EVALUATION OF MEAT QUALITY OF SPENT HENS FED ON BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE BASED DIET AND OF BREAD FROM WHEAT FLOUR ENRICHED WITH SPENT HEN MEAT POWDER.

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Food Science of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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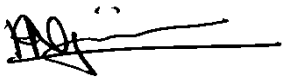

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DEDICATION

This work is dedicated to the Almighty God, my dear parents, my sisters and brother, my nephews and my daughter Lynerose Nafula for their inspiration, loving and selfless support.

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ABSTRACT

The utilization of insect protein in poultry feed is globally gaining momentum. However, the meat quality of hens fed on diet with black soldier fly larvae meal (BSFLM) as fishmeal (FM) substitute has received limited research attention. Off layers are usually sold at a very cheap price and hence the need to diversify their use. Wheat bread is among stable foods that are low in essential amino acids, thus enrichment is crucial. The birds were subjected to four diets –FM substituted with varying rations of black soldier fly larvae meal (T1-0% BSFLM-control, T2-25% BSFLM, T3-50% BSFLM, T4-75% BSFLM and T5-100% BSFLM). Fifteen birds per feeding group were slaughtered after 80 weeks and processed into meat powder. Refined wheat flour was then blended with 0%, 20%, 25%, 30% levels of the meat powder from hens fed on diet with 50% Black Soldier Fly Larvae Meal to produce high-valued bread products. The meat powders and bread from each treatment were subjected to proximate composition, mineral, amino acids, fatty acids and vitamin profiles analysis. The processed loaves of bread were further analysed for microbial quality and consumer acceptability. Results showed that the 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 Black Soldier Fly Larvae Meal. 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 Black Soldier Fly Larvae Meal incorporated diet. Crude protein, ash, Omega 3 fatty acids and vitamins (retinol, nicotinic acid, and pantothenic acid) were significantly increased in supplemented bread products. Limiting amino acids like lysine and threonine in enriched bread products increased by 3.0–4.5 and 1.8–3.1-folds, respectively. Iron, zinc, and calcium increased by 1.1, 1.2 and 3.0-folds in bread with 30% meat powder. Overall acceptability of breads prepared with 25 and 30% meat powder were highly ranked. These results provide valuable information on the applicability and suitability of meat powder from hen fed insect based meal (IBM) as novel ingredient in the development of new functional food products to address rampant global malnutrition.

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LIST OF ACRONYMS AND ABBREVIATIONS

AA	Amino Acid
AOAC	Association of Official Analytical Chemists
BCEU	Behavioural and Chemical Ecology Unit
BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
BSFLM	Black Soldier Fly Larvae Meal
Ca	Calcium
CP	Crude Protein
CF	Crude Fat
DM	Dry Matter
FAO	Food and Agriculture Organization of the United Nations
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FM	Fishmeal
Fe	Iron
GC-MS	Gas chromatograph coupled to mass spectrometry
IACUC	Institutional Animal Care and Use Committee
IB	Isa Brown
IBM	Insect Based Meal
icipe	International Centre of Insect Physiology and Ecology
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ILRI	International Livestock Research Institute
KALRO	Kenya Agricultural and Livestock Research Institute
KEBS	Kenya Bureau of Standards
LDPE	Low Density Polyethylene
LOD	Limit of detection
MBBU	Molecular Biology and Bioinformatics Unit
MUFA	Monounsaturated Fatty Acid
MS	Mass Spectrometry
NRC	National Research Council
n-3	Omega 3

n-6	Omega 6
P	Phosphorus
PEM	Protein –Energy Malnutrition
PUFA	Polyunsaturated Fatty Acid
RDA	Recommended Dietary Allowance
SBM	Soybean Meal
SHMP	Spent Hen Meat Powder
SSA	Sub-Saharan Africa
UFA	Unsaturated Fatty Acids
UPLC	Ultra- Performance Liquid Chromatography
USAID	United States Agency for International Development
VSRI	Veterinary Science Research Institute
Zn	Zinc

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Black Soldier Fly (*Hermetia illucens*) Larvae (BSFL) are a good source of protein (37-63%), with balanced amino acid profile (Tran *et al*., 2015) that are in line with the reference standards outline by World Health Organisation (Bessa *et al.*, 2020). For instance, it has high lysine levels contributing 6 to 8% of its crude protein. Further, they contain other constituents of nutritional relevance such as dry matter (35-45%), chitin (1-9%), phosphorous (0.6-1.5%) and fat (15-49%) (Makkar *et al*., 2014). According to Bessa *et al*. (2020) they contain iron (Fe) and zinc (Zn) content ranging between 2.1 to 3mg/100g and 6.8 to 15mg/100g, respectively which is superior to that of lean meat which has 1.1-1.8mg/100g iron and 4.2-4.6mg/100g zinc content. The latter researchers further reported that their calcium (Ca) amounts fall between 840 to 934mg/100g comparable to that of milk ranging 119 to 2900mg/100g. Therefore, its quality nutrient content places it in a good position in improving human diet, especially for individuals vulnerable or suffering from malnutrition that affects 23% of sub-Saharan Africa (SSA) population (FAO *et al.*, 2017). However its' consumption by human beings is still infeasible due to consumer perception, inadequate processing methods and safety concerns (Bessa *et al*., 2020).

In poultry, the use of such novel eco-friendly protein sources for partial or total replacement of expensive conventional protein sources (soybean and fishmeal) has been confirmed to be economical (Onono *et al.*, 2018) and has been recommended by organizations such Food and Agriculture organization of the United Nations (FAO) (Van Huis, 2013). References/ standards for the use of insects including Black Soldier Fly Larvae for food and feed have been framed in many countries while many others are in the process of developing the standards (Govorushko, 2019; Ssepuuya *et al*., 2017). For instance, Improving Livelihoods by Increasing Livestock production in Africa (ILIPA) project at International Center of Insect Physiology and Ecology (*icipe*) currently focus on commercial farming of insects as feed for poultry. In Kenya, Kenya Bureau of Standards (KEBS) approved new regulations that explicitize the specifications for dehydrate insect product intended to be used as animal feed in March 2018 (KEBS, 2018).

Nutritional value of Black Soldier Fly Larvae can therefore, potentially be availed indirectly through feeding birds which then can be consumed by humans. Black Soldier fly

larvae as poultry feed ingredient has been reviewed in general for broiler chicken (Vilela *et al*., 2021), broiler quails (Cullere *et al.,* 2018), laying quails (Zotte *et al*., 2019), Barbary partridge (Secci *et al.*, 2018) and laying hens (Sumbule, 2021). Even though almost all researchers globally agree that Black Soldier Fly Larvae are perfect replacers of traditional protein sources in poultry diets, no research has primarily focused on the effect of Black Soldier Fly Larvae diets on nutritional quality of spent hen meat. Despite lack of affordable, readily available and high quality feeds being the main challenge hindering growth in poultry industry, disposal of spent hens is also major challenge in poultry farming.

According to Lyon *et al*. (2003) spent hens have low weight, tough and chewy meat compared to broilers hence not preferred for whole muscle food prompting low market price (Semwogerere *et al*., 2019). This necessitates creation of new avenues for the utilization of spent hen meat which is of great concern in the egg industry especially in developing countries (Semwogerere *et al*., 2019). This meat however, plays an important role in culinary industries in developed countries; processed into comminuted or dried flour products where its inferior eating characteristics are masked (Kolawole, 2017). And such comminuted products can be used for enrichment of cereal based foods such as breads that are nutritionally imbalanced to obtain speciality bread. The information on hens' carcass quality therefore, becomes paramount with references to its economic benefits and the quality of hen meat-based processed products

In attempts to improve nutritional value of wheat bread which is a good carrier of functional ingredients, Umaraw *et al*. (2018) tested the effect of spent hen meat powder inclusion in whole wheat flour on proximate composition, textual profile, instrumental colour and physicochemical properties of resultant bread. The study by Cakmak *et al*. (2013) evaluated the effect of enrichment of white and whole wheat flour with chicken meat and its powder on rheological properties of dough, proximate composition, instrumental colour, crumb firmness, loaf volume and sensory evaluation of bread. Positive results regarding improved nutrition of wheat bread were reported with reference to the above studies.

However, storage, microbial and some nutrition aspects were not determined in the above studies. This study therefore, examines the effects of incorporating Black Soldier Fly Larvae meal in hens diet on proximate, minerals, vitamins, amino acids and fatty acids composition of spent hen meat and the effect of substituting white wheat flour with spent hen meat powder on breads proximate, minerals, vitamins, amino acids and fatty acids composition, sensory and microbial aspects.

1.2 Statement of the Problem

Black Soldier Fly Larvae meal presents a cheaper and valuable protein source economically ideal for productivity of layers. However, upon attaining a non-productive stage (spent hen), layers are often disposed as freebies. To maximize the profits accruing from the Black Soldier Fly Larvae fed-spent hens, their carcasses can be powdered and utilized in the developments of bakery products especially bread. Bread has low proteins, Essential Amino Acids, minerals and fats content and has been indicated to exacerbate malnutrition due to its wide consumption. Therefore, tapping the nutritional resource of Black Soldier Fly Larvae-fed spent hen into bread is a viable strategy towards revamping nutritional composition of bread to combat malnutrition as well as creating a better spent hen market. This study, evaluated the nutritional quality of SHMP from birds fed on Black Soldier Fly Larvae Meal based diet, formulated and evaluated the quality of bread enriched with the SHMP.

1.3 General Objective

To contribute towards food and nutrition security through production of bread from wheat flour enriched with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal.

1.4 Specific Objectives

i. To determine the effect of supplementing Black Soldier Fly Larvae meal in hens' diet on the nutritional properties of spent hens' meat.

ii. To determine the effect of enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal on the nutritional characteristics of the enriched bread

iii. To determine the effect of enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal on microbial safety of the enriched bread.

iv. To determine the effect of enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal on consumer acceptability of the enriched bread.

1.5 Null Hypotheses

i. Black Soldier Fly Larvae –supplemented diet had no significant effect on the nutritional properties of spent hen meat.
ii. Enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal had no significant effect on nutrition characteristics of the bread.
iii. Enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal had no significant effect on microbial safety of bread.
iv. Enriching wheat flour with spent hen meat powder from hens fed on Black Soldier Fly Larvae based meal had no significant effect on sensory properties and consumer acceptability of bread.

1.6 Justification

The study will generate information on the effect of Black Soldier Fly Larvae based diet on nutritional quality of spent hen meat. This information will be useful in promoting and enhancing the potential of Black Soldier Fly Larvae as source of protein and fat in chicken production and will also enhance the utilization of spent hen meat which is underutilized due to lack of information on their nutritional values. Utilization of spent meat powder in bread is expected not only to produce acceptable and nutritious bread with functional properties but also will provide a viable option for marketing spent hens meat. This could respond to Kenya Bottom –up Economic Transformative Agenda (BETA) –pillar 1: that target to increase agricultural value addition. Studies have shown that, wheat bread is protein and mineral deficient. However its demand has increased due to urbanization, increased population, consumer preference and convenience. Bread enrichment with animal proteins which are nutritious and readily available in Kenya is essential to improve the nutritional wellbeing of people who regularly consume it due to its convenience. It is also a viable option in helping to limit malnutrition in the world and the valorisation of unused poultry production, this could respond to United Nations Sustainable Development Goals (UN-SDGs) 2 and 3 that is Zero hunger and Good health and wellbeing, respectively.

CHAPTER TWO
LITERATURE REVIEW

2.1 Poultry Industry in Kenya

The poultry industry continues to expand rapidly every year due to increased demand for white meat and egg products (Poodari *et al.*, 2018). According to the authors, 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 (Kokoszynski *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 cross linkage (Okarini *et al*., 2013), 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 *et al*, 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 (Onono *et al.*, 2018; 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.

2.2 Insect Based Meal in Animal Diet

Insects have qualified as a high quality, efficient and potential alternative protein sources and have been a very popular research area in livestock and human nutrition (Do *et al*., 2020). Most edible insect species are collected from nature and used as food for human consumption or used to feed animals. Insect meal for poultry, has been investigated by various scientists and all come to conclusion that insects could replace 25- 100% of soy meal or fishmeal depending on the animal species (Henry *et al*., 2015; Makkar *et al*., 2014). Insects

like yellow meal worm, Black Soldier Fly Larvae and common house fly have been identified to be potential sources of protein and fat in animal ingredients (Biasato *et al*., 2016; Bovera *et al*., 2016; Maurer *et al*., 2016).

Globally, the regulations 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 (Lahteenmaki-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 (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 & 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).

