

**EFFECT OF BLACK SOLDIER FLY LARVAE MEAL
INCLUSION IN LAYER FEEDS ON THE QUALITY OF
THE EGGS**

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**Effect of Black Soldier Fly Larvae Meal Inclusion in Layer Feeds on
the Quality of the Eggs**

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the Degree of Master of Science in Food Science and Technology of
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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

I dedicate this work to my wife Alice Wangu, and my son Chaniel Kibali and my parents; Josphat Mwangi and Lucy Waitherero.

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ABBREVIATIONS AND ACRONYMS

AA	Arachidonic Acid
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
BHT	Butylated Hydroxytoluene
BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
BSFLM	Black Soldier Fly Larvae Meal
CF	Crude Fat
CP	Crude Protein
CRD	Completely Randomized Design
DCP	Dicalcium Phosphate
DHA	Docosahexaenoic Acid
DM	Dry Matter
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FID	Flame Ionization Detector
FM	Fish Meal

GC	Gas Chromatography
GDP	Gross Domestic Product
GHG	Greenhouse Gases
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
ICIPE	International Centre of Insect Physiology and Ecology
KARLO	Kenya Agricultural and livestock research organization
LA	Linoleic Acid
LDL	Low Density Lipoprotein
MCFA	Medium Chain Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
RH	Relative Humidity
SFA	Saturated Fatty Acid
SNK	Student–Newman–Keuls Test

ABSTRACT

The high cost of fish meal (FM), a conventional protein source in poultry feeds, presents a major challenge especially for the small-scale farmers. Black soldier fly larvae meal (BSFLM) is a promising nutrient rich alternative, but its effect on egg quality requires comprehensive evaluation. The current study aimed to provide the first comprehensive evidence of the nutrient quality of eggs harvested from layers fed diets containing black soldier fly larval meal (BSFLM) as a substitute for the fish meal (FM). This study utilized seventy-five Isa Brown layers (n=75) fed on BSF-based diet types that were nutritionally balanced consisting of 0% BSFLM+100% FM - (control); 25% BSFLM+75% FM; 50% BSFLM+50% FM; 75% BSFLM+25% FM and 100% BSFLM+0% FM). The research employed a completely randomized design (CRD) model, with the design assigning five (5) birds per replicate for each treatment throughout the entire egg laying phases in which each treatment was replicated three (3) times. This study revealed that increased inclusion levels of BSFLM in layers diets led to a significant increase in egg weight ($F_{4, 20} = 15.241, P < 0.001$) and yolk weight ($F_{4, 20} = 8.068, P < 0.001$). BSFLM inclusion in the layers diet did not significantly alter the n-6 (omega-6) and n-3 (omega-3) ratio ($F_{4, 20} = 1.438, P = 0.258$). The monounsaturated fatty acids (MUFA) content significantly increased with increasing levels of BSFLM ($F_{4, 20} = 41.054, P < 0.001$). Inclusion of BSFLM in the layers diet contributed to a significant increase in MUFA content and the egg weight, Haugh unit and shell thickness collectively improving the quality of the eggs.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Globally, food security is crucial in ensuring sustainable development in all nations. According to Belhadj Slimen, Yerou, Ben Larbi, M'Hamdi, and Najar (2023), it is estimated that by 2050 a total of 9 billion people will be occupying the Earth and this will contribute to a rise in demand by 70% for proteins of animal origin. Poultry meat and eggs can help to meet the protein requirements for the increased populations and forms a food source that has a production cycle that is short and the products are affordable (Sajid, Asghar, Tariq, Wilk, & Płatek, 2023).

Availability of feeds having all the dietary requirements for poultry production is a major challenge to the poultry industry (Oyegoke, Akintola, & Fasoranti, 2006). Traditionally, production of poultry relies on plants and fish meal as the source of proteins. Plant sources such as soy bean meal dominates due to high amino acid composition as well as high digestibility levels in comparison to other vegetable source of proteins (Khusro, Andrew, & Nicholas, 2012). However, vegetable sources are associated with unfavorable characteristics such as imbalance between non-essential and essential amino acids, high amounts of fiber and low palatability thus limiting their inclusion into the diet (Sánchez-Muros, Barroso, & Manzano-Agugliaro, 2014). Due to these conditions, the diet is completed by utilization of animal sources that have high protein value.

Poultry industries have used fish meal as the alternative source of proteins as well as other nutritional indispensable properties. However, production of the fish meal is determined by the catch thus varying at different times. In addition, marine environment has deteriorated over time leading to decreasing production of the fish meal which leads to price increase (Sánchez-Muros et al., 2014). This situation has brought to prominence the importance of seeking an alternative source of proteins from insects that are naturally consumed by the wild birds (Sánchez-Muros et al., 2014).

Recent studies identify different insects' species as a source of food for various animals. However, only few studies have focused on incorporation of insects into the diets of domestic animals. Some of these insects include caterpillars, grasshoppers, bees, winged termites among others (Khusro et al., 2012).

Studies have shown that insects are highly nutritious in terms of proteins, vitamins, minerals and fat with protein content ranging from 30% to 80% (Khusro et al., 2012). Moreover, the insects have a better conversion ratio which is as a result of their poikilothermic nature (Khusro et al., 2012). Since insects are cold blooded, they convert feed to protein much more efficiently. For instance, to produce a similar content of proteins, cattle feeds 12 times more than crickets, sheep feeds 4 times more than crickets. Also due to the high metabolic rates of insects, they have a significant amount of natural resources and energy (Anankware, Paarechuga, Oseckre, & Obengh, 2015).

The most promising insects for the industrial production include the Black soldier fly (BSF), the lesser mealworm, several grasshopper species, silkworm, the yellow mealworm, crickets and the housefly (van Huis, 2013). BSF larvae convert manure into a body mass that contains 35% fat and 42% protein (Sheppard et al., 2002; van Huis, 2013). This characteristic enables them to be an appropriate feed source for both fish and livestock (van Huis, 2013).

While BSFL is known to be a nutritious feed ingredient, comprehensive data on its impact on the end-product quality specifically the physical, nutritional and sensory characteristics of eggs from laying hen is lacking. By integrating these quality parameters, this study will provide the evidence that insects derived proteins are not just for survival of the layers but as a value adding intervention for commercial egg production. This study therefore aims to address these gaps by holistically evaluating the quality of eggs produced.

1.2 Problem Statement

The demand for the animal proteins globally is estimated to increase by 76% from 2005 to 2050 which will be as a result of rise in income and urbanization (Huis,

2015). Nevertheless, Livestock production is not sustainable currently due to their association with emission of at least 14% of the greenhouse gases. If appropriate measures are not taken, it is estimated that by 2050 greenhouse gases emissions from agricultural production will have increased by 80% (Huis, 2015). Besides, the poultry industry experiences significant resource challenge due to diversion of more than a third of cereal produced yearly to livestock production which would have been used to feed more than three billion people (Huis, 2015).

Currently, the poultry sector highly relies on fish meal to formulate feeds for domesticated birds (Elahi et al., 2020; Nyakeri, Ogola, Ayieko, & Amimo, 2017). Globally, its estimated that 14% of the total fish caught is directed to production of fish meal (Nyakeri et al., 2017). However, fish meal is becoming depleted faster and increasingly scarce in the midst of consistent rise in demand. This imbalance between supply and demand contributed to doubling of the fish meal prices between 2008-2013, (Nyakeri et al., 2017). This situation has increased the production cost subsequently leading to high market prices for eggs. The current situation has contributed to the reduction of protein intake and therefore increasing the occurrence of protein- energy malnutrition and the related diseases in the general population.

Insects, specifically BSFL has evolved as a source of protein that is sustainable and of high quality capable of partially or completely replacing traditional ingredients such as soy bean meal and fish meal. BSFL convert organic waste products into nutritious rich biomass (Oteri et al., 2021) (Makkar, Tran, Heuzé, & Ankers, 2014). BSF has been added in layer chicken in Kenya resulting to increased egg productivity. However, comprehensive data on BSF impact on egg quality such as physical, nutritional and sensory characteristics is lacking and this study aims to bridge this gap.

1.3 Objectives

1.3.1 General objective

To evaluate the effect of inclusion of black soldier fly larvae meal in layers chicken diet on the physical, chemical and sensory quality of eggs

1.3.2 Specific Objectives

- I. To evaluate the effect of inclusion of black soldier fly larvae meal at different inclusion rates to the layers feeds on the physical quality of eggs.
- II. To assess the effect of inclusion of black soldier fly larvae meal at different inclusion rates to the layers feeds on the nutritional quality of the eggs.
- III. To determine the effect of inclusion of black soldier fly larvae meal at different inclusion rates to the layers feeds on the sensory characteristics of eggs.

1.4 Hypothesis

Null hypothesis:

- The inclusion of black soldier fly larvae meal at different rates in the diet of laying hens will not affect the physical quality of eggs
- The inclusion of black soldier fly larvae meal at different rates in the diet of laying hens will not affect the nutritional quality of eggs.
- The inclusion of black soldier fly larvae meal at different rates in the diet of laying hens will not affect the sensory characteristics of eggs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

According to FAO (2024) the population of chicken globally in 2020 was above 33 billion in which 46% was from Asia. The demand for the poultry products is significantly increasing due to population growth, urbanization, and the rising income (Mottet & Tempio, 2017). Globally, In 2021, the production of eggs increased by 69% reaching 86 million tons as compared to the production of eggs in 2000 (FAO, 2023). Table 2.1 shows the world's top producing countries of eggs in 2021. The major producer of eggs globally in 2021 by country was China with a production of 34%, followed by India and United states of America each at 8% (FAO, 2023).

