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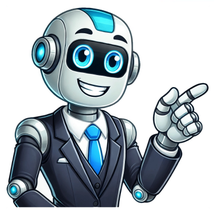

Background: Black soldier fly larvae are converters of organic waste into edible biomass, of which the composition may depend on the substrate. In this study, larvae were grown on four substrates: chicken feed, vegetable waste, biogas digestate, and restaurant waste. Samples of prepupae and substrates were freeze-dried and proximate, amino acid, fatty acid and mineral analyses were performed. Results: Protein content of prepupae varied between 399 and 431 g kg-1 dry matter (DM) among treatments. Differences in amino acid profile of prepupae were small. On the other hand, the ether extract (EE) and ash contents differed substantially. Prepupae reared on digestate were low in EE and high in ash (218 and 197 g kg-1 DM, respectively) compared to those reared on vegetable waste (371 and 96 g kg-1 DM, respectively), chicken feed (336 and 100 g kg-1 DM, respectively) and restaurant waste (386 and 27 g kg-1 DM, respectively). Prepupal fatty acid profiles were characterised by high levels of C12:0 in all treatments. Conclusion: Since protein content and quality were high and comparable for prepupae reared on different substrates, black soldier fly could be an interesting protein source for animal feeds. However, differences in EE and ash content as a function of substrate should be considered. © 2016 Society of Chemical Industry. Keywords: black soldier fly; fatty acid, amino acid; feed; protein; vegetable waste processing. As a library, NLM provides access to scientific literature. Inclusion in an NLM database does not imply endorsement of, or agreement with, the contents by NLM or the National Institutes of Health. Learn more: PMC Disclaimer | PMC Copyright Notice . 2022 Feb 25;17(2):e0263924. doi: 10.1371/journal.pone.0263924 Nutritional value of black soldier fly (Hermetia illucens) larvae (BSFL) processed by three different methods of treatment was compared. The resulting products were the spray-dried BSFL (SPR), oven-dried BSFL 1 (OVN1) and oven-dried BSFL 2 (OVN2). Proximate chemical composition, and profiles of amino acids, fatty acids, minerals, heavy metals, vitamins and nucleotides were analysed and compared. The tested BSFL meals were considered to have a good profile of essential amino acids (EAAs), with leucine, lysine, valine, and histidine being the dominant EAAs. Their content of saturated fatty acids exceeded that of the unsaturated fatty acids. Vitamins B1, B2, and C were also present in the samples. Minerals such as calcium, potassium, phosphorus, sodium, magnesium, zinc, iron, manganese and copper were found to be in adequate amounts in almost all the samples. Heavy metals in the BSFL meals were mostly below 1g kg-1. Nucleotides such as inosine monophosphate and uridine monophosphate occurred in all the BSFL meals. Other nucleotides, including guanosine monophosphate, adenosine monophosphate, xanthosine monophosphate, and cytidine monophosphate were detected in either or both of SPR and OVN2. In general, the nutritional value of the BSFL meals tested in the present study was influenced by the method of processing. Feeding farmed fish is a major challenge for development of aquaculture. Use of bulk quantities of wild fish from the ocean is one of the main concerns expressed about aquaculture. Sustainably increasing production through scientific and technological advances in finfish feeds is considered a necessary pathway to increasing the contribution of this sector to food security [1]. Aquafeeds that are nutritious and acceptable to the cultured fish should also comprise ingredients that are economically viable and are sourced from environmentally sustainable resources. There is an increasing number of investigations showing the importance of insect meal in enriching dietary intake of protein, polyunsaturated fatty acids and several other nutrients that play vital roles in growth and wellbeing of the aquaculture fish [2]. These qualities make the insect meal a suitable alternative to fish meal and oil. Black soldier fly larvae (BSFL, Hermetia illucens) have emerged as among the more environmentally sustainable choice for aquafeeds. BSFL meal contains a high level of protein, with the amino acid profile similar to fish meal and other nutrients that make it a well-balanced feed [3, 4]. H. illucens is widely distributed in warm and temperate regions and is a potential protein source. It has a short life cycle and is easy to breed and grow. Also, it does not require feeding at certain stages of its life cycle. BSFL can be reared on a wide variety of organic waste materials and thus, it provides a potential approach to reducing the volume of this waste [5]. Due to their small size, these insects require