Standardized ileal digestible amino acids and apparent metabolizable energy content in defatted black soldier fly larvae fed to broiler chickens

| Journal:          | Canadian Journal of Animal Science |
|-------------------|-----------------------------------|
| Manuscript ID     | CJAS-2018-0111.R2                 |
| Manuscript Type:  | Article                           |
| Date Submitted by the Author: | 31-Aug-2018 |
| Complete List of Authors: | Mwaniki, Zipporah; University of Guelph, Department of Animal Biosciences Kiarie, Elijah; University of Guelph, Department of Animal Biosciences |
| Keywords:         | Broiler chickens, Black soldier fly larvae meal, Standardized ileal digestibility of amino acids, AMEn |
Standardized ileal digestible amino acids and apparent metabolizable energy content in
defatted black soldier fly larvae meal fed to broiler chickens

Mwaniki Z. N. and E. Kiarie

Department of Animal Biosciences, University of Guelph, Guelph, ON, N1G 2W1

1 Presented in part at the Poultry Science Association 2018 annual meeting, San Antonio TX, USA, July 23-26
2 Correspondences: ekiarie@uoguelph.ca
Standardized (SID) ileal digestibility of amino acids (AA) and apparent metabolizable energy (AMEn) in defatted black soldier fly larvae meal (BFSLM) was determined in broiler chicks. A total of 180 d old male broiler chicks (Ross 708) were fed a commercial broiler starter diet to d 13 of age. On d 14, birds were weighed and placed in cages (10 birds/cage; n=6) and allocated one of 2 semi-purified cornstarch-based diets. The diets were: N free diet (NFD) for estimating endogenous AA losses and 20% CP test diet with BSFLM as the sole source of AA. All diets had 0.5% TiO$_2$ as an indigestible marker and the ratio of cornstarch to sucrose and soy oil in the test diet was identical to NFD to calculate AMEn by difference method. Birds were given feed and water ad lib. Excreta samples were collected on d 17 to 20 and ileal digesta on d 21. The SID of Lys, Met, Cys, Thr, Ile, Val were 86.3, 88.7, 72.8, 85.5 and 88.6%, respectively. Apparent retention of gross energy in BSFLM was 64.5 ± 2.27% and AMEn was 2,902 ± 101 kcal/kg DM. The data will aid in accurate incorporation of BSFLM in poultry feeding programs.

**Keywords:** broilers, black soldier fly larvae meal, SID of AA, AMEn

**ABBREVIATIONS USED:**

AA, amino acids; AID, apparent ileal digestibility; AMEn, apparent metabolizable energy corrected for nitrogen; AR, apparent retention; BFSLM, defatted black soldier fly larvae meal; NFD, Nitrogen free diet; SID, standardized ileal digestibility.

Feed costs account for more than 65% of variable cost of producing poultry products and the cost of energy and amino acids account for more than 90% of this cost (Kiarie et al. 2013). In the recent past, the global feed industry has seen soaring and volatile prices of traditional feedstuffs commonly used in livestock and poultry diets due to competition with the food and ethanol industries. Moreover, burgeoning human population and attendant demand for food,
especially high-quality animal protein will trigger an unprecedented increase in animal production. For example, the current animal protein production will need to increase 60% or more by 2050 (FAO 2011). This increase in animal protein demand will need enormous resources, the feed being the most challenging because of the limited availability of natural resources, climate change and food–feed–fuel competition (FAO 2011). This trend has clearly demonstrated the danger of relying on a limited pool of ingredients to formulate feeds and underscored the need to characterize the nutritive value of other feedstuffs with a potential to serve as alternatives to or complementary to traditional feedstuffs.