2.3 Black Soldier Fly (*Hermetia illucens*)

Black solder fly (BSF) belongs to stratiomydae family. Its common and widespread fly that is not regarded as pest species and do not transmit diseases (Newton *et al*., 2005). The adult fly do not enter living areas of people in such of food it depends on energy stored during the larval stage (Newton *et al*., 2005). The adult are robust with length between 15 and 20 mm. The larvae are well adapted to moist environment compared to wet or dry environment. They are well known to be inhabitants of nutrient rich ecosystems such as manure pile, outdoor toilets and composite heaps (Diclaro *et al*, 2012). With regard to its nutrient composition, it has been proven to provide protein with high biological value but which is dependent on rearing substrate of the larvae (Barragan-Fonseca *et al*., 2017). Its

crude protein content varying between 37 to 63% on dry matter with five day larvae having the highest protein content of about 61% while the older larvae to pre pupae giving lowest CP of around 40% (Barragan-Fonseca *et al*., 2017; Rachmawati *et al*., 2010). Chitin content range from < 1 to 9% on dry matter but majority of studies indicate an averaging of around 5-7% (Caligiani *et al*., 2018; Finke, 2013; Spranghers *et al*., 2017). Makkar *et al*. (2014) documented that its ash content is between 11- 28% on dry matter, 5-8% DM Calcium and 0.6-1.5% DM Phosphorus. Black Soldier Fly Larvae have significantly higher levels of calcium, manganese and iron than soybean meal, although lower potassium levels (Newton *et al*., 2005) and its crude fat content falls between 7- 39% on dry matter. However fat content can be reduced by diet manipulation and during meal processing i.e. defatting. Saturated fatty acids (SFA) accounts for the largest proportion of about 72% with lauric acid occupying 51.2% of total Fatty Acids (Finke, 2013).

2.4 Effects of Black Soldier Fly meal incorporation in Poultry Diet on Nutrient Composition of the Resultant Meat

Feed composition can affect or change strongly the characteristics of poultry meat, these makes feeding mode a crucial factor of meat quality (Jaturasitha *et al*., 2004; Jaturasitha *et al.*, 2008). The incorporation of different levels of either BSF larvae or pre-pupae meal as source of protein or fat in poultry diet has been noted to cause no significant effect ($P>0.05$) on proximate composition of Barbary partridge (Secci *et al*., 2018), quails (Cullere *et al*., 2018) and broiler meat (Balolong *et al*., 2020; Cullere *et al*., 2019; Pieterse *et al*., 2019).

Cullere *et al*. (2018) examined the effect of dietary inclusion of Black Soldier Fly Larvae meal in quails diet on the amino acids composition of quails meat. The authors reported significant increase in aspartic acid, glutamic acid, alanine, serine, threonine and tyrosine content of the meat but the content of unmentioned amino acids remained unaffected. Vilela *et al*. (2021) reported similar observations; increase in aspartic acid, glutamic acid and lysine levels of broiler meat, when Black Soldier Fly Larvae meal was incorporated in chicken diet. However, there is growing evidence that the increase or decrease in the proportion and content of individual amino acids in meat may not be necessarily predetermined by its amount in the 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 and transformed in the small intestine at different rate (Wu *et al*., 2014). Furthermore, amino acid

content in the diet does not correspond to their concentration in the blood circulation. Individual amino acids in the plasma have a different metabolic destiny which differs to that of amino acids in the tissue proteins (Wu *et al*., 2014).

According to Cullere *et al*. (2018), inclusion of Black Soldier Fly Larvae meal in quails diet led to significant increase in Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) contents of quails meat. Polyunsaturated fatty acids (PUFA) content decreased. PUFA of n-3 fraction showing the highest decrease resulting to increase in n-6/n-3 ratio. The total SFA content of the broiler meat increased significantly but PUFAs content decreased significantly with increasing levels of Black Soldier Fly Larvae meal in chicken diet (Cullere *et al*., 2019; Kim *et al*., 2020; Vilela *et al*., 2021). Furthermore, total MUFAs remained unaffected while n-6/n-3 ratio reduced (Vilela *et al*., 2021) and the ratio of Unsaturated Fatty Acids to Saturated fatty acids (UFAs /SFAs) also decreased (Kim *et al*., 2020). In conclusion, the alteration of Fatty acid (FA) profile of is possible, since they are monogastric animals; they absorb and deposit FA without any manipulation (Coetzee *et al*, 2002).

The variation in some 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). Cockcroft (2018), established that absorption of minerals such as Ca 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.

2.5 Wheat (*Triticum aestivum*) Production

Bread wheat accounts for 95% of total wheat produced in the world (approximately 750 million tons) and in SSA it is the main wheat type produced (Tadesse *et al*., 2018). Kenya is the fourth largest wheat producer after Ethiopia, South Africa and Sudan in SSA (Tadesse *et al*., 2018). In SSA wheat is currently very important cereal especially in urban areas and the demand far exceeds the production leading to importation (Tadesse *et al*., 2018). The increase in its demand is due to change in food habits, increased population and urbanization (Tadesse *et al*., 2018).

2.6 Bread Industry

Bread is a cereal food product that is made from dough with or without others ingredients which can be leavened or unleavened. It is considered a foreign food product that is important to many in SSA (Owade, 2016), staple food that is inexpensive, versatile and filling (Trinh *et al*., 2016). According to Adeleke *et al*. (2010), it has diverse recipe thus accepted by many people in the world. It is the most consumed wheat product followed by noodles worldwide (Verma *et al*., 2015). In Kenya, the demand, consumption and importance of bread in diets is on the rise (Owade, 2016). Even though different types of bread exist, white bread is the most consumed bread type due to its appealing sensory characteristics (Khoozani *et al*., 2020). It is made from white wheat flour, water, sugar, salt, yeast and fat (Okafor *et al*., 2012) but varying ingredients proportions results to product variation (Owade, 2016). The main ingredient in bread baking is wheat which is low in protein, vitamins, minor minerals and dietary fibre (Lu *et al*., 2018). Bread production method vary from country to country and the production has improved in terms of ingredient used (Owade, 2016). Development of speciality bread (bread with special features due to peculiar ingredients used in the manufacture beyond the essential ingredients) is the latest innovation in bread industry. Speciality bread includes fortified, high protein and enriched bread (KEBS, 2012). According to KEBS (2012), high protein bread must contain more than 22% protein on dry weight of the bread while enriched bread is bread that has been added macro or micro nutrients. Currently animal proteins are being used to produce high protein bread or enriched bread (Cercel *et al*., 2016; Desai *et al*., 2018; Monteiro *et al*., 2018; Osimani *et al*., 2018; Roncolini *et al*., 2019)

2.7 The Effect of Substituting Wheat Flour with Animal Meat on Nutrition of Bread

Umaraw *et al*. (2018) evaluated the quality characteristics of spent hen meat powder incorporated in whole wheat breads. The results revealed that increasing the level of meat powder had significant effects on physicochemical parameters and proximate composition. The values of moisture, protein, fat, ash and moisture retention increased significantly ($p>0.05$) when whole wheat flour was substituted. The effect of substituting white wheat flour with spent hen meat powder in preparation of white bread was not evaluated in this study. Storage stability, preservation aspect and microbial safety of the enriched bread were also not examined.

Cakmak *et al*. (2013) determined the effect of adding chicken meat powder and chicken meat at five different levels (10, 15, 20 25, and 30%) to white and whole wheat

baguette bread flour. Nutritional, sensory and quality characteristics of enriched bread samples were determined. Addition of chicken meat and chicken meat powder in bread caused an increase in protein content of sampled bread. From the study it was concluded that depending on storage conditions, the shelf life of the enriched breads would be no longer than 4-8 days due to high water activity. However the study recommended further studies on determination of microbial stability of enriched breads during storage.

Fagundes *et al*. (2018) reported Cobia (*Rachycentron canadum*) minced proteins incorporation in bread causes a protein increase in the range of 40 to 70% compared to reference bread. Cercel *et al*. (2016) supplemented wheat flour dough with fish protein and flour properties and bread characteristics were determined. The study reported that substitution of wheat flour with fish proteins improved the nutritional value of wheat flour bread but induced a decrease in loaf specific volume compared to non-enriched bread.

de Oliveira *et al*. (2017) added different ratios of Cinereous Cockroach (*Nauphoeta Cinerea*) flour in bread formulation. Chemical composition, colour, firmness and specific volume were analysed and compared to white and whole wheat bread. Bread enriched with 10% roasted Cinereous Cockroach flour had the best nutritional characteristics, differing slightly from white and whole wheat bread.

2.8 The Effect of Substituting Wheat Flour with Animal Meat on Sensory Properties and Consumer Acceptability of Bread

Umaraw *et al*. (2018) reported variable effects on sensory properties of bread when meat powder incorporation in wheat flour is high -colour, crust, crumb and flavour increases significantly ($p<0.05$) with increase in level of incorporation. Similar results were reported by Cakmak *et al*. (2013). Further, thirty per cent inclusion level of meat powder in brown bread gives a product of highest acceptability (Umaraw *et al.*, 2018). But on the contrary Adeleke *et al*. (2010) reported no significant effect on organoleptic properties of bread (taste, aroma, crust and crumb colour and overall acceptability) when Tilapia Fish Protein Flour (TFPF) was added in wheat flour.

2.9 The Effect of Substituting Wheat Flour with Animal Meat on Microbial Quality and Shelf Life of Bread

According to Berwal *et al*. (2013), meat is highly perishable thus food incorporated with meat are also highly perishable. Incorporation of meat in bakery products may affect their physicochemical stability during storage, leading to faster degradation process and short

shelf life (Monteiro *et al*., 2016). Negative changes in the colour, texture, lipid and protein oxidation of the bread formulations results from higher levels of tilapia waste flour inclusion (≥ 10%)) (Monteiro *et al*., 2019).

2.10 Research Gap

From the literature review, there is evidence that little has been done on nutritional quality of spent laying hens fed on diet supplemented with Black Soldier Fly Larvae. Also enrichment of bread using meat powder to obtain speciality bread has remained unexploited. Therefore areas considered as research gaps were mainly physico-chemical properties of spent laying hens fed on varying ratios of FM: Black Soldier Fly Larvae meal in hens diet, and effects of enriching bread flour with meat powder from hens fed on Black Soldier Fly Larvae based meal on chemical, sensory and microbial properties of bread. This study focused on nutritional analysis of spent laying hens fed on diet supplemented with Black Soldier Fly Larvae as source of protein and effects of enriching bread wheat flour with spent laying hen meat powder from hens fed on Black Soldier Fly Larvae based meal on chemical, sensory and microbial characteristics of bread.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Site

Feeding trials were conducted at 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). Meat powder processing, bread baking, chemical analyses; proximate composition, amino acids and fatty acids analysis, of hen meat and enriched bread, sensory tests and microbial analyses on breads were conducted at International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi (36° 45'Longutude, 1° 18' South Latitude), Kenya. Minerals and vitamins analysis were performed at International Livestock Research Institute (ILRI) Nairobi, Kenya.

3.2 Ethical Approval

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.

3.3 Experimental Design and Statistical Model

The experiment employed a completely randomized design (CRD). Diet formulated without BSFL meal (control-100% FM, 0% BSFL, Diet With 75 % FM, 25% BSFL, 50 % FM, 50% BSFL, 25 % FM, 75 % BSFL and 0 % FM, 100 % BSFL) represented the five factors in objective 1. Fifteen experimental hens per treatment (purposive sampling) were randomly collected and all the birds were slaughtered, processed into meat powder and the powder analysed in triplicates for proximate composition (moisture, crude protein, crude fat and carbohydrate contents), amino acids, minerals, vitamins and fatty acids profiling. The statistical model for this design was as follows;

$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

$Y_{ij} = \text{obsevation in terms of nutrition composition of meat powder}$

$\mu = \text{overall mean}$

$\alpha_i = \text{fixed effect of } i^{th} \text{ BLACK SOLDIER FLY LARVAE inclusion level}$

$\varepsilon_{ij} = \text{random error component}$

Bread formulated without meat powder (control-1000 gram wheat flour, 0 gram meat powder), bread with 800 gram wheat flour, 200 gram meat powder, bread with 750 gram wheat flour, 250 gram meat powder, bread with 700 gram wheat flour, 300 gram meat powder represented the factors of objective 2. The breads were then analysed in triplicates for proximate composition (moisture, crude protein, crude fat and carbohydrate contents), amino acids, minerals, vitamins and fatty acids profiling. And each of the experimental units obtained in the objective 2 were subjected to microbial determination and consumer acceptability test. The statistical model for this design was as follows;

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Y_{ij}

$= \text{obsevation in terms of nutrition composition or microbial load or sensory atribute of the bread}$

$\boldsymbol{\mu} = \text{overall mean}$

$\alpha_i = \text{fixed effect of i}^{th} \text{ meat powder inclusion level}$

$\varepsilon_{ij} = \text{random error component}$

3.3.1 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 5^{th} instar stage, the larvae were harvested and washed by dipping 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 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 (Maina *et al*., 2017; Munguti *et al*., 2006). All diets for the entire experimental period were formulated on one day at the beginning of the trials by Josiche General Traders Ltd.

3.3.2 Feeding Program

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.*, 2019). 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 80^{th} 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.

3.4 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 in the appendix A (Fig. 1). Briefly, after proper slaughtering and dressing, the meat was washed and put in a pan. After deboning and mincing, the meat was cookrd at 15 psi for 30 min. The meat was placed in an oven to dry at 60 °C for 20 hrs. The meat was then grounded into powder after cooling and packed into low density polyethylene (LDPE) bags and kept at temperatures below - 20 °C until further analyses were conducted.

3.5 Development of Bread Enriched with Hen Meat

Spent hens from treatment T4 were obtained and processed as describe in experiment 1 of this chapter. The baking ingredients: All-purpose fortified white wheat flour (brand – EXE), yeast, sugar, shortening and salt were purchased from a local supermarket.

3.5.1 Bread Formulation and Baking

Spent hen meat powder (SHMP) was used to substitute wheat flour in the ratios (wheat: SHMP w/w) of 800:200 (B_{20}), 750:250 (B_{25}), 700:300 (B_{30}) and 1000:0 SHMP (B_0) representing the control, resulting into four experimental variants. The straight dough method was used for bread making as illustrated by Oliveira *et al*. (2017) with sight modification.

