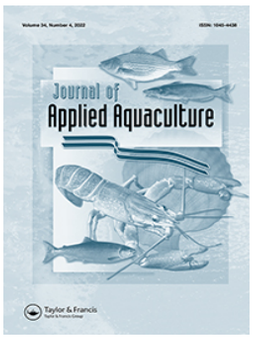

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Nutritional and microbial quality of extruded fish feeds containing black soldier fly (*Hermetia illucens* L) larvae meal as a replacement for fish meal for Nile Tilapia (*Oreochromis niloticus*) and African sharptooth catfish (*Clarius gariepinus*)

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ABSTRACT

The black soldier fly (*Hermetia illucens* L.) is a potential substitute of fish meal in feeds. However, information on the nutrition and safety of these feeds is inadequate. This study examined the quality of fish feed pellets extruded from blends formulated with and without black soldier fly larval meal (BSFLM). A further aim was to study the influence of extrusion processing types on feed composition. Two iso-proteinous feed blends containing 28% protein were formulated with 0% BSFLM (BSFLM0) and 75% BSFLM (BSFLM75). The feed blends were then cold- or hot-extruded (CE or HE) and the products analyzed for proximate composition, amino acids, fatty acid profiles and microbial content. The BSFLM75_HE pellets contained significantly higher levels of fat (15.6%), leucine (11.5 mg/g), and oleic acid (79.1 µg/g). Hot extrusion concentrated phenylalanine and leucine, increased polyunsaturated fatty acids and saturated fatty acids and decreased total viable counts, coliforms, yeast and molds, endospores and *Salmonella*.

KEYWORDS

Insects for feed; fish feed; hot extrusion; cold extrusion

Introduction

Fish is a good source of high-protein throughout the world and has significantly contributed to food and nutritional security in many developing nations (Chan et al. 2019). However, changes in climate, increasing water pollution, over-fishing, and mismanagement of our natural waters have resulted in a significant decline in the amount of captured fish (Coulthard, Johnson, and McGregor 2011). The focus has thus shifted to aquaculture, which has

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grown tremendously over the years and currently provides over half of the world's total fish consumption (FAO 2018). This growth in aquaculture has caused a rapid rise in fish feed demand in a bid to sustain the sector.

In many low- and medium-income countries, fish feeds are made by mixing locally available agricultural by-products with the aim of achieving a defined protein content. Plant sources of protein, such as soybean, cottonseed cake, and sunflower cake, have been used as major sources of protein in fish feeds. However, they contain anti-nutritional factors, such as proteinase inhibitor, tannins, phytic acid, gossypol, and antivitamins (Francis, Makkar, and Becker 2001; Vikas et al. 2012), and thus, fish do not fully benefit from their use. Fish meal has a balanced essential amino acid profile, no anti-nutritional factors, excellent palatability, and thus used as the primary source of protein in fish feeds (Hertrampf and Piedad-Pascual 2000; Vikas et al. 2012). However, fish meal is expensive as a raw material and results in high cost of feeds (Shipton and Hasan 2013). Therefore, the development and expansion of fish farming have been inhibited, especially in the developing countries,; hence,there is a need to explore more economical high-quality protein sources for a sustainable aquaculture sector.

Edible insects are a potential replacement for fish meal in the manufacture of fish feeds (Cottrell et al. 2020; Tran, Heuzé, and Makkar 2015). It has also been shown that fish farmers are aware of the benefits of using insects as feed and that they would be willing to purchase feeds containing insects (Chia et al. 2020). Among the edible insects, the black soldier fly (*Hermetia illucens* L.) larvae have gained much popularity due to their rapid multiplication and biomass production when fed organic wastes (Wang and Shelomi 2017). Black soldier fly has also found other potential uses depending on the stage of growth: for instance, as source of chitin – a natural fiber with multiple applications (Purkayastha and Sarkar 2020); management of organic wastes (Lalander et al. 2015) and feed for pig and poultry (Mutungi et al. 2019). However, an appropriate processing method is vital for quality meal to make feeds of high nutritional value. Some of the approaches that have been applied by various researchers to incorporate edible insects into feeds include drying followed by pulverization into a meal, which is then compounded with other ingredients (Mutungi et al. 2019). The compounded mash is then pressed into pellets using mechanical pressing tools. Unfortunately, such methods do not guarantee safety of the feed.