Insects have been proposed as a high quality, efficient and sustainable alternative protein source (De Marco et al. 2015; Marono et al. 2017; Dabbou et al., 2018; Mwaniki et al. 2018; Secci et al. 2018). Using insects as a protein source can contribute to global food security via feed or as a direct food source for humans (Schader et al. 2015). For example, in Canada, $27 billion worth of food ends up in landfills or composters each year (Parizeau et al. 2015). The nutrients in the organic waste could be recycled back for animal feeding through insect rearing (Rumpold and Schluter 2013; Makkar 2017). The insect species with the highest potential for large-scale production are the black soldier fly (BSF) (*Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*). Specifically, BSF larvae achieve high growth rate and excellent conversion of organic waste to produce a meal (BSFLM) with consistent amino acid concentration when raised on diverse substrates (Diener et al. 2009; Nguyen et al. 2015; Spranghers et al. 2017).

The use BSFLM as a component of diet has been reported for poultry (De Marco et al. 2015; Marono et al. 2017; Dabbou et al., 2018; Mwaniki et al. 2018; Secci et al. 2018). However, in general there is a dearth of information on digestible AA and AMEn data for BSFLM. Where
data do exist, most have been reported based on apparent ileal digestibility (AID) as opposed to standardized ileal digestibility (SID) estimates (De Marco et al. 2015; Schiavone et al. 2017). It has been suggested that SID estimates should be used in formulating poultry diets because these are additive in a mixture of feedstuffs compared with AID estimates (Angkanaporn et al. 1996). Moreover, formulating using SID of AA estimates results in diets that more closely match the birds’ requirements and reduce excess nutrients (Adedokun et al. 2007; Moughan et al. 2014; Adeola et al. 2016). We recently reported AA and gross energy composition of a commercial BSFLM approved for poultry feeding in Canada (Mwaniki et al. 2018). The present study determined SID of AA and AMEn value of this sample in a broiler chickens assay.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Guelph Animal Care Committee and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009).

Black soldier fly larvae meal and experimental diets

Defatted BSFLM (approximately 6% crude fat as fed) was procured from a commercial manufacturer and vendor (Enterra feed Corp., Vancouver, BC, Canada). The meal is a dry, powder product derived from larvae of the black soldier fly (Hermetia illucens) reared on pre-consumer recycled food collected from local farms, food processors and grocery stores. The meal is approved by the Canadian Food Inspection Agency for feeding poultry and its chemical composition was previously reported (Mwaniki et al. 2018). A nitrogen free diet (NFD, Table 1), corn starch-based diet was formulated to allow estimation of basal ileal endogenous N and AA losses for the calculation of SID of CP and AA (Adeola et al. 2016). The test BSFLM (as sole
A source of AA) containing diet was designed to contain 20% CP with the ratio of corn starch to sucrose to soy oil (the sole sources of energy in NFD) maintained constant to allow determination of AMEn in feed samples using the substitution method (Woyengo et al. 2010). All the diets contained TiO$_2$ (0.50%) as an indigestible marker and were fed as mash.

**Birds, housing and experimental procedures**

A total of 180 d old male broiler chicks (Ross 708) were placed in 12 cages and fed a commercial broiler starter crumbled diet to d 13 of age. The commercial broiler starter diet (Floradale Feed Mill Ltd., Floradale, ON, Canada) was corn, wheat and soybean meal based (3,000 kcal/kg of AME, 22% CP, 0.96% total Lys, 0.40% total Met, 0.80% TSAA, 0.53% available P, 0.97% Ca, and phytase at 500 phytase units/kg). On d 14, birds were weighed and placed in cages (10 birds/cage; n=6) and allocated to diets. The balance of chicks were transferred to Arkell general flock. Birds were given feed and water *ad lib*. Excreta samples were collected on d 17 to 20. On d 21, all birds were sacrificed for ileal digesta.

**Samples preparation and chemical analyses**

Daily excreta samples were pooled for each cage and oven-dried at 60°C, whereas ileal digesta samples were freeze-dried. Samples of diets, ileal digesta and excreta were finely ground in a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT) and thoroughly mixed for analysis. All samples were analyzed for DM, Ti and N. The samples were further analyzed as follows: diets for gross energy (GE), crude fat (CF), and AA contents; ileal digesta for AA contents; and excreta samples for GE, and CF contents. Dry matter determination was carried out according to standard procedures method 930.15 (AOAC 2005). Nitrogen was determined by the combustion method 968.06 (AOAC 2005) using a CNS-2000 carbon, N, and sulfur analyzer (Leco Corporation, St. Joseph, MI). The CP values were derived by multiplying
For Review Only

