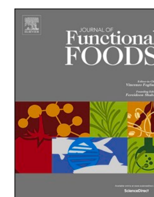
Journal of Functional Foods 101 (2023) 105430

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff



Nutritional quality of meat from hen fed diet with full-fat black soldier fly (*Hermetia illucens*) larvae meal as a substitute to fish meal

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A R T I C L E I N F O

Keywords:
Full-fat insect meal
Alternative feed source
Off layer meat quality
Food industry
Value-addition
Food security

A B S T R A C T

The utilization of insect protein in poultry feed is globally gaining momentum. However, the nutritional quality of meat from hen fed diet with black soldier fly larvae meal (BSFLM) as fishmeal (FM) substitute has received limited research attention. Our results revealed that feed substitution did not affect the proximate compositions of the meat products. Omega 3 fatty acids were uninfluenced ($P < 0.05$) whilst the total monounsaturated fatty acids progressively increased with increasing dietary inclusion of BSFLM. Lysine, methionine, and isoleucine were significantly higher ($P < 0.05$) in insect-fed hen meat products. The levels of zinc and B vitamins except B1 were proportionally enhanced in the chicken fed BSFLM incorporated diet. Thus, up to 75 % replacement of FM with BSFLM did not significantly compromise the meat quality. Meat from hen fed diet with BSFLM could be considered as promising and novel ingredient in the manufacturing of nutritious food products with healthy appeal for consumers.

1. Introduction

The poultry industry continues to expand rapidly every year due to increased demand for white meat and egg products. According to Pradeepa et al. (2019), over 2.6 billion spent hen meat is released into the market annually. Thus, the availability of the culled and spent hen meat has increased manifold accounting for 7 % of all poultry meat produced worldwide (Kokoszyński et al., 2016) with the rapid development of poultry layer industry. However, the spent hen meat is usually tough, less tender and has poor functional properties due to increased collagen content and linkage, striking factors that need to be altered to the benefit of the consumers. This explains why the spent hen meat is currently being considered more suitable for the pet industry and for processing value added and convenience meat products such as sausages, chicken soup, traditional delicacy recipes and animal feeds in the greater part of the developed and developing countries (Kolawole, 2017). This is because its hardness is not an obstacle for the production of most processed products, which use ground meat (Kondaiah & Panda, 1992).

Contrarily, in over 80 % of the countries in Africa, spent hens are sold in the informal market for domestic consumption in soups and stews (Ajuyah et al., 1992; Onono et al., 2015; Semwogerere et al., 2019), because a significant part of its population has limited access to fresh beef, and is only able to buy spent hen meat as a cheaper protein source. That notwithstanding, the prevailing high cost and scarcity of low-quality protein sources (particularly fish and soya bean meal) for poultry feed and exploding human population have necessitated the exploration for new and cheaper sustainable protein sources for increased layer chicken production for meat and eggs (FAO, 2014). A growing number of studies have tested the potential of different insect species as feed for different animals including layer chicken (FAO, 2014). Among the insect species that have been gaining attention as alternatives to the conventional feedstuffs for poultry, the black soldier fly (BSF) (*Hermetia illucens* L.) is certainly one of the most promising and well documented, due to its intrinsic nutritional value and, the possibilities of exploiting diverse waste substrates for mass production (Meneguz et al. 2018). This confirms why the utilization of *H. illucens*, as novel ingredient represents an emerging protein source relevant to farmers, feed companies, and feed marketers. Globally, the regulations

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https://doi.org/10.1016/j.jff.2023.105430
Received 12 May 2022; Received in revised form 15 January 2023; Accepted 29 January 2023
Available online 1 February 2023
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governing insect protein do not allow the use of all potential insect species as feed, particularly to ensure feed safety, as such each country has its own substantive and procedural rules for this purpose (Lähteenmäki-Uutela et al. 2021). Likewise, some legislations even regulate and specify feedstocks for rearing/breeding of insect species approved for protein source (Tanga et al. 2021). Nevertheless, these feed regulations are flexible and easily allow insect usage as ingredients in livestock and fish feed. However, there is lack of regulation in many countries, as well as lack of a stable and consistent set of regulations across international borders. In that respect, there is agreement to develop a common and unambiguous regulatory system around a scientific risk assessment approach to encourage investments, build trust, and normalize the industry (Van der Spiegel, 2016), especially among the public and private actors within the edible insect value chain (Allegretti et al., 2018). Therefore, global harmonization of policies and regulations would be a great strategy to widen the adoption and practice of insect farming and marketing of insect-based ingredients for animal feed.

Despite the above challenges, the use of BSF larvae protein partially or as complete substitute for the expensive fish and soya bean meal in animal feed has been widely recommended (Onono et al., 2018; van der Spiegel et al., 2013; van Krimpen and Hendriks, 2019). Up to now, the protein-energy dense BSF larvae meal has emerged as an excellent eco-friendly ingredient of choice with the most appealing nutritional characteristics for the animal feed industries (Dabbou et al. 2018). Consequently, research efforts have also been mostly directed towards this emerging BSF larvae meal ingredient, studying optimal incorporation levels intended for different poultry species of economic interest (Van Huis, 2013). Dietary inclusions ranging between 5 % and 20 % have been tested in broiler chickens (Dabbou et al. 2018; Schiavone et al. 2019), broiler quails (*Coturnix coturnix japonica*) (Cullere et al. 2016; Cullere et al. 2018), ducks (Gariglio et al. 2019), Barbary partridges (*Alectoris barbara*) (Loponte et al. 2017; Secci et al. 2018), broiler chicken (Onsongo et al. 2018, Mutisya et al. 2021; Mutisya et al., 2021), improved indigenous chicken and layer hen. Findings from previous studies have demonstrated that feeding avian species on BSF larvae significantly improves their general health status, growth performance, and meat quality traits both under on-farm and large-scale poultry production systems.

Despite the elaborate research on avian species fed insect-based feeds, none of these have focused on the nutritional quality of spent hen meat, which is considered as the by-product of egg industry (Pradeepa et al. 2019). Simultaneously the price of meat is also increasing which exerts a dire need to harvest every source of meat to reduce cost, maintain quality and meet the demand. Thus, spent hen meat continues to attract the meat industry as a cheaper source. In view of challenging task to utilize the spent hen meat, the present study focused on analysing the nutritional quality of meat from spent hen (Isa Brown layers) (i.e., best brown laying hen in the world), fed diets with full-fat BSF larvae meal as partial and complete substitute of fish meal (FM). The objective of this study was to evaluate the feasibility of improving the nutrient quality of meat of light and semi-heavy spent layer hens as future novel food ingredients in the manufacturing of better nutritional enriched food products with higher added value and economic benefits by using diets integrated with BSF larvae meal instead of the expensive and scarce fish meal, which are traditionally and widely used in feed formulations in the poultry industry.

2. Materials and methods

2.1. Ethical research

This research was approved by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO)-Veterinary Science Research Institute (VSRI); Muguga North upon compliance with all provisions vetted under and coded: KALRO-VSRI/IACUC028/16032022.

2.2. Feed ingredients for the manufacturing of the various diet types

The ingredients to formulate the various diet types for layer chicken were purchased from a well-known feed miller in the region, Josiche General Traders Ltd. (Nakuru, Kenya). The larvae of BSF used for the feeding experiment were obtained from the International Centre of Insect Physiology and Ecology (*icipe*), located in Nairobi, Kenya. The larvae were raised on barley spent grains obtained from the Kenya Breweries Limited. The conditions of the production facility were kept at 30 ± 2 °C, relative humidity of 60–70 % and 12: 12 (light: dark) photoperiod. At the 5th instar stage, the larvae were harvested and washed by deeping them in a container of boiling water (84 °C) for 3–5 min. Thereafter, the larvae were dried using stainless-steel trays in a food drying machine (Model: CT-C-III, Henan, China) at 120 °C for 2 hrs 30 min to ensure that the processed larvae were safe for incorporation into animal feed. The sterilized and dried BSF larvae were then ground into powder and mix up with other raw materials to formulate five diet types for the birds. Calculated estimate of the ingredients used in the formulated diets followed the nutrient requirements guidelines for laying hen (Swatson et al. 2003). Briefly, black soldier fly larvae meal (BSFLM) was used to replace fish meal (FM) partially or completely in the formulated diets for the trials. The FM:BSFLM ratios were 100:0, 75:25, 50:50, 25:75, and 0:100, resulting in five experimental diets (T1-100 %FM: 0 %BSFLM; T2-75 %FM: 25 %BSFLM; T3-50 %FM: 50 % BSFLM; T4-25 %FM: 75 %BSFLM and T5-0 %FM: 100 % BSFLM). The ingredient and chemical compositions [on dry matter (DM) basis] of layer mash were as described by Sumbule (2021). The diet formulation was based on BSFLM crude protein (CP) content of 46.8 % dry matter (Chia et al. 2019). Average CP content of fishmeal was 47.7 %, which is within the range commonly reported in Kenya against the expected CP content of $>$ 65 % DM (Munguti et al. 2006; Maina et al., 2017). All diets for the entire experimental period were formulated at once by Josiche General Traders Ltd.