Briefly, the dry ingredients: wheat flour for control or wheat flour-chicken meat powder blend (59.0 %), yeast (0.9 %), sugar (2.4 %) and salt (1.2 %) were whisked in a mixing bowl, transferred into a kitchen mixer (BJY-BM10, Berjaya, Malaysia) and mixed for 4 min on low speed followed by addition of water (35.4 %) and shortening (1.2 %). It was then mixed at full speed for 10 min to yield a consistent dough. Afterwards, the doughs (250 g) were molded in greased aluminum bread mold trays, covered in pans and placed in an oven at 30 °C for 95 min to ferment. The doughs were then transferred into a 200 °C preheated oven (BISTROT 665; BestFor®, Ferrara, Italy) and baked for 20 min. This process was repeated thrice for every treatment to give twelve experimental units. The breads were allowed to cool at room temperature for 5 min, and apportioned into two batches. First batch was immediately subjected to microbial assessment and the sensory tests while second batch was packed in sterile plastic bags (Zip loc bags, SC Johnson brand) and temporarily stored at -10 °C for later analysis.

3.6 Proximate Composition of Hen Meat Powder and Bread

Proximate analysis of meat powder and bread were done as per the Association of Official Analytical Chemists Method ((AOAC, 2012) methods).

3.6.1 Determination of Moisture and Dry Matter Content

Approximately 2 g of samples were weighed into clean, pre-dried and pre-weighed porcelain crucibles (W1). The joint weight of the crucible and the sample were recorded (W2) and the sample dried in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 135 ℃ for 2 h. The dried samples in the crucibles were then cooled in a desiccator for 30 min then weighed (W3). Percentage moisture content was calculated following the formular below:

$$MC\% = \frac{W2 - W3}{W2 - W1} \times 100$$

Where: MC= Moisture content; $W1$= weight of porcelain crucibles; $W2$=weight of crucible and wet sample; and $W3$= weight of crucible and dried sample.

3.6.2 Crude Protein

Crude protein content was determined using the improved Kjeldahl method 955.04. One gram g of sample of known dry matter content was weighed accurately into 250 mL digestion tubes with two tubes included for blank runs (with all reagents but no sample). A

catalyst mixture (15 g) in ratio of 9 K2SO4 to 1 CuSO4.5H2O was added to each tube and 15 mL of 98 % concentrated sulphuric acid $H2SO_4$ (96-98 %) was then added into each tube. Rack with tubes was including blank was inserted into digestion block heater. The sample was digested at 420 °C for 2 h until the liquid became transparent. It was then allowed to cool at room temperature. Thereafter the tubes were transferred separately to distillation and titration in an automatic Kjeldahl analyser (Velp UDK 159, Velp Scientifica, Europe) with a pre-set nitrogen-protein conversion factor of 6.25 (Finke, 2007) unit. The pre-defined method was selected and distillation and titration was started. The Kjeldahl analyser was connected to 40 % NaOH source to alkalize the digests and liberate ammonia. The liberated ammonia was harnessed by excess 4 % boric acid premixed with indicators (10 mL of bromocresol green and methyl red solution) to yield ammonium borate. Ammonium borate distillate was then titrated against 0.2 M HCl until a pink colour change was auto-detected by the analyser. The nitrogen and protein contents were calculated as follows;

$$\%\, Nitrogen\ = \frac{100\ \times (VT - VB) \times NA \times 14}{WS \times 1000}$$

Where:

VT; volume (mL) of standard acid used in titration; VB =volume (mL) of standard acid used in blank; NA= normality of acid (HCl); WS =Sample weight in g.

Protein content was calculated as percent nitrogen multiplied by 6.25.

3.6.3 Crude Fibre

Crude fibre was determined according to method no 985.29. The method involved accurately weighing 2 g flour sample of known dry matter content into a 500 mL round bottomed flask and adding of 100 mL boiling distilled water and 2.04 mol/l sulphuric acid solution. Boiling distilled water was again added to make the volume of the mixture up to 200 mL and the mixture was maintained at this volume while boiling for 30 min on a hot plate. Buchner funnel light packed with glass wool was used to filter the mixture and the residue was washed with boiling distilled water three times to remove traces of acid. The residue and the glass wool was transferred quantitatively back to the beaker and about 100 mL of boiling distilled water and 25 mL of 1.73 mol/l potassium hydroxide solution was added. Boiling distilled water was added to make the volume of the mixture up to 200 and this volume was maintained whilst boiling on a hot plate for 30 min. Glass wool was used again to filter the mixture and it was washed three times with boiling distilled water to

remove base traces. Small amounts of ethanol were used to further wash the residue. The residue and glass wool was transferred quantitatively to a pre-weighed porcelain dish (W_1) and dried in a forced draft air oven (WTB binder, Tuttlingen, Germany) at 105 °C for 2 h. The sample was cooled and was weighed in the porcelain dish (W_2) before igniting at 550 °C in a muffle furnace (Heraeus-Kundendienst, Düsseldorf, Germany) to constant weight. The sample was then cooled in the dish and the weight was recorded (W_3). The crude fibre content was calculated and was expressed as a percentage of the sample's dry matter content.

$$\%\ Crude\ Fibre = \frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

3.6.4 Total Ash

The ash content was determined using Method 923.03 in triplicates. Approximately 2 g of each sample was weighed into pre-weighed porcelain crucibles and was placed in a temperature controlled furnace preheated to 550 °C and was held at this temperature for 2 hours. The crucible was then transferred directly to a desiccator, was cooled and weighed. Ash content was reported as a percentage of the whole sample.

$$\%\ Ash\ = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

3.6.5 Crude Fat

Extraction cups were dried at 105 °C for 30 min and were cooled in a dessicator and weighed (W0). Sample (5 g) was weighed into extraction thimbles5 (Ws) and was covered with fat free cotton. The extractor (Soxhlet extractor -Velp SER 148, Velp Scientifica, Europe) was preheated and condenser cooling water turned on. Thimbles containing samples were attached to extraction columns and sufficient amount of solvent (Petroleum ether -70 mL for VELP extraction cups) was put into each extraction cup to cover test portion when thimbles are in boiling position. Cups were placed under extraction columns and secure in place, making sure that cups are matched to their corresponding thimble. Thimbles were lowered into solvent (Immersion) and boiled for 30 min. Thereafter, thimbles were raised out of solvent (Washing) and extract in this position for 60 min followed by distilling as much solvent as possible from cups (Recovery) to reclaim solvent and attain apparent dryness. Extraction cups with fats were removed from extractor and were placed in operating fume hood/open for about 30 min to finish evaporating solvent at low temperature. Extraction cups with fats were then dried in 105 °C oven for 30 min to remove the last traces of solvent and

moisture. Desiccators were cooled to room temperature and weighed (WF). The percentage fat was calculated following the formula below;

$$\%\ Crude\ Fat\ DM = \frac{Wf - W0}{Ws \times DM(fraction)} \times 100$$

Where: Wf= Weight of recovered fat and the extraction cup; $W0$= Weight of empty extraction cup; Ws=Weight of sample

3.6.6 Carbohydrate Content

Carbohydrate content will be determined using method described by Tekle. (2009). The following expression will be used:

$$\%\ \text{Carbohydrate} = 100 - [\text{protein} + \text{fat} + \text{ash} + \text{moisture}]$$

3.7 Mineral Analysis of Hen Meat and Breads

Mineral profiling was done as per the method of Campbell *et al*. (1992) and Horwitz *et al*. (2000). Inductively coupled plasma optical emission spectroscopy (ICP-OES) (optima 2100^{TM} DV ICP-OES, Perkin Elmer Massachusetts, United states) was used in quantification of mineral contents of the samples. Concentrated nitric acid (8 mL -67–69 % w/v, VWR Chemicals, Fontenay-sous-Bois, France) and 30 % hydrogen peroxide (2 mL w/w from Sigma- Aldrich, USA) were added in digestion tubes containing 0.5 g of homogenized meat powder or bread samples. 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 on an Inductively coupled plasma optical emission spectroscopy (ICP-OES) (optima 2100^{TM} DV ICP-OES, Perkin Elmer Massachusetts, United states). Serial dilution of the standard, CatNo.43843 (Sigma-Aldrich, USA), was performed using 2 % nitric acid to obtain calibration standards of 400, 800, 2000 and 4000 μg/l and analysed by the ICP-OES 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-285.213, Fe-259.939, Mn-257.61, Ca-317.933, P-213.617, Mo-202.031, K-766.49, Al-396.153, Cu-224.7, Co- 228.616, Zn- 213.857.

3.8 Ultra-performance Liquid Chromatography Tandem Coupled to Mass Spectrometry (LC-MS/MS) Analysis of Amino Acids

The amino acids were determined according to methods described by Cheseto *et al*., (2017). Into a 5 mL micro-reaction vial, 100 mg of SHMP and the developed breads were each mixed with 1.5 mL of 6 N HCl and capped after carefully adding nitrogen gas. This was followed by hydrolysis process; done for 24 h at 110 °C then *in vacuo* evaporation to dryness. The hydrolysates were reconstituted in 1mL of 0.01 % formic acid/acetonitrile (95:5) followed by vortexing, sonication and centrifugation for 30 s, 30 min and at 1400 rpm, respectively and the supernatant analysed 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 contained (A) water and (B) methanol (solvent B) both acidified with formic acid (0.01 %). MassLynx version 4.1 SCN 712 (Waters) was used to acquire data. The AAs were identified by comparing mass spectrometric data, retention time, and co-injection of the hydrolysate with those of an authentic standard mixture of AAs. External quantification was performed to measure the amounts of each AA present. The standard solution (AAS 18) of AAs was bought from Sigma-Aldrich (Chemie GmbH, Munich, Germany). This was done in triplicates using different samples batch. Tryptophan decomposes into ammonium during acid hydrolysis and due to this it was not determined. Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during hydrolysis thus, the amounts of these acids were determined as sum of those respective components (Vilela *et al*., 2021).

3.9 Fatty Acid Analysis of Hen Meat and Breads

FAs composition was determined as per method described by Cheseto *et al*. (2020). Briefly, 100 mg of the SHMP or bread samples were each methylated into different fatty acid methyl esters (FAMEs). 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 analyse FAs as fatty acid methyl esters (FAMEs) in the samples. The GC was fitted with a (5 %-phenyl)-methylpolysiloxane (HP5 MS) low bleed capillary column (30 m × 0.25 mm i.d., 0.25 μm; J&W, Folsom, CA, USA). The carrier gas was helium at a flow rate of 1.25 mL/min. The oven temperature, programmed from 35 °C to 285 °C with a rising rate of 10 °C/ min had the initial and final temperatures set to hold for 5 min and 20.4 min, respectively. The ion source and quadrupole mass selective detector temperatures were maintained at 230 °C and 180 °C, respectively. Acquisition of spectral masses from electron impact (EI) were at acceleration energy of 70 eV. Fragment ions were analysed over 40–550 *m*/*z* mass range in the full scan mode. The filament delay time was set at 3.3 min. Linear calibration curve of peak area vs. concentration which gave coefficient of determination (R^2 = 0.9997) was generated by analysing serial dilution of the authentic standard methyl octadecenoate (0.2–125ng/ μl) by GC-MS in full scan mode which gave the following equation$Y = 5E + 07 + 2E + 07$. 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.

3.10 Determination of Water-soluble Vitamins by Ultra- Performance Liquid Chromatography (UPLC)-Photo Diode Array detector (PDA)

Determination of water-soluble vitamins by UPLC was done as describe in the Thermo Fisher Scientific, 2010 (*Thermo Fisher Scientific*, 2010). Briefly, the 100 mg of each SHMP or bread sample was weighed into 50 mL falcon tubes, 25mL of distilled water was added, followed by ultra-sonication for 15 min, then resulting solution 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 made 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 15μg/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 2.6 *u*m polar C18– 100 mm x 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.

3.11 Determination of Fat-Soluble Vitamins by UPLC

Determination of fat soluble vitamins was done according to the method described by Hosotani (2003) and Bhatnagar-Panwar (2015). The samples were weighed (0.5 g) into 25 mL tubes, 6 mL ethanol with 0.1 % (BHT) added and followed by a 1 min homogenization. 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 ice-cooled. In each tube, 4 mL of deionized water was added and vortexed after which, 5 mL of hexane was added and vortexed again. The tubes were then centrifuged at 3000 rpm/5 min, then the upper phase (hexane) transferred into centrifuge tube using Pasteur pipette. The mix was extracted 3 more times with 4 ×3 × 3 mL hexane and the extract transferred into 25 mL tube. In the extract, 5mL of deionized water was added, vortex for 1 min and then centrifuged at 3000 rpm/5 min. The hexane layer was separated into a clean test-tube and evaporated under nitrogen in the N-Evap to complete dryness. The extract was then reconstituted in to 1mL of methanol: tetrahydrouran (85:15 v/v), vortexed, sonicated for 30 s and 0.8 mL transferred into clean vials for analysis on 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.