The ultimate objective of an aquaculture feed manufacturer should be to ensure that the feed produced is both safe and wholesome (Tacon and Metian 2008). Food safety risks, which may be associated with the use of commercial animal feeds, including compound aquaculture feeds, usually result from the possible presence of unwanted contaminants, either within the feed ingredients used or from the external contamination of the finished feed on prolonged storage. Major animal feed contaminants include *Salmonella*, mycotoxins,

veterinary drug residues, heavy metals, and persistent organic pollutants (Tacon and Metian 2008). These contaminants can have a direct negative effect on the health of the cultured target species, but more importantly, there is the risk that they may be passed along the food chain via contaminated aquaculture products to consumers. For instance, Lunestad et al. (2007) investigated the occurrence of *Salmonella* in fish feed and the implications for fish and human health. The authors described probable cross-contamination between fish feed factories and aquatic animals. Furthermore, *Salmonella* spp. related to poultry and swine were detected in the carcasses of native farmed fish in Brazil presenting the risk of foodborne-related salmonellosis transmitted by feeds (Dos Santos et al. 2019).

Extrusion is a robust aquafeed processing technique. The benefits include improvement of feed nutritional value through the destruction of anti-nutritional factors and improving feed digestibility (Nikmaram, Kamani, and Ghalavand 2015; Sorensen 2009). Other advantages include increased solubility of dietary fibers (Nikmaram, Kamani, and Ghalavand 2015) as well as the destruction of pathogenic microbes (Levic and Sredanovic 2010). Extrusion is classified into hot- and cold- extrusion depending on whether operating temperatures are above 100°C or at ambient temperatures, respectively (Choton et al. 2020). Hot extrusion makes it possible to produce water-stable floating aquafeeds for top feeders, such as Nile Tilapia (*Oreochromis nilotus* Linnaes, 1758), whereas cold extrusion produces sinking feeds for bottom feeders, such as catfish (*Clarius gariepinus* Burchell, 1822).

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. Irungu and coworkers determined the optimum extrusion conditions for the production of expanded floating aquafeeds using low-cost single screw extruder (Irungu et al. 2019). The authors also reported the effects of extrusion of fish feed blends containing black soldier fly pre-pupae meal on the physico-chemical properties, proximate composition, *in-vitro* protein digestibility and mineral composition (Irungu et al. 2018b, 2018a, 2018c). However, to the best of our knowledge, information on effects of both cold- and hot-extrusion on fatty acid and amino acid compositions, as well as the safety of the extruded feeds that contain black soldier fly meal, is missing. This study aimed to compare the nutritional and microbial quality of hot and cold extruded fish feed mixtures containing black soldier fly larvae. This knowledge is necessary to inform on the suitability of black soldier fly as a replacement of fish meal and also how extrusion affects the safety of fish feeds.

Materials and methods

Experimental materials

Blanched and sun-dried black soldier fly larvae (BSFL) samples were donated by Sanergy Limited in Nairobi, Kenya. The larvae were reared on a mix of latrine waste and saw dust. The self-harvesting stage (pre-pupae) was collected, blanched in boiling water for 6 min, and dried in a solar tent to constant weight in 4–5 days. The dried product was then packed in polythene bags and stored in a cool dry room. Other ingredients required for feed formulation (wheat pollard, maize germ, sunflower cake, cassava flour, and fish meal) were purchased from local approved stores in Luanda Market (Vihiga County, Kenya). All the ingredients were processed using a hammer mill and sieved through a 1 mm sieve. The proximate compositions of the sieved products were determined using standard methods (AOAC 2012), and were as reported by Irungu et al. (2018a).

Feed formulation and extrusion

Two iso-proteinous feeds were formulated based on the proximate composition of the ingredients, as detailed in Irungu et al. (2018a). Inclusion levels of each ingredient are given in Table 1. Extrusion cooking was conducted using a single screw extruder described by Irungu et al. (2019). Hot extrusion was achieved by heating the extruder barrel to 120°C, while cold extrusion was accomplished at 25°C. Feed conditioning time and die diameter were set at 100 seconds and 2 mm, respectively, according to the optimized extruder operating conditions for fish feeds (Irungu et al. 2019). Extruded feeds were solar-dried for six hours in order

Table 1. Inclusion levels of ingredients (%) and proximate composition (%) of the extruded pellets.

