Entomologia Experimentalis et Applicata
FOUNDED 1958

DOI: 10.1111/eea.12940

SPECIAL ISSUE: INSECTS IN PRODUCTION

Nutritional composition of black soldier fly larvae feeding on agro-industrial by-products

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Accepted: 13 March 2020

Key words: brewers' spent grain, brewer's yeast, molasses, *Hermetia illucens*, Diptera, Stratiomyidae, minerals, protein, organic side-streams, animal feed ingredients, black soldier fly, nutrition, agro industry, bioconversion

Abstract

Black soldier fly (BSF) larvae, *Hermetia illucens* L. (Diptera: Stratiomyidae), bio-convert organic side streams into high-quality biomass, the composition of which largely depends on the side stream used. In the present study, BSF larvae were reared on feed substrates composed of dried brewers' spent grains, each supplemented with either water, waste brewer's yeast, or a mixture of waste brewer's yeast and cane molasses to obtain 12 different substrates: barley/water, barley/yeast, barley/yeast/molasses, malted barley/water, malted barley/yeast, malted barley/yeast/molasses, malted corn/water, malted corn/yeast, malted corn/yeast/molasses, sorghum-barley/water, sorghum-barley/yeast, and sorghum-barley/yeast/molasses. The crude protein, fat, ash, and mineral contents of the BSF larvae fed each feed substrate were quantified by chemical analyses. The effect of substrate, supplementation, and their interaction on crude protein, fat, and ash contents of BSF larval body composition was significant. Calcium, phosphorus, and potassium were the most abundant macrominerals in the larvae and their concentrations differed significantly among substrates. These findings provide important information to support the use of BSF larval meal as potential new source of nutrient-rich and sustainable animal feed ingredients to substitute expensive and scarce protein sources such as fishmeal and soya bean meal.

Introduction

Animal feed is composed of major ingredients such as corn meal, wheat, rice, soybean, fishmeal, and fish oil; therefore, animal feed competes with human food requirements (Rana et al., 2009; van Huis et al., 2013). The growing human population and demand for food and feed places continuous pressure on the environment (Foley et al., 2011). The increasing demand for fishmeal and soybean meal as major protein sources in animal feeds has led to their scarcity and increasing market prices. Furthermore, feed costs represent about 70% of total aquaculture and livestock production (van Huis et al., 2013; Ssepuuya et al., 2017). It is, therefore, a matter of urgency to search for alternative and sustainable sources of protein for aquaculture and livestock. The potential of insect-based protein and other nutrients has attracted much interest from scientists and public organizations (van Huis, 2013, 2015, 2016; van Huis et al., 2013, 2015; van Huis & Vantomme, 2014; Dicke, 2018). Among several insect species recommended for animal feed, the black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae), has the highest potential for large-scale production (van Huis et al., 2013; Rumpold et al., 2018). Larvae of BSF convert low-grade organic side streams into high-quality protein and provide an innovative strategy for waste valorization (Nguyen et al., 2015; Surendra et al., 2016; Nyakeri et al., 2017; Spranghers et al., 2017; Meneguz et al., 2018). The Food and Agricultural Organization (FAO) recommends that insect species suitable for feed are those that can be mass-reared on an industrial scale, with a minimum reach of

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1, 000 kg per day of insect fresh weight (van Huis et al., 2013). However, meeting this recommendation requires a considerable amount of substrate for insect feeding. Moreover, nutritional composition of BSF larvae is influenced by the substrate used (Diener et al., 2009; Liland et al., 2017; Spranghers et al., 2017; Barragán-Fonseca et al., 2018; Wong et al., 2019).