2.3. Feeding program and experimental design

The feeding trials were conducted at the Poultry Research Unit in the Non-Ruminant Research Institute of the Kenya Agricultural and Livestock Research Organization (KALRO) located in Naivasha, Kenya (0°43′12.9″ S 36°25′42.7″ E). At the beginning of the experiment, one-day-old Isa Brown chicks were sourced from Kenchic Limited, Nairobi, Kenya. The birds were provided adequate care and their house conditions maintained following the procedures established by the Federation of Animal Science Societies (Lindahl et al., 2018). During the acclimatization phase, the chicks were kept together in a brooder, a round deep litter floor covered with a 7.6 cm-thick layer of wood shavings bedding and fitted with 250 Watts infra-red bulbs for heating. In the first 14-days, chicks were subjected to the control diet with 100 % fish meal (FM) as protein source, watered *ad libitum* and weighed after 14 days. Weighed chicken were transferred to different floor pens with cages (Each measuring 750 mm × 900 mm × 750 mm) capable of accommodating 5-layer chicken. Using a completely randomized design, the chicks were randomly assigned one of the five feeding regimes throughout the entire developmental feeding phase. The pens (constructed in a house with cemented floor and separated from each other using wire mesh) were equipped with plastic feeders (73 cm × 26 cm × 48 cm), 3 L plastic drinking containers and the in-house conditions maintained at 30 ± 1 °C with relative humidity (RH) of 70 ± 2 %. Lighting hours, from the initial 24 h in the first 4 weeks, were gradually decreased to 12 h dark and 12 h light cycle to facilitate adaptation to natural conditions by the end of developmental stage. The vitamins administration was through water while vaccination programme of the birds followed the generally agreed guidelines for the prevention of any disease-causing bacteria/virus that could build up by boosting the birds' immunity (Boccazzi et al. 2017).

High standards of hygiene including daily cleaning of drinkers, offering clean water and feeds every morning, using saw dust beddings (changeable every 3 weeks) and ensuring adequate ventilation, were maintained. Each experimental set-up was replicated nine times. At 80th week of the experiment, fifteen (15) experimental birds per treatment were randomly selected from the stock. The selected hens were humanely slaughtered by first electrically stunning them, and then slaughtering done by severing the jugular vein, and allowing the chicken to bleed out completely according to the recommended method (Odunsi et al., 2006). Feathers, feet, internal organs, head, neck and abdominal fat were removed and utilized in a separate study. Thigh, drumstick and breast were then separated, packaged and stored in a deep freezer (New Brunswick Scientific, England) at temperatures below −20 °C until use.

2.4. Spent hen meat powder

The processing of the spent hen meat from birds fed on the various diet types described above into powder has been illustrated below (Fig. 1). Briefly, after proper slaughtering and dressing, the meat was washed and put in a pan. After deboning and mixing, the liquor was separated just after slight boiling. The meat was placed on fire with continuous stirring and scraping with a flat bottom spoon until 6 % moisture was achieved (checked by placing in oven at 70 °C for 20 h). The meat was converted into powder after cooling and packed into low density polyethylene (LDPE) bags and kept at temperatures below −20 °C until further analyses were conducted.

2.5. Proximate composition analysis

Proximate composition of spent hen meat powder (SHMP) were determined according to the Association of Official Analytical Chemists Method (AOAC, 2012). Briefly, the moisture content was determined by drying the SHMP in an oven at 135 °C for 2 h. The nitrogen content was determined using an automatic Kjeldahl analyzer (Velp UDK 159, Velp Scientifica, Europe) and later converted to crude protein content using the conversion factor of 6.25. The ash content was determined using a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany), combusting the samples at 600 °C for 3 h. Crude fat was determined following the Randall Technique which is a modification of standard Soxhlet extraction using petroleum ether as extraction solvent in a Soxhlet extractor (Velp SER 148, Velp Scientifica, Europe). All parameters were determined in triplicate and expressed as a percentage of dry matter.

2.6. Mineral analysis of meat powders

Mineral profile was determined according to the method described by Campbell & Plank (1992) and Horwitz & George (2000). Inductively coupled plasma optical emission spectroscopy (ICP-OES) (optima 2100DV, Perkin Elmer, United states) was used to quantify minerals present in the samples. Concentrated Nitric acid (8 ml) and 30 % hydrogen peroxide (2 ml) were added in digestion tubes containing 0.5 g of homogenized meat powder. Digestion tubes were placed into a block digester (BD50/BD28, Seal Analytical Limited, US) for a programmed temperature digestion set as follows; 75 °C/ 30 min, 120 °C/ 20 min, 180 °C/ 20 min and 200 °C/ 10 min.

Upon cooling, the digests were carefully transferred to 50 ml Falcon tubes, topped up to the mark with 2 % Nitric acid and analyzed. Serial dilution of the standard was performed using 2 % Nitric acid to obtain calibration standards of 400, 800, 2000 and 4000 μg/l for external standard calibration. Calibration was performed using Perkin Elmer Winlab 32 software. Limit of detection (LOD) was 48.9 μg/100 g. The obtained data was used to calculate the final elemental concentration for each element in mg/100 g.

Operating conditions were as follows; RF power (W)-1450, Plasma gas flow rate (L min − 1)-45, Auxiliary gas flow rate (L min − 1)-0.2, Nebulizer gas flow rate (L min − 1)-0.8, Sample flow rate (L min − 1)-1.5, View mode- Axial, Read- Peak area, Source equilibration time (s)-10, Read delay (s)-10, Background correction- 2-point (manual point correction), Spray chamber- Scott type spray chamber, Nebulizer Cross-Flow GemTip Nebulizer (HF resistant), Detector CCD- CCD, Purge gas-Nitrogen, Shear gas- Air, Plasma gas- Argon and Wavelength (nm) – Mg-

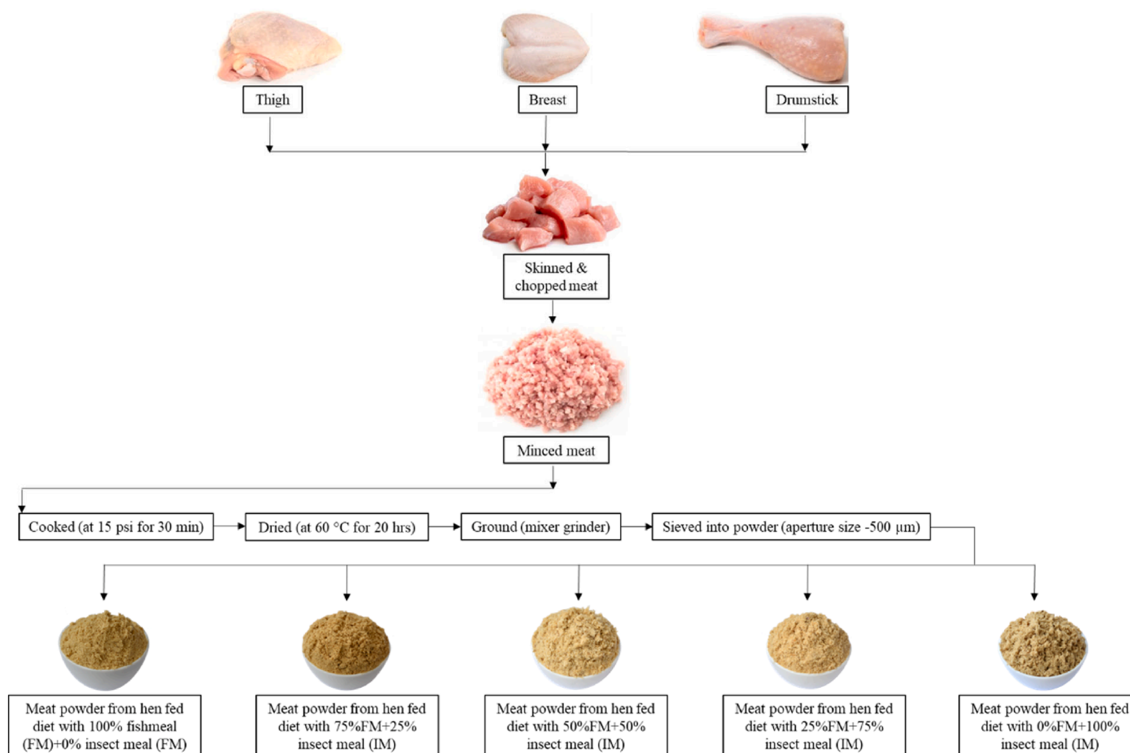

Fig. 1. Flow diagram showing how chicken meat powder was obtained. *Psi* – Pound per square inch; min – minutes & hrs- hours.