Ingredients/extruded pellets	BSFLM0_CE	BSFLM0_HE	BSFLM75_CE	BSFLM75_HE
BSFLM	0.00	0.00	21.75	21.75
Fish meal	29.00	29.00	7.25	7.25
Maize germ	19.00	19.00	19.00	19.00
Sunflower cake	19.00	19.00	19.00	19.00
Wheat Pollard	29.00	29.00	29.00	29.00
Cassava floor	4.00	4.00	4.00	4.00
Proximate Composition (dry weight basis)				
Crude protein	29.42 ± 0.99^{b}	31.48 ± 0.93^{a}	27.36 ± 0.12^{c}	27.03 ± 0.14^{d}
Crude fat	6.88 ± 0.38^{c}	4.93 ± 0.91^{d}	16.41 ± 0.37^{a}	15.6 ± 0.42^{b}
Crude fiber	9.97 ± 0.38^{bc}	9.28 ± 0.50^{c}	12.16 ± 0.18^{a}	10.18 ± 0.42^{b}
Ash	9.59 ± 0.42^{a}	9.05 ± 0.37^{ab}	8.66 ± 0.48^{b}	7.64 ± 0.26^{c}
Carbohydrate	44.14 ± 0.54^{b}	45.26 ± 0.12^{a}	35.40 ± 0.64^{d}	39.55 ± 0.45^{c}

BSFLM; Black Soldier Fly Larvae meal, BSFLM0_CE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are cold extruded, BSFLM0_HE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are hot extruded, BSFLM75_CE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are cold extruded, BSFLM75_HE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are hot extruded. Values for proximate composition are means ± standard error where means followed by the same letters in a row are not significantly different at $p < 0.05$

to attain a moisture content of 5%. Solar drier was designed to protect the feed from contamination by raising a wooden box of dimensions 1.2 m × 0.6 m × 0.2 m, on a slanting metal frame constructed to a height of 1 m on the air inlet end, and 1.2 m on the air exit end, respectively The top of the box was covered by a transparent plastic sheet, while a black polythene sheet lining on the inside enhanced absorption of solar radiation. Dried feeds were packaged in sterile polyethylene bags awaiting analyses.

Determination of proximate composition

Proximate composition of extruded feeds was carried out according to the AOAC (2012) methods: moisture content (934.01); crude fat (920.39); crude protein (2001.11); total fiber (942.05); total ash (945.46). Total carbohydrate content was determined by difference as 100 minus the summation of moisture content, crude fat, crude protein, total fiber, and total ash.

Amino acid profile analysis

The method for protein extraction was adopted from Maleknia and Johnson (2012). Briefly, ground samples (2 g each) were extracted for 1 h in ice cold 5 v/w 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.2, 2 mM dithiothreitol (DTT), 2.5% poly vinyl pyrrolidone (PVP), 0.5 mM ethylene diamine tetra acetic acid (EDTA), 1 mM benzamidine, 0.1 mM phenyl methane sulfonyl fluoride (PMSF) in a magnetic stirrer. The samples were filtered through KERLIX™ Gauze Bandage Rolls Sterile Soft Pouch 5.7 cm × 2.7 cm and centrifuged at 8000 rpm in Beckman centrifuge (Avanti J-25I; Beckman, CA, USA) for 30 min at 4°C to remove solid debris. Protein was precipitated between 45% and 80% $(NH_4)_2SO_4$, and the pellet recovered by centrifugation (Avanti J-25I; Beckman, CA, USA) at 21,000 rpm for 30 min at 4°C. The protein pellets were desalted in 20 mM HEPES–NaOH pH 8 containing 2 mM DTT using Sephadex G-25 gel filtration chromatography (PD-10 columns, GE Healthcare), and 10 mg of protein pellet were separately transferred into a 5 mL micro-reaction vial into which 2 mL of 6 N HCl were added and closed after careful introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110°C. For tryptophan analysis, 10 mg of the protein pellet from each of the samples were separately transferred into a 5 mL micro-reaction vial into which 2 mL of 6 N NaOH were added and then capped after careful introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110°C. After the hydrolysis, the mixtures were evaporated to dryness under vacuum. The hydrolyzates were reconstituted in 1 mL 90:10 water: acetonitrile, vortexed for 30 s, sonicated for 30 min, and then centrifuged (Avanti J-25I; Beckman, CA, USA) at 14,000 rpm, and the supernatant analyzed using

LC-Qtof-MS as described by Musundire et al. (2016). Analyses were conducted in duplicates.

Analysis of fatty acids

A methyl esterification reaction was performed on 5 g of each ground sample according to a protocol adapted from Christie (1993). A solution of sodium methoxide in methanol was prepared to give a concentration of 15 mg/mL. An aliquot of the solution (500 µL) was added to each ground sample, vortexed for 1 min and then sonicated for 5 min. The reaction mixture was incubated at 60° C for 1 h, thereafter quenched by adding 100 µL deionized water followed by vortexing for another 1 min. The resulting methyl esters were extracted using GC-grade hexane (Sigma–Aldrich, St. Louis, USA) and then centrifuged (Avanti J-25I; Beckman, CA, USA) at 14,000 rpm for 5 min. The supernatant was dried over anhydrous Na_2SO_4 and then analyzed using gas chromatography coupled to mass spectrometry (GC/MS). Fatty acids were identified as their methyl esters by comparison of gas chromatographic retention times and fragmentation patterns with those of authentic standards and reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 11. The analysis was replicated two times. Details of the GC/MS equipment and other instrumental parameters are as given in Edoh-Ognakossan et al. (2018).