Availability of sufficient amounts of feedstock is of paramount importance for sustainable insect production. Brewers' spent grain (BSG) is a by-product from beer production and a suitable protein source utilized as feed for livestock. However, the composition of BSG varies greatly depending on the grains used and the industrial processes and conditions such as fermentation, preservation, and temperature employed. Moreover, wet BSG contains 75–80% water and deteriorates rapidly because of bacterial and fungal growth, restricting their utilization as animal feed to the first few days after collection. Previous studies have demonstrated that feeding pigs on BSG stored for more than 1 week results in decreased feed intake and body weight loss (Aguilera-Soto et al., 2008; Thomas et al., 2010). Palatability of feed mixed with BSG has also been shown to decrease with storage time and any uneaten feed rapidly deteriorates, resulting in feed wasting. Fresh BSG left in open space spoils quickly and constitutes an environmental nuisance (Heuzé et al., 2017). Dehydrating or ensiling wet BSG can help to prevent spoilage, but these processes are time- and energy-consuming (Heuzé et al., 2017). Beer production results in more brewer's yeast than required for beer brewing. This is the second most common by-product of beer production, which is also used as a protein supplement in animal feed (Huige, 2006). Brewer's yeast has a short shelf life and its use as a feed supplement is limited. For prolonged use, drum-drying is required or it is mixed with BSG and then dried in a steam-tube drier. Again, these processes require machinery and energy, and are therefore not economical (Heuzé et al., 2018). Refining sugarcane results in a black syrup called molasses, which is rich in carbohydrates and minerals. In Kenya, this by-product is mainly used as a supplement in animal feed. However, when the production of molasses exceeds the demand for animal feed, it can cause environmental pollution, especially in water bodies if major spills or factory effluents drain into rivers or streams (M'Ndegwa, 2016).

In the context of the above concerns on utilizing BSG, brewer's yeast, and molasses in animal feeding, it is important to consider alternative ways of utilizing these by-products. Incorporating molasses and brewer's yeast in BSG could provide an alternative strategy to manage excess of by-products. There is limited information on the nutritional quality of BSF larvae reared on BSG (Meneguz et al., 2018). An in-depth study of the nutrient content quality of BSF larvae reared on brewery and sugar by-products can fill this important knowledge gap. Therefore, the aim of this study was to assess the nutritional content of BSF larvae reared on BSG, supplemented with either water, brewer's yeast, or brewer's yeast plus cane molasses, which are generated by the beer brewers and sugar companies in Kenya. In order to assess the nutritional quality for animal diets, the crude protein, fat, ash, and mineral contents of BSF larvae were analyzed. A previous study investigating the effect of nine semi-artificial diets demonstrated that body protein content was much less variable than body fat content (Barragán-Fonseca et al., 2019). Therefore, the null hypothesis we set out to test in this study was that predominantly body crude fat content depends on nutrient composition of the BSG-based feed substrates.

Materials and methods

Insect rearing and harvesting

Black soldier fly eggs were collected from the BSF colony at the Animal Rearing and Containment Unit of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. As substrate for the BSF larvae, four BSGs were obtained from the Kenya Brewery Limited, Nairobi, Kenya, resulting from the production of major beer brands, such as Tusker, Guinness, Senator, and Pilsner, as well as liquid brewer's yeast. The BSGs obtained were generated from barley (B), malted barley (MB), malted corn (MC), and sorghum plus barley (SB). Liquid cane molasses was obtained from Mumias Sugar Company, Kakamega, Kenya. Drying and preparation of substrates and their proximate compositions were previously described by Chia et al. (2018). In the present study, substrates for rearing BSF larvae were formulated as follows: 500 g of each BSG (moisture content 10%; Chia et al., 2018) was mixed with 800 ml of water to provide optimal moisture for larval feeding and growth. Another group of substrates consisted of 500 g of each BSG, mixed with 900 ml of liquid brewer's yeast. The last group of substrates consisted of 500 g of each BSG, mixed with 450 ml of liquid brewer's yeast plus 450 ml of liquid cane molasses.

Twenty batches of freshly laid BSF eggs (ca. 500 eggs per batch) were placed on the surface of each substrate in a 7-l plastic container in a temperature-controlled room at 28 ± 1 °C and 70 ± 2% r.h., as previously described by Chia et al. (2018). The experimental containers were screened with fine mesh and eggs hatched after 3–4 days. Larvae were provided with 500 g of freshly prepared substrates every 3 days until the fifth instar, recognizable by a beige color of the larvae; this occurred 2–3 weeks after egg hatching. At harvest, larvae were sieved and then manually separated from the substrate using forceps according to Spranghers et al. (2017). Harvested larvae were washed with water and frozen at 0 °C until further analysis. Before analysis, larvae were oven-dried at 60–70 °C, then ground to powder using a blender (500-W Trio Mixer Grinder; Preethi, Chennai, India).