Table 2.1: Top Countries Globally in Hen Egg Production in (2021)

Country	World total (%)
China	34
India	8
United states of America	8
Indonesia	6
Brazil	4
Mexico	3
Japan	3
Russian Federation	3
Others	31

Recently, there have been a major shift in which food from animal sources are more favored especially eggs, fish, milk and meat and this has been as a result of population growth, economic growth, high rate of urbanization, and the increased nutritional awareness (Shumo et al., 2019). Globally, it is estimated that by 2050, the human population will be close to 9.6 billion (Abd El-Hack et al., 2020; Anankware et al., 2015) in which the number of people living in urban areas will be approximately 70% while the income is projected to increase by 2% per annum (Mottet & Tempio, 2017). As a result of this, it is expected that the demand of food globally will increase by 70% leading to both environmental and food security issues (Shumo et al., 2019; Tahamtani et al., 2021). This increase in human population will lead to increase in demand for animal feeds and animal products (Tahamtani et al., 2021).

Eggs are a great source of minerals, essential vitamins and high quality proteins that are inexpensive (Zaheer, 2015). The global production and consumption of eggs and their usage as functional foods and the effects they have on human health have continued to expand due to the capacity they have to reduce the risks of various diseases apart from their affordability and their easy availability (Zaheer, 2015). The vision of the International Egg Commission is to ensure that there is a sustainable supply of food that is independent while ensuring there is self-sufficiency in terms of food for the people currently and also in the future (Zaheer, 2015). Undernutrition, have for long continued to be significant problem in developing countries, and therefore, poultry production have a major function to play in improvement of nutrition through provision of essential nutrients and high quality proteins that is affordable (Mottet & Tempio, 2017; Zaheer, 2015).

In the third world countries, poultry production have the potential to promote food security and alleviate poverty (Shumo et al., 2019). For example, poultry farming in Kenya plays a significant role in income generation in the rural areas and this contributes to the Gross Domestic Product (GDP) by more than 25% of the agricultural products and 8% of the Kenyan GDP (Shumo et al., 2019). Poultry keeping is a major livestock enterprise in Kenya that is favored by low capital and space requirements. The main commercial layers genotypes are Ross and Isa Brown

(Omiti & Okuthe, 2008). The sector has created employment in which around two million people are employed either in production, marketing, or in the supply of feeds, chicks and veterinary services. More than 50% of households in Kenya keeps chicken in which on average each house hold keeps 12 chickens (Omiti & Okuthe, 2008).

In relation to climate change, Globally, the total amount of greenhouse gases (GHG) emitted by poultry is approximately 11% of the total emissions by the livestock supply chain thus making poultry the smallest contributor of GHG among the livestock sector (Mottet & Tempio, 2017). In addition, Poultry production as a protein source have the lowest water footprint in comparison to the other livestock animals (Abd El-Hack et al., 2020).

However, poultry farming have been faced by high production cost due to high feed expenses that have been estimated to range between 65 to 75% of the overall portion of the production cost (Elahi et al., 2022; Khalifah et al., 2023). Among the poultry feed ingredients, proteins are very costly and therefore their inclusion in the animal feeds plays a significant role in determining the overall cost of the feeds (Oteri et al., 2021). Therefore, in order to achieve economic success in poultry production, there is the need to use nutritionally balanced diets that are of low cost, available and efficient (Elahi et al., 2022; Oteri et al., 2021).

Fish meal have been conventionally used in poultry production and contains high amount of proteins content, well-balanced essential amino acids and non-essential amino acids, a low content of carbohydrate, and have high digestibility and this nutrients plays a major role in maintaining the growth of the animal (Oteri et al., 2021). However, in the recent decades, there has been irregular supply of fish meal due to the shrinking global marine fish catches resulting from pollution of lakes and therefore the prices have significantly been increasing and thus the need to look for other sources of proteins (Oteri et al., 2021; Shumo et al., 2019). This have led researchers to explore more areas of unconventional sources of proteins that include industrial by-products, insects, seaweeds among others (Oteri et al., 2021).

Both fish and soy bean are poultry feed ingredients that are also directly suitable for human consumption thus leading to a high competition between food and feeds making them costly and eventually increasing the feed prices (Shumo et al., 2019). The rising sustainability concerns, revolving around fish meal and soy bean meal sourcing, escalating costs, and fluctuating availability, have made it necessary to explore other alternative sources of proteins. Continuous research is required in poultry diet formulation that have got well balanced nutritional properties in order to meet the high global demand of eggs that are sustainably produced and which are of high quality.

In order to deal with the previously mentioned issues, experts have recognized that use of insects meals in formulating poultry diets is a novel way that could improve food and feed security (Abd El-Hack et al., 2020). According to Hender, Siddik, Howieson, & Fotedar (2021), insects are suitable in feeding animals due to sustainable, efficient and good quality proteins that have not yet been fully exploited. Interests in insects have tremendously grown as feed ingredients for both aquatic and terrestrial animals (Oteri et al., 2021). In West Africa, people collect termites to feed poultry from the wild and in northern Ghana, farmers have a number of termitaria from which harvesting is done to meet the proteins need of poultry (Anankware et al., 2015). Worldwide, there are diverse insects' species that have been used as animal feeds. These includes the mealworms, black soldier flies, termites, common house fly larvae among others. In the Netherlands, super worm and the lesser meal worm larvae are used to feed pets, fish and avian (Anankware et al., 2015).

BSF naturally occurs in dense population in organic wastes for example in distillers waste, coffee bean pulp, fish offal among other wastes. Meal worms can be grown in waste products that are less nutritive and fed to broilers. Common house fly larvae are used as a source of proteins for poultry and contain 54% crude protein (Anankware et al., 2015). According to Marono et al. (2017), various insects such as common housefly and BSFL produces antimicrobial peptides that helps in defending themselves from potential pathogens that can be present in the organic waste or in the manure. These peptides have also been considered to be of benefit in poultry production (Marono et al., 2017).

Exploiting insects for use as feed does not directly compete with food production but reduces environmental impact from different production systems and also contributes to zero waste and circular economy (Oteri et al., 2021). According to Abd El-Hack et al. (2020) insects naturally form feeds for poultry. According to Elahi et al. (2022), chickens are found picking larvae and worms from litter, grass and soil when walking, thus the insects are naturally poultry feeds. Several researches have proven that, utilization of insect's meals in formulating feeds can enhance a sustainable supply chain in poultry production. Insects are the desirable and key participant for the sustainable future as a source of proteins for the poultry feeds. Amongst the abundant animal species found on the planet are insects in which more than 2000 species are edible insects which for so many years have been consumed as food (Khalifah et al., 2023). However, more consumers would like to utilize insects in poultry feeds rather than humans consuming them directly (Abd El-Hack et al., 2020).

The increased use of insects in animal nutrition have established that insects are highly advantageous as feeds due to their high content of essential amino acids, proteins and minerals (Abd El-Hack et al., 2020). According to Khalifah et al. (2023), insects have a variety of Polyunsaturated fatty acid PUFA, Monounsaturated fatty acid (MUFA) and Saturated fatty acid (SFA) just like the other animal species. Palmitic acid (C16:0) and oleic acid (C18:1) are the most predominant SFA and MUFA respectively in insects (Khalifah et al., 2023). Various factors affect the vitamins and micronutrients contents in insects which include seasons, the kind of substrate fed on, species and orders. Also there exists variation in nutritional composition of similar insects species if the study was conducted at different stages of the insects life cycle (Khalifah et al., 2023). According to Khalifah et al. (2023), the contents of minerals contained in the insects is enough to meet the nutritional needs of the animals. Various insects have been reported to contain large amounts of minerals such as zinc, iron, selenium, phosphorus, calcium, manganese, and magnesium. However insects have been found to be deficient in vitamin D, vitamin C, vitamin A, thiamine (B1) and niacin (B3) (Khalifah et al., 2023). Table 2.2 below shows the average nutritional composition of various insects in comparison to soy bean meal.

Table 2.2: The Average Nutritional Composition of Various Insects in Comparison to Soy Bean Meal (Dry Matter Basis).

Insect Name	Protein (%)	Fat (%)	Methionine (%) *	Lysine (%) *	Ca (%)	P (%)
Black soldier	42.3	33.5	2.1	5.7	3.2	0.9
House fly	52	18	2.2	6.1	0.47	1.6
Meal worm	45	30	1.5	5.4	0.27	0.78
Locusts	57.3	8.5	2.3	5.8	0.13	-
Silkworm	54	12	3	7	0.38	0.60
Grasshopper	47.71	12.21	-	-	-	-
Soy bean meal	44	0.9	0.65	2.95	0.32	0.65

* (% to 100% protein).

Source: (Khalifah et al., 2023)

Production of insects can be done in vertical systems and requires very limited amount of space as compared to the traditional sources of proteins and they only need little water therefore reducing their water usage. Insects are also beneficial to pollination of crops fed to animals and humans thus creating a food chain that have got a long term sustainability (Khalifah et al., 2023). For the production of the various insects species, organic side streams can be used in which the insects have been proven to have high conversion efficiency (Peer et al., 2021). Therefore, apart from using insects for feed, they are beneficial to the environment in that they are used to treat large amount of organic waste that can create environmental burden through generation of pollutants such as offensive odor (Abd El-Hack et al., 2020). Insects releases fewer Greenhouse gases (GHG) and ammonia and they are produced with minimum resources in comparison to other animals and they provide vitamins and high quality amino acids for both human and animal health (Khalifah et al., 2023). Naturally, numerous animals including free range poultry, fish and wild birds regularly consume insects as part of their diet. Consequently, the utilization of insects as a novel source of feeds have increasingly been used as an alternative protein source that is sustainable for poultry production and this has become affordable and feasible (Khalifah et al., 2023).

Among the insects, BSF has evolved among the promising candidates used as an alternative source of proteins in the poultry diet due to its sustainability capacity of

converting organic waste products into nutritious rich biomass (Makkar et al., 2014; Oteri et al., 2021). The BSF is reared on households and farm wastes and has been proven to significantly reduce the waste volume and the pollution potential (Abd El-Hack et al., 2020). BSFL voraciously consumes organic waste such as municipal organic waste, vegetable waste, decaying fruits, and animal manure among other types of wastes (Liu et al., 2017). During the last century in the early eighties and seventies, production of the BSFL on farm and food waste was examined extensively (Maurer et al., 2016). The main focus during this period was on waste reduction rather than on feed production which is the major focus through the available waste resources in a more sustainable and recycling context (Maurer et al., 2016).

