

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

F. G. Irungu · C. M. Mutungi · A. K. Faraj · H. Affognon ·  
C. Tanga · S. Ekesi · D. Nakimbugwe · K. K. M. Fiaboe

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**Abstract** Animal food sources provide human beings with minerals considerably in adequate quantities. Fish is an indispensable reliable source of nutrients, as aquaculture is a sector that is fast growing and which provides 50% of the world's fish production. However, fish production is hampered by the increasing costs of feeds due to the ever rising cost of fish meal, an integral component of fish feeds. Substituting fish meal with cheap, yet highly nutritious ingredients in fish feeds is therefore paramount. This study investigated the effects of substituting fish meal with adult cricket meal (ACM) and black soldier fly meal (BSFM) on minerals content of extruded fish feeds, where four levels of substitution (0, 25, 50 and 75%) were used. The effect of feed moisture content on minerals was also studied where 20 and 30% feed moisture levels were used. Leaching effects of the pellets were studied as well. The results showed a significant increase ( $P < 0.05$ ) in the levels of phosphorus and potassium as the level of fish meal substitution increased from 0 to 75%. On the other hand, iron and sodium levels reduced significantly ( $P < 0.05$ ) as the level of fish meal substitution increased. Magnesium content increased with increasing level of substitution with BSFM, but decreased with increasing level of substitution with ACM. Copper, zinc and manganese were not greatly influenced by levels of fish meal substitution. Diets that had zero substitution showed higher leaching effect for most minerals than diets that were substituted with 75% ACM or BSFM. This study found that both ACM and BSFM can be used to substitute fish meal in fish feeds and obtain adequate mineral profile and low leaching effect.

**Keywords** Fish meal · Substitution · Cricket · Black soldier fly larvae · Minerals · Extrusion

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F. G. Irungu (✉) · C. M. Mutungi · A. K. Faraj  
Department of Dairy and Food Science and Technology, Egerton University, P. O. Box 536-20115, Egerton, Kenya  
e-mail: gichuhofrancis@gmail.com

C. M. Mutungi · C. Tanga · S. Ekesi · K. K. M. Fiaboe  
International Centre of Insect Physiology and Ecology, P. O. Box 30772-00100, Nairobi, Kenya

C. M. Mutungi  
International Institute of Tropical Agriculture (IITA), Plot NO. 25, Mikocheni Light Industrial Area, Dar es Salaam,  
Tanzania

H. Affognon  
International Crops Research Institute for the Semi-Arid Tropics, BP 320, Bamako, Mali

D. Nakimbugwe  
Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bio-Engineering, Makerere  
University, P. O. Box 7062, Kampala, Uganda



## Introduction

Food insecurity is a threat to many nations, especially the developing countries. In fact, 10.9% of the world's population (795 million people) is undernourished. This situation is worse in the sub-Saharan Africa where Middle Africa and East Africa have 41.3 and 31.5% of their population being undernourished, respectively (FAO 2015). Animal food sources provide protein of high value as well as a variety of micronutrients such as minerals in adequate quantities (FAO 2012; Murphy and Allen 2003). In addition, nutrients from animal food sources are more bio-available than most plant sources (Trumpo et al. 2001). Fish is one such source that provides 17% of animal protein to the world's population (Halden et al. 2014). Given the forecasted 9 billion in world population by the year 2050, fish becomes an indispensable reliable source of nutrients as aquaculture is also fast growing (Msangi et al. 2013; Halden et al. 2014; FAO 2014). Fishmeal, an integral ingredient in fish feed is becoming extremely expensive due to fluctuations in its production and this has contributed to high cost of feeds (FAO 2012, 2013, 2014; Njagi et al. 2013). There is thus the need to identify better alternatives to fishmeal. Insects such as crickets (*Acheta domesticus*) and black soldier fly (*Hermetia illucens*) have that potential (FAO 2013; Bondari and Sheppard 1981; Makkar et al. 2014), due to their high nutritional profile (Henry et al. 2015; FAO 2010; van Huis 2013), high bio-conversion ratio (Barroso et al. 2014) and their ability to produce less greenhouse gases and to be reared on less land area (Oonincx et al. 2010).

