of the raw blends. Increasing the level of BSFM and ACM beyond 25 g/100 g resulted in significant (P<0.0001) decrease in ash content of pellets. Generally, feed moisture content did not significantly influence ash content of extruded products.

The NFE contents are shown in Figure 5. Interaction effect of type of insect meal and feed moisture content, as well as level of substitution and moisture content were highly

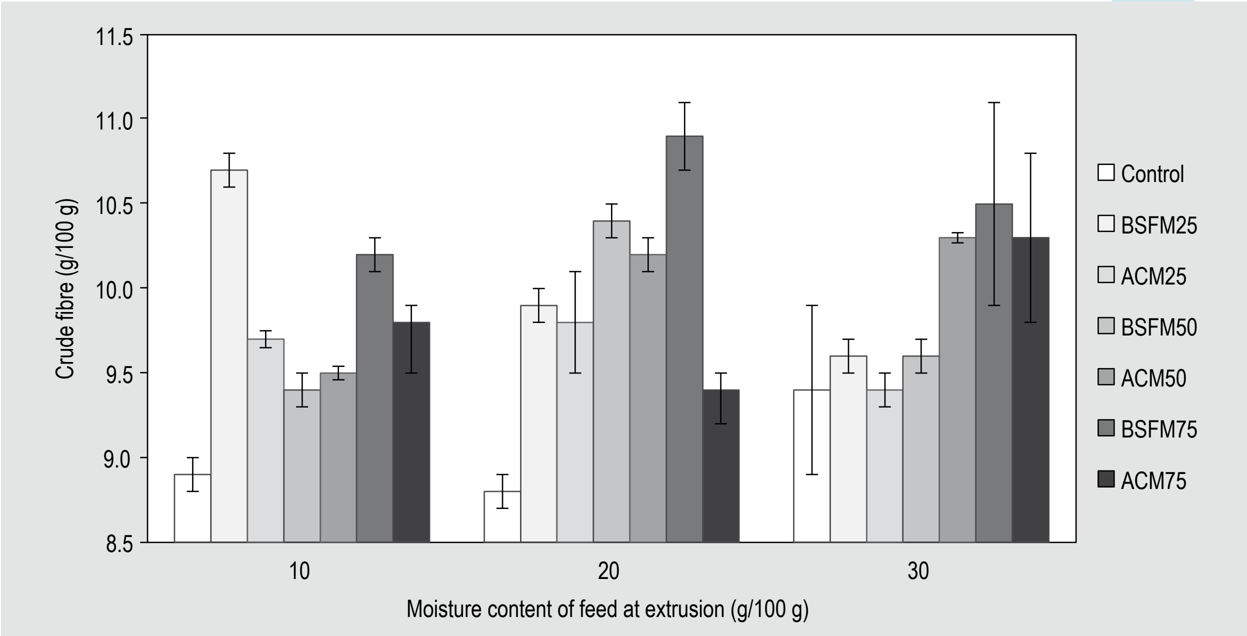

Figure 3. Crude fibre contents (g/100 g dry weight basis) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: contains FWSM but no insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.