In addition to their environmental sustainability, the last larvae stage of the BSFL called the prepupa have unique characteristics in that it contains high protein and fat content of 47% and 35% respectively on dry matter basis and therefore becoming a suitable source of feed for poultry, fish and pigs (Abd El-Hack et al., 2020). According to (Oteri et al., 2021), the BSFL larvae protein have a high biological value and the fat content contains fatty acids that are of high nutritional value and also have got a high ash content (11-15%) which have a high concentration of minerals. The protein present in the BSFL larvae is of high quality and comparable to fish meal in terms of the essential amino acids (Oteri et al., 2021). Depending on the type of diet fed on the BSFL, fatty acid and mineral composition can be influenced (Oteri et al., 2021). The kind of diet chosen to feed the BSFL determines the presence of EPA, DHA, alpha linolenic and linoleic acid since the larvae is not able to synthesize PUFA (Oteri et al., 2021). Undernourished populations have continued to increase in the developing countries and this could lead to higher risks of diseases outbreak either locally, nationally, or globally. Therefore, this leads to an urgent need for feed and feed sources containing significant amounts of micronutrients (zinc, calcium, iron), essential fatty acids and amino acids (Liu et al., 2017). In regard to this BSFL is a better solution through inclusion in the poultry diet due to its richness in nutritional composition.

2.2 Conventional Sources of Proteins

Over the years, the major sources of proteins in poultry diets have been soy bean and fish meal which has been used in supplementing proteins from vegetable sources such as soy bean meal (Frempong, Nortey, Paulk, & Stark, 2019; Khalifah et al., 2023). Both the soy bean meal and the fish meal have high similarities in terms of nutritional constituents thus making soy bean a preferable plant protein source for replacing fish meal (Frempong et al., 2019). However, the use of these traditional sources of protein have been faced with environmental concerns and shifts in Global supply chain (Frempong et al., 2019).

2.2.1 Fish Meal (FM)

FM is highly valued by farmers and nutritionists because it comprises of fat, minerals, vitamins and crude proteins that are highly digestible, (Frempong et al., 2019; Widjastuti, Wiradimadja, & Rusmana, 2014). FM contains high protein content ranging between 60 - 72% by weight (Cho & Kim, 2011). FM is a source of highly digestible essential fatty and amino acids and contains energy and high level of minerals such as calcium that enhances optimal growth, development and reproduction (Cho & Kim, 2011). The presence of a well-balanced long chain omega 3 fatty acids and essential amino acids distinguishes FM from other animal feeds ingredients (Cho & Kim, 2011). FM is also a good source of vitamins e.g. biotin, choline and vitamin A, D, E and B₁₂ and contains trace elements such as iodine and selenium (Cho & Kim, 2011). FM contains high amount of lysine and methionine which improves the growth of the animal and also enhances resistance to various diseases (Cho & Kim, 2011).

2.2.2 Soy Bean Meal

In comparison to other sources of proteins from plants, the soy bean meal has a significant feeding value due to its high content of proteins and essential amino acids that are required to meet the requirements for poultry nutrition. Previous studies have shown that supplementing soy bean meal with methionine have contributed to a

successful replacement of fish meal in broiler feeds leading to no difference in feed intake, feed efficiency and body weight gain (Frempong et al., 2019).

2.2.3 Challenges with Conventional Sources of Protein

In recent years, inclusion of fish meal in poultry diet has experienced challenges as a result of the limited availability of the FM, the high cost compared to the plant sources, and also the limited availability. In addition, FM contains trimethylamine that creates residual fish smell in both eggs and meat (Frempong et al., 2019). Utilization of soy bean meal also faces several challenges. Soy bean has been inadequate to meet the rising demand as a result of limited land and also the usage of soy bean oil in biofuels manufacturing (Frempong et al., 2019; Khalifah et al., 2023). Also, the production of soy bean meal has been influenced both quantitatively and qualitatively by climate change and global warming therefore creating an impact on the global food security (Abd El-Hack et al., 2020). In addition, the intensified production of soy bean have promoted unlawful acquiring of the land that leads to deforestation and other environmental consequences (Shumo et al., 2019). This have consequently led to increased cost of feeds, thereby raising concerns on whether poultry production is economically sustainable especially in the third world countries (Abd El-Hack et al., 2020).

Soy bean meal may have anti-nutritional factors for example phytic acids and trypsin inhibitors which may contribute to poor growth performance. However, proper thermal processing can help minimize or eliminate the anti-nutritional factors (Frempong et al., 2019). Land use and agriculture have contributed to a quarter of greenhouse gases emitted worldwide. For example, increase in production of soy bean has led to native forests deforestation thus influencing regulation of greenhouse gases. Therefore, this situation create the need for revolutionary sustainable alternatives in protein sources that are innovative, affordable, plentiful, effective and with minimal impact to the ecosystem in poultry production systems (Khalifah et al., 2023).

2.2.4 Insects as Alternative Protein Source in Poultry Nutrition

Insects are generally regarded as satisfactory nutrients source for instance; minerals, vitamins, energy, proteins and fat. In poultry nutrition, in order for the hen to experience a rapid growth over a short period of time, there should be a high supply of essential amino acids. This situation has made the insects an alternative source of proteins in feeding poultry (Khan, 2017). The following insects have been utilized a source of feeds for feeding the chicken; housefly, earthworm, mealworm, silkworm pupae, cricket, locust, grasshopper, as well as the black soldier fly.

Various studies have indicated that fish meal can partially be replaced in broiler feeding by housefly maggots. A study was carried out whereby the broiler feed was supplemented with 10-15% housefly larvae and this contributed to an increase in the growth performance as well as in the quality of the carcass. The content of the crude protein remained constant in the breast muscle whereas the level of trypsin and lysine content increased. This may have been contributed by the high amino acid profile, high protein digestibility (98.5%) and high crude protein content (63.99%) of the housefly larvae (Khan, 2017). Broiler fed with house fly larvae meal showed a significant greater portion of the breast muscle relative to the weight of the carcass in comparison to the chicken that were fed on the commercial diet.

Inclusion of the housefly larvae into the diet had no significant effects on the quality of the eggs in comparison to the control. The study showed that inclusion of 7.08% larvae together with 1.5 % fish meal and inclusion at 9.44% level without fish meal contributed to a significant difference in the decrease of the shell weight and the shell length of the eggs. The differences have been attributed to the low content of the calcium content in the housefly larvae meal. The study revealed that the house fly larvae meal can replace the dietary animal protein up to 50% supplied by the fish meal without negative impact on the shell strength and the egg productivity (Khan, 2017).

2.3 BSFL Production

Hermetia illucens (BSFL), originated from warm temperate, subtropical and tropical zones of America and is globally distributed mostly in the equatorial regions and its distribution areas includes, United states, Asia and Europe and Africa, and its classified under the Stratiomyidae family (Liu et al., 2017; Makkar et al., 2014). It has now spread in to the warmer and tropical regions of the world around 45 degrees N and 40 degrees S. The adult is black in color, wasp like and has a length of 15-20 mm long. The larva is about 6mm in width, 27 mm in length and weighs 220 mg during the last larval stage and they have a whitish, dull color. Single larvae can feed between 25 to 500 mg organic materials for instance decaying fruits and vegetables, animal manure, distiller grains and coffee bean pulp (Makkar et al., 2014).

The larvae mature in a period of two months under ideal conditions. However, if enough feed is not provided, the larval stages last for up to 4 months. The larvae stop moving, feeding and empties the digestive tract towards ending of the larval stage where by the prepupae is developed. The prepupae migrate into a protected and dry pupation site. The pupal stage can last for 14 days but may also vary up to 5 months. The adults now rely on the stored fat from the larvae stage since they do not feed (Makkar et al., 2014).

The Black soldier fly have been found to be a resistant species that is capable of surviving extreme environmental conditions for instance oxygen deficiency, food shortage and drought. These insects are advantageous over the others in that the adult do not feed and therefore they do not require any extra care and they are not potential disease carriers. Facilities used for the rearing activities utilize the prepupae migration behavior for self-collection whereby the larvae end up into a collecting vessel (Makkar et al., 2014).

Figure 2.1 below shows bioconversion of organic materials to poultry feed.

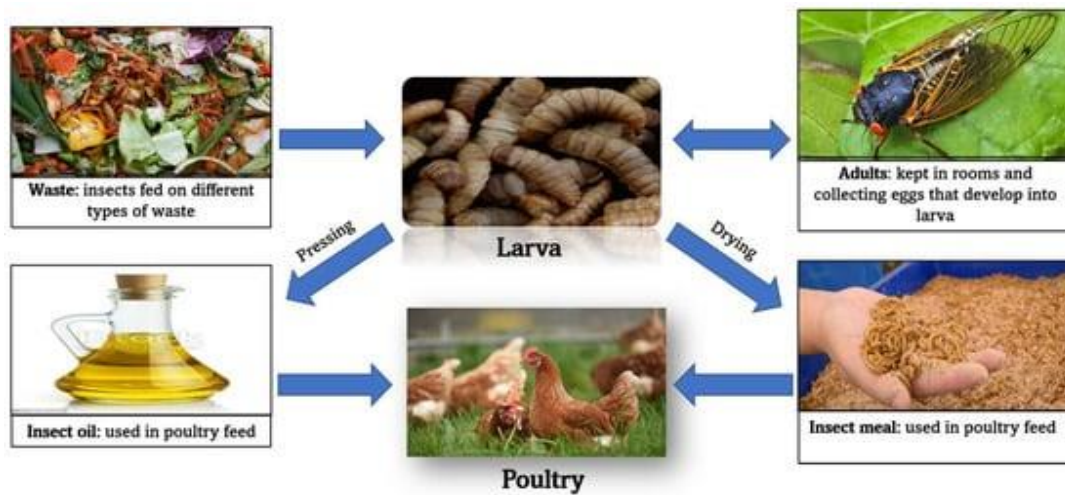


Figure 2.1: Bioconversion of Organic Materials to Poultry Feed (Khalifah et al., 2023)

Due to the fact that the nutritional composition of BSFL changes as the larvae develops, proper management should be carried out including maintaining low temperatures of 15°C to minimize the process of turning (transition in to the pupa stage) (Adnan, 2023). The BSF have four phases of life cycle which are egg, larvae, pupa and the adult stage. The prepupa is the last stage in development of the larvae and its referred to as a migratory stage in which the BSF prepupal prepares to keep large fat content for the purposes of supplying energy during the migration process and when pupating into an adult (Abd El-Hack et al., 2020).