Traditionally, insects have been processed using different methods. For example, after harvest, mopane caterpillar is normally sun dried with the aim of achieving higher shelf life (Mutungi et al. 2017). 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 sun drying, roasting, smoking, boiling as well as removal of unpalatable parts (Rumpold et al. 2014). 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. The 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  $\alpha$ -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, as the tannin and phytate content increased as a result of these treatments. 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 germinates* (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). Extrusion is a modern processing method that can be used to process insects and insects-based products such as fish feeds. It has the benefits of improving the nutritional value of end products, destroying undesirable enzymes and anti-nutritional factors (Nikmaram et al. 2015; Alam et al. 2016; Filipovic et al. 2010) and it has also been shown to enhance the apparent absorption of minerals (Razzaq et al. 2012; Alonso et al. 2001).

Minerals represent a minor proportion in the feeds' composition. However, they are required in almost every aspect of animal metabolism (Alonso et al. 2001; FAO 2017). They are classified either as macro elements, which are needed by the body in large quantities, or micro elements, needed by the body in small quantities (FAO 2017; Thompson et al. 2005). Macro elements include phosphorus, calcium, potassium, magnesium, sodium, chlorine and sulfur, while micro elements are composed of copper, zinc, manganese, iron, cobalt and iodine, among others (FAO 2017; Webster and Lim 2002). Fish requires these elements as they help in several functions such as transmission of nerve impulses, acid–base balance, as enzyme cofactors and activators, normal functioning of muscles, osmoregulation and in the formation of skeletal muscles (FAO 2017; Braga et al. 2016; Hassaan et al. 2013; NRC 1993). Fish may obtain some minerals such as calcium, sodium and potassium readily from the surrounding water environment (FAO 2017; NRC 1993), but cannot get adequate amounts of phosphorus, zinc, iron and copper from water (Oliva-Teles 2012; NRC 2011) and thus these must be supplied by the diet through nutritious feeds (Tang et al. 2012). The aim of this experiment was to study the effects of substituting fish meal with different levels of black soldier fly larvae and cricket meals on the mineral composition of extruded fish feeds. The effects of moisture content on the mineral profile of extrudates were also investigated.



## Materials and methods

### Research site

Extrusion of feeds was done using a small-scale single screw extruder at Bidii Fish Farmers, Luanda-Vihiga County, Kenya. Mineral analyses were carried out at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Kenya.

### Materials

Maize germ, sunflower cake, dried cassava chunks, wheat pollard and dried freshwater shrimps were purchased from a local vendor in Luanda town, Vihiga County, Kenya. Blanched and sun-dried adult house crickets (*Acheta domestica* L.) were purchased from farmers in Homa Bay County, Kenya, while blanched and sun-dried black soldier fly larvae (*Hermetia illucens* L.) were obtained from Sanergy Limited, Nairobi, Kenya, a commercial insect rearing farm. Each ingredient was then ground separately into fine meal using a hammermill (Model 4, ARTHUR H. THOMAS, Philadelphia, PA, USA) and finally passed through a 1.0 mm aperture sieve.

### Experimental design

A  $2 \times 4 \times 2$  factorial arrangement in a completely randomized design was used. The factors investigated were insect type (two 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) (four levels: 0, 25, 50 and 75%), and feed moisture content (two levels: 20 and 30% on wet weight basis). The experiment was replicated three times.

### Feed formulation

The quantities of the ingredients required to formulate iso-proteinaceous diets (26% protein; 5 kg formulation) and the amount of water needed to achieve a required moisture content (on wet weight basis) were calculated using Microsoft Excel<sup>®</sup> function on Windows 2007 and weighed using an electronic digital weighing scale (Model no.: 7765, Ashton Meyers, China), into a 20-L bucket. These were mixed manually for 2 min and then transferred to a multi-vane paddle mixer (Model: MX-25, Unitech, New Delhi, India) for further mixing for 2 min. The control diet and the insect-based formulations are shown in Table 1.

### Extrusion process

A single screw extruder (Model: DOLLY, Unitech, New Delhi, India), with a screw operating at a speed of 200 rpm was used. The extruder was equipped with a pre-conditioning chamber where blended ingredients were introduced through a manual hopper at a feed rate of 1 kg/min and preheated with steam at an inlet pressure of 4 bars for approximately 2 min. The mixture was then channeled into the extrusion jacket that housed a single screw with a barrel length and diameter of 55 and 6 cm, respectively. The extruder was set to operate at 120 °C and fitted with a die that had a diameter of 2 mm at the exit. 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, which were placed in cool and dry wooden locker boxes for further analysis.