less space if they were to be bred and farmed. It was also reported that the ammonia emissions associated with insect rearing are also much lower than the domestic livestock [6]. In a study conducted by Oonincx et al. [7], insects were reported to emit 80 times less ammonia compared to cattle on a weight for weight basis. This is significant because methane has 25 times impact on global temperature than carbon dioxide. Wang and Shelomi [8] have also highlighted this advantage with specific reference to BSFL. Apart from that, BSFL has also been bio-prospected for its potential as a source of antimicrobial peptides [9–12], chitin [13–15] and lipids [16]. In general, protein sources should meet certain conditions for their production, such as regular availability in quantity, economic value, non-competition with human resources and environmental sustainability. BSF seems to fit these criteria. At present, the BSFL meal is mainly utilized for livestock feed production rather than for human consumption [17, 18]. The animal feed industry world-wide is seeking alternative protein from sustainable sources. In addition to protein, BSFL also contains fatty acids and polysaccharides and possibly other substances of nutritional value. Currently, the use of BSFL is receiving more attention in aquaculture feed industry as an effort to reduce dependency on fish meal for protein and oil. A comprehensive biochemical analysis of feed stuffs is important for assessment of their nutritional value and sharing the information with companies and consumers. Studies on insect body composition show large differences between different species. In addition, the nutritional composition of insects can be significantly influenced by methods of their farming and processing for feed development [19]. Therefore, it is beneficial to investigate the nutritional composition of three different types of BSFL meals available in the local market. Fruit and vegetable wastes as substrates for rearing BSFL are widely used because of higher levels of long-term omega-3 fatty acids, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) that these products have been reported to achieve in the insect compared to other substrates sourced from terrestrial plants [20], and their bulk availability. In the present study, the nutritive values of three different types of BSFL were investigated. This enabled us to undertake focused studies for generation of comprehensive data through experimental trials. Proximate composition, and concentrations of amino acids, fatty acids, vitamins, minerals, nucleoside and nucleotide contents of the larvae meal were determined to understand if these chemical profiles are comparable to fish-based diets and will be able to support growth and health of the fed fish in culture systems. Three different types of BSFL were used in this investigation. These included spray-dried BSFL (SPR), oven-dried BSFL 1 (OVN1) and oven-dried BSFL 2 (OVN2). Both SPR and OVN1 were fed organic agroindustry by-products. BSFL were dried in an oven at 50°C for 24–36 hours until it is fully dried prior for grinding process. After that, the larvae were grinded into powder form using a blender. This physical form has been referred to as the BSFL meal in the manuscript. Lastly, the BSFL meal was sieved using a sieve to obtain homogenized powder and then tightly packed and stored at -5°C before being used for analysis. SPR and OVN1 meal were obtained from a BSF pilot farm located in Kuala Lumpur, Malaysia. Both SPR and OVN1 used BSFL fed with agroindustry by-products. SPR was dried by the help of spray-drying technique and BSFL was dried using an oven-drying method. Meanwhile OVN2 meal was obtained from an individual insect breeder located in Likas, Sabah, Malaysia. They were fed with food waste and then turned into BSFL powder in the laboratory using an oven-drying method at temperature of 60°C in an overnight treatment. Crude protein was determined by the Kjeldahl method using an automatic system (Kjeltec 2300). Crude lipid was gravimetrically measured by the ether-extraction method in a soxhlet extraction unit (Soxtec 2043). Crude ash content was determined as the residue remaining after incineration of samples at 550°C in a muffle furnace for 6 hours. Moisture was quantified by thermogravimetric method using an oven by drying samples at 105°C until constant weight [21]. For hydrolysis of lipids, the sample was refluxed with 1M solution of potassium hydroxide in 95% ethanol before being extracted with hexane-diethyl ether. The solvent-extracted samples were then subjected to centrifugation at 3,000 rpm for 10 minutes before being washed with water. Subsequently, the contents were dried by evaporation process in a rotary evaporator to obtain the free