The prepupa now moves to a suitable site that is dry to pupate in the last stages of larvae development (Liu et al., 2017). In the course of the dispersal phase, it becomes very easy to separate the larvae from the organic matter that it was feeding on and therefore the prepupae is easily harvested by directing them to collection bins to represent a pupation site they are searching for (Abd El-Hack et al., 2020). However, some prepupae should be left behind without harvesting so that they can produce a colony that will develop into pupae for the production chain to be sustained (Abd El-Hack et al., 2020). The adults are neither disease vectors nor pests and only utilize the fat stored during the larval development stage to survive and afterwards they don't feed on anything apart from water (Abd El-Hack et al., 2020; Liu et al., 2017). The female do not oviposit on the food but only oviposit around the edges of the

source of food for the larvae and therefore no pathogens transmission from the waste (Liu et al., 2017). Figure 2.2 represents a schematic diagram showing the life cycle of BSF.

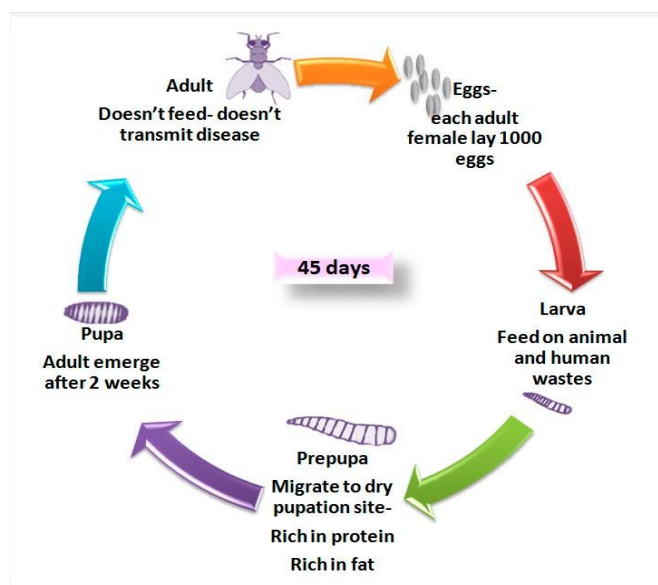


Figure 2.2: BSF Life Cycle (Abd El-Hack et al., 2020)

The BSFL reduces the population of houseflies and harmful bacteria and potentially reduces pollution from the animals and plants wastes by 50% and these wastes produces BSFL with approximately 30% fat and 40% proteins. Accumulation of lipids in the larvae is largely dependent on the type of feeding material used during the production process (Liu et al., 2017). BSFL has been extensively used as a source of feed for aquatic and terrestrial animals. Besides that, BSFL have the potential of being used in the production of biofuel thus making the technology of waste to energy to be more feasible (Liu et al., 2017).

2.4 Environmental Impact of BSFL

Exploration of Insects as an alternative source of proteins have been carried out in order to achieve global food security that is environmentally sustainable (Zhao et al., 2023). According to Elahi et al. (2022), BSFL consume food waste and animal manure and produces larvae rich in proteins and also producing fertilizer. The organic wastes are converted by insects into proteins, and also reduce methane

emission, odors and reduce excretion of total nitrogen. According to Zhao et al. (2023) BSFL have continued to catch more attention due to their nature of high nutritional value, low pathogenic risk, and effective biomass conversion. According to Campbell et al. (2020), BSFL is preferred for large scale farming due to its efficient ability to upcycle organic material into a protein source that is of high value thus enhancing the productivity and efficiency of food chain. Organic bi-products from the industries (such as breweries), facilitates large scale production of BSFL due to their production in large quantities (Campbell et al., 2020). According to de Souza Vilela et al. (2021), production of BSFL requires low use of rearing substrate, water, energy resources and space. Previous studies have reported that BSFL have the capacity to reduce mycotoxins (such as aflatoxin and ochratoxin) and pathogens (such as *E. coli* and *Salmonella sp.*) contaminations in waste materials (Zhao et al., 2023). BSFL can be processed as protein ingredients in animal feeds thus preventing anti-nutritional factors e.g. trypsin inhibitors from plant-based proteins from accumulating (Zhao et al., 2023).

2.5 Role of Feeds in Egg Production

Layers birds require provision of appropriate nutrition for the optimal production of eggs and body weight. Rations for the layers have been designed by nutritionists to meet the hens requirements in terms of vitamins, proteins, energy and minerals (Zaheer, 2015). Many studies have shown that fish meal can be substituted with BSFL in a range between 25% to 100% and this rationale could be related to larvae meal quality, diet formulation and the animal species (Oteri et al., 2021). In production of poultry eggs, the feedstuffs used should be in a position to improve or maintain the level of egg production, and the sensory attributes of the eggs that include appearance and the taste of the eggs (Hammershøj, Steinfeldt, Poultry, & Science, 2005). However, limited research has been carried out concerning how layers diet formulation with different materials affects the quality parameters of the eggs such as appearance, aroma and taste.

In other studies, different feed formulation have been seen to have a negative impact on the quality of eggs for example some fish products and rapeseeds have been noted

to induce off flavors and unwanted taste in hen eggs (Hammershøj et al., 2005). When consumers buy eggs in retail, one key parameter that they consider is the yolk color and this is influenced by the type of diet due to the presence of xanthophylls that enhance the color. Yolk color have been used to judge the quality of the eggs even though this have got no relationship with either cooking characteristics, nutritive value or even the freshness of the eggs (Hammershøj et al., 2005). The yolk color is derived from the red pigments (citraxanthin, canthaxanthin, astaxanthin) and yellow pigments (zeaxanthin, lutein, apo-ester) xanthophylls or carotenoids contained in the layers diet (Hammershøj et al., 2005). Color measurement for the yolk can be done using the Roche Yolk Color (15-grade fan) or using the L*, a*, b* color system (Hammershøj et al., 2005).

According to Zaheer, (2015), the composition of the eggs remains relatively stable in terms of essential amino acids, total proteins, and total lipids in reference to dietary change. However, other components like mineral contents, cholesterol, antioxidants, fatty acid composition, and carotenoids are highly affected by diet formulation (Zaheer, 2015). Other factors affecting the nutritional composition of the eggs include the prevailing environmental conditions, breed and the age of the birds (Zaheer, 2015). Factors like, storage, egg age, humidity, temperature and handling is vital for the overall quality of the eggs (Zaheer, 2015). Eggs are a good source of vitamins, both the water-soluble B vitamins (cobalamin (B12), biotin (b7), pantothenic acid (B5), folate (B9), riboflavin (B2), pyridoxine (B6), and thiamine (B1) and fat-soluble vitamins (A, D, E, K). The eggs are also a good source of minerals such as selenium, iron, sodium, magnesium, calcium, and phosphorus (Zaheer, 2015).

2.6 Nutritional Value of Black Soldier Fly Larvae

According to Makkar et al., (2014), fresh BSFL have a high dry matter content that ranges between 35-45%. In a previous study conducted by Al-qazzaz, (2016), the BSFL was found to contain high percentages of metabolizable energy measured at 696.3 kcal kg⁻¹. The nutrients composition of BSFL varies depending on the type of substances used for rearing them (Shah & Çetingul, 2022; Tschirner & Simon, 2015).

For example, the larvae fed on cattle manure contains 35% fat, swine manure 28%, food waste rich in oil 42-49%, and poultry manure 15-25% (Makkar et al., 2014). BSFL has been found to vary in terms of nutritional composition depending on the different larvae development stages (Abd El-Hack et al., 2020; Al-qazzaz, 2016; Barragan-Fonseca, Dicke, & van Loon, 2017). According to Shah and Çetingul (2022), the pre-pupae stage of the larvae contains up to 45-46 % protein content while the pupa stage contains approximately 56-57%. After the BSF eggs hatches, the protein content increases and then gradually decreases to a minimum level of 38% between 4th and the 12th day of the development stage and finally increases to 39.2% on the 14th day (Abd El-Hack et al., 2020). The protein content increases steadily reaching 46.2% for the early stages of the pupa and continues to increase in the adult stage reaching up to 57.6% (Abd El-Hack et al., 2020).

BSFL contains a high protein content (42% crude protein) in which the essential amino acids have a similar pattern compared to the fish meal (Hender et al., 2021; Tschirner & Simon, 2015). According to Lokaewmanee et al. 2023, the major fatty acids and amino acids present in BSFL are lauric acid and glutamic acid at 22.36 and 1215 mg/100 g respectively. Lauric acid has been reported to have some positive effects such as helping the BSFL to survive various environmental threats for example pathogenic microorganisms that are found in organic waste due to its strong antimicrobial properties (De Souza Vilela et al., 2021). In addition, lauric acid also have some beneficial characteristics towards modulating endometrial and breast cancer cells and have some anti-inflammatory effects (De Souza Vilela et al., 2021). However, lauric acid have been found to have negative impacts to the human body such as increasing LDL content leading to cardiovascular diseases (De Souza Vilela et al., 2021).