### Minerals analyses

Freshwater shrimps, cricket, black soldier fly larvae and extruded sample diets were 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



**Table 1** Inclusion levels of insects' meals in freshwater shrimp 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

*FSWM* freshwater shrimp meal, *BSFM* black soldier fly meal, *ACM* adult cricket meal, *BSFM25* black soldier fly meal substituting 25% of the protein supplied by *FSWM* in the control, *BSFM50* black soldier fly meal substituting 50% of the protein supplied by *FSWM* in the control, *BSFM75* black soldier fly meal substituting 75% of the protein supplied by *FSWM* in the control, *ACM25* adult cricket meal substituting 25% of the protein supplied by *FSWM* in the control, *ACM50* adult cricket meal substituting 50% of the protein supplied by *FSWM* in the control, *ACM75* adult cricket meal substituting 75% of the protein supplied by *FSWM* in the control

determined by atomic absorption spectrometry in an atomic absorption spectrophotometer (Model: AA-6300; S/N: A305243009165A; Shimadzu, Japan) after digestion in sulfuric 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) and measured through a wavelength of 880 nm.

#### Leaching activity

For each measurement, three replicates were performed. About 5 g of sample was 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 above.

#### Statistical analysis

Analysis was done using general linear model procedure (PROC GLM) of the Statistical Analysis System (SAS) software version 9.1.3 (SAS Institute Inc., USA) for analysis of variance (ANOVA). Means were separated using Tukey's HSD (honestly significant difference) test at  $P < 0.05$  level of significance.

## Results

The mineral profile of freshwater shrimp meal, adult cricket meal and black soldier fly meal

Phosphorus and potassium were significantly ( $P < 0.05$ ) higher in *BSFM* than in *FSWM*, though the two elements did not show significant ( $P < 0.05$ ) difference between *BSFM* and *ACM* (Table 2). Calcium was significantly ( $P < 0.05$ ) higher in *FSWM* than both in *ACM* and *BSFM*. Magnesium was significantly ( $P < 0.05$ ) higher in *BSFM* than in *FSWM* and *ACM*, while *FSWM* had significantly ( $P < 0.05$ ) higher magnesium than *ACM* (Table 2). Sodium, copper and zinc were higher ( $P < 0.05$ ) in both *BSFM* and *ACM* than in *FSWM*. Adult cricket meal showed significantly ( $P < 0.05$ ) higher amounts of manganese than *FSWM* and *BSFM*. Iron was higher ( $P < 0.05$ ) in *ACM* and *FSWM* than in *BSFM* (Table 2).



**Table 2** Mineral composition of freshwater shrimp meal (FWSM), adult cricket meal (ACM) and black soldier fly larvae meal (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 <sup>b</sup>	3932.4 ± 95.8 <sup>a</sup>	10,065.2 ± 962.9 <sup>b</sup>	2191.5 ± 35.7 <sup>b</sup>	21,246.1 ± 23.5 <sup>b</sup>	1512.5 ± 23.5 <sup>b</sup>	454.9 ± 14.7 <sup>b</sup>	1932.1 ± 95.6 <sup>b</sup>	922.2 ± 25.6 <sup>a</sup>
ACM	6542.5 ± 1246.7 <sup>ab</sup>	1600.7 ± 170.7 <sup>b</sup>	11,981.6 ± 957.6 <sup>ab</sup>	1467.7 ± 26.8 <sup>c</sup>	22,633.6 ± 97.4 <sup>a</sup>	1628.8 ± 30.6 <sup>a</sup>	566.4 ± 23.6 <sup>a</sup>	3126.1 ± 96.7 <sup>a</sup>	931.9 ± 24.8 <sup>a</sup>
BSFM	8579.7 ± 900.5 <sup>a</sup>	1971.4 ± 212.4 <sup>b</sup>	13,853.1 ± 978.7 <sup>a</sup>	3542.2 ± 12.5 <sup>a</sup>	22,546.9 ± 92.4 <sup>a</sup>	1609.4 ± 36.7 <sup>a</sup>	576.5 ± 14.7 <sup>a</sup>	1762.2 ± 86.7 <sup>b</sup>	845.1 ± 23.8 <sup>b</sup>

For each component, means followed by the same superscript letters along the same column are not significantly different at  $P < 0.05$

FWSM freshwater shrimp meal, BSFM black soldier fly meal, ACM adult cricket meal, P phosphorus, Ca calcium, K potassium, Mg magnesium, Na sodium, Cu copper, Zn zinc, Mn manganese, Fe iron



## Macro minerals as influenced by different diets extruded at various feed moisture contents

The level of phosphorus in control diets was significantly ( $P < 0.05$ ) lower than in all the insect-based diets (Table 3). As the level of insect substitution increased, so did the level of phosphorus. However, for each level of insect substitution, BSFM-based diets recorded higher values of phosphorus than ACM diets. Moisture content had a significant ( $P < 0.05$ ) effect on phosphorus only for the BSFM50 and BSFM75 diets. The highest level of phosphorus was recorded for the BSFM75 diet that had feed moisture content of 30%.