Proximate composition analysis

Larval samples were analyzed to determine their proximate composition, defined as dry matter, crude protein, fat, and ash contents according to AOAC (1990). Dry matter content of larval samples was assessed by oven drying the samples at 105 °C until constant weight, and water content was determined as the weight difference before and after oven-drying. For the crude protein content, the nitrogen content was determined following the Kjeldahl method and the value was multiplied by a conversion factor of 4.76 (Janssen et al., 2017) to obtain the crude protein value. Fat content of larvae was determined by diethyl ether extraction in a fat extraction unit (SER 148; Velp Scientifica, Usmate, Italy) following the Randall technique, which involves immersion of samples in a hot solvent (diethyl ether) to ensure rapid solubility, washing off the solvent after boiling, and recovery by evaporation and condensation of the solvent. Ash content was determined by ignition of samples at 550 °C in a muffle furnace. Three replicate samples were analyzed for each treatment.

Analysis of minerals

Mineral composition of BSF larvae was assessed by inductively coupled plasma emission spectrometry (ICP-AES). Sample preparation for mineral analysis involved incineration at 450 °C until a grey to reddish brown color of the ash was observed. The ash was then dissolved in a mixture of nitric acid (65%), hydrochloric acid (37%), and hydrogen peroxide (30%) (Manditsera et al., 2019). For each treatment, three subsamples from one biological replicate were analyzed for iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), sodium (Na), sulphur (S), magnesium (Mg), potassium (K), aluminum (Al), phosphorus (P), and calcium (Ca) contents. The Ca/P ratio was calculated as the ratio of the concentration of Ca to the concentration of P in the BSF larval samples.

Statistical analysis

Average crude protein, fat, ash, water, and the mineral contents of larvae were compared among substrates and supplements through two-way ANOVA ($\alpha = 0.05$), with least significant difference (LSD) test as post-hoc test. The following model was used:

$$Y = \mu + \text{subs} + \text{supp} + \text{subs: supp},$$

where Y is the observation, μ is the mean nutrient content of the larvae, 'subs' is the substrate on which larvae were reared, 'supp' is the supplement to the substrates and 'subs: supp' is the interaction between substrate and supplement. The relationship between the rearing substrates, crude protein, fat, ash, water and mineral contents was evaluated using principal component analysis (PCA). All statistical analyses of the data were implemented in R software (v.3.5.1).

Results

Proximate composition

The main effects of substrate and supplementation on protein, fat, and ash contents were significant, as was their interaction effect (Table 1). Larvae reared on SB supplemented with brewer's yeast had the highest protein content followed by those reared on SB supplemented with brewer's yeast plus molasses, whereas larvae reared on MB supplemented with brewer's yeast plus molasses had the lowest protein content. Overall, larvae reared on substrates supplemented with brewer's yeast had a higher protein content than those reared on water or yeast plus molasses-supplemented substrates. Fat content of larvae reared on substrates generated from barley, malted barley, and malted corn supplemented with brewer's yeast plus molasses was higher than when supplemented with water only or brewer's yeast only. Larvae reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses had the lowest fat content (Table 1). There were no significant effects of substrate and supplementation on larval water content. However, their interaction was significant (Table 1).

The first principal component (PC) in the PCA accounted for 64.6% of the variance in the data, whereas the second accounted for 21.6% (total of 86.2%) (Figure 1). The PCA results revealed a positive correlation between the crude protein and ash contents, whereas the fat content was negatively correlated to the crude protein content (Figure 1). Larvae reared on B, MB, and MC and supplemented with a mixture of brewer's yeast and molasses had a higher fat content than larvae reared on the other substrates, whereas SB supplemented with either brewer's yeast or a mixture of brewer's yeast and molasses resulted in higher values for larval crude protein and ash contents. Overall, larvae grown on brewer's yeast-based substrates had higher crude protein contents, whereas larvae fed on substrates supplemented with a mixture of brewer's yeast and molasses had higher fat contents compared to larvae fed on water-based substrates, except for larvae fed on SB supplemented with a mixture of brewer's

Table 1 Mean (± SD; n = 3) proximate composition (% dry matter) of black soldier fly larvae reared on substrates (B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley) composed of agro-industrial by-products, supplemented with water (W), brewer's yeast (Y), or brewer's yeast plus molasses (YM)