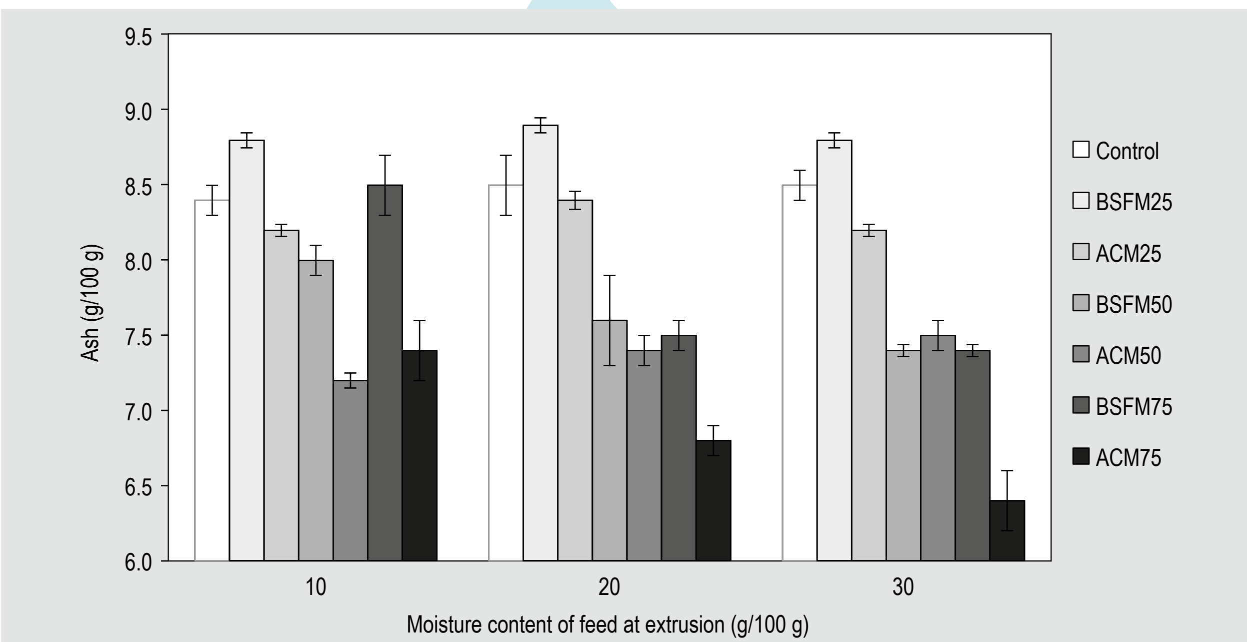

Figure 4. Ash contents (g/100 g dry weight basis) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: contains FWSM but no insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.

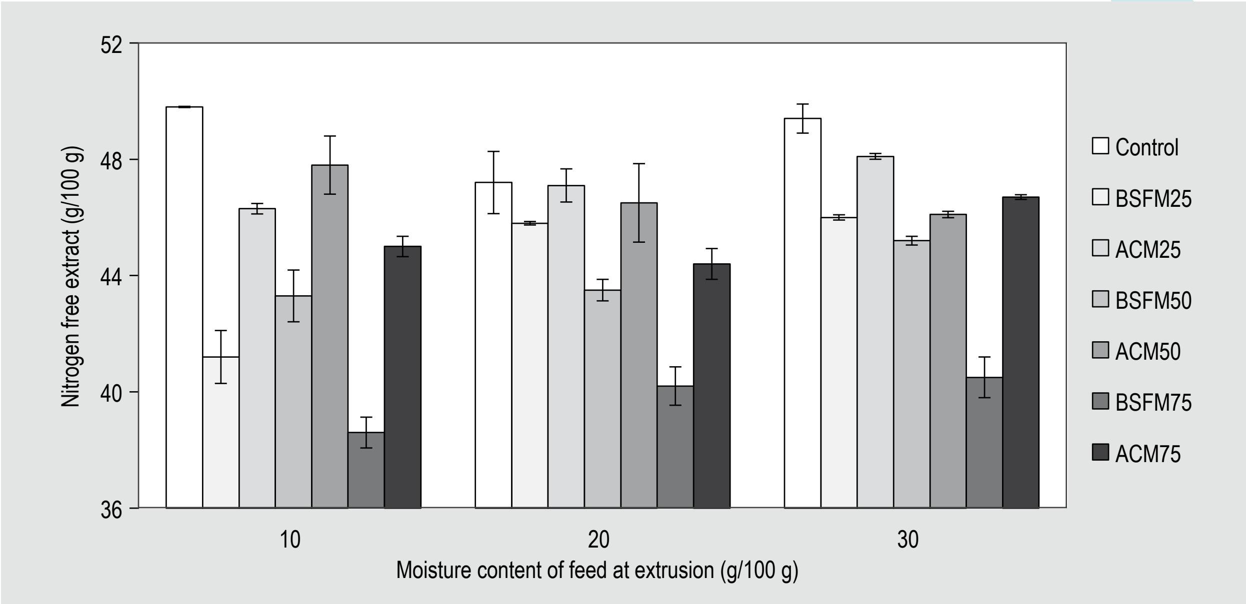

Figure 5. **Carbohydrate content contents (g/100 g dry weight basis) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: contains FWSM but no insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.**