The control diet that was extruded with feed moisture content of 20% gave the highest amounts of calcium (Table 3). This level, however, did not show significant ( $P < 0.05$ ) difference with the BSFM25 and ACM25 diets that were produced at 20% feed moisture content, or the control diet that had 30% feed moisture content. The increase in the level of insect substitution led to a corresponding decrease in calcium levels. However, BSFM-based diets had higher calcium levels than the corresponding ACM diets (Table 3). The moisture content was also found to have a significant ( $P < 0.05$ ) influence on calcium, where for each formulation calcium was slightly higher for the extrudates that had 20% feed moisture content than those that had a feed moisture content of 30%.

The level of potassium appeared to increase with increasing level of fish meal substitution for each insect-based diet (Table 3). This increase was significant ( $P < 0.05$ ), apart from the ACM25 diet that did not show significant ( $P < 0.05$ ) difference with the control diet. Another peculiar observation was that for each diet, the 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 a corresponding slight increase in the level of potassium (Table 3).

The amount of available magnesium seemed to increase as the level of fish meal substitution with BSFM increased (Table 3). On the contrary, increasing the level of fish meal substitution with ACM led to a slight decrease in the amount of magnesium. In addition, increasing the level of feed moisture content resulted in

**Table 3** Effects of different levels of freshwater shrimp meal substitution with different insect species extruded at different feed moisture contents on phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) of the extrudates

Diet	MC (%)	P (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)
Control	20	625.9 ± 19.9 <sup>e</sup>	15,063.8 ± 300.6 <sup>a</sup>	6220.8 ± 0.7 <sup>e</sup>	3287.6 ± 4.5 <sup>e</sup>	1268.3 ± 9.2 <sup>a</sup>
	30	649.4 ± 13.5 <sup>e</sup>	14,476.2 ± 114.6 <sup>ab</sup>	6229.4 ± 2.4 <sup>e</sup>	3318.9 ± 5.8 <sup>de</sup>	1157.8 ± 31.5 <sup>bc</sup>
BSFM25	20	712.6 ± 26.6 <sup>d</sup>	14,696.9 ± 420.8 <sup>ab</sup>	6335.4 ± 0.59 <sup>d</sup>	3436.1 ± 31.4 <sup>abcde</sup>	1223.9 ± 7.0 <sup>ab</sup>
	30	735.9 ± 39.8 <sup>d</sup>	10,752.5 ± 37.8 <sup>cd</sup>	6341.8 ± 3.8 <sup>c</sup>	3519.1 ± 4.4 <sup>ab</sup>	1107.6 ± 19.7 <sup>c</sup>
BSFM50	20	810.6 ± 10.1 <sup>b</sup>	13,077.1 ± 1412.7 <sup>abc</sup>	6359.5 ± 8.55 <sup>b</sup>	3451.9 ± 13.4 <sup>abcd</sup>	991.9 ± 44.4 <sup>d</sup>
	30	789.3 ± 6.7 <sup>c</sup>	10,386.2 ± 370 <sup>cd</sup>	6370.5 ± 10.4 <sup>b</sup>	3467.6 ± 10 <sup>abcd</sup>	804.7 ± 7.4 <sup>ef</sup>
BSFM75	20	804.2 ± 14.9 <sup>b</sup>	9646.2 ± 1053.9 <sup>cde</sup>	6362.5 ± 2.3 <sup>b</sup>	3491.6 ± 60.9 <sup>abc</sup>	492.1 ± 16.4 <sup>g</sup>
	30	840.9 ± 4.9 <sup>a</sup>	8855.8 ± 218.6 <sup>de</sup>	6385.9 ± 5.3 <sup>a</sup>	3597.5 ± 49.1 <sup>a</sup>	442.9 ± 5.3 <sup>g</sup>
ACM25	20	718.2 ± 2.3 <sup>d</sup>	14,613.2 ± 392.4 <sup>ab</sup>	6226.3 ± 3.6 <sup>e</sup>	3450.3 ± 42.4 <sup>abcd</sup>	1145.7 ± 18.4 <sup>bc</sup>
	30	715.9 ± 23.3 <sup>d</sup>	12,824.8 ± 409.3 <sup>bc</sup>	6243.1 ± 6.7 <sup>e</sup>	3383.8 ± 14.2 <sup>bcde</sup>	1049.6 ± 10.1 <sup>d</sup>
ACM50	20	749.4 ± 6.5 <sup>cd</sup>	8911.8 ± 1519.3 <sup>de</sup>	6359.7 ± 9.2 <sup>b</sup>	2956.6 ± 32.6 <sup>f</sup>	812.4 ± 33.6 <sup>ef</sup>
	30	769.9 ± 30.7 <sup>bc</sup>	4326.1 ± 39.2 <sup>f</sup>	6383.5 ± 8.4 <sup>a</sup>	2837.6 ± 38.4 <sup>f</sup>	717.9 ± 11.6 <sup>f</sup>
ACM75	20	782.1 ± 12.7 <sup>bc</sup>	6439.6 ± 392.4 <sup>ef</sup>	6385.3 ± 0.6 <sup>a</sup>	3334.8 ± 12.02 <sup>cde</sup>	878.2 ± 4.8 <sup>e</sup>
	30	767.8 ± 21.6 <sup>bc</sup>	4074.9 ± 357.7 <sup>f</sup>	6389.5 ± 3.3 <sup>a</sup>	2934.4 ± 17.8 <sup>f</sup>	766.09 ± 18.47 <sup>f</sup>