285.213, Fe-259.939, Mn-257.61, Ca-317.933, P-213.617, Mo-202.031, K-766.49, Cu-224.7, Co– 228.616, Zn- 213.857.

2.7. Ultra performance Liquid chromatography tandem coupled to mass spectrometry (LC-MS/MS) analysis of amino acids

The amino acid composition was determined as previously described by Cheseto et al. (2017). Into a 5 ml micro-reaction vial, 10 mg of meat powder was mixed with 1.5 ml of 6 N HCl and capped upon careful introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110 °C followed by *in vacuo* evaporation to dryness. The hydrolysates were reconstituted in 1 ml of 0.01 % formic acid/acetonitrile (95:5), vortexed for 30 s, sonicated for 30 min, and then centrifuged at 14000 rpm, and the supernatant analyzed using UPLC-MS/MS (0.2 μl). Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford,MA) fitted with an ACQUITY UPLC BEH C18 column (2.1 mm X 150 mm, 1.7 μm particle size; Waters Corp., Wexford, Ireland, oven temp 45 °C). The autosampler tray was cooled to 5 °C. The mobile phase comprised of (A) water and (B) methanol (solvent B) both acidified with 0.01 % formic acid.

The gradient system used was 0–2 min, 5 % B, 2–4 min, 40 % B, 4–7 min, 40 % B, 7–8.5 min 60 % B, 8.5–10 min 60 % B, 10–15 min, 80 % B, 15–19 80 % B, 19–20.5 min, 100 % B, 20.5–23 min, 100 % B, 23– 24 min 95 % B, 24–26 min, 95 % B. The flow rate was held constant at 0.2 ml/min. The UPLC was interfaced with an electrospray ionization (ESI) Waters Xevo TQ-S operated in full scan MS in positive ionization mode. Data were acquired over. The *m/z* range 40–2,000 with a capillary voltage of 0.5 kV, sampling cone voltage of 30 V, source temperature 150 °C desolvation temperature of 120 °C. The nitrogen desolvation flow rate was 800 L/h.

Data was acquired using MassLynx version 4.1 SCN 712 (Waters). The amino acids were identified by comparison of mass spectrometric data, retention time, and co-injection of the hydrolysate with an authentic standard mixture of amino acids. External quantification was used to determine the amounts of each amino acid present. The amino acid standard solution (AAS 18) was obtained from Sigma-Aldrich (Chemie GmbH, Munich, Germany). This was repeated three times using different samples batch. Tryptophan decomposes into ammonium during acid hydrolysis and due to this it was not determined. During hydrolysis asparagine and glutamine are hydrolysed into aspartic acid and glutamic acid respectively, therefore the amounts of these acids were determined as sum of those respective components (de Souza Vilela et al., 2021).

2.8. Fatty acid analysis of spent hen meat powders

Fatty acids composition of meat powders were determined as per method described by Christie (1993) and Cheseto et al. (2017). Briefly, 100 mg of the sample was methylated to form different FAs. This was followed by quenching process, then extraction of the resulting FAMEs and finally, drying the supernatant. Approximately 1000 μl of the supernatant was analysed using Gas chromatograph coupled to mass spectrometry (GC–MS) on a 7890A gas chromatograph coupled to a 5975C mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyze FAs as fatty acid methyl esters (FAMEs) in the samples.

Linear calibration curve of peak area vs. concentration which gave coefficient of determination ($R^2 = 0.9997$) was generated by analyzing serial dilution of the authentic standard methyl octadecenoate (0.2–125 ng/μl) by GC–MS in full scan mode which gave the following equation: $y = 5E + 07x + 2E + 07$ ($R^2 = 0.9997$). This equation was utilized during external quantification of different FAMEs in the samples. The FAMEs were identified by comparing mass spectral data and retention times with those of authentic standards where available and reference spectra published by library–MS databases: National Institute of Standards and Technology (NIST) 05, 08, and 11.

2.9. Determination of Water-soluble vitamins by Ultra- performance Liquid chromatography (UPLC)-Photo Diode Array detector (PDA)

Sample preparation: Determination of water-soluble vitamins by UPLC was done as describe in the Thermo Fisher Scientific, 2010. Briefly, the 100 mg of each sample was weighed into 50 ml facon tubes, 25 ml of distilled water was added, followed by ultra-sonication for 15 min, the solution was then filtered through 0.2 μm filters into UPLC vials. The vials were capped and loaded into the UPLC autosampler for analysis.

Preparation of standards: Stock solution of 1.0 mg/ml was prepared by dissolving the individual water-soluble vitamin standards in distilled water except for Vit B2 in (5 mM potassium hydroxide) and Vit B9 in (20 mM potassium hydrogen carbonate). A mix of all the standards was made then 4 calibration standards at a concentration of 2, 5, 10 and 15 μg/ml was prepared from the mix.

Chromatographic conditions were as follows; Instrument: Nexera Liquid chromatograph LC-30AC with Nexera column oven CTO-30A, detector: Diode Array Detector, column: Phenomenex Synergi 2.6um polar C18– 100 mm × 3.00 mm, column Oven temperature: 30 °C, flow rate: 0.4 ml/min, Column Flushing Solution: distilled water and LC program: Run Time (12 min), Mobile Phase A: 25 mM phosphate buffer and Mobile Phase B: 7:3 v/v Acetonitrile-Mobile phase A.

2.10. Determination of fat-soluble vitamins by ultra- performance liquid chromatography (UPLC)

Determination of fat soluble vitamins was done according to the method described by Hosotani & Kitagawa (2003) and Bhatnagar-Panwar et al. (2015). The samples were weighed (0.5 g) into 25 ml tubes, 6 ml ethanol with 0.1 % (BHT) was added and the samples were homogenized for 1 min. Approximately, 120 μl of Potassium hydroxide 80 % (W/V) was added, followed by vortexing then incubation at 85 °C/5 min. The tubes were then removed from water bath and immediately placed in ice to cool. In each tube, 4 ml of deionized water was added, vortexed and 5 ml of hexane also added and vortexed. The samples were centrifuged at 3000 rpm/5 min, then the upper phase (hexane) was transferred into centrifuge tube using Pasteur pipette. The mix was extracted 3 more times with 4 × 3 × 3 ml hexane and the extract placed into the 25 ml tube. In the extract, 5 ml of deionized water was added, vortex for 1 min and then centrifuged at 3000 rpm/5min. The hexane layer was recovered into a clean test-tube and evaporated under nitrogen in the N-Evap to complete dryness. The extract was then reconstituted in to 1 ml of methanol: tetrahydrouran (85:15 v/v), vortexed, sonicated for 30 s and 0.8 ml was transferred into HPLC vials.

HPLC Method; HPLC system: Shimadzu Nexera UPLC system linked to SPD -M2A detector, reverse phase gradient HPLC method, oven temperature-OFF, Injection volume 10 μl, Mobile phase A: methanol/*tert*-butyl methyl ether/water (85:12:3, v/v/v, with 1.5 % ammonium acetate in the water), Mobile phase B: methanol/*tert*-butyl methyl ether/water (8:90:2, v/v/v, with 1 % ammonium acetate in the water), total flow rate was 0.4 ml/min.

2.11. Statistical analysis

R software version 1.3.1093-1 (R Core Team, 2020) for windows was used for statistical analysis and Normality Test ($p \geq 0.05$ – Data is normal distributed) was tested using Shapiro –Wilk test. One-way Analysis of Variance (ANOVA) was performed to test the effect of dietary treatment on nutritional quality of SHMP and the means were considered significantly different at 5 % significant level ($p < 0.05$). To determine which means were significantly different between experimental treatments, the student Neuman Kuel' Test (SNK) Test was performed and the differences between the treatments were considered not significant at $p \geq 0.05$.

3. Results

3.1. Proximate composition of spent hen meat powder

The proximate composition (moisture content, crude ash, crude protein and crude fat) of the SHMP did not vary significantly across the various diet types (Table 1). However, crude protein accounted for the greater part of the proximate composition.

3.2. Mineral profile of spent hen meat powder

The mineral composition of the various meat type varied significantly, except for magnesium, phosphorus and iron (Table 2). However, potassium, phosphorous, calcium and copper were the most abundant minerals in meat from hen fed on the various diets.