	Treatment												Two-way ANOVA		
	B			MB			MC			SB					
Variable	W	Y	YM	W	Y	YM	W	Y	YM	W	Y	YM	Substrate	Supplementation	Substrate* supplementation
Crude protein	37.4 ± 0.62f	41.9 ± 1.25c	31.7 ± 0.08g	39.9 ± 0.34e	41.3 ± 0.22cd	29.9 ± 0.75h	40.6 ± 0.07de	39.8 ± 0.40e	31.8 ± 0.60g	40.3 ± 0.17e	45.7 ± 0.07a	44.6 ± 0.53b	<0.0001	<0.0001	<0.0001
Fat	33.2 ± 1.24d	22.5 ± 1.04g	49.0 ± 0.22a	21.1 ± 0.78g	17.1 ± 0.29h	39.3 ± 2.54c	25.5 ± 2.38f	21.1 ± 1.16g	42.3 ± 0.54b	29.7 ± 0.39e	9.5 ± 0.36i	11.4 ± 0.71i	<0.0001	<0.0001	<0.0001
Ash	8.3 ± 0.08g	7.2 ± 0.08h	6.7 ± 0.17i	9.7 ± 0.44d	8.7 ± 0.01f	9.1 ± 0.17ef	10.0 ± 0.41d	8.2 ± 0.05g	9.2 ± 0.26e	10.6 ± 0.48c	12.4 ± 0.06b	15.4 ± 0.11a	<0.0001	<0.0001	<0.0001
Water content	9.1 ± 0.40	10.8 ± 4.61	11.7 ± 0.46	10.3 ± 0.20	9.1 ± 0.21	10.1 ± 0.25	11.3 ± 0.24	10.4 ± 0.02	8.4 ± 0.70	9.9 ± 0.36	11.8 ± 0.20	12.1 ± 0.29	0.13	0.70	0.028

Means within a row followed by different letter are significantly different (LSD test: P<0.05).

yeast and molasses, which had a lower fat content (Table 1).

Mineral composition

Substrate, supplementation, and their interaction had significant effects on average concentration of macro- and microminerals in the larvae (Table 2). Calcium was the most abundant mineral in larvae across all substrates, followed by P, K, S, and Mg. All other minerals such as Na, Fe, Mn, and Zn were present at low concentrations. The concentration of Cu was lowest of all minerals in the larvae, independent of substrate on which they had been reared. Additionally, larvae reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses had significantly higher concentrations of all minerals compared to the other substrates investigated (Table 2). A similar result was obtained based on the PCA.

The first PC accounted for 56.9% of the variance in the data, whereas the second accounted for 17.9% (total of 74.8%) (Figure 2). The mineral content of larvae was positively related with the substrate SB supplemented with brewer's yeast or brewer's yeast plus molasses (Figure 2). Most other substrates – particularly B, MB, and MC supplemented with brewer's yeast or brewer's yeast plus molasses – resulted in lower larval mineral contents. The PCA clearly separates mineral content of larvae reared on water-supplemented, brewer's yeast-supplemented and brewer's yeast plus molasses-supplemented substrates, except for SB supplemented with brewer's yeast and brewer's yeast plus molasses (Figure 2).

Substrate, supplementation, and their interaction significantly affected Ca/P ratio in larvae (Table 2). Larvae reared on substrates supplemented with brewer's yeast only had lower Ca/P ratio than those reared on substrates supplemented with water or brewer's yeast plus molasses (Table 2).

Discussion

Our findings revealed that BSF larvae readily fed and grew on all the tested organic side streams (Chia et al., 2018) and converted them into nutrient-rich biomass. However, in contrast with our hypothesis, not only body fat content but also protein content of larvae was significantly influenced by the substrate type. Our results agree with previous reports that rearing substrate influences the nutritional composition of BSF larvae (Spranghers et al., 2017; Wang & Shelomi, 2017; Barragán-Fonseca et al., 2018). The supplementation of substrates with brewer's yeast, brewer's yeast plus molasses, or water had significant effects on several of the recorded nutritional parameters (protein, fat, ash, and minerals contents). The substrates