3.12 Experiment 3: Microbial Determination

The breads samples were collected after cooling and were evaluated for Total Viable Count (TVC), positive *Staphylococcus* coagulase, thermo-tolerant *coliforms* at 45 °C, *Salmonella, E. coli,* yeast and mould following the method described by (APHA, 2015). The Tuttnauer Autoclave-Steam sterilizer, model: 5075ELV-D (US) was used for sterilization. Petri-dishes were purchased from KOBIAN Scientific- China measuring 90 mm ×15 mm while for incubation purposes PHcbi cooled incubator model: MIR-554-PE –Japan was used.

3.12.1 Media Preparation and Serial Dilutions

Laminar flow chamber were pre-sterilized before use. Ten grams of sample were aseptically weighed and were transferred to a pre-sterilized stomacher bag (Bagmixer 400 W, Interscience, St. Nom, France) with 90 mL of sterile peptone water (0.85 % (wt/vol) NaCl, 0.1 % (wt/vol) (OXOID LP0034) and 8.5 g/L NaCl) under aseptic conditions. It was then stomached in a bag mixer for 3 min at the speed of 8. Serial dilution were made as per requirement by transferring 1 mL of this 10^{-1} dilution to sterile test tubes containing 9 mL of sterile 0.1 % peptone water and it were mixed to get dilutions of 10^{-2}, 10^{-3}, and so on.

3.12.2 Total Viable Count (TVC)

Plate count agar (PCA- Oxoid CM0463) was prepared per manufacturers instructions. Briefly, 23.5 g of plate count agar were suspended in 1000 mL of distilled water. It was then boiled to dissolve the agar completely and it was sterilized by autoclaving at 15 psi for 15 min. The final pH of the media was adjusted to 7.0 at 25 °C. Sterilized petri-dishes in duplicate were inoculated aseptically with 1mL of aliquots from appropriate dilutions. About 12-15 mL of plate count agar melted and maintained at 44-46 °C were poured gently in to wash dish and it were rotated in clockwise and anticlockwise directions to mix the media. The plates were incubated at 35 °C for 48 h. Plates with 30-300 colonies were counted. The number of colonies was multiplied by the reciprocal of the dilution and was expressed as log cfu/g.

3.11.3 Yeast and Mould Count (YMC)

About 39 g of potato Dextrose Agar (Oxoid Ltd., United Kingdom) were suspended in 1000 mL distilled water. It was then boiled too dissolve the agar completely and were sterilized by autoclaving at psi for 15 min. The sterilized cooled media were acidified with 10 mL of 10 % sterile tertanic acid to obtain a pH of 3.5. After addition of the acid, precaution were taken not to heat the medium to preserve solidifying properties of agar. To each dish, 10-15 mL of melted agar maintained at 44-46 °C was poured. The agar was allowed to solidify and the plates were kept in inverted position in an incubator maintained at 35 °C for 12 h. 0.5 mL aliquots from appropriate dilutions were split between two plates and inoculated aseptically in duplicates. Sterile L- spreaders were used to evenly distribute the inoculums over the surface of the plates. The plates were incubated at 25 °C for 5 days. Black, white, red and greenish coloured colonies appearing on the plates were counted and were expressed as log cfu/g.

3.12.4 *Staphylococcus aureus* Count

Braid parker (BP- (Oxoid CM1127,)) agar were prepared as per manufactures instructions. About 63 g of BP agar were suspended in 950 mL of distilled water. It was then boiled to dissolve the agar completely and it was sterilized by autoclaving at 15 psi for 15 min. After cooling to 44-46 °C, 50 mL of sterile egg yolk potassium tellurite emulsion (Oxoid CM0276) was added to the medium. About 10- 15 mL of the agar maintained at 44-46 °C were poured in to each dish. The agar was left to solidify and the plates were kept in inverted position in an incubator maintained at 35 °C for 75 h. Colonies which were circular, smooth, convex, grey-black in colour, 3-4 mm diameter and having both zone of precipitation 9 turbidity) followed by a clear halo zone of lysis with buttery to gummy consistencies were counted after 75 h of incubation. Plates showing 20-200 colonies were counted and total number of colonies from the two plates at the 1:10 dilution was multiplied by reciprocal of the dilution and was expressed as log cfu/g.

3.12.5 Thermo-Tolerant *coliforms* at 45°C

Coliform count was done using Violet Red Bile (VRB). About 45.53g of VRB were suspended in 1000 mL of distilled water and it was boiled for 2min. Sterilized petri-dish were inoculated aseptically with 1 mL of aliquots from appropriate dilution. About 12-15 mL of agar melted and maintained at 44-46 °C were poured gently into each dish and were rotated in to clockwise and anticlockwise directions to mix the media uniformly. The plates were incubated at 35 C for 48h. Plates showing 30-300 colonies were counted and the number of colonies were multiplied by the reciprocal of the dilution and were expressed as log cfu/g.

3.12.6 *Salmonella* Count

Enumeration of Salmonella species were done using Salmonella-Shigella agar. Approximately, 10 g of each sample were added to 90 mL of a sterile non-selective nutrient broth for a non-selective pre-enrichment at 35 °C for 24 h. The sample was then aseptically transferred into a sterile tetrathionate agar for a selective pre-enrichment at 35 °C for 18-24 h. Sample was then streaked on a sterile solidified salmonella-shigella agar (Selective differential agar) in a petri dish. The petri-dishes were then incubated at 35 °C for 24 h after which the colonies which are colourless or with black centres were examined. The colonies with such colour characteristics were then inoculated (stab butt and the slants streaked) into slants of triple sugar iron agar for 24 h at 35 °C.

3.12.7 *E. coli*

About 1 mL of each of the above dilutions was transferred on to sterilized petri-dishes in duplicates and 10-15 mL of media (Sorbitol MacConkey Agar) maintained at 44 -46 °C was added and rotated in clockwise and anticlockwise directions to mix. Plates were incubated in inverted position at 35 -37 °C for 24 h.

3.13 Consumer Acceptability Test of the Breads

Consumer acceptability test of the bread samples was performed according to Haber *et al*. (2019) with slight modification. Briefly, 60 naive panellists selected randomly among postgraduate students and staff of International Centre of Insect Physiology and Ecology, Nairobi, Kenya. The panellists were given instructions, guidelines and objectives of the test before commencement according to the institution's ethical requirement. Overall acceptability were evaluated on a five-point hedonic scale, in which 1 represented 'like very much' and 5 'dislike very much'. The samples were then assigned random codes and served to each panellist in identical containers at individual booths under room temperature conditions. Drinking water was also provided for panellists to rinse their mouths before and after tasting each bread sample.

3.14 Statistical Analysis

R software version 1.3.1093-1 (R Core Team, 2020) for windows was used for statistical analysis. The data was subjected to normality test ($p \geq 0.05$ – Data is normal distributed) using Shapiro –Wilk test. One-way Analysis of Variance (ANOVA) was performed to test (i) the effect of substituting fishmeal with Black Soldier Fly Larvae in hens' diet on proximate, minerals, vitamins, amino acids and fatty acids composition of spent hen meat (ii) the effect of substituting white wheat flour with SHMP on proximate, minerals, vitamins, amino acids and fatty acids composition, microbial content and consumer acceptability of resultant bread. The means were considered significantly different at 5% significant level ($p<0.05$). Student Neuman Kuel' Test (SNK) Test was performed for mean separation at $\alpha= 0.05$.

CHAPTER FOUR
RESULTS

4.1 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 (p<0.05) across the various diet types (Table 4.1). However, crude protein accounted for the greater part of the proximate composition.

Table 4.1: Comparison of Proximate Composition of spent hen meat powder (%) on dry matter basis

Parameter	Moisture	Crude Ash	Crude Protein	Crude Fat
T1	4.5 ± 0.50^{a}	7.5 ± 0.14^{a}	85.7 ± 0.64^{a}	8.1 ± 0.07^{a}
T2	4.2 ± 0.76^{a}	7.4 ± 0.10^{a}	85.8 ± 0.42^{a}	8.2 ± 0.82^{a}
T3	3.8 ± 0.29^{a}	7.2 ± 0.36^{a}	86.5 ± 0.21^{a}	8.2 ± 0.78^{a}
T4	4.3 ± 0.29^{a}	7.0 ± 0.32^{a}	85.6 ± 0.47^{a}	8.5 ± 0.58^{a}
T5	4.8 ± 0.29^{a}	7.0 ± 0.21^{a}	86.2 ± 0.48^{a}	8.6 ± 0.32^{a}

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: Black Soldier Fly Larvae ratios.

4.2 Mineral Profile of Spent Hen Meat Powder

The mineral composition of the various meat types varied significantly, except for magnesium, phosphorus and iron (Table 4.2). However, potassium, phosphorous, calcium and copper were the most abundant minerals in meat from hen fed on the various diets.

Table 4.2: Mineral Content of Spent Hen Meat

Mineral	**T1**	**T2**	**T3**	**T4**	**T5**	**P-value**	**RDA age 12-18 (mg/day)***
Iron (mg/100g)	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}	ns	13.5
Phosphorus (mg/100g)	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}	ns	1250
Manganese (µg/100g)	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}	p<0.01	
Zinc (mg/100g)	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}	p<0.05	8.5
Magnesium (mg/100g)	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}	ns	375
Potassium (mg/100g)	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}	p<0.001	3500
Sodium (mg/100g)	$183.\ 5 \pm 0.91^{c}$	242.5 ± 6.04^{a}	201.3 ± 9.91^{b}	167.1 ± 0.00^{d}	194.2 ± 8.10^{bc}	p<0.001	2000
Aluminum (mg/100g)	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}	p<0.001	
Copper (µg/100g)	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}	p<0.001	
Calcium (mg/100g)	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}	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: Black Soldier Fly Larvae 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: Black Soldier Fly Larvae ratios. *: (WHO, 2006).

4.3 Amino Acids Profile of Spent Hen Meat

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 Black Soldier Fly Larvae meal (Table 4.3). 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.

Table 4.3: Amino Acid Composition of spent hen meat powder in mg/g of Sample

Amino Acid (mg/g)	**T1**	**T2**	**T3**	**T4**	**T5**	**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]	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]	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]	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]	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]	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]	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]	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]	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							
Amino Acid	**T1**	**T2**	**T3**	**T4**	**T5**	**P-value**	**RDA during**
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]	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]	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]	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]	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]	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]	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]	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]	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]	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: Black Soldier Fly Larvae ratios; EAA-Essential Amino Acids; NEAA- Non Essential Amino Acids, [2]: Phenylalane + trysine, [3]: Metrionine +Cysteine.

4.4 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 4.4). Nine fatty acids were detected in meat products from hen diet with Black Soldier Fly Larvae meal but absent in meats from hen fed 100 % FM diet. Between meat products from hen fed diet with 100 % Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal as substitute of FM in the hen diets.

Table 4. 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}

RT	FAMEs	FA	ω-n (Δn)	T1	T2	T3	T4	T5
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 33 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

RT (min)	FAMEs	FA	ω-n (Δn)	T1	T2	T3	T4	T5
				MUFAs				
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-9t	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]

RT (min)	FAMEs	FA	ω-n (Δn)	T1	T2	T3	T4	T5
25.90	Methyl (10Z) nonadecenoate	(10Z)-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]
		Σ MUFA		2656.7	3214.0	3248.7	3277.7	3701.4
			PUFAs					
25.18	Methyl (9Z,12Z)-octadecadienoate	Linoleic acid	C18:2, n-6	1829.9 ± 22.51[a]	1779.4 ± 4.75[b]	1732.9 ± 4.86[c]	1667.7 ± 5.45[d]	1644.3 ± 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]

RT	**FAMEs**	**FA**	**ω-n (Δn)**	**T1**	**T2**	**T3**	**T4**	**T5**
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]
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]
		Σ PUFAs		2396.6	2330.3	2277.8	2205.1	2177.2
		ΣUFAs		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 row**s** 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: Black Soldier Fly Larvae 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.

4.5 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 Black Soldier Fly Larvae meal (Table 4.5). The B vitamins except B1 displayed significantly increasing trend with increasing levels of Black Soldier Fly Larvae meal in the hen diets. Retinol and nicotinamide were the most abundant vitamins.

Table 4.5: Vitamin profile (mg/kg) of spent hen meat powder

Vitamin (mg/Kg)	**T1**	**T2**	**T3**	**T4**	**T5**	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}	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}	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}	p<0.001
Vitamin 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}	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}	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}	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}	p<0.001
Vitamin B9	968.8 ± 68.73^{a}	-	206.9 ± 9.77^{b}	-	-	p<0.001

Values are expressed as mean ± SE of triplicate determinations. Different small superscript letters following each other within row**s** 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: Black Soldier Fly Larvae ratios

4.6 Proximate Composition of Enriched Bread

Moisture, crude protein, crude ash, crude fat and carbohydrate content of breads were significantly different (p<0.05) across the breads (Table 4.6). Moisture content of bread increased by 1.1 folds between bread without SHMP and bread containing 30% SHMP. The crude protein, crude ash and crude fat increased by 2.0-2.4, 1.6-2.0, and 1.3-1.7 folds in chicken meat powder-breads, respectively whereas carbohydrates reduced by 0.7 folds compared to the reference bread.