significant (P<0.0001 and P=0.0043, respectively). Overall, compared to the raw blends, NFE increased by 23.9% in the pellets extruded from the control formulation, and by 20.3 and 14.7% in the pellets extruded from ACM and BSFM containing formulations, respectively. For the majority of the blends (ACM25, BSFM25, BSFM50, BSFM75 and ACM75), NFE content increased linearly as the level of feed moisture increased.

Effects on *in-vitro* protein digestibility

The *in-vitro* digestibility of processed pellets is presented in Figure 6. Only pellets processed at 20 and 30 g/100 g moisture were evaluated for this parameter as those processed at 10 g/100 g moisture did not have optimal physical properties (Irungu *et al.*, 2018). The interaction of type of insect meal, level of substitution and moisture content was significant (P<0.0001). Pellets extruded from ACM containing blends exhibited higher IVPD than the BSFM containing ones at 20 g/100 g feed moisture. In addition, ACM containing pellets exhibited significantly lower (ACM25: P=0.0002; ACM50: P=0.0225; ACM75: P=0.0215) IVPD when the feed moisture content was increased from 20 to 30 g/100 g, a trend which was also observed for the pellets processed from the control formulation. On the contrary, BSFM containing pellets showed higher IVPD when the extrusion moisture was increased to 30 g/100 g.

4. Discussion

The high contents of NFE determined in the ingredients of plant origin are due to the fact that cell wall of plants is mainly composed of carbohydrate while that of animals is composed of proteins and lipids. Furthermore, plants store their energy in form of carbohydrates such as fructans and starch (McDonald *et al.*, 2010). On the other hand, animal tissues store their energy in form of lipids, which explains the higher contents of crude fat in BSFM, ACM and FWSM (Finke, 2002; Makkar *et al.*, 2014). Both BSFM and ACM have high fibre contents than FWSM mainly due to the presence of chitin, a non-starch polysaccharide that is found in insects (Tran *et al.*, 2015), and for this reason increasing the substitution level of FWSM with BSFM and ACM increased the crude fibre content of the feeds. The higher amounts of crude ash and crude protein determined in BSFM, ACM and FWSM means that these ingredients are also good sources of minerals and dietary protein. Slight differences in the base compositions of the various raw blends formulated for extrusion can be attributed to the differences in the composition of ACM and BSFM relative to that of FWSM. While for practical reasons these variations could not be completely eliminated, our study provided important insights on the effect of extrusion on nutritional quality of processed product.