Assessment of microbiological quality

Microbial determinations were performed according to AOAC (2012) methods. Ten grams of each sample were added to 90 mL of 2% buffered peptone water (CM0009, Oxoid, UK) and mixed for 40 seconds into a homogeneous mass using a stomacher laboratory homogenizer (BagMixer® 400 CC, Buch and Holm, Herlev). From the homogenate, serial dilutions up to 10^{-7} were prepared, and 1 mL of each dilution transferred into duplicate pre-labeled disposable plastic Petri dishes containing the appropriate media. Total viable counts (TVC) were determined by plating onto Plate Count Agar (CM0325, Oxoid, UK) followed by incubation at 37°C for 48 h. Coliforms were enumerated on Violet Red Bile Agar (M049, HiMedia, India) incubated at 37°C for 24 h. *Staphylococci* were cultured on Baird Parker Agar (CM0275, Oxoid, UK) containing 50 mL egg yolk and 3 mL of 3.5% potassium tellurite solution (SR0054, Oxoid, UK) in 950 mL of buffered peptone water incubated at 35–37°C for 48 h. Yeasts and molds were enumerated on acidified Potato Dextrose Agar (CM0139, Oxoid, UK) plates incubated at 25°C for 5 days. Endospores were cultured on nutrient agar (CM0003, Oxoid, UK) incubated at 55°C for 48 h, while *Salmonella* counts determined on *Salmonella Shigella* (SS) Agar (CM0099, Oxoid, UK) incubated at 35°C for 24 h. Only the plates with visible

colony counts, between 30 and 300, were counted. Counts were log-transformed and reported as log colony-forming units per gram (log cfu/g) of the sample.

Statistical analysis

Data were analyzed for variance using the Generalized Linear Model (PROC GLM) procedure of the Statistical Analysis System (SAS 9.1; SAS Institute Inc., Cary, North Carolina), and differences between individual treatment means were determined using Duncan's multiple range test at $P < .05$. Two factor analysis was performed to test the effect of extrusion method (hot or cold) and feed blend (BSFLM0, BSFLM75) and the interaction effects on the response variables.

Results and discussion

Effects of feed blend and extrusion method on proximate composition

Proximate composition of different extruded feeds is given in Table 1. Feeds that had 0% BSFLM had significantly higher crude protein than those containing 75% BSFLM (BSFLM75). However, all BSFLM-based pellets contained similar protein levels as the un-extruded mixture whose protein content was 28%. Crude fat content of the BSFLM-containing pellets produced either by cold or hot extrusion was significantly higher than that of pellets containing 100% fishmeal (BSFLM0). In addition, the crude fat was higher in the cold extruded pellets containing BSFLM. Likewise, the BSFLM-containing pellets had significantly higher crude fiber than their counterparts. Hot extrusion of BSFLM75 resulted in ash content that was significantly lower as compared to all other products. The ash contents of the cold-extruded BSFLM0 and hot-extruded BSFLM75 did not differ significantly. The BSFLM-free pellets (BSFLM0) contained significantly higher carbohydrate contents compared to those containing BSFLM (BSFLM75).

Fish requires protein for optimal growth, lipids as a source of energy and carrier of nutrients, fiber for regulation of bowel movement, and carbohydrate as a source of cheap energy (McDonald et al. 2010). Fish meal has a significantly higher protein content than black soldier fly Larvae meal (Irungu et al. 2018a), and this explains why the BSFLM0 blend produced feeds with higher protein content than BSFLM75. The high crude fat in BSFLM75 pellets is attributable to the higher fat content of black soldier fly larval meal. Black soldier fly larvae accumulate a large amount of fat that is used as a sole source of energy throughout its life cycle (Ushakova et al. 2016). This gives the insect a preferential advantage over other insects that need to be fed on a continuous basis until their use as feed.

The higher fiber content of BSFLM75 pellets is related to the high fiber content of the black soldier fly meal. The exoskeleton of insects contains chitin which is often measured as fiber (Tran, Heuzé, and Makkar 2015). On the contrary, the ash and carbohydrate contents in fish meal are reported to be considerably higher than in black soldier fly larvae (Irungu et al. 2018a), which explains the higher ash and carbohydrate contents in the BSFLM0 pellets. Proximate composition of the extruded products indicates that BSFLM can be used to substitute fish meal in fish feeds up to 75% substitution level without compromising the basic nutritional requirements for fish, as outlined in FAO (2017).