the assayed N values by a factor of 6.25. Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC). Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). For AA analyses, samples were prepared by acid hydrolysis according AOAC (2005, method 982.30) as modified by Mills et al. (1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (wt/vol) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam, Germany). Samples for analysis of sulfur containing AA (Met and Cys) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Titanium content was measured on a UV spectrophotometer following the method of Myers et al. (2004). The NDF and ADF contents were determined according to Van Soest et al. (1991) using Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). For BSFLM samples, the amount of protein linked to acid detergent fiber (ADF) was determined and was used to estimate the amount of chitin, according to Marono et al. (2015), Chitin (%) = ash free ADF (%) – ADF-linked protein (%). The ADF-linked protein was derived from the concentration of N in ADF residue.

Calculations and statistical analysis

The AID, SID of CP and AA and apparent retention (AR) of components in experimental diets and BSFLM sample were calculated according to Adeola et al. (2016). The SID content of CP and AA content for BSFLM were calculated using the following equation:

\[
\text{Digestible AA content} (\%) = \left[ \left( \text{SID of AA for BSFLM, } \% \right) \times \left( \text{AA content in BSFLM, } \% \right) \right]/100.
\]

The AR of dry matter, crude fat and gross energy in BSFLM were determined by substitution
method (Woyengo et al., 2010) with NFD as the basal diet using the following equation: \(D_A = D_B + \frac{(D_D - D_B)}{P_A}\), where \(D_A\) = AR of component (%) in BSFLM; \(D_B\) = retention of AR in NFD; \(D_D\) = AR of component in BSFLM containing diet; and \(P_A\) = proportion (decimal percentage) of BSFLM in BSFLM containing diet. The AME content for BSFLM was calculated using the following equation:

The AME content (kcal/kg) = \(((\text{AR of GE for BSFLM, %}) \times (\text{GE content in BSFLM, kcal kg}^{-1}))\)/100.

The AMEn content for BSFLM was calculated from AME as described by Woyengo et al. (2010) using the following equation:

\[
AMEn (\text{kcal kg}^{-1}) = AME - (8.22 \times \text{ANR}),\]

where ANR = apparent N retained (g kg\(^{-1}\) of feed intake).

The AA and GE content in BSFLM were from Mwaniki et al. (2018).

Data were reported as average and standard deviation (SD).

**Results and discussion**

Analyzed AA concentrations in BSFLM sample were: 2.79, 5.65, 2.45, 3.91, 3.30, 0.92, 2.17, 2.32, 3.46, 3.90, 5.27, 0.41, 6.84, 3.06, 3.42, 2.57, and 2.83% DM for Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr, respectively (Mwaniki et al. 2018). The concentration of NDF and ADF in BSFLM sample was 38.7 and 12.6% DM, respectively. Insects have been reported to contain variable and significant amounts of fiber measured as crude fiber, NDF and ADF (Barker et al. 1998; Marono et al. 2015). Analyses of six
*Hermetia illucens* samples showed concentration ranging from 6.1 to 36.3% DM NDF and 4.7 to 9.3% DM ADF (Marono et al. 2015). In plant-based feedstuffs, NDF is composed of cellulose, lignin, and hemicelluloses (Van Soest et al. 1991). Although, insects contain significant amounts of both ADF and NDF, the mono sugar components that make up these fractions are largely unknown (Finke 2007). Finke (2007) showed a significant amount of amino acids (9.3 to 32.7% by weight) associated with ADF fractions of several insect species. As ADF is part of NDF, the amount of protein linked to ADF were included both in the CP and NDF; for this reason, the sum of ash + CP + crude fat + NDF was shown in several insect samples to be higher than 130/100 g (Marono et al. 2015). The fiber in insects is represented by chitin, a linear polymer of β-(1-4) N-acetyl-D-glucosamine units structurally similar to cellulose [(linear polymer of β-(1-4)-D-glucopyranose units)]. Because the ADF fraction has been shown to contain N, Marono et al. (2015) proposed a method for calculating chitin concentration. Based on this method, the concentration of chitin was 7.48% DM for BSFLM sample used in the present study. The amount of chitin in BSFLM has been reported to vary from 1.7 to 9.7% DM (Diener et al. 2009; Kroeckel et al. 2012; Marono et al. 2015).