3.3. Amino acids profile of spent hen meat powder

The levels of essential (isoleucine, leucine and methionine) and non-essential (proline and aspartic acid) amino acids (EAA and NEAA, respectively), varied significantly across the meat types from hen fed diets with different levels of BSFLM (Table 3). Leucine and lysine (EAA) and (glutamic acid and aspartic acid) (NEAA) were the most predominant amino acids among the meat types. All the meat types showed significantly lower levels of methionine and cystine. The ratio of EAA to NEAA and EAA/Total AA were not influenced by experimental diet. However, the ratio of EAA/Total AA was<40 % in all the meat products.

3.4. Fatty acid profile of spent hen meat powder

A total of 39 fatty acids were detected in SHMP samples with saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) contributing 18, 9 and 12 parts, respectively (Table 4). Nine fatty acids were detected in meat products from hen diet with BSFLM but absent in meats from hen fed 100 % FM diet. Between meat products from hen fed diet with 100 % BSFLM and 100 % FM, total SFAs, MUFAs and UFAs was observed to increase by 1.4, 1.4 and 1.2-folds, respectively, with reduced total PUFAs (0.9-folds). There was considerable variation between the MUFAs (36–40.2 %), SFAs (31.-36.2 %) and PUFAs (23.6–32.4 %) in the meat products. Palmitic acid (63–69 %), oleic (85–93 %) and linoleic acid (79–76 %) of SFAs, MUFAs and PUFAs were the dominant fatty acids that were recorded in the meat products. Methyl (9Z,12Z,15Z)-octadecatrienoate (ALA), methyl (5Z, 8Z,11Z,14Z,17Z)- eicosapentaenoate (EPA), methyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosahexaenoate (DHA) and Methyl (7Z, 10Z, 13Z, 16Z, 19Z)-docosapentaenoate were the main omega-3 PUFAs identified, however, no significant variation was observed. The PUFA/SFA and n-6/n-3 ratios of the spent hen meats were positively correlated with the increasing levels of BSFLM as substitute of FM in the hen diets.

Table 1
Comparison of proximate composition of spent hen meat powder (%) on dry matter basis.

Parameter	T1	T2	T3	T4	T5	$F_{(4,10)}$	p-value
Moisture	4.5 ± 0.50^a	4.2 ± 0.76^a	3.8 ± 0.29^a	4.3 ± 0.29^a	4.8 ± 0.29^a	1.95	ns
Crude Ash	7.5 ± 0.14^a	7.4 ± 0.1^a	7.2 ± 0.36^a	7.0 ± 0.32^a	7.0 ± 0.21^a	1.92	ns
Crude Protein	85.7 ± 0.64^a	85.8 ± 0.42^a	86.5 ± 0.21^a	85.6 ± 0.47^a	86.2 ± 0.48^a	0.49	ns
Crude Fat	8.1 ± 0.07^a	8.2 ± 0.82^a	8.2 ± 0.78^a	8.5 ± 0.58^a	8.6 ± 0.32^a	2.85	ns

Values are expressed as mean ± standard error (SE). Same small superscript letters following each other within a row indicates no significant difference ($p < 0.05$). ns:not significant;T1 (control)- 100:0, T2 −75:25, T3 −50:50, T4-25:75 and T5-0:100 representing FM: BSFL ratios.

3.5. Vitamin profile of spent hen meat powder

The vitamins: α-tocopherol, ɣ-tocopherol, retinol, vitamins B1, B2, B3, B5 and B9 expressed significant ($p < 0.05$) variability across the meat types derived from hens fed diets with BSFLM (Table 5). The B vitamins except B1 displayed significantly increasing trend with increasing levels of BSFLM in the hen diets. Retinol and nicotinamide were the most abundant vitamins.

4. Discussion

4.1. Proximate composition of spent hen meat powders

The proximate compositions of meat derived hen subjected to diet with varying inclusion levels of BSFLM was similar to that from hen provided diet with FM. These findings corroborate earlier reports (Balolong et al., 2020; Cullere et al., 2018; Pieterse et al., 2019; Uushona, 2015). The slight increase in crude protein observed in meat from hen fed diet with BSFLM might be directly attributed to the high protein and energy levels in the feed formulation, which is consistent to the observation by Secci et al. (2018). The results of the proximate composition of the SHMP from hens fed diet with BSFLM is comparable to meat from broiler chicken fed on commercially available industrial feeds containing fish meal (Aslam et al., 2000; Gokulakrishnan, 2014). Given that the protein contents in meat from spent hen fed diet with BSFLM ranged from 86 to 87 %, it is certainly one of the most promising offers of an alternative with health promoting benefits compared to broiler meat, due to its intrinsic nutritional values. Thus, protein fraction of the BSFLM represents a promising ingredient quantitatively and nutritionally, with possible application in layer hen feeding which could alleviate the pressure on conventional overexploited feed sources. Proteins are known for being an essential element in body cell membranes and an obligatory precursor that could aid in nutrient synthesis and degradation, metabolic functions, essential in maintaining muscle mass and strength (Roncolini et al., 2019) in animals.

4.2. Mineral profile of spent hen meat powders

The dominant elements reported in this study have previously been reported as dominant elements in chicken meat (Chen et al., 2016). The present study exhibited no variation in the levels of most minerals except for Ca and K with change in dietary formulation. The uninfluential impacts of dietary change on certain minerals in this study corroborate findings witnessed in previous studies (Cockcroft, 2018; Fernando et al., 1998; Pieterse et al., 2019; Uushona, 2015). Variation in minerals may be predetermined by factors such as bioavailability, antagonistic or synergistic interactions, physical and chemical properties and coexistence and co-involvement with other components in physiological and metabolic processes as suggested by Zajac et al. (2020). Furthermore differences in other factors such as diet composition, muscle type, gender, age, breed and physical activity of chicken have also been proved to cause changes in the mineral levels in birds (Chen et al., 2016; Cockcroft, 2018; Geldenhuys et al., 2013; Kokoszyński et al., 2016; Lin et al., 2014; Pieterse et al., 2019; Uushona, 2015).

Cockcroft (2018), established that Ca absorption into a bird's muscle is jointly regulated nutritionally and physiologically and as such, dietary source may not be of great influence if bird's Ca requirement is already met. But, Uushona (2015) attributed these differences in the Ca levels of chicken tibia bone to higher bioavailability of Ca content in diet.

In addition, Ca, Na, Zn and Fe contents were higher compared to that reported in broilers in previous studies (Chen et al., 2016; Pieterse et al., 2019). We estimated that 2 g of SHMP from hens fed 50 % FM and 50 % BSFLM (T3) can contribute 90 % of Fe, 92 % of P, 115 % of Zn, 35 % of

Table 2
Mineral content of spent hen meat powder.

Mineral	T1	T2	T3	T4	T5	$F_{(4,10)}$	P-value	RDA age 12–18 (mg/day) *
Iron (mg/100 g)	4.2 ± 0.03[a]	3.5 ± 0.01[a]	6.1 ± 4.17[a]	3.1 ± 0.82[a]	3.4 ± 0.70[a]	2.37	ns	13.5
Phosphorus (mg/100 g)	600.0 ± 23.58[a]	551.5 ± 19.62[a]	572.2 ± 52.77[a]	545.4 ± 40.3[4a]	607.9 ± 7.66[a]	4.36	ns	1250
Manganese (µg/100 g)	104.7 ± 4.41[ab]	91.1 ± 0.06[b]	124.5 ± 24.23[a]	86.6 ± 0.41[b]	103.6 ± 12.9[ab]	8.47	p < 0.01	
Zinc (mg/100 g)	4.7 ± 0.65[b]	5.1 ± 0.03[ab]	4.9 ± 0.32[ab]	5.4 ± 0.27[a]	5.0 ± 0.08[ab]	4.23	p < 0.05	8.5
Magnesium (mg/100 g)	69.3 ± 9.27[a]	63.4 ± 4.47[a]	64.7 ± 1.08[a]	66.5 ± 0.42[a]	70.5 ± 3.61[a]	2.18	ns	375
Potassium (mg/100 g)	654.8 ± 10.49[ab]	650.9 ± 17.25[ab]	573.0 ± 29.03[c]	604.7 ± 7.65[bc]	698.7 ± 32.61[a]	29.87	p < 0.001	3500
Sodium (mg/100 g)	183. 5 ± 0.91[c]	242.5 ± 6.04[a]	201.3 ± 9.91[b]	167.1 ± 0.00[d]	194.2 ± 8.10[bc]	118.02	p < 0.001	2000
Aluminum (mg/100 g)	1.2 ± 0.07[d]	1.7 ± 0.17[c]	2.3 ± 0.13[b]	2.7 ± 0.19[b]	4.6 ± 0.23[a]	348.15	p < 0.001	
Copper (µg/100 g)	236.1 ± 12.8[b]	243.1 ± 1.43[ab]	286.2 ± 22.62[a]	292.1 ± 42.62[a]	297.8 ± 23.20[b]	21.29	p < 0.001	
Calcium (mg/100 g)	262.2 ± 53.38[ab]	181.6 ± 1.99[b]	339.3 ± 66.77[a]	177.3 ± 3.42[b]	229.5 ± 11.47[ab]	17.89	p < 0.001	1200

Values are expressed as mean ± SE of triplicate determinations. Different small superscript letters following each other within a row are significantly different ($p < 0.05$).The ratio of FM: BSFL T1 (control, 100:0), T2 (75:25), T3 (50:50), T4 (25:75) and T5 (0:100). ns:not significant;T1 (control)- 100:0, T2 −75:25, T3 −50:50, T4-25:75 and T5-0:100 representing FM: BSFL ratios. *: (WHO, 2006).