Effects of the extrusion on protein content were not definitive, where hot extrusion gave significant higher protein in BSFLM0-based pellets than cold extrusion, while in BSFLM75, hot extrusion resulted in significant lower protein than cold extrusion. An increase in extrusion temperature was also found to result in a reduction of moisture content of the extrudates (Irungu et al. 2019). This could have concentrated the proteins in BSFLM0-containing pellets that were processed through hot extrusion resulting in higher protein content. On the other hand, high temperature may have promoted maillard reactions resulting in complexion of some free amino acids and carbonyl compounds found in black soldier fly larval meal thus the lower protein content in BSFLM75 that was hot extruded.

Generally, cold extrusion resulted in higher fat, fiber and ash contents than hot extrusion for both BSFLM0 and BSFLM75 pellets, while hot extrusion gave higher ($P > .05$) carbohydrate contents than cold extrusion. Hot extrusion could have favored formation of solid fat bridges by altering their glass transition temperatures (Tumuluru 2014) resulting into low fat contents of the feeds. The reduction of fiber content at hot extrusion could be caused by the partial degradation of non-starch polysaccharides at high shear pressure and high temperature increasing their solubility and breaking of covalent and non-covalent bonds between polysaccharides and cell wall constituents (cellulose, pectin, hemicellulose) as well as loss of polymer side chains (Meng et al. 2010; Oryschak et al. 2010).

Effects of feed blend and extrusion method on amino acid profile

Effects of BSFLM substitution levels and extrusion regimes on the quantities of amino acids are shown in Table 2. The BSFLM75 pellets contained significantly lower levels of glutamic acid, valine, methionine and isoleucine but significantly higher levels of leucine. For both BSFLM0 and BSFLM75 blends, hot extrusion resulted in significantly higher levels of leucine and phenylalanine but a decrease in proline, valine, methionine and isoleucine. Tyrosine levels did not differ with extrusion regime.

Amino acids are crucial in fish nutrition as they are required for normal physiological function and optimal metabolism. Deficiencies in essential

Table 2. Amino acid profile (mg/g protein) of test diets as influenced by substitution levels of fish meal by black soldier fly larvae meal and cold and hot extrusion processing regimes.

Diet	Essential Amino Acids							Non-essential amino acids		
	Arg	Met	Leu	Ile	Phe	Tyr	Val	Glu	Pro	Hyp
BSFLM0_CE	8.0 ± 0.3 ^{c}	7.4 ± 0.1^{a}	5.8 ± 0.1^{d}	11.5 ± 0.2^{a}	7.9 ± 0.1^{b}	5.8 ± 0.3^{a}	10.0 ± 0.0^{a}	10.4 ± 0.2^{b}	11.6 ± 0.2^{a}	11.3 ± 0.2^{a}
BSFLM0_HE	41.8 ± 0.4^{a}	6.4 ± 0.0^{b}	6.0 ± 0.0 ^{c}	7.1 ± 0.2^{b}	9.7 ± 0.3^{a}	5.7 ± 0.3^{a}	6.7 ± 0.2^{b}	16.1 ± 0.3^{a}	6.4 ± 0.4 ^{c}	5.6 ± 0.2 ^{c}
BSFLM75_CE	22.5 ± 0.3^{b}	6.3 ± 0.7^{bc}	6.8 ± 0.3^{b}	5.9 ± 0.2 ^{c}	6.0 ± 0.2^{d}	6.7 ± 0.2^{a}	6.6 ± 0.3^{b}	nd	7.2 ± 0.2^{b}	6.6 ± 0.3^{b}
BSFLM75_HE	22.1 ± 0.1^{b}	5.7 ± 0.2 ^{c}	11.5 ± 0.5^{a}	5.5 ± 0.1^{d}	6.6 ± 0.2 ^{c}	6.7 ± 0.7^{a}	6.1 ± 0.1 ^{c}	5.9 ± 0.1 ^{c}	6.7 ± 0.1 ^{c}	6.2 ± 0.1^{b}

BSFLM; Black Soldier Fly Larvae meal, BSFLM0_CE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are cold extruded, BSFLM0_HE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are hot extruded, BSFLM75_CE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are cold extruded, BSFLM75_HE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are hot extruded, Arg; Arginine, Met; Methionine, Leu; Leucine, Ile; Isoleucine, Phe; Phenylalanine, Tyr; Tyrosin, Val; Valine, Glu; Glutamic acid, Pro; Proline, Hyp; Hydroxyproline. Means followed by the same letter within a column are not significantly different.