Extrusion was done at a barrel temperature of 120 °C and feed exited through a 2 mm die

For each component, means followed by the same superscript letters along the same column are not significantly different at  $P < 0.05$

MC moisture content, P phosphorus, Ca calcium, K potassium, Mg magnesium, Na sodium, Control 0% insect substitution of FWSM, BSFM25 black soldier fly meal substituting 25% of the protein supplied by FWSM in the control, BSFM50 black soldier fly meal substituting 50% of the protein supplied by FWSM in the control, BSFM75 black soldier fly meal substituting 75% of the protein supplied by FWSM in the control, ACM25 adult cricket meal substituting 25% of the protein supplied by FWSM in the control, ACM50 adult cricket meal substituting 50% of the protein supplied by FWSM in the control, ACM75 adult cricket meal substituting 75% of the protein supplied by FWSM in control



higher amounts of magnesium for the control and BSFM diets, but resulted in lower levels of magnesium for the ACM diets (Table 3).

Increasing the level of fish meal substitution with insect meal beyond 25% resulted in lower levels of sodium, where BSFM50, BSFM75, ACM25, ACM50 and ACM75 diets gave significant ( $P < 0.05$ ) lower amount of sodium than the control diet (Table 3). Moreover, increasing the amount of feed moisture content from 20 to 30% resulted in a significant ( $P < 0.05$ ) reduction in the amount of sodium (Table 3) for all the diets, apart from BSFM75 whose reduction was not significant ( $P < 0.05$ ).

#### Micro minerals as influenced by different diets extruded at various feed moisture contents

Copper levels in control diets did not show significant ( $P < 0.05$ ) difference compared with BSFM75, ACM25 and ACM50 diets that were extruded with 30% feed moisture content (Table 4). The ACM75 diet extruded at both 20 and 30% feed moisture gave the highest values of copper that significantly ( $P < 0.05$ ) differed from the other formulations. Moisture content showed significant ( $P < 0.05$ ) influence on the level of copper, in that, for each diet, copper levels increased with increasing moisture content (Table 4).

The control diet that had 20% feed moisture content did not have significant ( $P < 0.05$ ) difference compared with BSFM25 (20% MC), BSFM50 (20% MC), BSFM75 (30% MC), ACM25 (30% MC) and ACM50 (both 20 and 30% MC) in amount of zinc (Table 4). Similarly, the control diet that had 30% feed moisture content did not show significant ( $P < 0.05$ ) difference in amount of zinc, compared with BSFM50 and ACM75 diets that were also extruded at 30% feed moisture content as well as BSFM75 that had 20% feed moisture content. ACM75 diet with 20% feed moisture content gave the highest level of zinc that had significant ( $P < 0.05$ ) difference with all other formulations.