Table 3
Amino acid composition of spent hen meat powder in mg/g of sample.

Amino Acid (mg/g)	T1	T2	T3	T4	T5	$F_{(4,10)}$	P-value	RDA during pregnancy (mg/Kg/day)[1]
Essential amino acids								
Phenylalanine	32.6 ± 1.28[a]	32.5 ± 0.87[a]	31.9 ± 0.51[a]	31.6 ± 0.79[a]	31.6 ± 0.78[a]	0.94	ns	44[2]
Isoleucine	34.6 ± 0.53[c]	34.8 ± 0.52[bc]	35.1 ± 0.98[bc]	36.2 ± 0.98[ab]	36.7 ± 0.32[a]	6.08	p < 0.01	25
Leucine	69.0 ± 1.56[a]	68.6 ± 1.33[a]	69.7 ± 1.56[a]	74.0 ± 5.16[a]	71.7 ± 1.12[a]	2.18	ns	56
Valine	37.9 ± 2.04[a]	37.4 ± 0.95[a]	37.8 ± 1.42[a]	36.7 ± 1.08[a]	34.9 ± 1.07[a]	2.44	ns	
Histidine	31.3 ± 2.16[a]	31.6 ± 0.93[a]	32.1 ± 1.26[a]	31.2 ± 1.42[a]	30.7 ± 1.26[a]	0.34	ns	18
Methionine	19.2 ± 0.19[b]	19.5 ± 0.15[ab]	19.8 ± 0.15[a]	19.4 ± 0.15[ab]	19.6 ± 0.13[a]	6.27	p < 0.01	25[3]
Lysine	65.2 ± 0.02[b]	66.0 ± 0.19[a]	66.0 ± 0.26[a]	66.0 ± 0.26[a]	66.1 ± 0.23[a]	9.89	p < 0.01	51
Threonine	36.9 ± 2.04[a]	36.1 ± 3.24[a]	36.2 ± 4.30[a]	37.0 ± 4.42[a]	38.6 ± 2.23[a]	0.49	ns	26
Total EAA	326.7 ± 4.71	326.5 ± 3.24	328.6 ± 4.59	332.1 ± 7.70	329.9 ± 3.35			
Non-essential amino acids								
Tyrosine	22.9 ± 1.06[a]	23.3 ± 0.53[a]	23.8 ± 1.16[a]	23.1 ± 0.89[a]	23.4 ± 0.39[a]	0.25	ns	
Proline	45.6 ± 0.68[c]	47.3 ± 0.81[b]	49.1 ± 0.46[a]	50.0 ± 0.43[a]	49.8 ± 0.45[a]	25.45	p < 0.001	
Glycine	30.7 ± 1.44[a]	31.6 ± 1.84[a]	30.9 ± 1.67[a]	30.6 ± 1.50[a]	31.5 ± 1.01[a]	0.25	ns	
Alanine	46.4 ± 0.83[a]	43.9 ± 0.93[a]	45.1 ± 1.55[a]	45.2 ± 1.11[a]	44.8 ± 0.58[a]	2.20	ns	
Cystine	19.4 ± 0.79[a]	22.1 ± 2.90[a]	22.9 ± 1.76[a]	23.4 ± 0.75[a]	23.4 ± 1.09[a]	3.03	ns	
Glutamic acid	114.3 ± 1.39[a]	113.7 ± 1.01[a]	113.8 ± 0.89[a]	113.1 ± 0.89[a]	113.7 ± 2.15[a]	0.27	ns	
Aspartic acid	74.1 ± 0.40[a]	72.0 ± 0.90[b]	70.7 ± 0.40[bc]	70.4 ± 0.68[bc]	68.7 ± 1.23[c]	14.39	p < 0.001	
Serine	26.8 ± 1.57[a]	28.1 ± 0.79[a]	28.2 ± 2.06[a]	28.5 ± 1.45[a]	30.8 ± 0.87[a]	2.99	ns	
Arginine	59.3 ± 0.92[a]	59.4 ± 1.41[a]	59.8 ± 1.60[a]	57.8 ± 1.65[a]	57.5 ± 1.48[a]	1.15	ns	
Total NEAA	439.5 ± 4.55	441.4 ± 5.49	443.4 ± 6.71	442.1 ± 2.21	444.5 ± 2.24			
Total AA	766.2 ± 8.32	767.9 ± 6.73	772.0 ± 5.01	774.2 ± 6.19	774.3 ± 3.81			
EAAs/NEAAs	0.74 ± 0.01	0.74 ± 0.01	0.74 ± 0.02	0.75 ± 0.02	0.74 ± 0.01			
EAA/Total AA	42.6 %	42.5 %	42.7 %	42.9 %	42.7 %			

Values are expressed as mean ± SE of triplicate determinations. Different small superscript letters following each other within rows are significantly different ($p < 0.05$). ns:not significant;T1 (control)- 100:0, T2 −75:25, T3 −50:50, T4-25:75 and T5-0:100 representing FM: BSFL ratios; EAA-Essential Amino Acids; NEAA- Non Essential Amino Acids.

Mg, 33 % of K, 20 % of Na and 57 % of Ca RDAs for 12–18 year old school-going child (WHO, 2006).

4.3. Amino acid profile of SHMP

The increase or decrease in the proportion of amino acids in the present study may not be necessarily predetermined by its content in diet, with discrepancies being specifically large for arginine, cysteine, glutamate, glutamine, glycine, histidine, methionine, proline and serine (Wu et al., 2014). This is because individual amino acids are catabolized/ transformed and deposited in the intestine at different rate depending on several factors (Wu et al., 2014). However, de Souza Vilela et al. (2021) and Cullere et al. (2018), also reported an increase in some amino acids compared to control when BSFLM was included in broiler and quails diet, respectively.

The dominant and scarce amino acids in the present study have also been reported to be dominant/ scarce in chicken meat by de Souza Vilela et al. (2021), Haščík et al. (2016), Haščík et al. (2020) and Yirmaga (2017). Furthermore, the levels of individual amino acids of SHMP are within the ranges of amino acids profile of chicken broiler meat (de Souza Vilela et al., 2021; Haščík et al., 2016, 2020; Yirmaga, 2017). Nevertheless, our results differ from the results reported by Zotte et al. (2020) who compared the amino acid profile of slow-growing indigenous chickens with that of commercially used hybrid from alternative farming systems such as organic and free-range. Here the content of all amino acids analyzed under this different feeding regimes reported by Zotte et al. (2020) were considerably lower compared to the results presented in the current study. On contrary, Salah et al. (2019), reported higher content of each individual amino acids analyzed when broiler diets were supplemented with synbiotic and/or organic acids. All the examples, confirms the role played by feeding regimes on the amino acids profile of chicken meat.

In this study, from the appreciable levels of free amino acids; asparagine, threonine, serine, glutamic acid, glycine, and alanine, connotes the critical contribution they might play in the sensory attributes of hen meat products from hen fed diet with BSFLM, particularly associated to taste (Bachmanov et al., 2016). However, the concentration of these amino acids did not vary significantly with increased integration of BSFLM in the diets. Dietary alterations using BSFLM on the stability of amino acid composition in meat, that are main precursor of bitter taste and flavor (valine, isoleucine, leucine, phenylalanine, methionine, arginine, and proline) have been reported in other studies by

Table 4
Fatty acids composition (μg/g) of spent hen meat powder analyzed by gas chromatography coupled to mass spectrometry.