amino acids may result in to reduced growth rate, low weight gain, impaired immunity, skeletal deformations, cataracts and high fish mortality (Shefat and Karim 2018). Fish feeds should therefore be formulated and processed in a manner that minimizes loss of essential amino acids and improves their bioavailability. Fish meal contains higher amounts of glutamic acid, valine, methionine and isoleucine than black soldier fly (Liu and Wu 2020), which is why the BSFLM0 pellets contained higher levels of these amino acids. Quantities of individual amino acids in black soldier fly depend on the stage of development at harvest. The optimal stage is the prepupae where the majority of nutrients are accumulated within the insect biomass (Liu et al. 2017). In the present study, we used prepupae whose amino acid profile is comprised of high levels of methionine, glutamic acid, tyrosine and proline but low levels of lysine. However, feeding substrates have a huge impact on the amino acid profile given that varied substrates have varied chemical compositions

Hot extrusion might have activated the reactive side chains of proline, valine and isoleucine with carbonyl groups of reducing sugars formed by hydrolysis of starch at high temperature and shear force during extrusion, thus the reduction of these amino acids may have resulted from maillard reactions contributing to reduction in amino acids during extrusion (Paes and Maga 2004). On the other hand, reactive chains of leucine and phenylalanine may have resisted maillard reactions during hot extrusion, and instead, this extrusion regime probably favored the existence of these amino acids resulting into their increased amounts. Given the methionine content in the hot-extruded pellets, one of the essential amino acids in fish (Hertrampf and Piedad-Pascual 2000), was low, there is a need to investigate what extrusion conditions would reverse this trend for the production of floating feeds. However, other essential amino acids in fish, such as lysine, cysteine, tryptophan and threonine, were not studied in the present work in order to make a conclusive judgment.

Effects of feed blend and extrusion methods on fatty acids profile

The fatty acid profiles of feeds were influenced by substitution of fish meal with black soldier fly meal and extrusion regimes and is given in Table 3. The most abundant fatty acids in all the test samples in descending order were linoleic acid (C18:2 n-6; 37.14–50.08 μg/g), lauric acid (C12:0; 1.93– 91.14 μg/g), palmitic acid (C16:0; 7.96– 40.19 μg/g), stearic acid (C18:0; 4.20– 10.63 μg/g) and palmitoleic acid (C16:1 n-7; 1.14– 5.82 μg/g). Oleic acid (C18:1 n-9) was not detected in BSFLM0_CE while the highest levels were determined in BSFLM75_HE (79.08 μg/g). C18:1 n-4 and C24.0 were only found in the hot extruded pellets. The total monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) were highest in BSFLM75_HE (MUFAs: 115.73 μg/g;

Table 3. Fatty acid profile (µg/g extract) of test diets as influenced by substitution levels of fish meal by black soldier fly larvae meal and cold and hot extrusion processing regimes.

	BSFLM0_CE	BSFLM0_HE	BSFLM75_CE	BSFLM75_HE
C12:0	1.93	3.73	42.59	91.14
C14:0	nd	1.32	8.14	16.88
C16:1 (n-7)	1.14	1.40	3.66	5.82
C16:0	7.96	19.77	24.23	40.19
C16:3 (n-3)	1.68	nd	nd	nd
C17:0	0.37	nd	0.67	nd
C18:1 (n-9)	nd	47.70	43.16	79.08
trC18:1(n-9)	61.08	nd	nd	nd
C18:1 (n-4)	nd	6.51	nd	28.39
C18:0	4.20	6.51	6.97	10.63
trC18:2 (n-7)	nd	nd	3.51	nd
C18:2 (n-6)	50.08	47.78	37.14	46.80
C19:0	3.16	nd	nd	nd
C20:1(n-7)	nd	2.22	nd	nd
C22:0	nd	1.94	nd	nd
C24:0	nd	3.00	nd	1.94
Total MUFAs	62.22	57.83	46.82	115.73
Total PUFAs	51.76	47.78	30.65	46.8
Total SFAs	17.62	36.27	82.6	162.03

BSFLM0_CE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are cold extruded, BSFLM0_HE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are hot extruded, BSFLM75_CE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are cold extruded, BSFLM75_HE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are hot extruded, MUFAs- Monounsaturated fatty acids, PUFAs-Polyunsaturated fatty acids, SFAs- Saturated fatty acids, nd- not detected

SFAs: 162.03 µg/g), whereas the BSFLM0_CE contained the highest amounts of total polyunsaturated fatty acids (PUFAs) (51.76 µg/g).