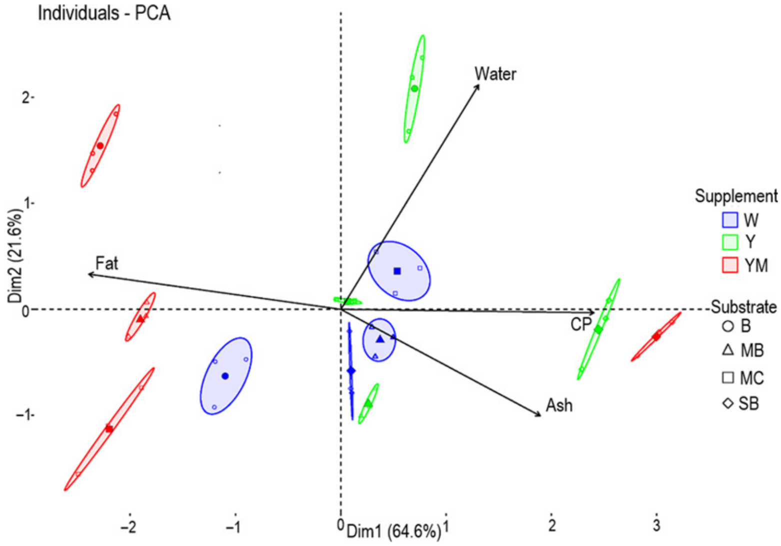

Figure 1 Principal component analysis of proximate composition of black soldier fly larvae reared on substrates (B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley) composed of agro-industrial by-products, supplemented with water (W), brewer's yeast (Y), or brewer's yeast plus molasses (YM). Substrates depicted on the same side of the vertical axis as the vector of each of the content variables water, crude protein (CP), fat, or ash have a high value for this variable, whereas substrates depicted on the opposite side of the vertical axis have a low value for this variable. Each treatment was replicated 3×. Solid symbols represent the centroid values of the substrate+supplement combinations. [Colour figure can be viewed at wileyonlinelibrary.com]

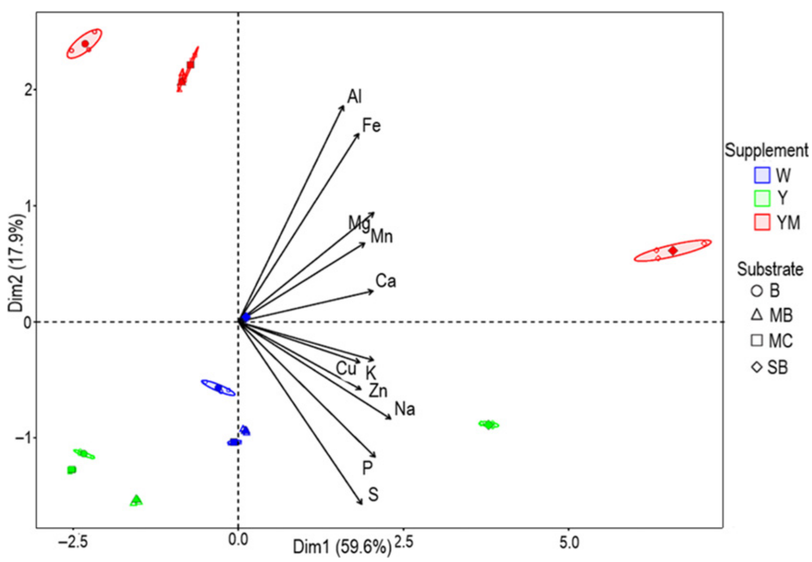

Figure 2 Principal component analysis of mineral composition of black soldier fly larvae reared on substrates (B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley) composed of agro-industrial by-products, supplemented with water (W), brewer's yeast (Y), or brewer's yeast plus molasses (YM). Substrates depicted on the same side of the vertical axis as the vector of each of the minerals have a high value for this variable, whereas substrates depicted on the opposite side of the vertical axis have a low value for this variable. Each treatment was replicated 3×. Solid symbols represent the centroid values of the substrate+supplement combinations. [Colour figure can be viewed at wileyonlinelibrary.com]

on which larvae were reared in the present study were generated from a combination of agricultural products. The nutritional contents of these substrates are reported in Chia et al. (2018). It should be noted that the nutrient content of the substrates and the larvae were not analyzed at the same time. Therefore, it is difficult to directly relate the