For BSFM diets, there was no definite trend on how different levels of substitution as well as moisture content influenced manganese (Table 4). However, ACM diets showed slight increase in the levels of manganese as feed moisture content was increased from 20 to 30%. In addition, ACM75 diets gave the highest

**Table 4** Effects of different levels of freshwater shrimp meal substitution with different insect species extruded at different feed moisture contents on copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) of the extrudates

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

Extrusion was done at a barrel temperature of 120 °C and feed exited through a 2 mm die

For each component, means followed by the same superscript letters along the same column are not significantly different at  $P < 0.05$

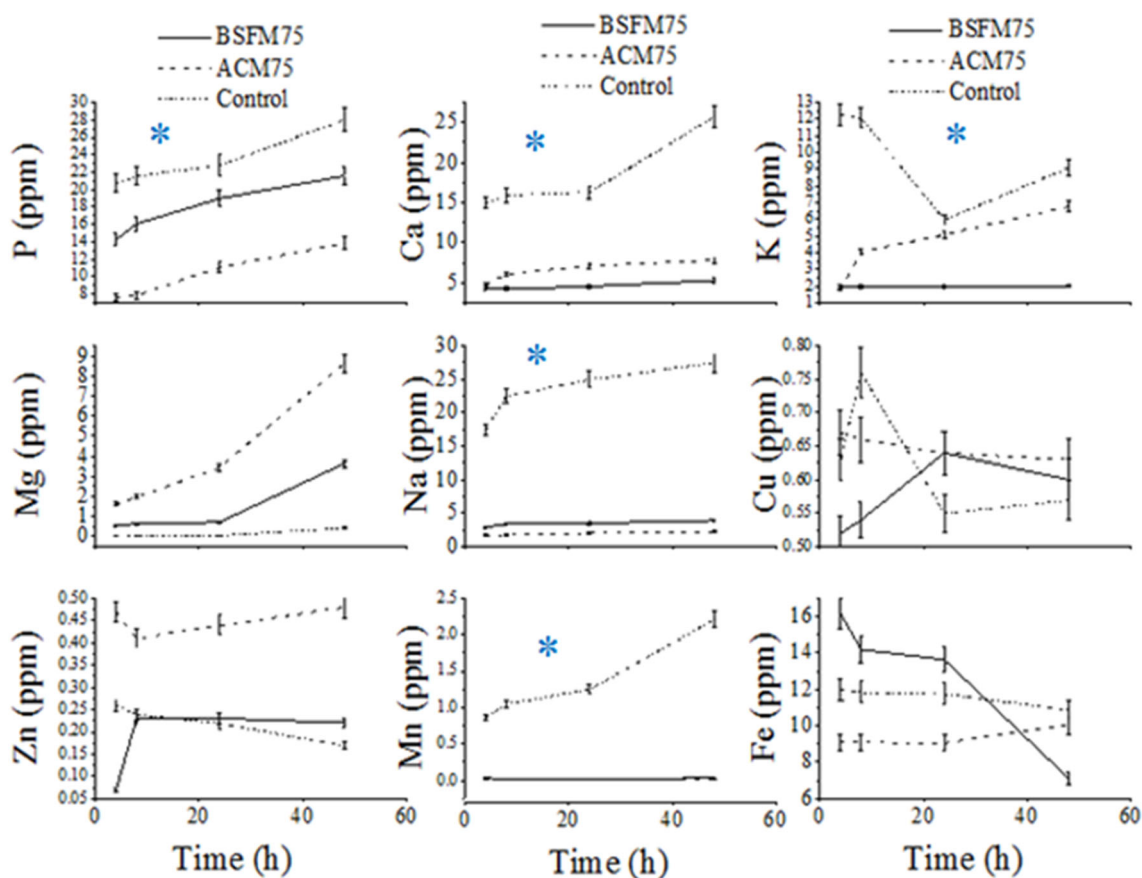
MC moisture content, Cu copper, Zn zinc, Mn manganese, Fe iron, Control 0% insect substitution of FWSM, BSFM25 black soldier fly meal substituting 25% of the protein supplied by FWSM in the control, BSFM50 black soldier fly meal substituting 50% of the protein supplied by FWSM in the control, BSFM75 black soldier fly meal substituting 75% of the protein supplied by FWSM in the control, ACM25 adult cricket meal substituting 25% of the protein supplied by FWSM in the control, ACM50 adult cricket meal substituting 50% of the protein supplied by FWSM in the control, ACM75 adult cricket meal substituting 75% of the protein supplied by FWSM in the control

values of manganese that did not differ significantly ( $P < 0.05$ ) with BSFM50 diets as well as the BSFM75 diet that were extruded at 20% feed moisture content (Table 4).