High levels of C12.0 in the BSFLM-containing pellets indicate that black soldier fly larvae contain this fatty acid in significant high levels, and this finding is in agreement with other studies (Ewald et al. 2020; Truzzi et al. 2020). This abundance gives black soldier fly a preferential advantage as a feed ingredient given that C12:0 has been documented to offer nutraceutical benefits to animals, such as providing an anti-inflammatory role in the intestines (Truzzi et al. 2020). Dominance of C16:0 and C18:0 in BSFLM75 diets agrees with Ushakova et al. (2016) who reported that fatty acids in black soldier fly larvae are highly saturated in the decreasing order of C12:0, C18:0, C16:0, and C14:0. Fatty acids can be exposed to positional or geometric isomerism at high temperature. This can cause changes in the position of the double bond from the native *cis* form to *trans* or shift the double bond from one carbon position to another (Larque, Zamora, and Gil 2001). Thus, hot extrusion may have caused geometric isomerism in C18:1 n-4 and C24:0 making these fatty acids more abundant in hot extruded pellets than in cold extrusion.

Polyunsaturated fatty acids (PUFAs) are normally categorized as essential fatty acids (EFA) in fish, because they not only act as good sources of energy but also contribute to structure and function of cellular membrane. The most important fatty acids for freshwater warm fishes, such as Nile Tilapia, are the C18 PUFAs. Of particular interest is linoleic acid (C18:2 n-6) (Taşbozan and

Gökçe 2017; Tocher 2010). There were significant amounts of this fatty acid (C18:2 n-6) in the hot-extruded BSFLM75 pellets, which implies that black soldier fly larvae are a better source of this essential fatty acid needed by Nile Tilapia and hot extrusion would allow it to be readily available. The exceedingly high levels of saturated fatty acids in BSFLM75 pellets is explained by the fact that black soldier fly larvae store large amounts of saturated fats that resist oxidation (Ushakova et al. 2016). High values of monounsaturated fatty acids (MUFAs) in the hot-extruded BSFLM75 were possibly due to oxidation of unsaturated fats during extrusion.

Effects of feed blend and extrusion method on microbial quality

Figures 1 and 2 show the microbial loads of the extruded feeds. The cold-extruded BSFLM75 contained significantly higher loads than the other feeds. Hot extrusion resulted in significant reduction of the studied microbes in both BSFLM0 and BSFLM75 pellets. For *S. aureus*, hot extrusion significantly reduced the counts in pellets containing black soldier fly larval meal (Figure 1).

Black soldier fly larvae were harvested after rearing on agricultural wastes that likely favored growth of some microbes. This also could have contributed to the increased microbial counts that resisted shear forces exerted by extruder operating at ambient temperatures (cold extrusion). However, hot extrusion had a positive effect on reduction of microbes which could be attributed to the

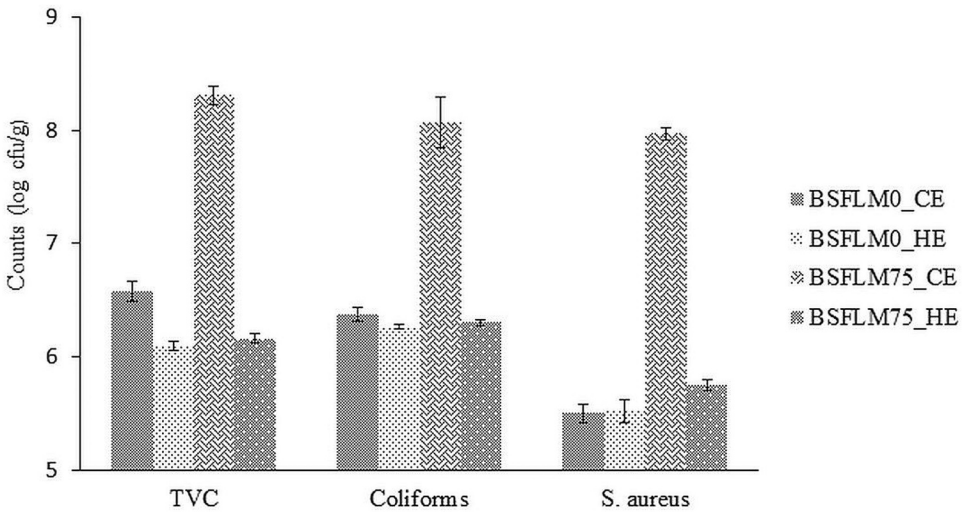

Figure 1. Effects of cold and hot extrusion processing regimes of different test diets on total viable counts (TVC), coliforms and Staphylococcus aureus. BSFLM0_CE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are cold extruded, BSFLM0_HE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are hot extruded, BSFLM75_CE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are cold extruded, BSFLM75_HE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are hot extruded.