Table 4.6: Proximate composition (%) of enriched bread on dry mater basis

Parameter	B_0	B_{20}	B_{25}	B_{30}	$F_{(3,8)}$	P-value
Moisture	36.5 ± 0.50^{b}	38.8 ± 0.29^{a}	40.0 ± 1.00^{a}	40.0 ± 0.50^{a}	20.63	0.001
Crude protein	12.7 ± 0.39^{d}	25.1 ± 0.30^{c}	27.3 ± 0.27^{b}	31.0 ± 0.97^{a}	603.5	0.001
Crude ash	3.5 ± 0.38^{c}	5.7 ± 0.08^{b}	6.1 ± 0.39^{ab}	6.9 ± 0.40^{a}	51.15	0.001
Crude fat	3.9 ± 0.03^{d}	4.9 ± 0.02^{c}	5.8 ± 0.10^{b}	6.7 ± 0.06^{a}	1186.6	0.001
Crude fiber	0.8 ± 0.01	0.8 ± 0.08	0.9 ± 0.04	0.9 ± 0.05	2.3	ns
Carbohydrate	79.0 ± 0.67^{a}	63.5 ± 0.30^{b}	59.9 ± 0.18^{c}	54.4 ± 0.65^{d}	1333.3	0.001

Values are presented as means ± SD of triplicate determinations. Means followed by similar letters are not significantly different at p<0.05. Breads made from B_0: bread 1000g white wheat flour (Control); B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP; ns: not significant.

4.7 Amino Acid Profile of the Breads

Blending white wheat flour with spent hen meat powder caused significant variations (p<0.05) in the levels of all the amino acids examined (Table 4.7). Leucine and isoleucine were the most dominant essential amino acids whereas proline and glutamic acid were the predominant non-essential amino acids in all the bread types.

Table 4.7: Amino acid profile (mg/g dry matter) of bread

Amino acid	B_0	B_{20}	B_{25}	B_{30}
Phenylalanine	5.2 ± 0.02^{d}	8.5 ± 0.04^{c}	11.4 ± 0.07^{b}	12.5 ± 0.04^{a}
Isoleucine	6.8 ± 0.56^{d}	10.8 ± 0.23^{c}	13.8 ± 0.23^{b}	15.9 ± 0.09^{a}
Leucine	9.0 ± 0.05^{d}	15.2 ± 0.04^{c}	20.2 ± 0.03^{b}	23.2 ± 0.04^{a}
Methionine	2.6 ± 0.04^{d}	3.7 ± 0.09^{c}	4.3 ± 0.21^{b}	4.8 ± 0.11^{a}
Valine	4.7 ± 0.10^{d}	7.7 ± 0.09^{c}	9.6 ± 0.07^{b}	10.7 ± 0.04^{a}
Histidine	2.2 ± 0.11^{d}	3.4 ± 0.10^{c}	5.8 ± 0.10^{b}	7.2 ± 0.1^{a}
Lysine	2.5 ± 0.09^{d}	7.5 ± 0.11^{c}	9.4 ± 0.21^{b}	11.2 ± 0.27^{a}
Threonine	3.3 ± 0.28^{d}	6.0 ± 0.19^{c}	8.2 ± 0.27^{b}	10.2 ± 0.09^{a}
Total EAA	36.3	62.8	82.7	95.7
Tyrosine	4.8 ± 0.19^{c}	5.8 ± 0.21^{b}	6.3 ± 0.24^{b}	7.4 ± 0.07^{a}
Proline	19.6 ± 0.55^{c}	24.1 ± 1.03^{b}	26.8 ± 0.58^{a}	27.6 ± 0.13^{a}
Glycine	4.6 ± 0.30^{d}	6.3 ± 0.35^{c}	7.5 ± 0.20^{b}	8.4 ± 0.30^{a}
Alanine	4.5 ± 0.28^{d}	6.5 ± 0.11^{c}	7.8 ± 0.40^{b}	9.7 ± 0.16^{a}
Cystine	3.8 ± 0.21^{c}	4.6 ± 0.52^{b}	5.4 ± 0.21^{a}	6.3 ± 0.10^{a}
Glutamic acid	35.6 ± 1.01^{b}	41.6 ± 2.01^{a}	42.6 ± 1.01^{a}	42.6 ± 2.01^{a}
Aspartic acid	4.6 ± 0.30^{b}	7.5 ± 0.48^{a}	8.1 ± 0.16^{a}	8.0 ± 0.21^{a}
Serine	5.7 ± 0.16^{d}	6.3 ± 0.20^{c}	7.1 ± 0.26^{b}	7.9 ± 0.11^{a}
Arginine	4.5 ± 0.36^{d}	8.9 ± 0.22^{c}	10.5 ± 0.19^{b}	12.2 ± 0.10^{a}

Amino acid	**B_0**	**B_{20}**	**B_{25}**	**B_{30}**
Total NEAA	87.7	111.6	122.1	130.1
Total AA	124.0	174.4	204.8	225.8
EAA/AA	29%	36%	40%	42%
EAA/NEAA	0.4	0.6	0.7	0.7

Values are presented as means ± SD of triplicate determinations. Breads made from B0: bread 1000g white wheat flour (Control); B20: 800g white wheat flour + 200g SHMP; B25: 750g white wheat flour + 250g SHMP; B30: 700g white wheat flour + 300g SHMP; means followed with similar letter are not significantly different, AA: amino acid, NEAA: non-essential amino acids, EAA: essential amino acids.

4.8 Fatty Acid Profiles of the Breads

A total of forty-eight fatty acids (FAs) were identified in the experimental breads; saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) contributed 26, 14 and 8 components, respectively (Table 4.8). The SFAs, MUFAs and PUFAs accounted for 26-34%, 16-17% and 49-54% of the total FAs, respectively in all the breads. Methyl (9Z,12Z,15Z)-otadecatrienoate (ALA), methyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosahexaenoate (DHA) and methyl (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoate (EPA) were the main omega 3 FAs detected and were significantly ($p<0.05$) higher in the chicken enriched breads than the control breads. The ratios PUFA/SFA and n-6/n-3 ranged 4.7-4.9 and 1.4-2.3, respectively.

Table 4.8: Fatty acid profile (µg/g dry matter) of bread

RT (Min)	FAMEs	B_0	B_{20}	B_{25}	B_{30}
		Saturated Fatty Acids (SFAs)			
13.87	Methyl octanoate	1.3 ± 0.36^{b}	3.8 ± 0.73^{a}	4.3 ± 0.43^{a}	4.7 ± 0.48^{a}
15.33	Methyl nonanoate	0.4 ± 0.03^{c}	0.7 ± 0.06^{b}	0.8 ± 0.03^{b}	0.9 ± 0.03^{a}
16.49	Methyl decanoate	3.7 ± 0.10^{d}	6.5 ± 0.10^{c}	11.8 ± 0.92^{b}	13.8 ± 1.01^{a}
17.77	Methyl undecanoate	-	1.1 ± 0.17	1.9 ± 1.00	1.8 ± 0.43
18.46	Methyl 10-methyl undecanoate	-	3.6 ± 0.58^{b}	3.7 ± 0.53^{b}	4.8 ± 0.40^{a}
18.91	Methyl dodecanoate	122.4 ± 4.90^{c}	205.0 ± 11.76^{b}	220.1 ± 2.95^{ab}	226.9 ± 4.37^{a}
19.70	Methyl 11-methyl-dodecanoate	2.9 ± 0.59^{b}	5.5 ± 0.71^{a}	6.8 ± 1.04^{a}	6.8 ± 0.64^{a}
19.79	Methyl 10-methyl dodecanoate	5.8 ± 0.25^{d}	8.7 ± 0.27^{c}	9.9 ± 0.43^{b}	11.9 ± 0.56^{a}
20.12	Methyl tridecanoate	4.6 ± 0.13^{c}	8.4 ± 0.48^{b}	9.1 ± 0.48^{b}	12.3 ± 1.02^{a}
20.79	Methyl 12-methyl tridecanoate	5.3 ± 0.86^{c}	13.4 ± 0.61^{b}	18.5 ± 4.65^{ab}	21.7 ± 1.52^{a}
21.24	Methyl tetradecanoate	64.6 ± 22.24^{d}	308.7 ± 6.47^{c}	382.4 ± 10.33^{b}	530.1 ± 29.51^{a}
21.76	Methyl 4,8,12-trimethyl tridecanoate	2.8 ± 0.10^{d}	3.8 ± 0.10^{c}	4.6 ± 0.26^{b}	8.7 ± 0.37^{a}
21.89	Methyl 13-methyl tetradecanoate	22.1 ± 4.36	29.4 ± 6.63	32.5 ± 4.52	33.5 ± 4.62
21.98	Methyl 12-methyl tetradecanoate	12.0 ± 4.29^{b}	38.2 ± 2.04^{a}	46.4 ± 4.50^{a}	45.6 ± 3.10^{a}
22.29	Methyl pentadecanoate	28.4 ± 7.81^{b}	76.5 ± 31.07^{a}	100.3 ± 12.38^{a}	104.9 ± 9.57^{a}
23.50	Methyl hexadecanoate	1085.0 ± 12.64^{b}	2184.3 ± 13.42^{a}	2203.0 ± 29.03^{a}	2229.6 ± 4.89^{a}
23.75	Methyl 14-methyl hexadecanoate	7.8 ± 1.82	8.5 ± 0.42	7.8 ± 0.50	8.6 ± 1.02

RT (Min)	FAMEs	B_0	B_{20}	B_{25}	B_{30}
23.97	Methyl 15-methyl hexadecanoate	26.8 ± 0.92^{b}	41.5 ± 4.33^{a}	44.4 ± 0.64^{a}	47.5 ± 1.33^{a}
24.26	Methyl heptadecanoate	0.3 ± 0.25^{c}	5.6 ± 0.37^{b}	6.2 ± 0.69^{ab}	6.9 ± 0.58^{a}
25.52	Methyl octadecanoate	122.7 ± 10.77^{c}	357.4 ± 4.40^{b}	370.9 ± 7.18^{ab}	379.0 ± 4.50^{a}
26.98	Methyl eicosenoate	38.2 ± 6.81^{b}	79.6 ± 5.24^{a}	79.5 ± 3.96^{a}	92.3 ± 3.9^{a}
27.80	Methyl heneicosanoate	7.9 ± 3.77^{b}	18.3 ± 5.08^{a}	23.9 ± 1.68^{a}	24.2 ± 0.97^{a}
28.59	Methyl docosanoate	12.6 ± 1.73^{b}	25.2 ± 1.24^{a}	30.2 ± 3.01^{a}	31.1 ± 4.26^{a}
29.35	Methyl tricosanoate	6.9 ± 1.73^{b}	15.9 ± 1.68^{a}	16.0 ± 1.85^{a}	18.2 ± 1.48^{a}
30.13	Methyl tetracosanoate	7.8 ± 2.01^{b}	17.5 ± 0.86^{a}	17.0 ± 2.29^{a}	16.7 ± 1.72^{a}
32.04	Methyl hexacosanoate	18.5 ± 1.70	16.9 ± 2.52	16.5 ± 1.95	18.5 ± 0.91
	Monounsaturated Fatty Acid (MUFAs)				
20.95	Methyl (11*Z*)-tetradecenoate	14.8 ± 0.97^{b}	15.6 ± 2.21^{ab}	16.1 ± 1.55^{ab}	17.5 ± 0.89^{a}
21.08	Methyl (9*Z*)-tetradecenoate	28.0 ± 1.79^{b}	40.1 ± 10.34^{b}	64.2 ± 3.47^{a}	66.5 ± 4.89^{a}
21.69	Methyl 10-undecenoate	2.8 ± 0.34^{b}	6.1 ± 1.39^{ab}	6.0 ± 1.21^{ab}	5.6 ± 1.64^{a}
22.12	Methyl (9*E*)-dodecenoate	4.3 ± 0.15^{b}	7.1 ± 0.66^{a}	8.4 ± 0.96^{a}	8.5 ± 1.17^{a}
22.14	13-Methyl (9*E*)-tetradecenoate	0.9 ± 0.46^{b}	3.2 ± 0.50^{a}	3.2 ± 0.19^{a}	3.2 ± 0.23^{a}
23.19	Methyl (9*Z*)-hexadecanoate	352.4 ± 10.17^{c}	843.5 ± 9.65^{b}	861.5 ± 5.24^{b}	935.4 ± 16.56^{a}
24.11	Methyl (10*Z*)-heptadecenoate	5.0 ± 0.97^{b}	12.5 ± 1.36^{a}	13.2 ± 1.32^{a}	14.4 ± 0.85^{a}
24.67	Methyl (9*E*)-Octadecenoate	2.9 ± 0.16^{b}	3.8 ± 0.67^{ab}	3.5 ± 0.40^{ab}	4.2 ± 0.47^{a}