BSFL can be used in feeds either as full fat or can be defatted in which the protein content has been found to increase to 66% and this value is high in comparison to full fat BSFL (Shah & Çetingul, 2022). According to Schiavone, De Marco, et al., (2017), when the BSFL is defatted, the protein value increases surpassing the protein content of the soy bean meal. The defatting process of BSFL can be done through the mechanical process of cutting the larvae followed by pressing them to facilitate the

leaking of the intracellular fat. Also, the defatting process can be done through a chemical process by extracting the insect meal using petroleum ether (Schiaivone, De Marco, et al., 2017). Abd El-Hack et al. (2020) reported that BSFL that was partially defatted contained 55.3 % crude protein content, while the BSFL that was highly defatted recorded a crude protein content of 65.5%.

Abd El-Hack et al. (2020) further reported that the crude protein content of the BSFL closely compares to that of the fish meal and meat. Various authors have reported the protein content of BSFL and the lowest content have been documented is 35-36% which however is higher than protein from plant based sources such as sunflower and linseed meal (Shah & Çetingul, 2022). Insects have high levels of methionine, lysine, and arginine and also contains high amount of histidine that is four times greater in comparison to fish meal (Shah & Çetingul, 2022). Schiaivone, De Marco, et al. (2017) reported that BSFL have an amino acid composition that is rich in lysine and methionine (22.3-9.05 g/kg DM) and is superior in comparison to soy bean. In terms of non-essential amino acids, BSFL contains large amounts of tyrosine, proline, and alanine as compared to the fish meal and soy bean meal (Shah & Çetingul, 2022). The table 2.3 below shows the contents of BSF crude proteins, crude fat, amino acids and ash contents.

Table 2.3: Crude Protein, Amino Acids, Crude Fat, Ash Contents in (BSF)

CP %	CF %	Ash %	Amino Acids % DM					
			LYS	MET	THR	ARG	VAL	ILE
39.9–43.1	21.8–38.6	2.7–19.7	2.34–2.57	0.71–0.87	1.54–1.68	1.99–2.03	2.41–2.82	1.72–1.91
43.6	33.1	15.5	2.62	0.74	1.78	2.65	2.79	2.03
36.2	18	9.3	2.75	0.54	1.95	2.98	2.28	2.1
40.7	15.6	19.7	2.9	1.3	2	3.27	2.6	2.17
36.9	34.3	17.3	2.23	0.9	1.52	1.94	2.2	1.72
55.3	18	9.9	2.1	0.65	1.7	2.2	2.7	1.9
54.8	15.6	7.7	2.1	0.66	2.04	1.73	3.8	2.34
65.5	4.6	9.3	2.5	0.86	2.2	2.7	3.5	2.4
62.7	4.7	8	4.14	1.33	2.37	-	5.13	3.18
57.5	7	-	3.3	0.92	2.32	2.79	3.47	2.44

CP = crude protein, CF = crude fat, LYS = lysine, MET = methionine, THR = threonine, ARG = arginine, VAL = valine, ILE = isoleucine. Source: (Abd El-Hack et al., 2020)

Insects lipids have been reported as a less expensive and suitable alternative to coconut oil, soy bean oil, fish oil, and palm kernel oil used in animal feeds (Gasco et al., 2019). The amount of ether extract and the fatty acid composition in insects varies depending on the rearing substrate used, development stage, species, sex, and the method of processing used (Gasco et al., 2019). Also, composition of the fatty acid in the larvae largely depends on the composition of the fatty acid in the diet. BSF larvae that were fed on manure obtained from the cows contained 16% palmitic acid, 0.2% omega-3-fatty acid, 32% oleic acid, and 21% lauric acid while the following proportions were respectively 11%, 3%, 12% and 43% for larvae that were fed on 50% cow manure and 50% fish offal. The lipid content was found to increase from 21% to 30% dry matter. Utilization of BSF larvae that are fed on waste material that contains omega-3- fatty acids is a better way to enrich the biomass (Makkar et al., 2014).

In addition, the composition of FAs and fats in BSFL are also influenced by the different stages of the life cycle. The ether extract have been found to increase rapidly reaching the value in the early prepupa development stage (Liu et al., 2017). BSFL pupa stage have been reported to reach 28-30% with lauric acid which is a SFA contributing 35-50% of the total amount of the fatty acids contained in the BSFL (Shah & Çetingul, 2022). According to Gasco et al., (2019), the lipid content of the BSFL varies between 15-49% on the basis of dry matter (DM) in which the author reported that the highest proportion of the total fatty acid is contributed by the SFA that reach up to 44.5 %. The most abundant SFAs in BSFL are palmitic acid (C16:0), lauric acid (C12:0) and myristic acid (C14:0) (Gasco et al., 2019).

For the MUFA content, oleic acid have a high content ranging between 79.7-266.0 g/kg and for the PUFA linoleic acid (omega-6) forms the highest content ranging between 38.0-314.0 g/kg (Gasco et al., 2019; Lu et al., 2022). According to the figures reported by Lu et al. (2022), the figures for SFA, MUFA, n-6 PUFA, and n-3 PUFA are as follows;362.0-782.9 g/kg, 85.5-287.0 g/kg, 80.0-314.0 g/kg and 9.8-36.0 g/kg respectively. BSFL contains high amounts of myristic fatty acids in comparison to soy bean meal (Shah & Çetingul, 2022). According to Hender et al. (2021), BSFL larvae oil which is a byproduct resulting from processing BSFL

contains high fat content especially the SFA in which the highest percentage among the total fatty acids is represented by lauric acid (C12:0) (21.4-49.3%) and therefore more research is needed in utilization of BSFL oil in animals diet. The substrate used in feeding BSFL affects the composition of the BSFL oil for example the use of fish offal as a substrate increases the n-3 PUFA in the oil while use of seaweed has the potential to enrich the larvae with iodine and EPA (Hender et al., 2021).

BSFL have been reported to contain MUFA in moderate amounts, 15% PUFA, and high amount of saturated fatty acids (C14:0), (C12:0), and (C16:0) (Shah & Çetingul, 2022). According to the figures reported by (Shah & Çetingul, 2022), the levels of alpha-linolenic acid (1.6%) and linoleic acid (31.4%) were high at the first week of the larvae stage and decreased to 1.5% and 7% respectively at the 14th day of the larvae development stage (Shah & Çetingul, 2022). These figures compares with those reported by Xiu Liu et al. (2017), in which the values of the PUFA content decreased with the larvae development of the BSF as shown in Figure 3 below. Essential fatty acids forms the basis of synthesizing long chain PUFAs, for examples arachidonic acid, EPA, and DHA (Liu et al., 2017). Furthermore, they are significant in maintaining the nerve impulse transmission, brain function, and cell membrane in normal conditions. The essential fatty acids are also critical during cell division, transferring of atmospheric oxygen to the plasma and in hemoglobin synthesis. The PUFAs such as omega-3 and omega-6 fatty acids help in preventing cancers and cardiovascular diseases (Liu et al., 2017). Figure 2.3 below shows alteration of SFA and PUFA in the BSF dry mass during metamorphosis.

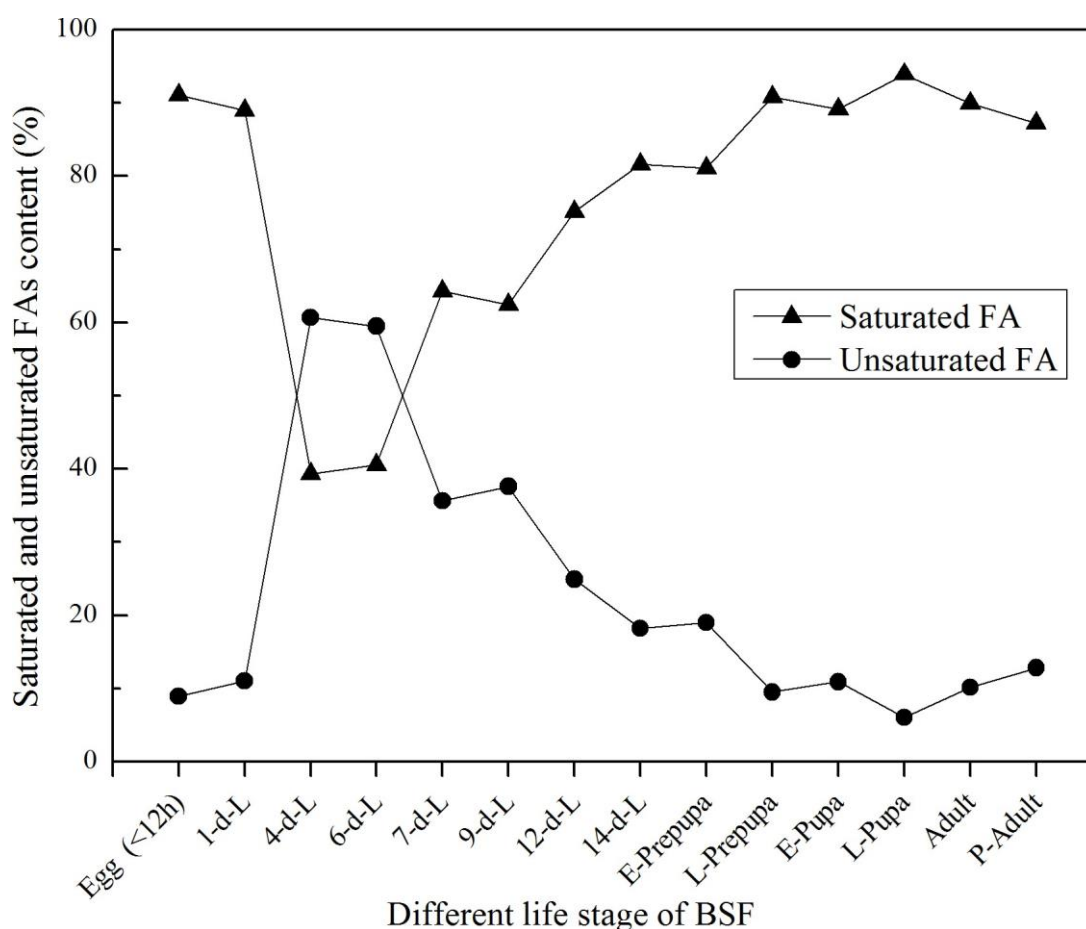


Figure 2.3: Alteration of SFA and PUFA in the BSF Dry Mass During Metamorphosis (Liu et al., 2017)

Micronutrients such as vitamins and minerals play a critical role in poultry production for their nutritional value. Insects contain essential micronutrients that are present in significant amounts such as vitamin E, phosphorus, calcium and zinc which are required in poultry growth and production. Further studies are required to investigate the bioavailability of minerals that are derived from insects such as cadmium, iron and zinc (Abd El-Hack et al., 2020). The ash content of the BSFL varies between 11 -28% dry matter and are rich in phosphorus (0.6-1.5% dry matter) and calcium (5-8% dry matter) (Makkar et al., 2014). According to Lu et al. (2022), the most abundant mineral in BSFL is calcium with the highest figure reported being 35.7 g/kg.