The analyzed chemical composition of the NFD and BSFLM containing diets is shown in Table 2. The AID and SID of CP in BSFLM was 80.7 and 84.6%, respectively (Table 3). Among the indispensable AA, Arg had the highest AID (88.7%) and His had the least AID (54.6%). The AID for Lys and Met were higher than for the whole BSFLM sample (46 and 42%, respectively) (De Marco et al. 2015) but comparable with values reported for defatted BSFLM (80 and 81%, respectively) (Schiavone et al. 2017). The crude fat content of BSFLM fed in the present study was 7.01% DM in previous evaluation (Mwaniki et al. 2018) and was lower than values of 15-35% DM reported for non-defatted (whole) BFLSM (Makkar et al. 2014; De Marco et al. 2015).
but comparable to defatted BSFLM samples (Marono et al. 2017; Schiavone et al. 2017). Defatting has been shown to increase concentration of crude protein from 40-44% DM in whole BSFLM (Makkar et al. 2014; Spranghers et al. 2017) to a high of 65.5% DM (Schiavone et al. 2017). The concentration of CP of BSFLM sample tested in the present study was 57.5% DM (Mwaniki et al. 2018). Based on the present observations and previous reports it can be interpreted that defatted BSFLM has high concentration of CP than non-defatted samples.

The SID of Lys, Met, Cys, Thr, Ile, Val were 86.3, 88.7, 72.8, 85.5 and 88.6%, respectively (Table 3). The SID CP content was 47.5 ± 0.29% DM (Table 3). The SID content of Leu (3.48 ± 0.01% DM) and His (3.45 ± 0.08% DM) were the highest whereas for Met (0.82 ± 0.03% DM) and Cys (0.30 ± 0.02% DM) were the lowest. The SID content of Lys, Met, and Thr was 2.85, 0.82 and 1.98% DM which were higher or comparable to values for soybean meal (2.80, 0.60 and 1.70% DM) (Adedokun et al. 2008; Bandegan et al. 2010; Ullah et al. 2016), fermented soybean meal (2.67, 0.77 and 2.04% DM) and pea protein isolate (3.49, 0.43 and 1.73% DM) (Frikha et al. 2013). Moreover, with few notable exceptions values for Lys, Met and Thr were respectively higher or comparable with fish meal (1.62, 0.67 and 1.06% DM) (Ullah et al. 2016), feather meal (1.25, 0.46 and 2.74% DM) and poultry by-products (2.89, 0.90 and 1.71% DM) (Bandegan et al. 2010), meat and bone meal (2.05, 0.57, 1.24% DM) and animal protein blends (2.09, 0.55 and 1.45% DM) (Rochell et al. 2013). Previous broiler chicken studies indicated that defatted BSFLM had higher or comparable digestible AA concentration to typical animal and plant protein sources used in poultry feed (De Marco et al. 2015; Schiavone et al. 2017).