RT (Min)	FAMEs	ω-n (Δn)	B_0	B_{20}	B_{25}	B_{30}
25.14	Methyl (9*Z*)-octadecenoate		393.2 ± 4.49^{c}	428.7 ± 5.08^{b}	431.7 ± 3.05^{b}	447.0 ± 1.65^{a}
25.97	Methyl (10*Z*)-nonadecenoate		33.1 ± 1.77^{c}	71.8 ± 4.49^{b}	73.8 ± 4.00^{ab}	83.1 ± 5.57^{a}
26.15	Methyl (10*Z*)-nonadecenoate		14.4 ± 2.73^{c}	36.3 ± 1.09^{b}	36.9 ± 2.57^{b}	46.9 ± 5.62^{a}
26.82	Methyl (11*Z*)-eicosenoate		102.6 ± 17.52^{b}	202.9 ± 25.73^{a}	206.5 ± 12.02^{a}	226.6 ± 12.47^{a}
28.41	Methyl (13*Z*)-docosenoate		11.0 ± 0.96^{b}	32.0 ± 3.97^{a}	33.0 ± 3.32^{a}	38.3 ± 2.91^{a}
29.95	methyl (15*E*)-tetracosenoate		5.3 ± 1.77^{c}	17.2 ± 2.48^{b}	16.7 ± 1.77^{b}	22.5 ± 1.92^{a}
	Polyunsaturated Fatty Acids (PUFAs)					
24.80	Methyl (6*Z*,9*Z*,12*Z*)-octadecatrienoate	C18:3, n-6	11.4 ± 1.36^{c}	23.0 ± 1.27^{b}	23.6 ± 1.50^{b}	27.5 ± 0.72^{a}
25.18	Methyl (9*Z*,12*Z*)-octadecadienoate	C18:2, n-6	2661.5 ± 23.58^{d}	3683.6 ± 7.32^{c}	3843.7 ± 23.45^{b}	4187.8 ± 23.43^{a}
25.83	Methyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate	C18:3, n-3	469.0 ± 6.92^{d}	665.9 ± 5.22^{c}	704.6 ± 2.69^{b}	774.3 ± 6.91^{a}
26.21	Methyl (9*Z*,11*E*,13*E*)-octadecatrienoate	C18:3, n-6	35.9 ± 1.69	34.0 ± 2.54	33.4 ± 2.62	35.6 ± 1.50
26.44	Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoate	C20:4, n-6	189.0 ± 79.03^{b}	281.8 ± 22.95^{ab}	327.8 ± 28.50^{a}	334.1 ± 9.97^{a}
26.62	Methyl (8*Z*,11*Z*,14*Z*)-eicosatrienoate	C20:3, n-6	109.5 ± 21.44	113.1 ± 4.25	105.1 ± 12.06	124.6 ± 11.86
28.05	Methyl (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosahexaenoate	C22:6, n-3	111.3 ± 38.1	131.8 ± 26.96	141.4 ± 8.56	185.5 ± 14.26
28.16	Methyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate	C20:5, n-3	43.5 ± 4.93	46.9 ± 4.37	50.9 ± 3.91	52.3 ± 4.07

Table 4.8: Fatty acid profile (µg/g dry matter) of bread … continued

∑ SFA	1610.8	3484.0	3668.5	3901
∑ MUFA	970.7	1720.8	1774.7	1919.7
∑ PUFA	3631.1	4980.1	5230.5	5721.7
∑UFA	4601.8	6700.9	7005.2	7641.4
∑ n-6	3007.3	4135.5	4333.6	4709.6
∑ n-3	623.8	844.6	896.9	1012.1
n-6/n-3	4.8	4.9	4.8	4.7
PUFA/SFA	2.3	1.4	1.4	1.5
Total FA	6212.6	10184.9	10673.7	11542.4

Breads made from B_0: bread 1000g white wheat flour (Control) +0g SHMP; B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP, Mean ± SE (standard error) of triplicate determinations; means followed with different letters are significantly different, FA: fatty acid, 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.

4.9 Vitamin Profile of the Breads

The levels of all the vitamins examined except ɣ-tocopherol varied significantly (p<0.05) with the increasing levels of chicken powder in the breads (Table 4.9). The values of retinol, nicotinic acid and pantothenic acid increased by 1.4-4.1, 1.8-2.1 and 1.4-1.5-folds, respectively between the control breads and those with chicken powder added. Additionally, the levels of the vitamins; ascorbic acid, α- tocopherol, ɣ- tocopherol and riboflavin were significantly higher in the control breads than the breads with chicken powder included.

Table 4.9: Concentration of vitamins (mg/kg dry matter) of breads

Vitamin	**B_0**	**B_1**	**B_2**	**B_3**	**P-value**
Retinol	10.0 ± 0.05^{d}	14.0 ± 0.09^{c}	30.0 ± 0.11^{b}	41.0 ± 0.34^{a}	0.001
Ascorbic acid	128.9 ± 13.68^{a}	97.6 ± 4.12^{b}	93.3 ± 11.29^{b}	89.2 ± 2.26^{b}	0.01
α- tocopherol	64.0 ± 0.29^{a}	16.0 ± 0.18^{b}	15.0 ± 0.02^{b}	12.0 ± 0.14^{b}	0.001
ɣ- tocopherol	13.0 ± 0.10	12.0 ± 0.08	11.0 ± 0.05	8.0 ± 0.07	0.001
Riboflavin	6.3 ± 0.59^{a}	5.9 ± 0.38^{a}	5.5 ± 0.80^{b}	4.5 ± 0.14^{b}	0.05
Nicotinic acid	66.2 ± 3.79^{b}	113.7 ± 7.50^{b}	137.2 ± 9.97^{a}	138.7 ± 12.02^{a}	0.001
Pantothenic acid	445.9 ± 39.63^{b}	613.7 ± 39.38^{a}	649.7 ± 41.03^{a}	657.7 ± 34.82^{a}	0.001

Breads made from B_0: bread 1000g white wheat flour (Control) +0g SHMP; B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP, Mean ± SE (standard error) of triplicate determinations; means followed with different letters are significantly different.

4.10 Mineral Profile of the Breads

The incorporation of chicken meat powder into the bread formulation mixes significantly (p<0.05) influenced the content of all minerals identified with the exception of cobalt which was below the detection limit (Table 4.10). The predominant minerals in the breads were sodium, potassium, phosphorus, magnesium and calcium especially in the breads enriched with chicken powder.

Table 4.10: Mineral profile (mg/100g dry matter) of breads

Mineral (mg/100g)	B_0	B_{20}	B_{25}	B_{30}	RDA age 12-18 (mg/day)*	P-value
Iron	2.5 ± 0.19^{b}	2.8 ± 0.12^{ab}	3.0 ± 0.18^{a}	3.0 ± 0.09^{a}	13.5	0.05
Phosphorus	20.1 ± 0.85^{b}	32.5 ± 0.83^{a}	35.0 ± 2.90^{a}	35.1 ± 0.34^{a}	1250	0.001
Manganese	0.4 ± 0.02^{c}	0.5 ± 0.02^{c}	0.6 ± 0.02^{b}	0.7 ± 0.03^{a}	-	0.001
Zinc	3.5 ± 0.02^{c}	3.8 ± 0.17^{bc}	3.9 ± 0.08^{ab}	4.1 ± 0.09^{a}	8.5	0.01
Magnesium	18.7 ± 0.98^{c}	22.3 ± 0.72^{b}	23.0 ± 0.97^{b}	28.0 ± 0.62^{a}	375	0.001
Molybdenum	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	-	ns
Potassium	162.1 ± 5.36^{c}	212.2 ± 8.16^{b}	228.2 ± 8.16^{ab}	245.5 ± 5.65^{a}	3500	0.001
Sodium	215.0 ± 5.80^{d}	232.1 ± 5.62^{c}	286.1 ± 11.85^{b}	318.9 ± 12.38^{a}	2000	0.001
Aluminum	0.7 ± 0.02^{d}	1.6 ± 0.05^{c}	1.9 ± 0.13^{b}	5.9 ± 0.32^{a}	-	0.001
Copper	0.1 ± 0.01^{c}	0.1 ± 0.02^{bc}	0.2 ± 0.01^{ab}	0.2 ± 0.00^{a}	-	0.01
Calcium	16.3 ± 1.06^{d}	32.5 ± 1.77^{c}	38.9 ± 2.51^{b}	48.9 ± 1.10^{a}	1200	0.001

Breads made from B_0: bread 1000g white wheat flour (Control) +0g SHMP; B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP, Mean ± SE (standard error) of triplicate determinations; means followed with different letters are significantly different.

4.11 Microbial Levels in Breads Enriched with chicken meat powder

All samples showed no countable Total Viable Counts, *Staphylococcus aurous*, Yeast and mould, coliforms, *Salmonella spp* and *E. coli* as indicated in Table 4.11.

Table 4.11: Microbial Levels in Breads Enriched with chicken meat powder

	TVC (Counts)	*Staphylococcus aureus* (log10 cfu/g)	Mould and yeast count (log10 cfu/g)	Coliforms (log10 cfu/g)	*Salmonella spp* (log10 cfu/g)	*E. coli* (log10 cfu/g)
B0	<30	ND	ND	ND	ND	ND
B20	<30	ND	ND	ND	ND	ND
B25	<30	ND	ND	ND	ND	ND
B30	<30	ND	ND	ND	ND	ND

B_0**:** Breads made from 1000g white wheat flour (Control) +0g SHMP; B_{20}: Breads made from 800g white wheat flour + 200g SHMP; B_{25}: Breads made from 750g white wheat flour + 250g SHMP; B_{30}: Breads made from 700g white wheat flour + 300g SHMP; TVC: Total Viable Count.

4.12 Overall acceptability of the Breads Enriched with Chicken Meat Powder

The mean sensory scores of the breads are as shown in Table 4.12. Chicken meat powder significantly affected the scores of overall acceptability.

Table 4.12: Mean Sensory Scores of Breads Enriched with chicken meat powder

Sensory Attribute	**B_0**	**B_{20}**	**B_{25}**	**B_{30}**
Overall Acceptability	3.8 ± 0.86^b	3.8 ± 0.87^b	4.3 ± 0.58^a	4.5 ± 0.53^a

Values are presented as means ± SD of triplicate determinations. Means followed by similar letters are not significantly different at $p<0.05$. Breads made from B_0: bread 1000g white wheat flour (Control); B_{20}: 800g white wheat flour + 200g SHMP; B_{25}: 750g white wheat flour + 250g SHMP; B_{30}: 700g white wheat flour + 300g SHMP; ns: not significant.

CHAPTUR FIVE
DISCUSSION

5.1 Proximate Composition of Spent Hen Meat Powder

The proximate compositions of meat derived hen subjected to diet with varying inclusion levels of Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal is comparable to meat from broiler chicken fed on commercially available industrial feeds containing fish meal (Aslam *et al*., 2000).

Given that the protein contents in meat from spent hen fed diet with Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal 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.

5.2 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 (Kokoszynski *et al*., 2016; Lin *et al*., 2014; 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 % Black Soldier Fly Larvae meal (T3) can contribute 90 % of Fe, 92 % of P, 115 % of Zn, 35 % of Mg, 33 % of K, 20 % of Na and 57 % of Ca RDAs for 12–18 year old school-going child (WHO, 2006).

5.3 Amino Acid Profile of Spent Hen Meat Powder

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, Cullere *et al*. (2018) and Vilela *et al*. (2021) also reported an increase in some amino acids compared to control when Black Soldier Fly Larvae meal 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 Vilela *et al*. (2021) and Yirmaga (2017). Furthermore, the levels of individual amino acids of SHMP are within the ranges of amino acids profile of chicken broiler meat (Vilela *et al*., 2021; 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 analysed 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 analysed 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 Black Soldier Fly Larvae meal, particularly associated to taste (Bachmanov *et al*., 2016). However, the concentration of these amino acids did not vary significantly with increased integration of Black Soldier Fly Larvae meal in the diets. Dietary alterations using Black Soldier Fly Larvae meal on the stability of amino acid composition in meat, that are main precursor of bitter taste and flavour (valine, isoleucine, leucine, phenylalanine, methionine, arginine, and proline) have been reported in other studies by Bachmanov *et al*. (2016), except for isoleucine and methionine. Furthermore, AAs that enhance the savoury 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 & Sclafani, 2011, 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 Black Soldier Fly Larvae meal by consumers would open new avenues for uses of these amino acids as flavour, nutritive, and therapeutic agents.

5.4 Fatty Acid Composition Spent Hen Meat

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 *et al*., 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 *et al*., 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 Black Soldier Fly Larvae meal, 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 (Schiavone *et al.*, 2019; Vilela *et al*., 2021). 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 Panel on Dietetic Products, Nutrition, 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 Black Soldier Fly Larvae meal in the chicken diet (Cullere *et al*., 2018; Vilela *et al*., 2021). This has been a major drawback in the adoption of Black Soldier Fly Larvae in whole substitution of conventional protein sources such as fish and soya bean in poultry diet. However, fatty acid profile of Black Soldier Fly Larvae meal can be modified by modulating rearing substrate (Spranghers *et al*., 2017; Tschirner *et al*., 2015). For example, the concentration of SFAs in meat can be reduced by increasing levels of n-3 in diet. Furthermore, defatting of Black Soldier Fly Larvae meal has also been suggested as a viable option of reducing the amount of fatty acids in the meal (Kim *et al*., 2020; Wang *et al*., 2017).

In the present study, the relatively low amount of PUFAs especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in Black Soldier Fly Larvae meal (Secci *et al*., 2018) may probably explain why SHMP from hens fed on diet containing Black Soldier Fly Larvae meal had lower levels of PUFA. Furthermore, the negative association between PUFA levels and Black Soldier Fly Larvae meal dietary levels concur with the results reported in several published works in literature (Vilela *et al*., 2021). PUFA concentration in Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal) may have been introduced in the SHMPs by Black Soldier Fly Larvae meal in the diets. The dominant SFAs, MUFAs and PUFAs reported in the present study mirrored that of the diet Black Soldier Fly Larvae meal 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 *et al*., 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; 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 *et al*., 2002). The significant reduction in concentration of arachidonic acid in meat from hen fed diet with increasing inclusion levels of Black Soldier Fly Larvae meal up to 100 % represents great benefit to the consumer as reported by Vilela *et al*. (2021). To assess the impact of diet on cardiovascular health PUFA/SFA ratio is the most commonly used ratio (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 *et al*., 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 Panel on Dietetic Products, Nutrition, 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 *et al*., 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 Vilela *et al*. (2021) reported a ratio ranging between 17.6 and 11.2 on meat from broiler chicken fed diet with Black Soldier Fly Larvae meal. 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 Black Soldier Fly Larvae meal 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 *et al*., 2002).