Table 2 Mean (± SD; n = 3) mineral composition (g kg^{-1} dry matter) of black soldier fly larvae reared on substrates (B = spent barley; MB = spent malted barley; MC = spent malted corn; SB = spent sorghum and barley) composed of agro-industrial by-products, supplemented with water (W), brewer's yeast (Y), or brewer's yeast plus molasses (YM)

		Treatment											
		B			MB			MC			SB		
	Mineral	W	Y	YM	W	Y	YM	W	Y	YM	W	Y	YM
Macrominerals	Ca	20.73 ± 0.208e	9.62 ± 0.121j	17.4 ± 0.20f	24.2 ± 0.20cd	14.83 ± 0.153h	16.57 ± 0.058g	23.73 ± 0.208d	13.23 ± 0.058i	20.47 ± 0.153e	28.07 ± 0.404b	24.53 ± 0.379c	31.27 ± 0.643a
	P	10.53 ± 0.153g	11.30 ± 0.173e	7.83 ± 0.135j	11.73 ± 0.058d	12.37 ± 0.058c	9.60 ± 0.053i	11.70 ± 0.100d	11.07 ± 0.058f	10.13 ± 0.058h	12.37 ± 0.058c	17.0 ± 0.10a	15.63 ± 0.322b
	Ca/P ratio	1.97 ± 0.014e	0.85 ± 0.014i	2.22 ± 0.040b	2.06 ± 0.010c	1.20 ± 0.013h	1.73 ± 0.007f	2.03 ± 0.035cd	1.20 ± 0.006h	2.02 ± 0.017d	2.27 ± 0.033a	1.44 ± 0.015g	2.00 ± 0.013de
	K	8.20 ± 0.173h	10.53 ± 0.058d	8.7 ± 0.10g	9.77 ± 0.058e	11.63 ± 0.058c	10.40 ± 0d	9.53 ± 0.058f	11.50 ± 0c	10.4 ± 0.10d	9.80 ± 0e	16.27 ± 0.153b	17.07 ± 0.208a
	Mg	3.07 ± 0.055e	3.38 ± 0.053d	3.16 ± 0.093e	3.40 ± 0.012d	3.38 ± 0.044d	4.09 ± 0.021c	3.4 ± 0.06d	2.89 ± 0.021f	4.11 ± 0.006c	3.48 ± 0.068d	4.23 ± 0.010b	4.89 ± 0.135a
	Na	1.18 ± 0.018e	0.77 ± 0.003i	0.62 ± 0.010k	1.32 ± 0.004c	0.95 ± 0.005f	0.65 ± 0.002j	1.25 ± 0.008d	0.89 ± 0.004g	0.84 ± 0.006h	1.18 ± 0.004e	1.53 ± 0.017b	1.94 ± 0.025a
	S	4.02 ± 0.068e	3.99 ± 0.046e	2.93 ± 0.038h	4.14 ± 0.035cd	4.05 ± 0.006de	2.98 ± 0.006gh	4.16 ± 0.047c	3.87 ± 0.006f	3.06 ± 0.015g	4.18 ± 0.015c	5.55 ± 0.038a	4.99 ± 0.156b
Microminerals	Cu	0.016 ± 0.0002a	0.010 ± 0.0001f	0.009 ± 0.0003h	0.015 ± 0.0003cd	0.010 ± 0.0002g	0.014 ± 0.0001d	0.014 ± 0.0001d	0.010 ± 0.0001gh	0.010 ± 0.0001g	0.011 ± 0.0001e	0.015 ± 0.0001b	0.016 ± 0.0002a
	Fe	0.24 ± 0.004d	0.18 ± 0.002g	0.36 ± 0.010c	0.21 ± 0.002e	0.16 ± 0.005h	0.37 ± 0.005c	0.20 ± 0.004f	0.15 ± 0.003h	0.36 ± 0.011c	0.25 ± 0.004d	0.43 ± 0.002b	0.50 ± 0.008a
	Mn	0.25 ± 0.003c	0.09 ± 0.002k	0.19 ± 0.002g	0.22 ± 0.001d	0.11 ± 0.001j	0.195 ± 0f	0.21 ± 0.002e	0.12 ± 0.002i	0.18 ± 0.001h	0.26 ± 0.002b	0.21 ± 0.002e	0.32 ± 0.006a
	Zn	0.19 ± 0.004d	0.19 ± 0.002d	0.13 ± 0.001i	0.171 ± 0f	0.20 ± 0.002b	0.15 ± 0.001h	0.20 ± 0.002c	0.15 ± 0.001g	0.15 ± 0.001g	0.13 ± 0.002i	0.18 ± 0.001e	0.32 ± 0.007a