The AR of crude fat was 93.1% (Table 4) and was comparable to values of more than 90% reported for non-defatted and defatted BSFLM samples fed to broiler chickens and quails.
(De Marco et al. 2015; Cullere et al. 2017; Schiavone et al. 2017). The AR of CP, NDF and ADF were 47.0, 44.3 and 28.5 %, respectively. Moderate and variable retention of protein and energy in broilers fed BSFLM has been attributed to the negative effects of chitin (De Marco et al. 2015; Schiavone et al. 2017). Broiler chickens fed chitin derived from crustacean shell waste (37.3% CP) digested approximately 50% of chitin protein (Hossain and Blair 2007). Marono et al. (2015) demonstrated that in vitro CP digestibility was negatively correlated to the chitin content. Surprisingly, chickens have been shown to produce chitinase in the proventriculus and hepatocytes (Suzuki et al. 2002). The AR of GE in BSFLM was 64.5 ± 2.27% and the AMEn was 2,902 ± 101 kcal kg\(^{-1}\) DM (Table 4). Majority of insect meal research has focused on whole insect meal and manufacturers have started defatting the meal mechanically or chemically to increase protein fraction, improve meal keeping quality and to create fat stream for other value-added applications. The typical crude fat content in whole BSFLM is 15-35 % DM (Makkar et al. 2014) and defatting processes produce meal with crude fat content of as low as 5% DM depending on fat extraction procedures (Schiavone et al. 2017). De Marco et al. (2015) reported a higher level of AMEn (3,967 kcal kg\(^{-1}\) DM) in full-fat BSFLM (34.3% DM crude fat). Defatted BSFLM samples were determined to have AMEn of 3,554 kcal kg\(^{-1}\) DM (18.0% DM crude fat; 55.3% DM CP) and 2,354 kcal kg\(^{-1}\) DM (4.6% DM crude fat; 65.5% DM CP) (Schiavone et al., 2017). This suggested importance of crude fat concentration on assigning accurate AMEn values of BSFLM in practical poultry feed formulation.

**Conclusions**

Successful application of BSFLM in poultry rations will depend on expanding digestible nutrients and energy database to document variability in poultry. The present data generated information to allow accurate incorporation of BSFLM in practical poultry diets. The BSFLM
should be evaluated for optimal and economical inclusion levels in practical poultry diets. It is noteworthy, SID content of Met and Cys was the lowest suggesting needs to fortify Met in practical poultry formulation. Moreover, the implication of chitin on nutrients utilization and potential physiological impact need to be determined.

Acknowledgments

Authors thankful for amino acids analysis support by laboratory of Professor C. M. Nyachoti (University of Manitoba). Technical assistance by C. Zhu, I. Wilson, and D. Vandenberg appreciated. Funded by Ontario Ministry of Agriculture, Food and Rural Affairs, National Sciences and Engineering Research Council of Canada-Discovery program and McIntosh Family Professorship in Poultry Nutrition.

References

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2008. Standardized Ileal Amino Acid Digestibility of Plant Feedstuffs in Broiler Chickens and Turkey Poults Using a Nitrogen-Free or Casein Diet. Poult. Sci. 87: 2535-2548.

Adedokun, S. A., C. M. Parsons, M. S. Lilburn, O. Adeola, and T. J. Applegate. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86: 2590-2597.

Adeola, O., P. C. Xue, A. J. Cowieson, and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. Anim. Feed Sci. Technol. 221: 274-283.

Angkanaporn, K., V. Ravindran, and W. L. Bryden. 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. Poult. Sci. 75: 1098-1103.

AOAC. 2005. Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg, MD.

Bandegan, A., E. Kiarie, R. L. Payne, G. H. Crow, W. Guenter, and C. M. Nyachoti. 2010. Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. Poult. Sci. 89: 2626-2633.

Barker, D., M. P. Fitzpatrick, and E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17: 123-134.
CCAC 2009. Guidelines on the care and use of farm animals in research, teaching and testing. Pages 1–168. Canadian Council on Animal Care, Ottawa, ON, Canada. https://www.ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf. Accessed March 2018.

Cullere, M., G. Tasoniero, V. Giaccone, G. Acuti, A. Marango, and A. Dalle Zotte. 2017. Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. Animal 12: 640-647.

Dabbou, S., G. M. Biasato, I. Capucchio, M. T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., and A. Schiavone. 2018. Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. J. Anim. Sci. Biotechnol. 9:49.

De Marco, M., S. Martinez, F. Hernandez, J. Madrid, F. Gai, L. Rotolo, M. Belforti, D. Bergero, H. Katz, S. Dabbou, A. Kovitvadhi, I. Zoccarato, L. Gasco, and A. Schiavone. 2015. Nutritional value of two insect larval meals (Tenebrio molitor and Hermetia illucens) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. Anim. Feed Sci. Technol. 209: 211-218.

Diener, S., C. Zurbrugg, and K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag. Res. 27: 603-610.