RT (min)	FAMEs	FA	ω-n (Δn)	T1	T2	T3	T4	T5
		SFAs						
14.39	Methyl octanoate	Caprylic acid	C9:0		0.8 ± 0.04^{b}	0.9 ± 0.06^{b}	0.9 ± 0.16^{b}	1.5 ± 0.17^{a}
16.81	Methyl decanoate	Capric acid	C10:0	1.5 ± 0.12^{c}	1.0 ± 0.02^{d}	1.5 ± 0.16^{c}	2.0 ± 0.21^{b}	2.3 ± 0.31^{a}
18.93	Methyl dodecanoate	Lauric acid	C12:0	23.1 ± 1.44^{c}	152.0 ± 1.94^{b}	157.2 ± 1.94^{a}	159.5 ± 0.71^{a}	160.9 ± 1.10^{a}
20.19	methyl tridecanoate	Tridecylic acid	C13:0		3.1 ± 0.34^{a}	3.2 ± 0.04^{a}	3.3 ± 0.23^{a}	3.5 ± 0.48^{a}
20.84	Methyl tetradecanoate	Myristic acid	C14:0	85.1 ± 6.54^{c}	96.6 ± 7.48^{c}	104.6 ± 4.80^{c}	178.3 ± 7.53^{b}	203.9 ± 10.81^{a}
22.29	Methyl pentadecanoate	Pentadecylic acid	C15:0	12.2 ± 0.59^{e}	17.7 ± 1.4^{d}	22.5 ± 1.06^{c}	26.5 ± 1.65^{b}	30.3 ± 1.80^{a}
23.48	Methyl hexadecanoate	Palmitic acid	C16:0	1611.5 ± 1.29^{e}	1667.7 ± 2.75^{d}	1946.5 ± 1.19^{c}	1998.0 ± 1.64^{b}	2237.1 ± 1.60^{a}
24.29	Methyl heptadecanoate	Margaric acid	C17:0		42.3 ± 0.66^{a}	46.3 ± 1.09^{a}	41.5 ± 1.70^{a}	43.1 ± 0.28^{a}
25.32	Methyl octadecanoate	Stearic acid	C18:0	457.6 ± 1.4^{c}	455.7 ± 1.0^{c}	457.5 ± 0.61^{c}	463.7 ± 0.42^{b}	467.2 ± 1.44^{a}
26.12	Methyl nonadecanoate	Nonadecylic acid	C19:0	12.4 ± 0.57^{a}	11.7 ± 0.44^{a}	12.2 ± 0.6^{a}	11.7 ± 0.49^{a}	13.3 ± 1.20^{a}
26.98	Methyl eicosenoate	Arachidic acid	C20:0	56.0 ± 0.71^{b}	57.5 ± 0.69^{b}	57.5 ± 1.54^{b}	60.6 ± 0.92^{a}	62.1 ± 0.65^{a}
27.80	Methyl heneicosanoate	Heneicosylic acid	C21:0		9.3 ± 0.95^{a}	8.3 ± 1.17^{a}	9.4 ± 0.46^{a}	9.6 ± 0.21^{a}
28.59	Methyl docosanoate	Beheric acid	C22:0	23.2 ± 0.84^{a}	21.9 ± 0.93^{a}	22.5 ± 1.33^{a}	21.8 ± 0.49^{a}	23.3 ± 0.93^{a}
29.37	Methyl tricosanoate	Tricosylic acid	C23:0	14.9 ± 0.62^{a}	13.1 ± 0.67^{ab}	11.6 ± 0.39^{b}	11.7 ± 0.36^{b}	14.6 ± 1.17^{a}
30.13	Methyl tetracosanoate	Lignoceric acid	C24:0	19.5 ± 0.84^{a}	19.6 ± 1.75^{a}	19.6 ± 1.58^{a}	18.8 ± 0.30^{a}	18.8 ± 0.34^{a}
32.06	Methyl hexacosanoate	Cerotic acid	C26:0	15.2 ± 0.84^{a}	15.5 ± 0.76^{a}	15.8 ± 1.07^{a}	15.8 ± 0.70^{a}	16.7 ± 0.52^{a}
21.91	Methyl 13-methyltetradecanoate	Methyl 13-methylmyristate	Iso-methyl-C14:0		6.7 ± 0.7^{a}	7.3 ± 0.62^{a}	6.7 ± 1.28^{a}	6.4 ± 0.92^{a}
23.95	Methyl 15-Methyl hexadecanoate	15-methyl exadecenoic acid,	Iso-methyl C16:0		14.2 ± 0.3^{c}	14.5 ± 0.18^{c}	17.2 ± 0.68^{b}	19.0 ± 0.72^{a}
		∑ SFA		2332.3	2606.1	2909.5	3047.2	3333.5
21.08	Methyl −9Z-tetradecenoate	Myristoleic acid	C14:1, n-5		18.7 ± 0.57^{a}	18.1 ± 0.15^{a}	19.7 ± 1.45^{a}	20.5 ± 1.70^{a}
23.12	Methyl (9Z)-hexadecanoate	Palmitoleic acid	C16:1, n-7	52.8 ± 2.87^{b}	235.1 ± 13.19^{a}	245.1 ± 9.08^{a}	236.9 ± 6.34^{a}	261.5 ± 10.40^{a}
28.41	Methyl 13Z-docosenoate	Erucic acid	C22:1, n-9	16.5 ± 0.66^{b}	16.6 ± 1.57^{b}	19.2 ± 0.56^{b}	17.2 ± 1.4^{ab}	21.9 ± 2.80^{a}
29.95	methyl *(15E)*-tetracosenoate	Nervonic acid	C24:1, n-6	19.5 ± 0.86^{a}	14.6 ± 1.74^{b}	11.0 ± 2.57^{c}	10.7 ± 2.05^{c}	10.3 ± 1.36^{c}
25.21	Methyl (9E)-octadecenoate	Elaidic acid	C18:1, n-9 t	43.4 ± 1.26^{e}	47.1 ± 0.94^{d}	51.0 ± 1.76^{c}	56.4 ± 1.35^{b}	62.1 ± 1.45^{a}
25.00	Methyl (9Z)-octadecenoate	Oleic acid	C18:1, n-9	2473.1 ± 8.04^{c}	2751.7 ± 9.57^{b}	2771.1 ± 10.50b	2795.7 ± 31.08^{b}	3179.1 ± 22.58^{a}
26.89	Methyl (11Z)-icosenoate	Gondoic acid	C20:1, n-9	51.4 ± 3.28^{d}	67.8 ± 3.04^{c}	71.7 ± 5.08^{bc}	77.4 ± 2.81^{ab}	80.6 ± 3.33^{a}
24.09	Methyl (10Z)-heptadecenoate	(10*Z*)-Heptadecenoic acid	C17:1 (n-7)		28.6 ± 1.17^{a}	27.5 ± 0.81^{a}	28.5 ± 1.00^{a}	29.9 ± 0.62^{a}
25.90	Methyl (10Z) nonadecenoate	(10*Z*)-Nonadecenoic acid	C19:1 (n-9)		33.9 ± 0.96^{a}	34.1 ± 0.77^{a}	35.3 ± 0.64^{a}	35.7 ± 1.04^{a}
		∑ MUFA		2656.7	3214.0	3248.7	3277.7	3701.4
25.18	Methyl (9Z,12Z)- octadecadienoate	Linoleic acid	C18:2, n-6	1829.9 ± 22.51^{a}	1879.4 ± 4.75^{b}	1932.9 ± 4.86^{c}	1867.7 ± 5.45^{d}	1844.3 ± 3.51^{e}
24.81	Methyl (6Z,9Z,12Z)-Octadecatrienoate	γ- linolenic acid	C18:3, n-6	62.9 ± 1.04^{a}	60.0 ± 1.07^{ab}	59.9 ± 1.58^{ab}	58.4 ± 2.30^{b}	58.8 ± 1.61^{b}
25.45	Methyl (9Z,12Z,15Z)-octadecatrienoate	α- linolenic acid	C18:3, n-3	202.4 ± 3.03^{a}	252.1 ± 1.81^{a}	299.9 ± 2.28^{a}	298.4 ± 2.36^{a}	298.4 ± 1.43^{a}
26.28	Methy (9Z,11E,13E)-octadecatrienoate	α -Eleosteric acid	C18:3, n-5	7.4 ± 0.26^{c}	10.3 ± 0.18^{b}	10.6 ± 0.50^{b}	11.9 ± 1.3^{ab}	12.9 ± 0.72^{a}
26.44	Methyl (5Z,8Z,11Z,14Z)-eicosatetraenoate	Arachidonic acid	C20:4, n-6	56.3 ± 5.01^{a}	47.8 ± 0.17^{b}	46.0 ± 3.34^{b}	44.1 ± 2.13^{b}	44.1 ± 1.34^{b}
26.59	Methyl (8Z, 11Z, 14Z)- eicosatrienoate	Dihomo-y-linolecnic acid	C20:3, n-6	36.0 ± 0.51^{a}	34.5 ± 1.28^{a}	31.5 ± 1.28^{b}	29.4 ± 0.51^{c}	26.2 ± 1.48^{d}
	Methyl (11Z, 14Z) -eicosadienoate	Eicosadienoic acid	C20:2, n-6	139.10 ± 3.07^{a}	137.9 ± 6.25^{a}	136.3 ± 4.94^{a}	134.0 ± 0.84^{a}	132.5 ± 2.07^{a}
28.095	Methyl (5Z, 8Z,11Z,14Z,17Z)-eicosapentaenoate	Eicosapentaenoic acid	C20:5, n-3	22.3 ± 1.53^{a}	20.9 ± 1.51^{a}	21.5 ± 0.98^{a}	21.6 ± 1.27^{a}	21.3 ± 1.83^{a}
	Methyl (13Z, 16Z)- docosadienoate	Docosadienoic acid	C22:2, n-6	5.2 ± 0.01^{a}	5.2 ± 1.10^{a}	5.0 ± 0.97^{a}	5.0 ± 0.21^{a}	5.0 ± 0.30^{a}
28.09	Methyl (7Z,10Z,13Z,16Z)-docosapentaenoate	Ozubondo acid	C22:5, n-6	3.4 ± 0.33^{a}	3.3 ± 1.01^{a}	3.0 ± 0.85^{a}	2.9 ± 0.23^{a}	3.0 ± 0.34^{a}