5.5 Vitamin Composition of Spent Hen Meat Powder

There was increment in the levels of α- tocopherol, nicotinamide, vitamin B2 and vitamin B5 in meat products from hen fed diet with Black Soldier Fly Larvae meal 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 Black Soldier Fly Larvae meal, 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.

5.6 Proximate Composition of Enriched Bread

The moisture content of bread directly corresponds to the amount of water absorbed during mixing of the dough. The higher moisture content of the chicken powder enriched

breads may be attributable to the presence of increased levels of water-binding substances such as protein and fibre mainly consisting of more engaging hydrophilic groups (Adeleke *et al*, 2010; Bhatt *et al*., 2015; Kurek *et al*., 2016; Monteiro *et al*., 2019). In this study, chicken meat powder-breads expressed significantly higher protein content relative to the control breads, diluting the significant effect of hydrophobicity of >35% amino acids in wheat gluten (Iwaki *et al*., 2021) hence accounting for the disparity in moisture contents. Similar observation was made in other studies involving wheat bread enrichment with spent hen meat powder (Verma *et al*., 2015), chicken meat powder (Cakmak *et al*., 2013), cheese (Malomo *et al*., 2012), grasshopper powder (Haber *et al*., 2019) and strip loin beef powder (Kulcu *et al*., 2019). In contrast, Monteiro *et al*. (2019) reported decrease in moisture content with increasing level of tilapia waste flour in wheat bread, possibly due to the dominating hydrophobic components of the formulation mix.

Further, a progressive increase in these proximate components correlated with the increasing levels of the incorporated chicken powders. This signifies that chicken meat powder-breads reflected the amounts of protein, ash and fat in the varying levels of chicken meat powder incorporated. A similar phenomenon manifested in breads enriched with house cricket powder (Bawa *et al*., 2020; Kowalczewski *et al*., 2021), *Tenebrio molitor* powder (Roncolini *et al*., 2019) and lupin (Plustea *et al*., 2022), linking the elevated proximate parameters to the added ingredients. In the present study, chemical characterization indicated that chicken powder contains excellent levels of protein (86.5%), ash (7.2%) and fat (8.2%) (Table 4.1). These findings therefore suggest that enrichment of bread with insect-based meal (IBM)-fed chicken meat powder depicts nutritional relevance pertinent to human nutrition. Consumption of these breads can easily contribute to the achievement of protein Recommended Dietary Allowance (RDA) (WHO *et al*., 2007) and satisfy the functional body requirements such as growth, health and growth improvement (Bawa *et al*., 2020).

The low carbohydrate levels in the chicken meat powder-breads is indicative of the dilution effects of the low carbohydrate chicken ingredients since, wheat flour, the key ingredient in bread-baking, comprises 50-80% of carbohydrates (Dewettinck *et al*., 2008; Umaraw *et al*., 2018). Such observation was replicated by other researchers enriching wheat breads with low carbohydrate ingredients such as fish flour (Chambó *et al*., 2018; Zebib *et al*., 2020) and mushroom powder (Okafor *et al*., 2012).

5.7 Amino Acid Profile of the Breads

The concentration of individual amino acid in bread increased with increasing levels of meat powder inclusion, with histidine, lysine and threonine recording the highest margins of 1.5-3.3, 3.0-4.5 and 1.8-3.1 folds, respectively for the essential amino acids, and glycine, alanine and arginine registering 1.4-1.8, 1.4-2.2- and 2.0-2.7-folds increment, respectively for the non-essential amino acids. This trend demonstrates the amino acids quality and superiority of the substrate relative to the reference breads, purely made of wheat. Such a tendency also emerged in other studies where wheat bread was formulated with quality protein ingredients (Desai *et al.*, 2021; Osimani *et al.*, 2018).

Wheat flour is deficient in certain essential amino acids necessitating enrichment to revamp its nutritional quality (Desai *et al*., 2021). The amino acids concentrations in the enriched breads appear to reflect the levels in the chicken meat powder used in the formulations. For instance, escalated leucine levels in the breads may have been derived from chicken powder utilized for enrichment since it has been reported the most abundant essential amino acid in high protein animal products (Mafu *et al*., 2022). The abundance of glutamic acid and proline in wheat flours and breads has previously been reported elsewhere (Osimani *et al.*, 2018). Notably, despite the significant differences in glutamic and aspartic levels between control breads and those enriched with chicken powder, no significant variations were discernible in the latter. It can therefore be postulated that some of these amino acids are utilized by yeast fermenters or thermo-degraded during baking (Ogur, 2014).

The marked increase in the levels of certain amino acids especially alanine and serine may also be hypothesized to rise from hydrolytic breakdown of peptides by yeast fermenters and activated flour proteases in the doughs into free amino acids (Ogur, 2014; Roncolini *et al*., 2019). Some of these free amino acids, particularly lysine are reactants in Maillard reactions causing the browning of bread crumps and yielding aromatic compounds to the detriment of their biological value (Ogur, 2014). Therefore, controlled fermentation and cooking parameters tenable to the biological activity of such essential amino acids should be considered. However, in this study, lysine levels rose steadily with increasing levels of chicken powder in the baked product. This may be due to delayed denaturation of lysine as result of hydrophobic proteins interaction in the formulation mix (Miranda-Ramos *et al*., 2020). Further, the supplemental effects of the other amino acids as reactants in the browning reactions may have limited their excessive utilization. In this study, the limiting amino acids

in cereals, lysine and threonine, recorded 3.0-4.5 and 1.8-3.1-folds higher levels, respectively, between control and enriched breads signifying a strong correlation with chicken powder addition. This justifies the aim of this study to develop nutritious breads with well-balanced essential amino acid profiles. Other researchers have also succeeded in correcting the amino acids imbalances in wheat breads through enrichment with nutritionally superior ingredients (Desai *et al*., 2021; Ogur, 2014; Osimani *et al*., 2018; Roncolini *et al*., 2019). The ratio of Essential Amino Acids/Non-essential Amino Acids (EAA/NEAA) followed the order B_{30} $>B_{25}>B_{20}>B_0$ with breads containing chicken meat powder attaining ≥0.6, a ratio indicating good amino acid source (FAO/WHO, 1973). Bread with 25% and 30% chicken meat powder met the FAO/WHO requirements of essential amino acids, accounting for ≥40% of the total amino acids (FAO/WHO, 1973).

5.8 Fatty Acid Profiles of the Breads

Lipids improve the nutritional value, contribute to stability of flours and baked products during storage, forestall bread staling, influence the baking and functional properties of doughs, and release hydroperoxides which improve the aroma and flavour of the baked products (Kowalczewski *et al*., 2021; Osimani *et al*., 2018). The amounts of the SFAs, MUFAs and PUFAs in the breads with chicken powder increased 2.2-2.4, 1.8-2.0 and 1.4-1.6-folds relative to the control breads exposing the influence of addition of chicken powder in the formulation mix. Methyl hexadecanoate (palmitic acid), methyl tetradecanoate (myristic acid) and methyl octadecanoate (stearic acid) of the SFA, methyl 9Z-hexadecanoate (myristoleic acid) and methyl (9Z) octadecenoate (oleic acid) of the MUFAs, and methyl (9Z,12Z)-octadecadienoate (linoleic) and methyl (9Z,12Z,15Z)-octadecatrienoate (α-linolenic) of the PUFAs were the most predominant fatty acids. Likewise, Belichovska *et al*. (2020) identified linoleic acid, oleic acid and palmitic acids as the most prevalent FAs in chicken, particularly with regards to drumstick and breast parts, which were considered in this study. Furthermore, these fatty acids were the dominant profiles in the baking ingredients wheat flour (Giaretta *et al*., 2017; Nikolit *et al*., 2008; Osuna *et al*., 2016) and chicken meat powder (Table 4.8) suggesting that the fatty acid of the breads reflected the peculiarities of the FA profile of the ingredients. Such a trend was revealed by other authors who formulated breads integrated with novel ingredients (Osimani *et al*., 2018; Plustea *et al*., 2022).

The FA profile is susceptible to influence by the dietary intake as demonstrated by Panda *et al*. (2015). Chicken used in this study were fed on black soldier fly larvae which is

known to possess excellent profiles of unsaturated fatty acids (UFAs) derived from their feeds (Ewald *et al*., 2020). Of greater interest are the omega 3 eicosapentaenoic acid (EPA) which is related to cardiovascular health and docosahexaenoic acid (DHA) which is associated with the formation and functionality of the nervous and visual tissues (Osuna *et al*., 2016). The UFAs increased by 1.5-1.7-folds between control breads and those enriched with chicken powder, indicating the significant amount of UFA remained stable upon baking, making the enriched bread healthier. Similar observation was demonstrated when fish powder (Desai *et al*., 2018) and *kinako*/chia (Giaretta *et al*., 2017) were incorporated in bread. The ratio PUFA/SFA is an indicator of food healthiness (Osuna *et al*., 2016).

The PUFA/SFA ratios of the breads in this study exceeded 0.45, the minimum recommended threshold for a healthy food, associated with blood pressure reduction and prevention of hypertension in human body (Giaretta *et al*., 2017; Osuna *et al*., 2016). The notable high n-6/n-3 in the control breads can be linked to the predominance of n-6 fatty acids in cereal grains. That notwithstanding, the n-6/n-3 ratios of all the breads were compliant with the ratios of between 1 to 5, depicting cardio-friendliness, as recommended by food agencies, scientific societies, and national and international organizations (Desai *et al*., 2018).

5.9 Vitamin Profile of the Breads

Wheat is naturally scarce in lipid which negatively affects their content of fat-soluble vitamins such as vitamins A (Dewettinck *et al*., 2008), However, other lipophilic vitamins like E and K are known to be less abundant in meat products but abundant in plant-based products (Marangoni *et al*., 2015). This may explain why retinol, a precursor of vitamin A and the tocopherols, precursors of vitamin E, were relatively lower in the control breads and chicken powder enriched breads, respectively. On the other hand, the progressive increase in retinol, nicotinic acid and pantothenic acids with the rising levels of chicken powder inclusion manifests the contribution of the latter in boosting the levels of such micronutrients deficient in wheat breads.

Hydrophilic vitamins such as nicotinic and pantothenic acids are common to animal products like poultry meat and are able to withstand cooking conditions owing to their thermal stability (Marangoni *et al*., 2015), hence their escalated levels in the baked breads formulated with chicken meat powder. Ascorbic acid and riboflavin are also prevalent in cereals than in animal products hence, replacing wheat flour with the chicken meat powder

may have diluted their concentrations in the enriched bread. The vitamins nicotinamide, thiamine, pyridoxine and cobalamin were the least abundant evidenced by their non-detection. Their levels may have been affected by cooking time, pH, temperature and mixing process of used of dough (Dewettinck *et al*., 2008). Due to the paucity in information regarding vitamins contents of breads enriched with chicken powder, we could not compare our data with any other.

5.10 Mineral Profile of the Breads

The levels of all the minerals except cobalt positively correlated with increasing levels of chicken meat powder. Spent hen meat powder used in this study had high content (7.2%) of ash (Table 4.10) which may have translated to the increased minerals levels. This is concurrent with related studies which indicated enhancement in the mineral levels of bread incorporated with pumpkin, mushroom and fish flours (Kiharason *et al*., 2017; Ndung'u *et al*., 2015; Zebib *et al*., 2020). The abundant minerals have previously been reported in black soldier larvae (Shumo *et al*., 2019) fed to chicken used in this study. Further, the levels of these minerals in animal products depends on their concentrations in the dietary sources (Shumo *et al*., 2019) In the current study, iron, phosphorous, zinc, copper and calcium increased 1.0-1.1, 1.6-1.7, 1.1-1.2, 1.2-1.5 and 2.0-3.0-folds between the control breads and the enriched breads, respectively. Iron is crucial in haemoglobin synthesis and co-factor for enzymes (Carocho *et al*., 2020). The concentrations of zinc and iron in the breads containing 30% chicken powder can be estimated to contribute 48.2% and 22.5%, respectively of the recommended daily intake of minerals for a person aged between 12-18 years (WHO, 2006). Copper also plays a role in haemoglobin synthesis, redox reaction and cuproenzymes (Carocho *et al*., 2020). Their levels in the breads enriched with 30% chicken powder can be estimated to contribute 0.18 mg of 5mg/day copper daily intake for adults (EFSA, 2006).

5.11 Microbial Levels in Breads Enriched with Chicken Meat Powder

Bacteria and fungi were not detected from the freshly baked breads (Table 4.11). Microbial characteristics of the breads were therefore compliant with the permissible microbial levels as prescribed in the Food and Drug Administration (FDA) circular on microbiological quality of baked products. Elevated baking temperatures which subdue most microorganisms and fungal spores and hygienic post-baking handling largely contribute to products with low microbial counts (Saranraj *et al*., 2012). The lack of detection of

Salmonella sp. and *E. coli* suggest no feacal contamination of the breads produced hence safe for consumption.