FAO. 2011. World Livestock 2011 – Livestock in food security, Rome.

Finke, M. D. 2007. Estimate of chitin in raw whole insects. Zoo Biology 26: 105-115.

Frikha, M., D. G. Valencia, A. de Coca-Sinova, R. Lázaro, and G. G. Mateos. 2013. Ileal digestibility of amino acids of unheated and autoclaved pea protein concentrate in broilers1. Poult. Sci. 92: 1848-1857.

Hossain, S. M., and R. Blair. 2007. Chitin utilisation by broilers and its effect on body composition and blood metabolites. Brit. Poult. Sci. 48: 33-38.

Kiarie, E., L. F. Romero, and C. M. Nyachoti. 2013. The role of added feed enzymes in promoting gut health in swine and poultry. Nutr. Res. Rev. 26: 71-88.

Kroekel, S., A. G. E. Harjes, I. Roth, H. Katz, S. Wuertz, A. Susenbeth, and C. Schulz. 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (Hermetia illucens) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (Psetta maxima). Aquaculture 364-365: 345-352.

Makkar, H. P. S. 2017. Opinion paper: Food loss and waste to animal feed. Animal 11: 1093-1095.

Makkar, H. P. S., G. Tran, V. Henze, and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197: 1-33.

Marono, S., R. Loponte, P. Lombardi, G. Vassalotti, M. E. Pero, F. Russo, L. Gasco, G. Parisi, G. Piccolo, S. Nizza, C. Di Meo, Y. A. Attia, and F. Bovera. 2017. Productive performance and blood profiles of laying hens fed Hermetia illucens larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. Poult. Sci. 96: 1783-1790.

Marono, S., G. Piccolo, R. Loponte, C. Di Meo, Y. A. Attia, A. Nizza, and F. Bovera. 2015. In vitro crude protein digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with chemical composition traits. Italian J. Anim. Sci. 14: 3889.

Mills, P. A., R. G. Rotter, and R. R. Marquardt. 1989. Modification of the glucosamine method for the quantification of fungal contamination. Can. J Anim Sci 69: 1105-1106.

Moughan, P. J., V. Ravindran, and J. O. B. Sorbara. 2014. Dietary protein and amino acids-Consideration of the undigestible fraction1. Poult. Sci. 93: 2400-2410.
Mwaniki, Z., M. Neijat, and E. Kiarie. 2018. Egg production and quality responses of adding up to 7.5% defatted black soldier fly larvae meal in a corn–soybean meal diet fed to Shaver White Leghorns from wk 19 to 27 of age. Poult. Sci. 97: 2829-2835.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82: 179-183.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2015. Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environ. Entomol. 44: 406-410.

Parizeau, K., M. von Massow, and R. Martin. 2015. Household-level dynamics of food waste production and related beliefs, attitudes, and behaviours in Guelph, Ontario. Waste Manage. 35: 207-217.

Rochell, S. J., D. L. Kuhlens, and I. W. A. Dozier. 2013. Relationship between in vitro assays and standardized ileal amino acid digestibility of animal protein meals in broilers1. Poult. Sci. 92: 158-170.

Rumpold, B. A., and O. K. Schluter. 2013. Potential and challenges of insects as an innovative source for food and feed production. Innov. Food Sci. Emerg. 17: 1-11.

Schader, C., A. Muller, H. Scialabba Nel, J. Hecht, A. Isensee, K. H. Erb, P. Smith, H. P. Makkar, P. Klocke, F. Leiber, P. Schwegler, M. Stolze, and U. Niggli. 2015. Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. J. R. Soc. Interface 12: 20150891.

Schiavone, A., M. De Marco, S. Martinez, S. Dabbou, M. Renna, J. Madrid, F. Hernandez, L. Rotolo, P. Costa, F. Gai, and L. Gasco. 2017. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (Hermetia illucens L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. J. Anim. Sci. Biotechnol. 8: 51.

Secci, G., F. Bovera, S. Nizza, N. Baronti, L. Gasco, G. Conte, A. Serra, A. Bonelli, and G. Parisi. 2018. Quality of eggs from Lohmann Brown Classic laying hens fed black soldier fly meal as substitute for soya bean. Animal: 1-7.