An increase in level of fish meal substitution with BSFM resulted in a decrease in final iron content, where BSFM75 diets had significantly ( $P < 0.05$ ) lower levels of iron than the control (Table 4). On the other hand, substitution with ACM resulted in increased levels of iron as the level of substitution increased with all the ACM diets giving significantly ( $P < 0.05$ ) higher levels of iron than the control and BSFM diets. It was also noted that increasing feed moisture content gave a corresponding increase in the amount of iron for all the diets, though this increase was only significant ( $P < 0.05$ ) for the BSFM50 and BSFM75 diets (Table 4).

### Leaching activity

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 good mineral profile and have also shown better physico-chemical properties in comparison to other treatments (Irungu et al. 2018). Figure 1 shows the leaching activity of minerals that were studied over a period of 48 h. Phosphorus, calcium, potassium, sodium and manganese were highly leached in the control diet (as shown in Fig. 1 with asterisks), where, during the period studied, the control showed significantly ( $P < 0.05$ ) higher leaching effect as compared to the ACM75 and BSFM75 diets. The leaching of magnesium and zinc was significantly ( $P < 0.05$ ) higher with the ACM75 diets than with BSFM75 and control diets throughout the 48 h study period. At 4 h, BSFM75 had significantly ( $P < 0.05$ ) lower leached



**Fig. 1** Leaching activity of minerals (*P* phosphorus, *Ca* calcium, *K* potassium, *Mg* magnesium, *Na* sodium, *Cu* copper, *Zn* zinc, *Mn* manganese, *Fe* iron) 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% moisture content. Parameters with an asterisk show that the control diet gave significant ( $P < 0.05$ ) higher leaching effect than ACM75 and BSFM75 diets





copper levels than ACM75 and the control, both of which were not significant at the time. However, at 48 h, all the three diets had no significant ( $P < 0.05$ ) difference in the amount of leached copper. At 4, 8 and 24 h, BSFM75 had significantly ( $P < 0.05$ ) higher amounts of leached iron levels than the control and ACM75 diets. This scenario changed at 48 h where BSFM75 showed significantly ( $P < 0.05$ ) low amounts of leached iron than the control and ACM75 diets, 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 trend from 24 to 48 h with regard 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.

## Discussion

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

Phosphorus and calcium are important minerals required by the fish for healthy bone formation as well as in enhancing the 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 the calcium–phosphorus complex (Chavez-Sanchez et al. 2000); therefore, both are discussed together. The high values of phosphorus recorded in BSFM-based diets can be attributed to the fact that BSFM had the highest amounts of phosphorus in comparison to ACM and FWSM (Table 2). 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 2). 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 and leached during the extrusion process. 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 2005; Tang et al. 2012). As such, BSFM- and ACM-based diets are good sources of phosphorus, as both resulted in higher levels of phosphorus than the control (100% freshwater shrimps) diet. However, none of the study diets resulted in minimum requirements for phosphorus for tilapia (*Oreochromis Niloticus* [L]) and catfish (*Clarias gariepinus* [Burchell]), which is 5000 ppm and 4500 ppm, respectively (FDKS 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 (Hassaan et al. 2013; FAO 2016), the Ca/P ratio of these diets on growth and development of fish need to be studied.

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; NRC 1993). The increase in potassium as the 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 the 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, which allows phosphorus to become bio-available. Razzaq 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 and 2600 ppm, respectively, and thus all our study diets meet these requirements.

Magnesium is required by fish, as it is an essential cartilage and bone component and also stimulates muscle contraction (Jahnen-Dechent and Ketteler 2012; FAO 2017). The decrease in the amount of magnesium as FWSM was substituted with BSFM is due to the higher magnesium content that is found in BSFM than in FWSM (Table 2). Likewise, ACM has lower magnesium content than FWSM (Table 2), the reason why diets substituted with ACM had lower magnesium content than the control diet. The fiber in ACM could have been

more susceptible to restructuring due to the extrusion process to an extent of promoting chelating effects that could have rendered magnesium unextractable, and thus the low magnesium content in ACM diets (Cheftel 1986). According to FAO (2017), the minimum magnesium requirements for tilapia and catfish are 600–800 and 400 ppm, respectively, and all our study diets 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 the surrounding water and feeds must supply the demand (FAO 2017).