BSFL contains a high amount of minerals (phosphorus, iron, calcium and zinc) and Vitamin E that have a significant role in human nutrition (Shah & Çetingul, 2022). Vitamin E helps in protecting the body against heart diseases caused by free radicles since vitamin E is an antioxidant that is fat soluble (Liu et al., 2017). The pupa stage of the BSFL larvae have a high vitamin E content (6.7 mg/100 g) as compared to the pre-pupa stage that contains (3.3 mg/100 g) (Shah & Çetingul, 2022). Minerals such as iron, sodium and zinc, have been reported to be abundant in the mature stage while phosphorus and calcium have been reported to be in double quantities in pre-pupa stage (Shah & Çetingul, 2022). Also Xiu Liu et al. (2017) reported that iron, sodium and zinc were highest in the mature larvae stage in comparison to the early pre pupal stage as shown in the Table 4 below. According to Xiu Liu et al. (2017), the concentration of most minerals and vitamin E content was found to be highest in in the early pre-pupa stage and in the mature larvae. Therefore, both prepupa stage and the mature larvae development stage have been found to be the most economically effective with the highest level of nutrients (Liu et al., 2017). According to Xiu Liu et al. (2017), the high mineral level in BSFL can help in the production of animal products to be consumed by humans in countries where deficiencies in micronutrients is prevalent.

Table 2.4: Mineral and Vitamin Content of BSFL

Vitamin and mineral	Mature larvae (14th day)	E-prepupa
Vitamin E (mg/100 g)	6.7±0.64	3.3±0.42
Calcium (mg/100 g)	2900.0±13.57	3000.0±18.45
Phosphorus (mg/100 g)	350.0±6.11	620.0±9.85
Sodium (mg/100 g)	100.0±2.48	50.1±1.41
Iron (mg/100 g)	200.0±4.27	7.5±0.95
Zinc (mg/100 g)	61.4±1.71	3.3±0.29

Mineral and Vitamin content of BSFL (Liu et al., 2017)

Besides the type of feeding material used in feeding the BSFL, the different larvae development stages, and the method of processing employed, other factors affect the nutritional composition of the BSFL and they include, conditions such as humidity,

moisture content, temperature, pH and sunlight. Temperature and humidity have an effect on the incubation stage, development and BSFL life span. The ideal conditions for BSFL are as follows; relative humidity should range between 40-70% while the temperatures should range between 26-40°C (Lu et al., 2022). High relative humidity leads to excessive wetness of the diet making it difficult for the larvae to consume it and obtain enough oxygen supply (Makkar et al., 2014). BSFL grows better in alkaline conditions in comparison to acidic conditions and the pH of 6-9 is considered ideal (Lu et al., 2022). Figure 2.4 below summarizes the nutritional composition of BSFL.

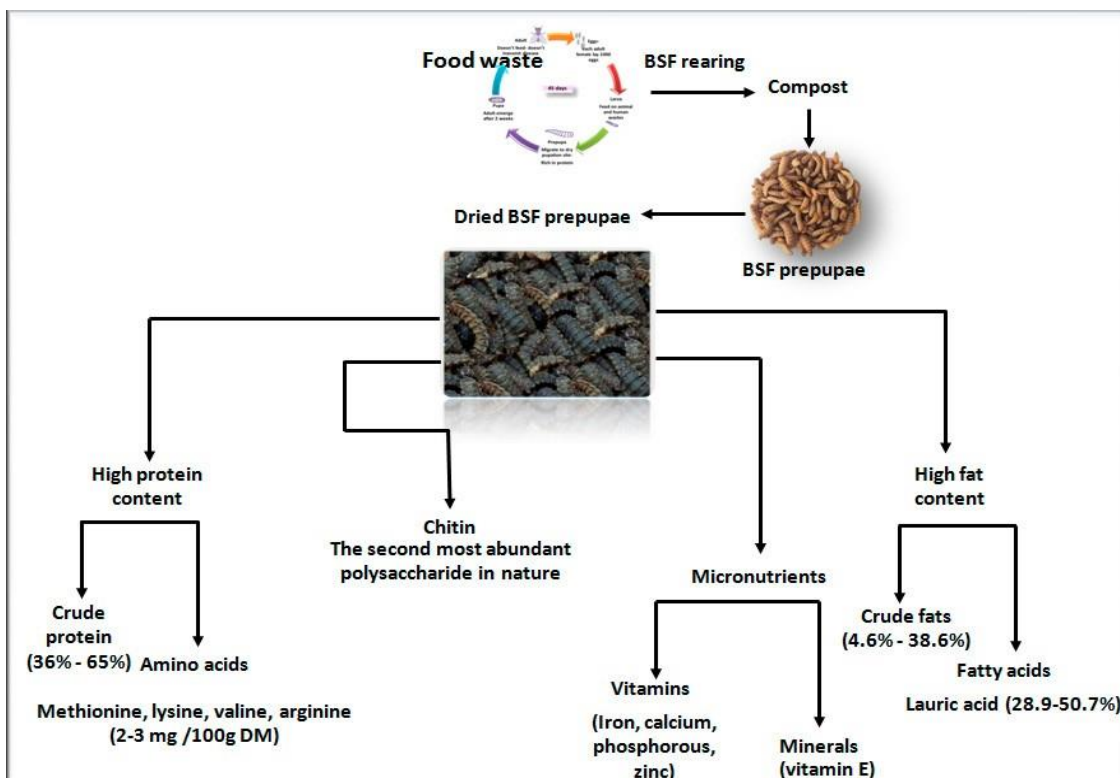


Figure 2.4: Nutritional and Chemical Composition of BSFL (Abd El-Hack et al., 2020).

2.7 Application of BSFL in Poultry Production

2.7.1 Effects on Laying Performance

Research from several studies clearly shows that inclusion of BSFLM in the layers diet does not have an adverse effect on the laying performance. Research conducted

across various strains such as Bovans, Hy-line Brown, and Lohmann Leghorn layers identified that inclusion of BSFLM in the layers diet did not show significant differences in laying percentage (Kim et al., 2022; Maurer et al., 2016; Tahamtani et al., 2021). In order to have high production of eggs, adequate nutrition is necessary for the layers hen and one of the critical nutrients is the Sulphur amino acids (Domingues et al., 2012). For the layers hens to have an optimal performance, they requires supply of proteins based on essential amino acids requirements which are lysine and methionine + cystine (Domingues et al., 2012).

However, Mwaniki, Neijat, and Kiarie (2018) reported data on daily production that showed a quadratic response after BSFLM inclusion in the layers diet. For example, the hen daily production for the 0%, 5%, 7.5% inclusion rate was 89.4%, 84.8%, and 87.8% respectively. Mwaniki, Neijat, and Kiarie (2018) suggested that daily egg production and egg size are mainly influenced by the amino acid and energy intake from the layer's feeds. At 5%, Mwaniki, Neijat, and Kiarie (2018) identified a reduction in daily egg production in layers despite the diet having comparable amounts of amino acids and energy with the other diets and therefore was not able to give a conclusive reason on the observed reduction suggesting that laying percentage might be sensitive to certain nutrients intake levels.

Conversely, Marono et al. (2017), reported a decline in production performance of layers hen fed on defatted BSFLM in replacement of the soy bean meal highlighting that production performance of layers is associated with feed intake in which a higher feed intake results into a high productive performance. According to Marono et al. (2017), feed intake can be influenced by the color and the flavor of the feeds in which layers hens avoid diets containing ingredients that are unusual compared to the diets having basic nutrients obtained from the natural feed materials.

2.7.2 Feed Intake and Feed Conversion Ratio (FCR)

Xu Liu et al. (2021) conducted a study on effects of adding different amounts of BSFLM in Xuefeng black-bone layers diet on layers performance. The different levels of inclusion of BSFL diet in the layers diet were 0% (control), 1%, 3%, and 5% and the diets were isoenergetic and isonitrogenous. Xu Liu et al. (2021)

identified that supplementation of layers diet with BSFL diet at 3% and 5% lead to a significant increase in the feed intake and on the other hand reduced the feed conversion ratio during the experimental period. Xu Liu et al. (2021), suggested that the increase in feed intake was as a result of the palatability of the layers diet that contained the BSFL.

Kim et al. (2022) conducted a study to establish whether dietary BSFL oil could be used to replace soy bean oil as an alternative source of fat in layers hen diet. In this study, Hy-line Brown layers (25 weeks) were assigned to either experimental diets replacing soy bean oil with either 50% BSFL or 100% BSFL or a control diet. Addition of the larvae in the layers diet did not have a negative impact on the feed intake, and feed conversion ratio. Feed intake has been found to be influenced by the nature of processing the BSFL. High temperature processing of the BSFL contributes to lipid oxidation which brings about smell differences thus influencing the feed intake by the layers (Kim et al., 2022).