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, and S. De Smet. 2017. Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. J. Sci. Food Agr. 97: 2594-2600.

Suzuki, M., W. Fujimoto, M. Goto, M. Morimatsu, B. Syuto, and T. Iwanaga. 2002. Cellular Expression of gut chitinase mRNA in the gastrointestinal tract of mice and chickens. J. Histochem. Cytochem. 50: 1081-1089.

Ullah, Z., G. Ahmed, M. U. Nisa, and M. Sarwar. 2016. Standardized ileal amino acid digestibility of commonly used feed ingredients in growing broilers. Asian-Austral. J. Anim. Sci. 29: 1322-1330.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy. Sci. 74: 3583-3597.

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci. 89: 1182-1189.
Table 1. Composition of N-free and experimental diet, as fed basis

| Ingredient                        | N free | Insect meal |
|-----------------------------------|--------|-------------|
| Corn starch                       | 76.78  | 52.87       |
| Black fly soldier larvae meal     | -      | 34.48       |
| Sucrose                           | 8.25   | 5.68        |
| Cellulose                         | 5.00   | -           |
| Soy oil                           | 2.50   | 1.72        |
| Mono calcium phosphate            | 2.32   | 1.71        |
| Potassium carbonate               | 1.77   | 0.85        |
| Limestone fine                    | 1.29   | 0.66        |
| Vitamin trace minerals premixa    | 1.00   | 1.00        |
| Titanium dioxide                  | 0.50   | 0.50        |
| Salt                              | 0.38   | 0.38        |
| Magnesium oxide                   | 0.17   | 0.15        |
| Sodium bicarbonate                | 0.04   | -           |
| Calculated provisions AME (kcal kg\(^{-1}\)) | 2,872  | 3,178  |
| Crude protein (%)                 | -      | 20.0        |
| Crude fat (%)                     | 2.40   | 5.79        |
| Ca (%)                            | 0.88   | 0.88        |
| Available P (%)                   | 0.43   | 0.42        |
| Na (%)                            | 0.16   | 0.22        |
| Cl (%)                            | 0.23   | 0.23        |
| Mg (%)                            | 0.10   | 0.10        |
| K (%)                             | 1.00   | 1.00        |

*aProvided per kg of premix: vitamin A (retinol), 880 KIU; vitamin D3 (cholecalciferol), 330 KIU; vitamin E, 4,000 IU; vitamin K3 (menadione), 330 mg; vitamin B1 (thiamin), 400 mg; vitamin B2 (riboflavin), 800 mg; vitamin B3 (niacin), 5,000 mg; vitamin B5 (pantothenic acid), 1,500 mg; vitamin B6 (pyridoxine), 300 mg; vitamin B9 (folic acid), 100 mg; vitamin B12 (cyanocobalamin), 1200 mcg; biotin, 200 mcg; choline, 60,000 mg; Fe, 6000 mg; Cu, 1000 mg; I, 1 mg, Se, 30 mg
Table 2. Analyzed chemical composition of experimental diets, % as fed

|                          | N free | BSFLM |
|--------------------------|--------|-------|
| Dry matter (%)           | 92.8   | 89.7  |
| Crude protein (%)        | 0.40   | 19.1  |
| Gross energy kcal kg⁻¹   | 3,529  | 3,894 |
| Crude fat (%)            | 3.82   | 3.60  |
| Neutral detergent fiber (%) | 4.95  | 9.96  |
| Acid detergent fiber (%) | 4.95   | 5.20  |
| Indispensable AA (%)     |        |       |
| Arg                      | 0.05   | 0.94  |
| His                      | 0.00   | 1.89  |
| Ile                      | 0.01   | 0.89  |
| Leu                      | 0.02   | 1.33  |
| Lys                      | 0.01   | 1.12  |
| Met                      | 0.02   | 0.33  |
| Phe                      | 0.03   | 0.76  |
| Thr                      | 0.03   | 0.81  |
| Val                      | 0.02   | 1.19  |
| Dispensable AA (%)       |        |       |
| Ala                      | 0.02   | 1.36  |
| Asp                      | 0.02   | 1.80  |
| Cys                      | 0.03   | 0.13  |
| Glu                      | 0.05   | 2.56  |
| Gly                      | 0.01   | 1.07  |
| Pro                      | 0.01   | 1.48  |
| Ser                      | 0.03   | 0.96  |
| Tyr                      | 0.01   | 1.09  |
Table 3. Apparent and standardized ileal digestibility and standardized ileal digestible content of crude protein and amino acids in BSFLM fed to broilers