(*continued on next page*)

Table 4 (*continued*)

RT (min)	FAMEs	FA	ω-n (Δn)	T1	T2	T3	T4	T5
28.07	Methyl (4Z,7Z,10Z,13Z,16Z,19Z)-Docosahexaenoate	Cervonic acid	C22:6, n-3	18.2 ± 1.29[a]	18.0 ± 0.38[a]	17.9 ± 1.51[a]	17.5 ± 0.79[a]	17.5 ± 0.32[a]
28.10	Methyl (7Z, 10Z, 13Z, 16Z, 19Z)-docosapentaenoate	Sardine	C22:5, n-3	13.5 ± 2.10[a]	13.2 ± 1.05[a]	13.1 ± 0.89[a]	13.1 ± 0.11[a]	13.1 ± 0.09[a]
		∑ PUFAs		2396.6	2330.3	2277.8	2205.1	2177.2
		∑UFAs		5053.3	5544.3	5526.5	5482.8	5878.6
		PUFA/SFA		1.0	0.9	0.8	0.7	0.7
		n-6		2132.8	2068.0	2014.6	1941.6	1913.9
		n-3		256.4	252.1	252.6	251.7	250.3
		n-6/n-3		8.3	8.2	8.0	7.7	7.6

Values are expressed as mean ± SE of triplicate determinations. Different small superscript letters following each other within rows are significantly different ($p < 0.05$). T1 (control)- 100:0, T2 −75:25, T3 −50:50, T4-25:75 and T5-0:100 representing FM: BSFL ratios; RT-Retention time; SFAs-saturated fatty acids; MUFAs-monounsaturated fatty acids; PUFAs-polyunsaturated fatty acids; UFAs-Unsaturated fatty acids; n-6-omega-6 fatty acids; n-3-omega-3 fatty acids.

Table 5
Vitamin profile (mg/kg) of spent hen meat powder.

Vitamin (mg/Kg)	T1	T2	T3	T4	T5	$F_{(4,10)}$	P-value
α-tocophenol	0.9 ± 0.05[b]	0.9 ± 0.08[b]	0.9 ± 0.05[b]	0.9 ± 0.07[b]	1.3 ± 0.11[a]	34.44	p < 0.001
ɣ-tocophenol	1.2 ± 0.03[a]	1.1 ± 0.08[b]	1.0 ± 0.03[b]	0.7 ± 0.10[c]	0.4 ± 0.03[d]	72.89	p < 0.001
Retinol	231.4 ± 2.75[a]	218.5 ± 7.60[a]	218.0 ± 12.65[a]	150.6 ± 15.19[b]	122.9 ± 4.79[c]	73.14	p < 0.001
Nicotinamide (Vit B3)	316.0 ± 17.16[c]	341.9 ± 11.23[bc]	359.1 ± 15.97[bc]	367.0 ± 12.28[b]	404.7 ± 21.2[a]	13.38	p < 0.001
Vitamin B1	31.2 ± 3.67[a]	22.0 ± 1.02[b]	19.9 ± 2.39[bc]	16.9 ± 0.98[c]	16.0 ± 1.11[c]	24.79	p < 0.001
Vitamin B2	7.9 ± 0.13[c]	8.6 ± 0.78[c]	14.5 ± 1.51[b]	30.8 ± 3.38[a]	31.2 ± 2.37[a]	101.45	p < 0.001
Vitamin B5	125.7 ± 8.90[c]	125.9 ± 5.14[c]	133.3 ± 7.14[c]	170.3 ± 7.30[b]	251.7 ± 15.03[a]	99.03	p < 0.001
Vitamin B9	968.8 ± 68.73[a]	–	206.9 ± 9.77[b]	–	–	361.34	p < 0.001

Values are expressed as mean ± SE of triplicate determinations. Different small superscript letters following each other within rows are significantly different ($p < 0.05$). T1 (control)- 100:0, T2 −75:25, T3 −50:50, T4-25:75 and T5-0:100 representing FM: BSFL ratios.

Bachmanov et al. (2016), except for isoleucine and methionine. Furthermore, AAs that enhance the savory or umami taste (aromatic amino acids) of meat also were not significantly altered. These amino acids are known and have been reported to play multiple roles in their free form and are building blocks of proteins. According to Bachmanov et al. (2016) protein content of food can be predicted on the basis of the taste of amino acids, which are often present in free form in protein-containing foods. Consistent with this, most amino acids are known have a taste, which makes some of them important as taste-active components in food (Bachmanov et al. 2016). Once ingested, amino acids and their metabolites have been widely reported to generate signals that affect appetite and satiety (Ackroff and Sclafani 2011; Ackroff and Sclafani 2013; Uematsu et al. 2009). Further studies to have a better understanding of the mechanisms involved in processing different amino acids from meat derived from poultry fed diet with BSFLM by consumers would open new avenues for uses of these amino acids as flavor, nutritive, and therapeutic agents.

4.4. Fatty acid composition

Monogastric animals absorb dietary fatty acids and deposit it in their tissues without manipulation, hence fatty acids composition of their meat reflects that of their diet (Cao et al., 2012; Coetzee & Hoffman, 2002). This implies that, essential fatty acid (EFA) content of poultry can be manipulated through dietary means so as to produce meat that is more healthy for the benefit of consumers (Coetzee & Hoffman, 2002). But, for effective modification of dietary fatty acid into the meat, it is necessary to feed the birds with manipulated diet for a reasonable feeding time before slaughter (Uushona, 2015). In the present study the hens were fed on the manipulated diet for a reasonable feeding period (60 weeks), which was enough period to initiate changes in the FA profile of the meat.

The increase in SFA and UFAs in this study might be attributed to the integration of BSFLM, which are very rich source of saturated fatty acids (Secci et al., 2018; Uushona, 2015) and UFAs (Shumo et al., 2019). Similar results were observed earlier in related studies (de Souza Vilela et al., 2021; Schiavone et al., 2017). On the other hand, the increase of MUFAs was most likely due to desaturation and elongation activities of lauric acid, myristic acid and palmitic acid. The desaturation and elongation activities are performed by stearoyl-CoA desaturase (Δ9 desaturase) which is a rate-limiting lipogenic enzyme that is up- regulated and down-regulated by low fat high carbohydrate diets and dietary addition of PUFA, respectively (Ntambi, 1999).

SFA and *cis*-MUFA are synthesized by the body thus they are not much important in human diet. Moreover, intake of imbalanced SFA is not recommended as it is positively related to cardiovascular diseases (Liu et al., 2020). Thus, SFAs intake should be as low as possible as recommended by European Food Safety and Authority (EFSA, 2016). The findings of this study confirm that healthiness (food with smaller fraction of SFA and greater fraction of PUFA) of the SHMP reduced with the proportional increase of BSFLM in the chicken diet (Cullere et al., 2018; de Souza Vilela et al., 2021). This has been a major drawback in the adoption of BSFL in whole substitution of conventional protein sources such as fish and soyabean in poultry diet. However, fatty acid profile of BSFLM can be modified by modulating rearing substrate (Spranghers et al., 2017; Tschirner & Simon, 2015). For example, the concentration of SFAs in meat can be reduced by increasing levels of n-3 in diet. Furthermore, defatting of BSFLM has also been suggested as a viable option of reducing the amount of fatty acids in the meal (Kim et al., 2020; Wang & Shelomi, 2017). Furthermore, the concentration of SFAs in meat can be reduced by increasing levels of n-3 in diet.