Fish need sodium for maintenance of acid base balance and for regulation of osmotic pressure (FAO 2017; Webster and Lim 2002). The sodium in BSFM and ACM may not be readily available as in FWSM, probably due to differences in their structural formation, and this could explain why substituting FWSM with BSFM and ACM resulted in low amounts of sodium. 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, thus rendering sodium unextractable (Alonso et al. 2001). In addition, high feed moisture content may have lowered the glass transition temperature, leading to the formation of solid bridges (Tumuluru 2014) that resulted in dense products that made sodium unavailable. There is little information on the minimum sodium requirements for both tilapia and catfish and thus it is impossible to conclude whether our study diets (especially insect-based diets) can successfully substitute fish meal and meet the sodium demand for these fishes.

Copper is directly involved in enzymes activity such as dopamine hydroxylase and cytochrome oxidase (Watanabe et al. 1997) and also acts as catalyst for some enzymes (Camire et al. 1990). The higher values of copper in the ACM75 diet than BSFM and control diets is due to the higher copper amounts that were found in ACM in comparison to BSFM and FWSM (Table 2). 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 Waals forces (Tumuluru 2014) and thus increasing the availability 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%. Wheat, one of the ingredients used, has also been shown to influence copper levels (Singh et al. 2007). All the study diets met the minimum requirements for copper in tilapia (6 ppm), as outlined by KEBS standards (FDKS 2010), as well as the minimum requirements for catfish (5 ppm), as specified by FAO (2017). Thus, the ACM75 diet can be used to substitute FWSM and still obtain the same or higher values of copper.

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 2010), while the minimum requirement for catfish is 20 ppm (FAO 2017). The fact that all the study formulations were between 100 and 200 ppm and that the control diet did not differ from BSFM50 and ACM75 demonstrate that BSFM and ACM can be used to substitute FWSM up to 50 and 75%, respectively, with regard to zinc. The extrusion process has little effect on zinc composition, as the moisture content did not show significant influence on its content. Other studies have also documented the non-significant influence of extrusion on zinc and its bio-availability (Guy 2001; Kang and Chenoweth 2000).

Manganese is required in fish for normal brain functioning as well as for the metabolism of carbohydrates and lipids (Watanabe et al. 1997). The minimum requirements for manganese in feeds intended for tilapia and catfish are 12 and 2.4 ppm, respectively (FAO 2017). Findings from our 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 met the minimum requirements for manganese that is required by catfish. Manganese is one of the elements that is least affected by the extrusion process and its parameters (Razzaq et al. 2012; Singh et al. 2007), partly due to its electronic configuration that renders it less susceptible to the formation of chelates (Alonso et al. 2001). This explains why feed moisture content did not influence the manganese content of the final products.

Iron is necessary for hemoglobin 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 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 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) (FAO 2017; FDKS 2010). The 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 could be attributed to the fact that moisture and high temperature of the extrusion (120 °C) increased the abrasion of the extruder barrel, screw and the die, thereby increasing the final iron content. Similar phenomena have been reported by others (Razzaq et al. 2012; Guy 2001; Alonso et al. 2001; Camire et al. 1990).

#### Leaching activity

Leaching activity is determined by water stability of feeds, where pellets with low water stability exhibit higher leaching effect (Saalah et al. 2010; Ayadi et al. 2011). Irungu et al. (2018) had reported that 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 diet had higher leaching effect for most of minerals than the ACM75 and BSFM75 diets. Our findings are in agreement with those of Haghbayan and Mehrgan (2015) and Hardy (2002), who reported that fish meal in fish feed increased the nutrient load in wastewater and thus contributed to higher leaching effect. 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 substitute fish meal with either ACM75 or BSFM75 meals, as the two lead 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 contribute 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 the BSFM75 diets and copper and zinc in the ACM75 and control diets, respectively, could be due to formation of complexes by the macro minerals rendering these micro elements unavailable with time.

#### Conclusion

Both adult cricket meal and black soldier fly larvae meal can be used to substitute freshwater shrimp meal up to 75% in fish feeds and supply both tilapia and catfish with adequate quantities of minerals such as potassium. Further studies should investigate the bio-availability of minerals for fish from these diets.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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