5.12 Overall acceptability test of the Breads Enriched with Chicken Meat Powder

Over acceptability were highly ($p<0.05$) rated for the breads enriched with 25% and 30% chicken powder. The panellists may have preferred the dark colour of bread crumbs and crust as depicted in breads enriched with 25% and 30% chicken powders. Similar findings were reported by Umaraw *et al*. (2018) on bread fortified with chicken powders. The darkening in colour may have yielded golden brown colour which is a characteristic colour of bread crusts that consumers are accustomed to. Flavour preference of the breads correlated with the chicken powder inclusion levels. This may be due to fermentation-mediated release of free amino acids from the enriching substrate which may have contributed flavour enhancement of the product compared to the control bread. The panellists may have relied on such attributes to gauge the acceptability of the products.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were made from the study findings:

i. Spent hen meat powered from hens fed on Black Soldier Fly Larvae based meal had high content of lysine, methionine, isoleucine, and zinc but had low content of PUFAs compared to the SHMP from hens fed on FM based meal.
ii. The incorporation of IBM-fed hen meat powder into wheat bread formulation increased the content of; Protein, amino acids, retinol, nicotinic acid, pantothenic acid, iron, zinc and calcium of the bread.
iii. Processing of SHMP and baking of bread drastically suppresses *Staphylococcus aurous, Salmonella spp.* and yeast and moulds counts to undetectable levels.
iv. Bread made by substituting 30% of WF with 30% SHMP was the most liked by consumers.

6.2 Recommendations

The following recommendation was made from the study conclusions:

i. The substrate used to rear Black Soldier Fly Larvae that will be used in hen diet should contain high levels of PUFA.
ii. The necessary protocol be initiated to have the enriched bread adopted by entrepreneurs in confectionary and bread industry to address the food security.
iii. Pre-processing of SHMP prior to incorporation into products should be encouraged to ensure end-products of high sanitary levels.
iv. Bread made by substituting 30% of WF with 30% SHMP should be adopted for bread formulation since it had high sensory score resulting to high consumer acceptability score.

6.3 Further Research

1. Further research to be conducted to confirm the impact of defatted Black Soldier Fly Larvae meal on SHM quality, which may help to reduce the

amount of saturated fatty acids in resultant meat which are undesirable quality that is not acceptable to consumers.

2. Further research to be conducted to determine volatile compounds produced in bread enriched with SHMP after fermentation and baking which may promote desirable or undesirable changes in bread.

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APPENDICES

Appendix A: Chicken Meat Powder Processing

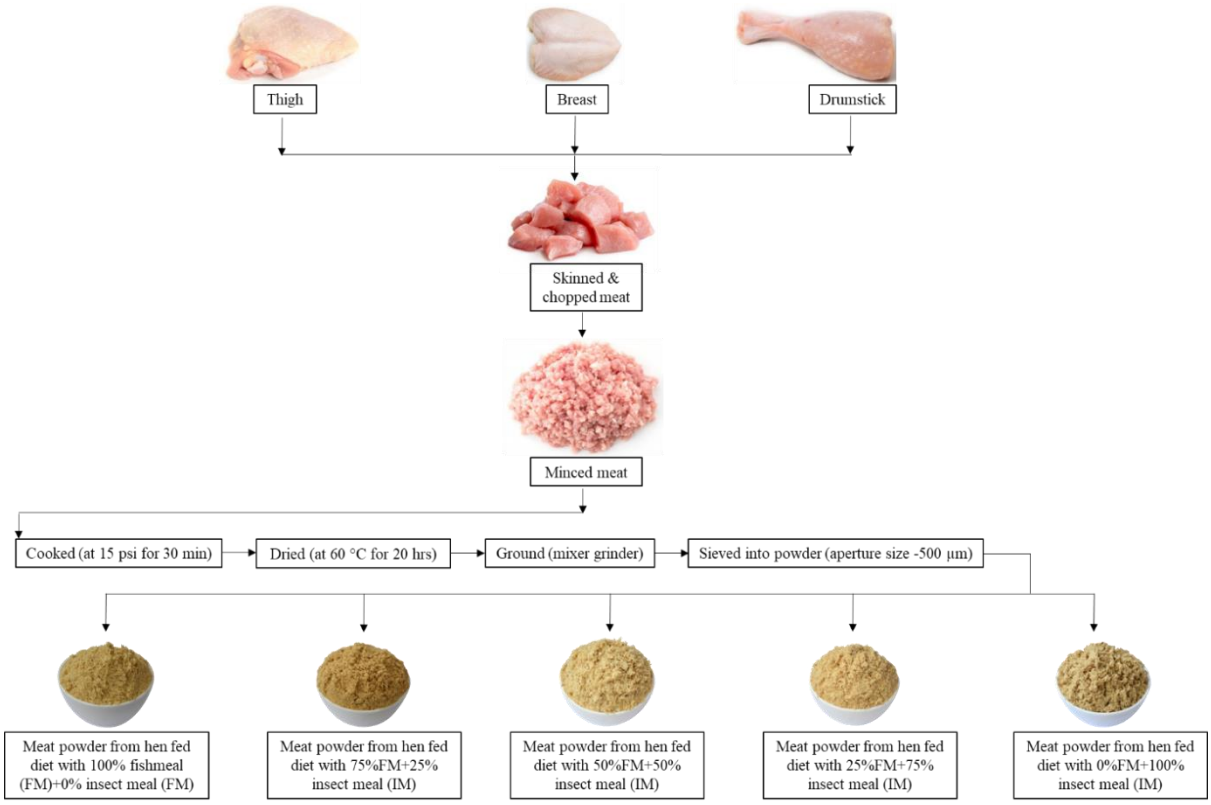

Figure 1: Flow diagram showing how chicken meat powder was obtained. *Psi* – Pound per square inch; min – minutes; hrs- hours.

Appendix B: Baked Breads

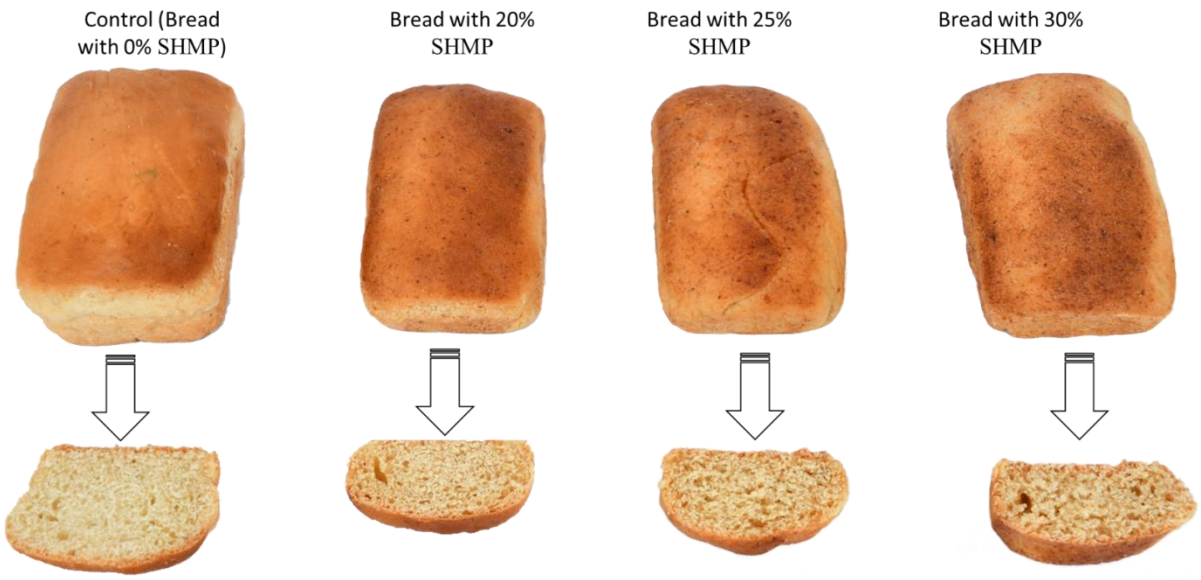

Figure 2: Pictures of the Baked Breads

Appendix C: Sensory Evaluation Questionnaire

Panelist Code: _____

Date: __ __ 2021

A) CONSENT FORM

You are invited to participate in a research study on the perception of chicken meat-based bakery product. Keenly read through this form and ask any questions for clarity before agreeing to be enrolled in this study. This exercise is purely voluntary thus, promptly notify us of any allergenicity or intolerance to chicken meat-based food products so that you are excluded from this study. The results of your assessment as a panelist will be kept strictly confidential. Kindly fill in your details in the section below.

I, (Name)……., have read the information pertaining to my involvement in this study and comfortably confirm that my

concerns have been addressed to satisfaction. I hereby give my voluntary consent for participation in this study.

Gender

Male **[ ]** Female **[ ]**

Age Bracket

Less or equal to 20 **[ ]** 21-25 **[ ]** 26-30 **[ ]** 31-35 **[ ]** 36-40 **[ ]** 41 and above **[ ]**

Signature: _____

B) SENSORY EVALUATION

INSTRUCTIONS

You have been provided with **five (4) coded** samples of chicken meat powder -based breads. Please take a sip of water to cleanse your palate before and after tasting each sample. Taste the samples and hold in the mouth while chewing for 5 sec. Please look and taste each of the (4) coded bread samples. Rate each of the coded sample against the scale of 1-5 provided below. Each number in the scale denotes the degree of likeness. Put the appropriate number in the table against each attribute with reference to the scale below:

- 1-Like very much
- 2-Like
- 3- Neither like nor dislike
- 4- Dislike
- 5-Dislike very much

Attributes	**Sample codes**			
	BRA	**BRB**	**BRC**	**BRD**
Overall acceptability				

Additional comments

.....

.....

.....

Thank you for participating in the study.

Appendix D: Abstract of Publications

1st Publication

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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ARTICLE INFO

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

ABSTRACT

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.

2nd Publication

Heliyon 9 (2023) e20506

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Unravelling the nutritional and health benefits of wheat bread enriched with meat powder from laying hen fed diet with insect (*Hermetia illucens*) meal

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ARTICLE INFO

Keywords:
Insect meal
Functional ingredients
spent hen meat powder
Food fortification
Novel foods
Food security

ABSTRACT

Wheat bread is among stable foods that are nutritionally imbalanced, thus enrichment is crucial. We evaluated the nutritional impact of high-valued wheat bread enriched with varying levels of meat powder from hen fed diet with insect (*Hermetia illucens*)-based meal. Crude protein and ash in bread increased with increasing inclusion of meat powder. Limiting amino acids like lysine and threonine in enriched bread products increased by 3.0–4.5 and 1.8–3.1-folds, respectively. Omega 3 fatty acids were significantly enhanced in bread fortified with meat powder. Vitamins (retinol, nicotinic acid, and pantothenic acid) were significantly increased in supplemented bread products. Iron, zinc, and calcium increased by 1.1, 1.2 and 3.0-folds in enriched bread with 30% meat powder. Colour, flavour and overall acceptability of breads prepared with 25 and 30% meat powder were highly ranked. Our findings demonstrate that meat powder (i.e., from hen fed insect-based diets) enrichment would provide added health and nutritional benefits to bread products without having adverse effects on any functional or sensory properties. Thus, this could be a novel strategy and trend for improving bread products, that might generate increasing demand for a healthier consumer-oriented lifestyle.

Appendix E: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 222775

Date of Issue: 28/January/2021

RESEARCH LICENSE

This is to Certify that Ms. Marcasy Phanice Makokha of Egerton University, has been licensed to conduct research in Nairobi, Nakuru on the topic: Quality Evaluation of Bread Enriched with Meat Powder from Spent Laying Hens Fed on Black Soldier Fly (Hermetia illucens) Larvae Supplemented Diet for the period ending : 28/January/2022.

License No: NACOSTI/P/21/8780

222775

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Appendix F: Samples of Statistical Data Analysis Output

R Studio Output for amino acid Lysine

```
> aoviron<-aov(lysine~treat, data = iron)
> summary(aoviron)
            Df Sum Sq Mean Sq F value  Pr(>F)
treat        4 1.6228  0.4057    9.89 0.00167 **
Residuals   10 0.4102  0.0410
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> print(model.tables(aoviron, "means", se=TRUE), digits = 3)
Tables of means
Grand mean

65.85867

 treat
treat
   1    2    3    4    5
65.2 66.0 66.0 66.0 66.1

Standard errors for differences of means
        treat
        0.165
replic.     3
> SNK.test(aoviron, "treat", console = T)
```

```
Student Newman Keuls Test
for lysine

Mean Square Error:  0.04102

treat,  means

    lysine        std r   Min   Max
1 65.20333 0.02309401 3 65.19 65.23
2 66.04333 0.19035055 3 65.86 66.24
3 66.01000 0.26851443 3 65.77 66.30
4 65.97667 0.21126603 3 65.78 66.20
5 66.06000 0.22715633 3 65.90 66.32

Alpha: 0.05 ; DF Error: 10

Critical Range
         2         3         4         5
0.3684635 0.4533232 0.5059204 0.5442406

Means with the same letter are not significantly different.
```

```
    lysine groups
5 66.06000      a
2 66.04333      a
3 66.01000      a
4 65.97667      a
1 65.20333      b
>
```

FAMEs Peaks of Bread from Chemstation Output