In the present study, the relatively low amount of PUFAs especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in BSFLM (Secci et al., 2018) may probably explain why SHMP from hens fed on diet containing BSFLM had lower levels of PUFA. Furthermore, the negative association between PUFA levels and BSFLM dietary levels concur with the results reported in several published works in literature (de Souza Vilela et al., 2021; Kim et al., 2020). PUFA concentration in BSFLM can be improved through manipulation of the larval diet since they are also monogastric animals (Khan, 2018; Schiavone et al., 2019). Nine undetected FAs in the control diet (T1- diet without BSFLM) may have been introduced in the SHMPs by BSFLM in the diets. The

dominant SFAs, MUFAs and PUFAs reported in the present study mirrored that of the diet BSFLM which is almost entirely composed of palmitic acid, stearic acid, myristic acid, lauric acid, oleic acid, linoleic acid and α linoleic acid (Cullere et al., 2018; Schiavone et al., 2017; Surendra et al., 2016). In fact, 89.5 % and 7.8 % of its PUFA is linoleic acid and α-linolenic acid (Schiavone et al., 2017). Furthermore, synthetase reaction that occurs within the tissue of a chicken produces primary SFAs products such as free palmitic acid (main product), myristic acid, lauric acid, traces of stearic acid and MUFA of the n-9 series, usually oleic acid (Coetzee and Hoffman, 2002). Predominant FAs in SHMP witnessed in the present study have also been reported as the main FAs in chicken meat by various researchers (Ajuyah et al., 1992; Jung et al., 2011; Yirmaga, 2017). However, values of individual FA of the above-mentioned studies might be higher or lower than those of the present study, which can be attributed to the different avian species, age and diet used. Inflammation processes can be increased by consumption of high amounts of arachidonic acid. The higher arachidonic acid contents across meat types may be due to desaturation and elongation activities of excess amounts of linoleic acid stored in tissues and complex lipids of hens (Coetzee and Hoffman, 2002). The significant reduction in concentration of arachidonic acid in meat from hen fed diet with increasing inclusion levels of BSFLM up to 100 % represents great benefit to the consumer as reported by de Souza Vilela et al. (2021).

To assess the impact of diet on cardiovascular health PUFA/SFA ratio is the most commonly used index (Liu et al., 2020). Interestingly, the PUFA/SFA ratio of the chicken meat products remained higher than the ideal recommended minimal ratio of 0.45 in human diet (Pieterse et al., 2019), hence concurring with the previously reported ratios ranging between 0.5 and 1.0 in cooked broiler meat fed on diets containing different levels of BSF pre-pupae meal (Pieterse et al., 2019), 0.78–0.85 in fresh chicken meat from laying hens fed with graded levels of microalgae supplementation (Liu et al., 2020) and 1.05, 0.68, 0.65, 0.61 and 1.36 values of guinea fowl, ostrich fan fillet, pick duck and broiler chicken meat, respectively (Geldenhuys et al., 2013).

The omega-6 to omega-3 (n-6/n-3) ratio is a key index for balanced synthesis of eicosanoid in the human body (Abedi & Sahari, 2014). According to Lakshani et al. (2016) this index determines the beneficial effect of PUFA in human body with a recommended ratio of 4.0 (EFSA, 2016). The incorporation of the correct n-6/n-3 ratio in human diet is very important because it reduces plasma lipids, thus preventing coronary heart disease (Coetzee & Hoffman, 2002). The n-6/n-3 indices for the different meat products reported in the present study are higher than the recommended value. However, the ratios fall within the range of 2.3–12.3 and 1.9–12.8 for thigh and breast muscle, respectively, of laying hen fed on diet supplemented with microalgae (Liu et al., 2020). On the contrary, a study by Salah et al.(2019) discovered a 6/n-3 ratio ranging 4.1–4.5 on breast meat from broiler chicken fed on diet supplemented with synbiotic and/ or organic acids whereas de Souza Vilela et al.(2021) reported a ratio ranging between 17.6 and 11.2 on meat from broiler chicken fed diet with BSFLM. On seldom occasions, the n-6 and n-3 fatty acids suppress the metabolism of each other due to competitive interaction occurring between linoleic and α- linolenic acids. This stiff competition for substrates and biosynthesis enzymes between n-3 and n-6 PUFAs might have led to significant reduction of total n-6 PUFAs with increasing levels of BSFLM inclusion in the diets, culminating into low n-6/n-3 ratio. Therefore, a more balanced eicosanoid metabolism can be achieved by increasing the proportion of n-3 in animal diet which prevents linoleic acid from forming long chain n-6 PUFAs (Coetzee & Hoffman, 2002).

4.5. Vitamin composition

There was increment in the levels of α- tocopherol, nicotinamide, vitamin B2 and vitamin B5 in meat products from hen fed diet with BSFLM can be attributed to the vitamin-rich resource present in the insects. The cause of the reduction in the levels of ɣ-tocopherol, retinol, and vitamin B1 in the various meat types remain unknown and warrant further research to substantiate. However, it known that animals are incapable of synthesizing vitamin E (α- tocopherol and ɣ-tocopherol) *in vivo* hence largely rely on dietary sources for their metabolic needs (Bou et al., 2009). This partly explains the considerable variation observed for tocopherols, which might be attributed to negligible levels in the various intake by the hen. Due to lack of studies on SHMP from layer hen fed diet with BSFLM, we couldn't find any related study to compare our results with; moreover, cause of increase or decrease in the composition of individual vitamins still needs to be further investigated.

5. Conclusion

Partial substitution of dietary fishmeal protein with BSFLM seems to be a suitable feeding strategy for layer chicken production. Total substitution of fishmeal in hen diets led to increased SFAs and MUFAs, but partial substitution up to 75 % had no adverse effects on the nutritional quality of the meat. From a nutritional point of view, spent hen meat derived from birds fed diet with BSFLM would be an excellent and cheaper source of high-quality protein with balanced amino acids, vitamins, minerals, omega 3 and omega 6 fatty acids, irrespective of the composition of the insect-based feeds. Therefore, value addition and effective utilization of spent hen meat derived from hen fed insect-based feed can be enhanced by processing the meat powder into ready-to-use convenience products for millions of vulnerable people worldwide especially in underdeveloped and developing countries to supply protein-energy rich ingredients to consumers who are suffering from severe malnutrition problem, due to acute shortage of animal protein in their diets. However, when considering the fatty acid profile of the spent hen meat, the inclusion levels of BSFLM in the layer diet should be carefully examined to minimize the effects of unwanted variability of nutrient changes, especially in the case of 100 % BSFLM substituted diets. Further experiments would be useful to confirm the impact of defatted BSFLM on poultry meat quality, which can help to reduce the undesirable quality properties that are not acceptable to consumers. On this background, spent hen meat powder from birds fed diets containing BSFLM can be safely used to formulate nutritious food products that are nutritionally well-balanced. Additional studies are urgently needed to establish the effect of BSFLM substitution in poultry feed on sensory properties and consumers' acceptance of poultry meat product.

Funding

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: Australian Centre for International Agricultural Research (ACIAR) (ProteinAfrica –Grant No: LS/2020/154), the Rockefeller Foundation (WAVE-IN—Grant No: 2021 FOD 030); Bill & Melinda Gates Foundation (INV-032416); the Curt Bergfors Foundation Food Planet Prize Award; Norwegian Agency for Development Cooperation, the Section for research, innovation, and higher education grant number RAF–3058 KEN–18/0005 (CAP–Africa); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Federal Democratic Republic of Ethiopia and the Government of the Republic of Kenya. The first author, Marcasy P. Makokha, was financially supported by the Center of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) of Egerton University, Kenya. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The views expressed herein do not necessarily reflect the official opinion of the donors.*

Data Availability Statement

All datasets presented in this study are included in the article and can be availed by the authors upon reasonable request.

CRediT authorship contribution statement

Marcasy P. Makokha: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Visualization. **Patrick S. Muliro:** Conceptualization, Validation, Writing – review & editing, Supervision. **Peninah N. Ngoda:** Validation, Writing – review & editing, Supervision. **Changeh J. Ghemoh:** Conceptualization, Methodology, Investigation, Validation, Writing – review & editing. **Cheseto Xavier:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – review & editing. **Chrysantus M. Tanga:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Visualization, Project administration, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank all the technical staff of ICIPE and KALRO for their commitment and technical support, which was instrumental in contributing consistently to the realization of this study.

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