|                | Digestibility (%) | Digestible content (%) |
|----------------|-------------------|------------------------|
|                | Apparent | Standardized<sup>a</sup> | SD | SD |
| CP             | 80.7     | 84.6                  | 0.508 | 47.5 | 0.285 |
| Indispensable AA |         |                       |     |     |    |
| Arg            | 88.7     | 92.0                  | 0.780 | 2.56 | 0.022 |
| His            | 54.6     | 61.0                  | 1.343 | 3.45 | 0.076 |
| Ile            | 86.3     | 89.6                  | 0.581 | 2.19 | 0.014 |
| Leu            | 85.8     | 88.9                  | 0.284 | 3.48 | 0.011 |
| Lys            | 83.9     | 86.3                  | 0.725 | 2.85 | 0.024 |
| Met            | 85.3     | 88.7                  | 3.210 | 0.82 | 0.030 |
| Phe            | 86.6     | 89.7                  | 1.551 | 1.95 | 0.034 |
| Thr            | 78.2     | 85.5                  | 0.619 | 1.98 | 0.014 |
| Val            | 85.0     | 88.6                  | 0.266 | 3.07 | 0.009 |
| Dispensable AA |         |                       |     |     |    |
| Ala            | 86.2     | 88.7                  | 0.486 | 3.46 | 0.019 |
| Asp            | 77.7     | 80.9                  | 0.899 | 4.26 | 0.047 |
| Cys            | 57.4     | 72.8                  | 3.870 | 0.30 | 0.016 |
| Glu            | 85.1     | 88.3                  | 0.483 | 6.04 | 0.033 |
| Gly            | 74.8     | 78.3                  | 1.054 | 2.40 | 0.032 |
| Pro            | 86.6     | 90.1                  | 0.380 | 3.08 | 0.013 |
| Ser            | 81.3     | 87.3                  | 0.816 | 2.24 | 0.021 |
| Tyr            | 87.1     | 88.8                  | 0.324 | 2.52 | 0.009 |

<sup>a</sup>Calculated by correcting values for apparent digestibility for basal endogenous losses from birds fed N-free diets: 8.75, 0.35, 1.38, 0.33, 0.47, 0.31, 0.13, 0.27, 0.67, 0.49, 0.39, 0.64, 0.23, 0.92, 0.42, 0.58, 0.65 and 0.21 g kg<sup>-1</sup> DM intake for crude protein, Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr, respectively.

<sup>b</sup>Calculated by multiplying standardized ileal digestibility (%) by the concentrations of the crude protein and AA in BSFLM reported by Mwaniki et al. (2018).
Table 4. Apparent retention of components and AME in BSFLM fed to broiler chickens

| Item                                | Mean  | SD    |
|-------------------------------------|-------|-------|
| Dry matter (%)                      | 75.8  | 1.130 |
| Crude fat (%)                       | 93.1  | 0.640 |
| Crude protein (%)                   | 47.0  | 2.610 |
| Neutral detergent fiber (%)         | 44.3  | 7.351 |
| Acid detergent fiber (%)            | 28.5  | 12.10 |
| Gross energy (%)                    | 64.5  | 2.270 |
| AME, kcal kg⁻¹ as fed               | 3,206 | 113.1 |
| AMEn, kcal kg⁻¹ as fed              | 2,820 | 98.04 |
| AME, kcal kg⁻¹ DM                   | 3,288 | 116.0 |
| AMEn, kcal kg⁻¹ DM                  | 2,902 | 100.9 |