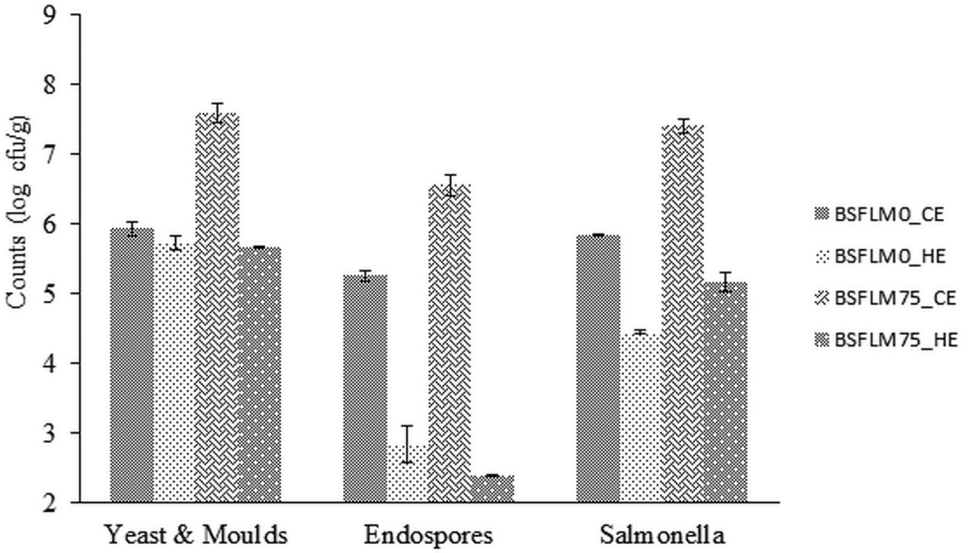

Figure 2. Effects of cold and hot extrusion processing regimes of different test diets on Yeasts and moulds, Endospores and Salmonella. BSFLM0_CE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are cold extruded, BSFLM0_HE; Black Soldier Fly Larvae meal substitute 0% of fish meal and feeds are hot extruded, BSFLM75_CE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are cold extruded, BSFLM75_HE; Black Soldier Fly Larvae meal substitute 75% of fish meal and feeds are hot extruded.

high destructive temperatures (120°C). It has also been reported that macromolecular transformations, such as gelatinization of starch during extrusion, may immobilize microbial cells by microencapsulating them with polymerized starch (Rathore et al. 2013). Our findings are in agreement with Campbell et al. (2020) who reported a significant reduction in TVC in BSFLM that were thermal processed.

Hot extrusion was effective in destruction of microbes that are known to be heat-resistant. Endospores have a thick layer of peptidoglycan which is found between the outer proteinase layer and the spore core. The core exists in a dehydrated form and its purpose is to house the cell, which is protected from heat denaturation (Popham 2002). However, high temperatures coupled with high pressure could have caused disruptions of peptidoglycan exposing the cell to heat destruction. In addition, high extrusion temperature and pressure could have induced germination of the endospores to the vegetative form (Paidhungat et al. 2002), thus making them susceptible to high temperature destruction.

Relatively higher counts of *Salmonella* were observed. This could be associated with post-extrusion contamination. The survival of *Salmonella* after high-temperature extrusion could be associated to the contamination of the ingredients with the house strain of *Salmonella* (Nesse et al. 2003), which has

acquired heat resistance. Such strains undergo modification in the fatty acid composition of the cell membrane due to heat stress. *Salmonella* is an enteric microorganism that may also find its way into animal feeds via the contaminated ingredients or the end product during handling (Jones 2011). It is also possible for *Salmonella* recontamination of extruded feeds to be caused by the cooling air, dust from milling equipment and condensed water inside the electric cooler (Davies and Wray 1997; Jones 2011). In this study, a solar dryer was used, and the feeds could therefore have been contaminated by circulating air and feed contact surfaces. *Salmonella* is a major food borne zoonotic pathogen and has a huge impact on economy and health of both animals and humans. It is therefore paramount for feed manufacturers to ensure that regular and effective cleaning protocol is adopted and executed.

Conclusion

This study has offered insights on how substitution of fish meal with black soldier fly larvae and extrusion processing can enhance the nutritional and microbial quality of fish feeds. The pellets from hot-extruded black soldier fly larvae meal contained significantly higher levels of linoleic acid – an essential fatty acid for many fish species and also other important unsaturated fatty acids. In addition, processing of fish feeds by hot extrusion reduced microbial counts, including heat-resistant endospores. However, handling procedures potentially resulted in post-processing contamination. Further studies should be carried out to investigate how extrusion variables can be optimized to maximize retention of essential amino acids and fatty acids.

Disclosure statement

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