Mwaniki, Neijat, & Kiarie (2018) conducted research on the effects of formulating layers diet with 0%, 5% and 7.5% of defatted Black soldier fly larvae meal (BSFLM) in a soy bean meal diet on egg quality and production from shaver white leghorns between 19 to 27 weeks of age. Feed conversion ratio increased linearly with increase in BSFL in the layers diet while feed intake had both linear and quadratic increase with BSFLM inclusion at 7.5% having the highest value in comparison to 0% and 5% BSFLM level. The author of this study reported that, the increase in feed intake could have been contributed to the high fiber content contained in the BSFLM diet in form of chitin. Composition of feeds in terms of nutritional composition and the balance of nutrients is critical in determining the voluntary intake of feeds by poultry birds (Mwaniki et al., 2018).

Generally, in cases where factors such as health, genotype, and the composition of ingredients (such as fiber) are standardized, the layers hen fed ad libitum have been reported to consume feeds until they meet their requirements for the first limiting nutrients and this in most cases is the energy yielding nutrients (Mwaniki et al., 2018). Availability of nutrients and energy in poultry diets is affected by the dietary

fiber present and therefore the birds feeding on the fibrous diet are required to consume large amount of that diet (Mwaniki et al., 2018). Previous studies reported that feed conversion ratio, feed intake and egg production were unaffected by replacing 100% soy bean meal with defatted BSFL (Mwaniki et al., 2018). Maurer et al. (2016) conducted a study involving feeding leghorn laying hens with partly defatted BSFL dried meal fed with vegetarian by-products by replacing the soy bean by 0% (control), 12%, and 24%. The results obtained showed that inclusion of BSFL in the layers diet did not have a significant effect on feed intake, feed efficiency, in comparison to the control diet. The variations in the data reported could have been contributed by the use of different strains of layers at different age groups (Mwaniki et al., 2018). Different studies have reported contrary results and this could be as a result of several factors that include the level of inclusion of the BSFL diet, the animal age, and the stage of development of the insect.

2.7.3 Physical Properties of Eggs (Egg Weight)

Adnan (2023), conducted a study on feeding layers hen (Bovan white hens), with live or dried BSFL with an objective of determining the impact on egg quality. The researcher replaced 20% of the daily intake of the dry matter with dried and live BSFL. Adnan (2023) established that feeding hens with the larvae had no significant effect on egg weight. Tahamtani et al. (2021), conducted a study to establish the effects of feeding layers hens with varying amount of live BSFL on egg quality. The study involved feeding 40 Bovans layers individually with 4 dietary treatments of the live larvae provided at 0%, 10%, 20%, and ad libitum. The author also reported that there was no significant difference noted between the treatments on egg weight.

Maurer et al. (2016) reported no significant effect on egg weights on a study conducted involving feeding leghorn laying hens with partly defatted BSFL dried meal. This study also reported that yolk weights were not significantly affected by diet change. Lokaewmanee et al. (2023) studied the effect of live BSFL inclusion in the Charoan Pokphad Brown layers and reported that there was no significant effect on egg weight. Mwaniki, Neijat, & Kiarie (2018) reported However, reported data

that showed a quadratic response for egg weight suggesting that egg size is mainly influenced by the amino acid and energy intake from the layer's feeds.

2.7.4 Physical Properties of Eggs (Shell Thickness)

Egg shell thickness is critical in minimizing breakages thus reducing economic losses. Adnan (2023) established that feeding hens with the larvae had no significant effect on shell breaking strength, and egg shell thickness. Several other studies reported no significant difference between the treatment on egg shell thickness (Heuel, Sandroock, et al., 2021; Kim et al., 2022; Lokaewmanee et al., 2023; Star et al., 2020; Tahamtani et al., 2021). However, (Mwaniki et al., 2018) highlighted that Inclusion of the BSFL meal in the diet linearly increased the shell thickness. Improvement of the egg shell qualities could have been contributed by the improved absorption of calcium in the gut as well as improved metabolism of calcium (Mwaniki et al., 2018). (Liu et al., 2021) reported that feeding layers with BSFL diet at the 56th day of the experiment did not affect the egg quality negatively. However, at 28th day, egg shell thickness significantly decreased meaning BSFL had an adverse impact on the quality of the eggs when the experiment was at an early stage and later improved towards the 56th day. Improvement in shell thickness could have been as a result of increased circulation of the level of plasma calcium in BSFLM fed layers in comparison to the control. Therefore, improved absorption and metabolism of calcium in BSFL fed layers could have led to production of stronger eggshells (Liu et al., 2021).

2.7.5 Physical Properties of Eggs (Albumin Height and Haugh Units)

Several studies reported no significant effect on albumin thickness (Adnan, 2023; Heuel, Sandroock, et al., 2021). Other studies reported no significant effect on quality parameters such albumin index and Haugh unit (Kim et al., 2022; Lokaewmanee et al., 2023; Mwaniki et al., 2018; Star et al., 2020). Liu et al. (2021) reported that feeding layers with BSFL diet at the 56th day of the experiment did not affect albumin height negatively. However, at 28th day, albumin height significantly decreased and later improved towards the 56th day BSFL suggesting that BSFL inclusion had a negative effect on eggs quality at early stages of the experiment.

Internal quality parameters of the egg such as albumen index and Haugh units may be influenced by the level of the sulfur amino acids (Domingues et al., 2012). Taurine, homocysteine, methionine and cysteine are sulphur containing amino acids in which only methionine and cysteine are incorporated in protein diets (Brosnan & Brosnan, 2006). In layers, methionine is referred to as the first limiting amino acids while lysine is referred to as the second limiting amino acid (Heuel et al., 2021).

2.7.6 Sensory Characteristics and the Yolk Color

The sensory characteristics of the egg yolk, specifically yolk color, is a key driver of perceived nutritional value and consumer preference. Several studies reported higher yolk color that increased with age and further suggested that the darker yolk color obtained could have been due to the high content of lutein and B-carotene in the BSFL meal (Adnan, 2023; Liu et al., 2021; Mwaniki et al., 2018). According to Adnan (2023), the high lutein content in BSFL eggs is beneficial to the health of humans since it has been reported to improve cognitive ability in aged people, protect against stroke and heart disease. Kim et al. (2022). Found that the yolk color was intensified by addition of larvae oil in the diet. The presence of the high crude fat extract in the BSFL oil diet have been found to enhance the absorption of carotenoids in the eggs. However the results reported by Kim et al. (2022). Showed that crude fat was constant in the different experimental diets and therefore the intensified red yolk color was as result of carotenoids pigments such as β -carotene, lutein, and zeaxanthin in the BSFL oil and BSFL. Lokaewmanee et al. (2023) reported contradicting results whereby egg yolk color decreased with increase of BSFL in the diet thus concluding that the larvae had less pigments. Variations in yolk color results reported by different studies could be attributed to the kind of rearing substrate used in the production of the larvae.

2.7.7 Fatty Acids and Cholesterol

Diet fed to the layer's birds have the capacity to alter the egg yolk fatty acid composition (Lokaewmanee et al., 2023). Xu Liu et al. (2021) reported that the yolk content from layers reared on BSFLM contained a high content of MUFA (47%) followed by SFA (37%) and finally PUFA (19%). Xu Liu et al. (2021) identified that

incorporation of the dietary BSFLM in the hen diet modified the profile of the fatty acid whereby the PUFA content of the egg yolk increased significantly and reported that this could have been as a result of the increase in proportion of the C20:2 and C18:2n6c fatty acids. Lokaewmanee et al. (2023) reported a significant effect on the yolk fatty acid composition for example C20:3n6, DHA C22:6n3 and linolenic acid C18:3n6.

However, Kim et al. (2022), found out that the total PUFA content reduced in the eggs and in the layer's hen abdominal fat. Similar results have been reported whereby the PUFA contents i.e. n-6 and n-3 PUFA contents decreased in the eggs (Bejaei & Cheng, 2020; Chatzidimitriou et al., 2022). These discrepancies could be as a result using different rearing substrates and using larvae at different stages. Therefore, fatty acid composition of the BSFL can be improved through supplementing the rearing substrate by using high PUFA content or by utilizing the larvae when it is at an early development stage (Kim et al., 2022). For example, during the 6th day of the larvae development stage, linoleic acid level is found to be at its maximum (31.4%) while lauric acid content is found to be at its minimum on the 4th day of larvae development stage (7.6%) (Kim et al., 2022).

According to Chatzidimitriou et al. (2022) replacing soy bean meal completely with defatted BSFLM increased the MUFA contents in the eggs. Similar results were reported by Bejaei and Cheng (2020) who identified that BSFL fat produced yolk lipids which had higher MUFA content, in comparison to yolk lipids produced by layers fed on the soy bean meal diet (control). Bejaei & Cheng (2020) reported similar results of increased MUFA content in the yolk fat after supplementation of layers diet with soy bean meal.

Results obtained by Zawisza et al. (2023) showed that SFA content, myristic acid, and lauric acid increased significantly in the eggs from layers fed 10% and 15% BSFL levels. This results compares to those reported by B. Kim et al., (2022) whereby the SFA content increased with increase in BSFLM in the diet. Bejaei and Cheng (2020) and Chatzidimitriou et al. (2022) also noted an increase in C14:0 and C12:0 but this did not affect the egg total lipid content. Despite the increase in lauric

acid in the egg yolk, the content analyzed still remained at a low level of 0.0267-0.08 g/100 g of the overall fatty acid content (Zawisza et al., 2023). Previous studies have reported that transfer of lauric acid to the egg yolk from the insect-based diet was below 1%. However, the transfer of the palmitic and myristic acid have been reported to be approximately 100% and 30% respectively (Zawisza et al., 2023).

Inclusion of BSFLM in the diet lead to a decrease in cholesterol level in the egg yolk as compared to the control group (Zawisza et al., 2023). However previous studies, have reported no significant difference in cholesterol level in the egg yolk after feeding the layers with the BSFLM and concluding that the level of cholesterol in the eggs depend on intra organismal transformation and not on the diet (Zawisza et al., 2023). Also BSFL contains chitin which have been reported to lower the amount of the cholesterol in the BSFL eggs (Zawisza et al., 2023).