The main effects of treatments 'substrate' and 'supplementation' as well as their interaction effect were significant for all minerals (two-way ANOVA: P<0.0001). Means within a row followed by different letters are significantly different (LSD test: P<0.05).

two results. However, in both cases (substrate and larvae), significant differences were observed in nutrient content among treatments. Furthermore, the development time of BSF larvae reared on these substrates was significantly different among substrate combinations and supplements (Chia et al., 2018). The significant interaction between substrate and supplementation reveals that the main effect of substrate on the nutrient content of the larvae is dependent on the type of supplementation or vice versa. The main effects of substrate and supplementation on water content were not significant, but their interaction was significant. This means that there was no overall effect of either substrate or supplementation on water content of the larvae.

The crude protein content of larvae in our study ranged between 30 and 46%. These values are within the range of crude protein values for BSF larvae reported in the literature (Liland et al., 2017; Spranghers et al., 2017; Meneguz et al., 2018). When larvae were reared on SB supplemented with brewer's yeast or brewer's yeast plus molasses, the resulting crude protein values were higher than obtained for the other substrates investigated. The lowest fat content values were also recorded on these substrates. Furthermore, the addition of brewer's yeast, which contains high crude protein and ash (Chollom et al., 2017) might have contributed to the high nutritional content of the larvae reared on SB. Low fat contents have previously been reported in BSF larvae with high crude protein contents (Finke, 2013; Musundire et al., 2016; Meneguz et al., 2018). This indicates that these substrates are most suitable for rearing BSF larvae.

Minerals have structural, physiological, catalytic, and regulatory functions in the body (Andrieu, 2008; Suttle, 2010). The levels of Ca, Cu, Mg, Na, Mn, Fe, and Zn in BSF larvae from our study are comparable to those reported by Spranghers et al. (2017), whereas the levels of P, K, and S were higher than the levels reported by Spranghers et al. (2017). The mineral levels in BSF larvae from our study are also similar to the levels reported by Tschirner & Simon (2015) for BSF larvae reared on various substrates. Furthermore, Makkar et al. (2014) reported higher and similar levels of Ca and P, respectively, in BSF larvae compared to our study. The differences between studies might be due to differences in life stage of the BSF analyzed. For instance, Spranghers et al. (2017) reported values for BSF pre-pupae, whereas we analyzed fifth instars. Overall, the mineral levels in BSF larvae in the present study are in compliance with the requirements of poultry, pigs, and fish (NRC, 1994, 1998; Davis & Gatlin, 1996). Therefore, BSF larvae reared on agro-industrial substrates used in the present study are promising alternatives to fishmeal and soybean meal in terms of mineral content.