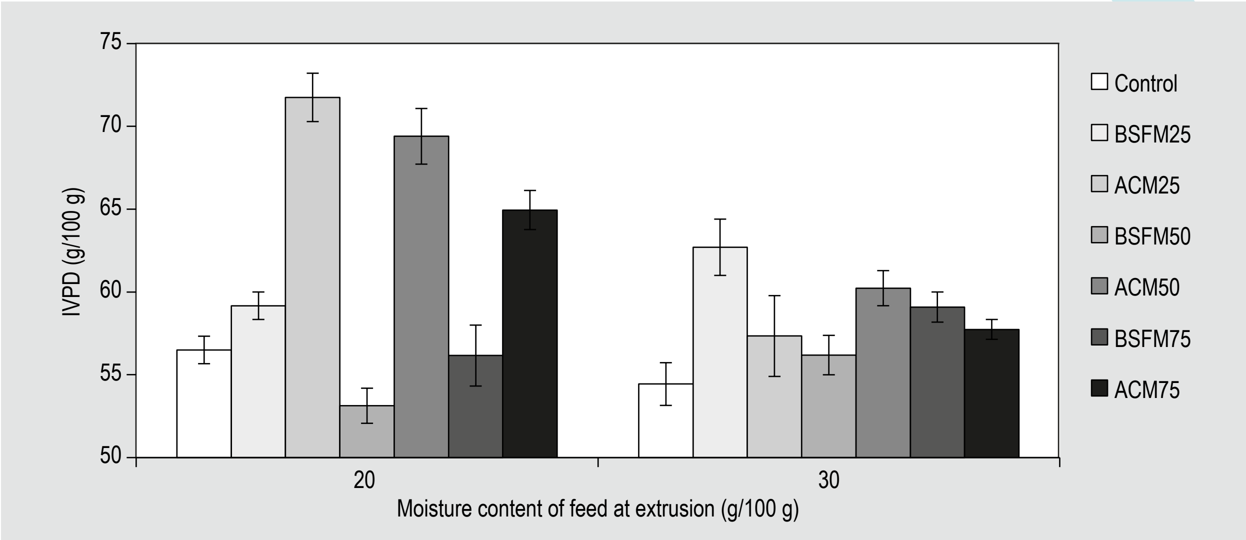

Figure 6. ***In vitro*** **protein digestibility (IVPD) of pellets extruded from feed mixtures containing varying levels of adult cricket meal (ACM) or black soldier fly meal (BSFM) as substitute for fresh water shrimp meal (FWSM) at different moisture levels. Control: contains FWSM only and no insect meal; BSFM25: black soldier fly meal substitutes 25 g/100 g of the protein supplied by FWSM in control; BSFM50: black soldier fly meal substitutes 50 g/100 g of the protein supplied by FWSM in control; BSFM75: black soldier fly meal substitutes 75 g/100 g of the protein supplied by FWSM in control; ACM25: adult cricket meal substitutes 25 g/100 g of the protein supplied by FWSM in control; ACM50: adult cricket meal substitutes 50 g/100 g of the protein supplied by FWSM in control; ACM75: adult cricket meal substitutes 75 g/100 g of the protein supplied by FWSM in control.**

The net effects of extrusion on proximate composition were increase in apparent crude protein and NFE and decrease in ether extract and fibre contents. The increase in protein content observed especially for ACM and control formulations as feed moisture content was increased could be due to enhanced hydration that reduces absorption of thermal energy. The more a food material absorbs thermal energy during extrusion, the higher the amount of proteins that are lost due to denaturation or re-orientation of proteins to form insoluble structures (Onyango, 2005). The fluctuating trend in crude protein content of BSFM formulations at 10 and 20 g/100 g moisture could be related to the interaction of a number of factors including formation of insoluble lipid-protein interactions, usually attributed to the binding of oxidised unsaturated lipids to proteins (Ladikos and Lougovois, 1990) and the formation of maillard reaction products (Guy, 2001). The decrease in amount of crude fat during extrusion as feed moisture increased from 10 to 30 g/100 g can be attributed to the formation of starch-lipid complexes that resist lipid extraction (Guy, 2001; Onyango *et al.*, 2004); higher moisture promotes starch gelatinisation hence the formation of these complexes is also enhanced. According to Guy (2001) 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 as well as starch-lipid complexes that are not digestible at normal fibre analytical procedures. In the present study, the contents of crude fibre generally decreased suggesting that the solubilisation effects were predominant. Mogilevskaya *et. al.* (2006), for instance, have argued that the pressure and shear developed during extrusion induces plastic deformation which leads to dispersion of chitin, increasing the specific surface area of its particles, thus causing chemical conversions and degradation of the polymer.