fatty acids. The fatty acid methyl esters (FAMEs) were prepared with methanolic sulphuric acid. The fat was dissolved in toluene and 1% sulphuric acid in methanol before storage overnight. After that, water containing 5% sodium chloride was added. The FAMEs were then washed with 2% potassium carbonate and then dried by passing through a short column of anhydrous sodium sulphate. FAMEs were then diluted with hexane before injection into a Gas Chromatography column. Vitamins A, B1 and B2 were analysed using a High-Performance Liquid Chromatography detector (HPLC, Waters Alliance, model E2695 PDA), while Vitamin C was determined by HPLC (Agilent model 1200 Series DAD) detector. The sample was subjected to grinding before being extracted for carotenoids using tetrahydrofuran that contained butylated hydroxytoluene (BHT) in the presence of sodium sulphate and calcium carbonate for a few times followed by HPLC processing. Vitamins B1 (Thiamine) and B2 (Riboflavin) were extracted from the sample by acid hydrolysis followed by enzymatic hydrolysis. The aqueous extract was injected onto a reverse phase HPLC column. The fluorescence of riboflavin was measured, and the thiamine was determined after post-column derivatisation with alkaline potassium ferricyanide that converts the thiamine into thiochrome. For vitamin C (ascorbic acid), the sample was homogenised in 3% metaphosphoric acid. The homogenate was filtered, and a sample of the extract was chromatographed on RP C18 column by means of HPLC. Evaluation was then performed by differentiating the peak area against the ascorbic acid standard. The phosphorus content of the sample was analysed using the Inductively Coupled Plasma—Optical Emission (ICP-OES). For digestion of the sample, the organic matter was removed using concentrated nitric acid as the oxidising agent. Concentrated hydrochloric acid was then added before the sample was digested in a block digester until a clear solution was obtained. The solution so prepared was nebulised into argon plasma, where all the components were vaporized. The phosphorus element was then atomized and excited, and the emitted radiations were measured at various wavelengths simultaneously. The samples were subjected to hydrolysis using hydrochloric acid under total hydrolysis condition. A measured small amount of the amino acid solution was then derivatized with AccQ-Fluor Reagent together with aminobutyric acid as the internal standard. The amino acid derivatives were analysed using HPLC (Waters–Alliance e2695) with Fluorescence detector (2475-waters). The amount of amino acid (ng) was determined by external standard calibration with aminobutyric acid (AABA) to compensate for variation in derivatization between the samples. The mineral contents of the BSFL were analysed using an ICP-OES (Perkin Elmer Optima 5300 DV ICP-OES, USA). Before the analysis, the BSFL was digested using nitric acid and hydrochloric acid. Dried sample of 0.1 g was weighed and transferred into a dry digestion flask. After that, 5 ml of nitric acid (69% concentration) was added into the flask and the contents were heated on a heating mantle in a fume hood until the mixture started to boil and white precipitate was obtained. The flask was then cooled to room temperature, and this was followed by addition of 3 ml of hydrochloric acid and heating until green colour of the sample turned yellow, orange or red. After the colour was changed, the flask was heated again for 10 minutes to prevent the colour reversal. The sample was then cooled to room temperature and poured into a 100 ml volumetric flask. Distilled water was added until 100 ml volume and the contents were allowed to stand until analysis in an ICP-OES. The contents of nucleotides in the three BSFL and the BSFL diets were determined using the UPLC-TQ-MS/MS method. Dried sample powder measuring 1 g was sonicated with 50mL distilled water at room temperature and extracted by centrifugation at 13,000 rpm for 10 min. The supernatant was then kept at 4°C and filtered using a 0.22 µm membrane filter prior to the injection. Individual standards were also prepared by dissolving in 10mL of distilled water, kept at 4°C and filtered using a 0.22 µm membrane filter before injection. Subsequently, the chromatographic analysis was accomplished using Waters Acquity UPLC system (Waters, Milford, MA, USA) equipped with an Acquity UPLC HSS T3 c18 column (1.8 µm, 2.1 mm x 150 mm). The column temperature was maintained at 35°C. The flow rate of the mobile phase was 0.3 mL/min. and the injection volume was 1 µL. Data on proximate composition, and concentration of minerals and heavy metals were subjected to one-way analysis of variance (ANOVA) and Duncan’s post-hoc test for determining the significance of difference at p