Although all macrominerals are important, Ca and P are the most abundant macrominerals in the body. Calcium and P are functionally related, they are particularly implicated in bone formation and eggshell formation (Pelicia et al., 2009; Li et al., 2017; Yang, 2019; Zotte et al., 2019). A deficiency or an excess of either of these minerals affects the utilization of the other. Animal feed is often deficient of these two minerals (McDowell, 2003; Suttle, 2010) and requires supplementation to meet dietary requirements. In the present study, Ca and P were the predominant macrominerals recorded in BSF larvae. An optimal Ca/P ratio is important in reducing nutritional secondary hyperparathyroidism in insectivorous animals such as cattle egrets, reptiles, amphibians, and cats. This is a metabolic bone disease that results from insufficient dietary intake of Ca or excessive intake of P in the diet (Krook et al., 1963; Phalen et al., 2005; Lock, 2017; Boykin, 2019). The Ca/P ratio in the present study is lower than the 8.4 value for BSF larvae reported by Makkar et al. (2014). However, the values found in our study largely fall within the recommended range (1–2) for animal diets (Olson & Hale, 2001; Pelicia et al., 2009; Makkar, 2014; Stewart, 2017; Li et al., 2017; Zotte et al., 2019). In addition, our values are similar to the Ca/P ratio in fishmeal and higher than values for soybean meal, housefly maggot meal, mealworm, locust meal, house cricket, Mormon cricket, silkworm, and pupae meal (Makkar et al., 2014). Our data agree with previous reports that BSF larvae contain a higher concentration of Ca than of P (Dierenfeld & King, 2008; Klaphake, 2010; Finke, 2013; Boykin, 2019). Larvae reared on BSG supplemented with brewer's yeast yielded significantly lower Ca/P values compared to those reared on substrates supplemented with water or brewer's yeast plus molasses. Brewer's yeast has been shown to contain lower levels of Ca than of P (Onofre et al., 2017). This might have contributed to lower Ca concentrations and high P concentrations in the larvae and may explain the low Ca/P ratio. However, Ca/P values are within the recommended range, indicating that the addition of brewer's yeast to the substrates can still result in the production of high-quality BSF larvae with well-balanced Ca/P ratio, which will contribute to the total dietary Ca/P requirement of farmed animals. Furthermore, our results show that Ca is the most abundant essential mineral in the larvae. This is consistent with previous reports (Makkar et al., 2014; Li et al., 2017; Spranghers et al., 2017; Wang & Shelomi, 2017; Schmitt et al., 2019). Overall, our data demonstrate that larvae reared on these substrates represent a promising alternative source of minerals for animal feeds.

In livestock feed formulation, ingredients are selected based on their nutrient content, availability, palatability, and cost (www.poultryhub.org/nutrition/feed-formulation/). Key nutrients in feed ingredients are amino acids (contained in proteins), vitamins, and minerals. Fats and protein provide energy needed to support metabolic processes and growth, whereas minerals are needed for bone formation, enzyme activation, and egg shell development in laying hens. Against this background and from an overall evaluation of our data, BSF larvae reared on BSG can successfully provide protein-rich meal for feed. Furthermore, the high content of Ca, P, and other essential microminerals such as Fe and Zn recorded in the present study represents a high potential of the larvae as a feed component in livestock feed. The nutrient-rich larvae are suitable for several purposes, one of which is animal feeding (van Huis, 2013; Liland et al., 2017; Spranghers et al., 2017). The inclusion of BSF larval meal can therefore minimise Ca supplementation in diets (Dierenfeld & King, 2008). Although all the substrate combinations tested would produce high-quality larvae in terms of nutrient composition, substrates generated by sorghum and barley, and supplemented with brewer's yeast or brewer's yeast plus molasses appear to be more suitable as rearing substrates and result in larvae with higher levels of crude protein and minerals. Our findings show that BSF larvae may be mass-produced on these substrates for animal feed, providing an alternative strategy for managing agro-industrial side streams, when the conventional uses of these substrates such as direct feeding to livestock are not sufficient.

Acknowledgements

SYC was financially supported by the Netherlands Organization for Scientific Research, WOTRO Science for Global Development (NWO-WOTRO) (ILIPA – W 08.250.202). We would like to thank Njowe Kieran Bong Yisa, David Mutegi Marikah, Isaiah Erick Rachami, and Alexandre Villela for their substantial contribution during sample analysis and data processing in this study. We sincerely acknowledge the financial support for this research provided by NWO-WOTRO, the Canadian International Development Research Centre (IDRC), the Australian Centre for International Agricultural Research (ACIAR) (INSFEED – Phase 2: Cultivate grant no: 108866-001), the Rockefeller Foundation (SiPFeed – Grant no: 2018 FOD 009), and the Federal Ministry for Economic Cooperation and Development (BMZ) (ENTONUTRI – 81194993) through the International Centre of Insect Physiology and Ecology (*icipe*). We also gratefully acknowledge the *icipe* core funding provided by UK Aid from the Government of the United Kingdom, the Swedish International Development Cooperation Agency (Sida), the Swiss Agency for Development and Cooperation (SDC), BMZ, Germany, and the Kenyan Government. The views expressed herein do not necessarily reflect the official opinion of the donors.

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