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 (Filipovic *et al.*, 2010; Levic and Sredanovic, 2010; Sørensen, 2007) which in turn increases protein digestibility. This explains the high values of protein digestibility that were reported in this study. The generally lower digestibility of BSFM-based extrudates could be due to the effect of fat. For example, fat inhibits the activity of the enzyme pepsin. The increase in protein digestibility from 0 (control) to 25 g/100 g level of substitution may be due to the positive associative effects of incorporating insect meal into the fed blend (McDonald *et al.*, 2010), where the high quality of protein in BSFM and ACM enhanced the activity of enzyme pepsin to digest the substrate at the low substitution levels. On the other hand, the decrease in protein digestibility of ACM-based pellets as level of substitution was increased from 25 to 75 g/100 g may have been due to increase in chitin content, as chitin is able to bind digestible nutrients such as proteins and also resists enzymatic attack (Onyango, 2005). This argument could also be given for the low IVPD observed with the BSFM-based pellets since BSFM also has high chitin content (Tran *et al.*, 2015). With respect to feed moisture, the decrease in protein digestibility of the ACM-based pellets as the feed moisture was increased from 20 to 30 g/100 g could be attributed to the ability of high moisture to promote formation of protein-polyphenol complexes under high extrusion temperatures (Onyango *et al.*, 2004); the complexes resist digestibility by hindering enzyme access to active sites.

The findings of this study have implications for manufacture of feeds for fish species that are commonly farmed in small-scale aquaculture; they indicate that good quality ready-to-use feeds can be processed by extrusion of feed blends containing insect meals as substitute protein sources. Catfish and tilapia are common fish species in small aquaculture systems. The minimum protein requirement for tilapia and catfish is 28-32 and 40-42 g/100 g, respectively (FAO, 2017). All the ACM and BSFM products processed in the present study 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 (Davis *et al.*, 2009). In fish feeds, lysine and methionine are the limiting amino acids and their minimum requirements are 1.2 and 0.5 g/kg for tilapia and 4.49 and 3.2 g/kg for catfish, respectively (FAO, 2017). Black soldier fly pre-pupae and adult cricket have methionine levels of 9.04 and 10.52 g/kg DM, respectively (Vrabec *et al.*, 2015). Adult cricket has lysine content of 11 g/kg (Finke 2002) while BSFM was shown to contain significant high amount of lysine (Dahiru *et al.*, 2016) to an extent that its inclusion in feeds does not necessitate further fortification and this means that both BSFM and ACM are good protein substitutes to FWSM 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 of such changes. The minimum requirements for crude fat for tilapia and catfish are 10 and 10-12 g/100 g crude fat, respectively (FAO, 2017). The extruded BSFM-based pellets can satisfactorily meet these requirements, whereas the ACM-based pellets can meet half of the requirement. Fibre supplies fish with no nutrients or energy but may play a vital role in regulating movement of bowel (Robinson *et al.*, 2006). The FAO (2017) has put the maximum crude fibre requirement for tilapia at 8-10 g/100 g, thus the fibre contents of the processed products were within the limits. 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 reduce the digestibility of nutrients by binding them and increasing the viscosity of the digested matter in the intestines. Thus, any effort to reduce the chitin content of BSFM and ACM while substituting for FWSM in fish feeds would be beneficial. Carbohydrates dietary requirement in fish have not been demonstrated but if they are not supplied in the formulation, fish will catabolise proteins and fats for energy and thus these nutrients will not be able to perform their primary functions. Even so, a maximum carbohydrate content of 40 g/100 g for tilapia has been suggested (FAO, 2017) due to the consideration of energy to protein ratio balance, which is well met by the products extruded in this study. There are no set requirements for ash content in tilapia and catfish feeds, although a feed with considerably high ash content shows that the feed has high amounts of minerals. However, some organic material may be present in ash and thus this parameter may not give a good representation of the mineral composition in feeds (McDonald *et al.*, 2010); there is need for further analysis to determine the mineral composition using the atomic absorption spectrometry.

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