However, Kim et al. (2022) reported contradicting results whereby the cholesterol content reduced in eggs produced by BSFLM diets and this could be as a result of the presence of chitin. Chitin has been associated with reducing the absorption of lipids accompanied by a decline in cholesterol level in the blood (Kim et al., 2022). Medium chain fatty acids have been proven to increase the levels of cholesterol in the serum as well as the HDL cholesterol. However, even though BSFL oil have high level of MCFA, Kim et al. (2022), reported that MCFA in BSFL oil diet could not influence the cholesterol levels in the eggs due to the nature of lipid metabolism and the lack of chitin.

The figure 2.5 below shows a summary of utilization of BSFL in layers hen production and effects on quality of the products.

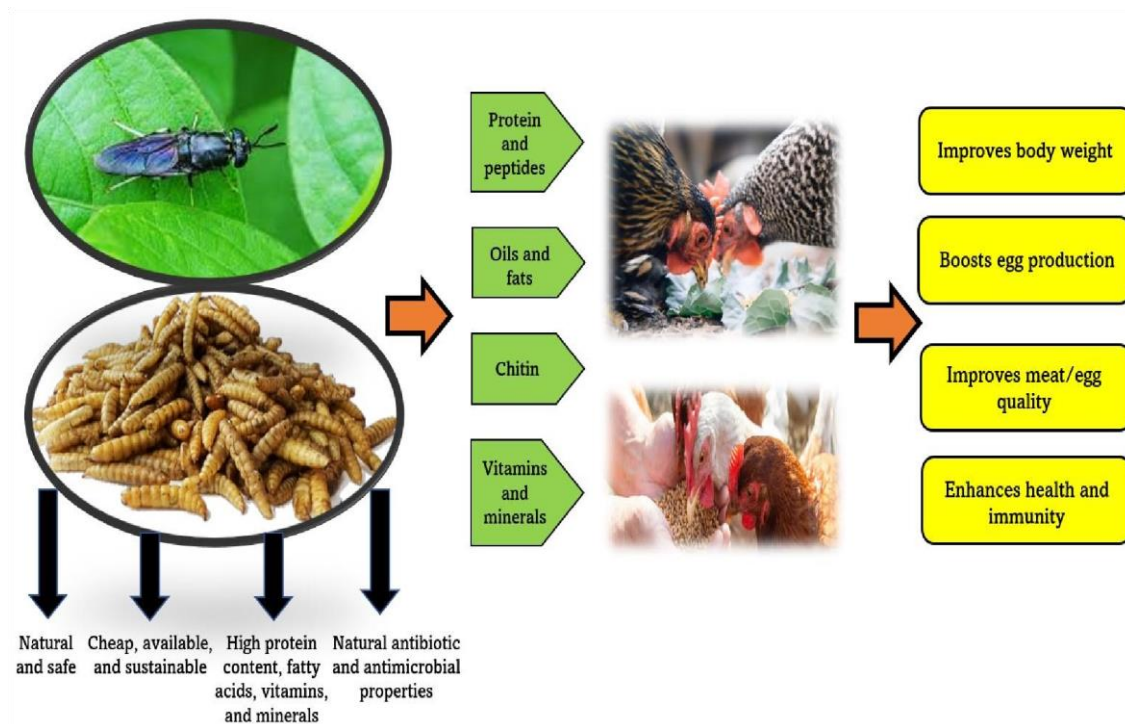


Figure 2.5: BSFL Nutritional Characteristics and their Use in Poultry Production (Khalifah et al., 2023)

2.8 Use of BSFL Safety Considerations

Production of insects can be interfered with by chemical pollution which may include pesticides, heavy metals and veterinary medications (Khalifah et al., 2023). Insects may contain chemical pollutants as a result of accumulation from the feeding substrate or the insects itself can synthesize a natural toxin (Khalifah et al., 2023). One of the safety considerations in the BSFL eggs is the heavy metals concentration in the eggs which can potentially risk the consumer's health. Heavy metals can get in to the BSFL larvae at the different stages of growth of the larvae and prepupae through dietary consumption (Kim et al., 2022). According to Kim et al. (2022), the presence of heavy metals that were undesirable for example Cd, As, Hg, F, Pb, and Al after feeding layers with BSFL oil was at acceptable levels thus concluding that the larvae oil is a safe ingredient for use in layer hens diet. According to Khalifah et al. (2023), heavy metals concentration in insects reduces during the molting stage and during metamorphosis and this leads to a lower concentration in the adults. In regards to this, the accumulation of heavy metals in insects is determined by the

rearing substrate, type of the heavy metal and the insect species (Khalifah et al., 2023). In reference to hormones, pesticides residuals and veterinary medication, previous studies have reported that they are naturally broken down as the insects continues to develop therefore minimizing their risk of contamination to the animal feeds (Khalifah et al., 2023). However Maurer et al. (2016), reported that cadmium bioaccumulation presents a major risk which processing cannot address.

According to Chatzidimitriou et al. (2022), C12:0 and C14:0 have been debated on their negative effect on the human health. These fatty acids have been contemplated to raise the low-density lipoprotein (LDL) and enhance insulin resistance, inflammation, and blood coagulation. The ratio of LDL to high density lipoprotein (HDL) predicts the risk of cardiovascular diseases and the aim is to reduce the amount of LDL. Although C10:0, C12:0, and C14:0 have been reported to increase the total cholesterol and LDL, other studies have reported that C12:0 has the potential to increase the HDL. Also, although supplementing layers diet with BSFL have significantly raised the amount of C14:0 and C12:0 in the BSFLM eggs in comparison to the control, the actual level in the eggs is lower in comparison to other foods that are common. The significant increase of the MCFA is because the initial amount in the control eggs is very low and therefore the high level of increase. In conclusion, although C14:0, C12:0 and SFA have been reported to increase significantly in the BSFL eggs, they were not high enough to challenge the health of a consumer (Chatzidimitriou et al., 2022)

According to Maurer et al. (2016), previous studies have shown that insects are capable in transmitting pathogens to poultry. According to Khalifah et al. (2023), insects have the capacity to carry parasites orally and therefore spreading them. Thus, making them a potential microbiological risk. Parasites oocytes of *Ascaris suum*, *Eimeria nieschulzi* and *Eimeria tenella* eggs were reported in the BSFL stomach in a previous study which may be transmitted it feeds formulated with the larvae (Khalifah et al., 2023). Therefore, there is need to develop suitable protocols that will help in reducing parasitic and microbial risks (such as heat treatment) thus ensuring food and feed safety (Khalifah et al., 2023; Maurer et al., 2016).

According to De Souza Vilela et al. (2021), even though PUFA and mainly omega 3 fatty acids are desirable in meeting the need for human health, lipid oxidation may be increased by PUFA and mostly the omega 3 fatty acids thus affecting the shelf life and the organoleptic characteristics of the fatty products.

2.9 Limitation of using BSFL Larvae in Animal Feeds

Although BSFL is a sustainable source of proteins for animal feed production, its utilization has been limited by factors such as lower palatability, reduced digestibility, potential accumulation of toxic materials and relatively low PUFA levels (Hender et al., 2021). In addition, high fat content in BSFL may lead to milling difficulties by the feed industry due to decreased pellet stability, excessive use of energy and susceptibility to lipid oxidation.

2.10 Consumer Acceptance of BSFL Products

According to Lu et al. (2022), consumer acceptance of animal products produced by use of BSFL fed animals should be considered. A previous study conducted through a questionnaire showed that, 67% of all the study participants accepted the use of BSFL for use in the animal feeds (Liu et al., 2017). However, the figures shows that there is still a gap in the acceptance of insects in animal feeds by the consumers and therefore the need to ensure there is improvement in consumers perception towards insect fed animal products (Liu et al., 2017). However, in regards to the significantly increased SFA and decreased PUFA, the author suggested the further studies are required to establish the impact that this fatty acid modification will have on the consumers health (De Souza Vilela et al., 2021). In comparison to the total amount of fatty acids consumed by a human in the diets, the impact from the BSFL diet may be negligible but however, future studies needs to focus on further investigation on consumers' perception (De Souza Vilela et al., 2021).

2.11 Conclusion

The available research confirms the suitability of inclusion of BSFL in layers diet with BSFL containing a high protein content (42% crude protein) in which the essential amino acids have a similar pattern compared to the fish meal (Hender et al., 2021; Tschirner & Simon, 2015). However, several discrepancies inhibits large scale adoption specifically the egg quality characteristics for example the yolk color that significantly varies across different studies depending on the birds strain and the larvae diet (Kim et al., 2022; Lokaewmanee et al., 2023). In addition Tahamtani et al. (2021), identified no significance difference on egg weight after BSFL inclusion in the diet while Mwaniki, Neijat, and Kiarie (2018) reported data that showed a quadratic response for egg weight. Therefore, to address these inconsistencies and to substantiate global findings within the local context, the current study evaluates the effects of BSFLM inclusion in Isa Brown chicken.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Feeding Regime of Laying Hen and Eggs Collection

3.1.1 Layers Experiment

Seventy-five Isa Brown layers (n=75) obtained from Kenchic Limited in Kenya were randomly selected from the growers experiment at 20 weeks and subjected to BSF-based diet types consisting of 0, 25, 50, 75 and 100% inclusion levels of BSFLM until the age of 80 weeks. The layers were introduced to the 5 treatment diets 7 days after the acclimatization. A completely randomized design (CRD) model was used, and five (5) birds were assigned per replicate for each treatment throughout the entire egg laying phases in which each treatment was replicated three (3) times. The quantity of feeds given to the layer hens ranged between 110 to 150 g on a daily basis for up to 80 weeks. The hens were treated humanely, and ethical clearance (KALRO-VSRI/ IACUC019/30082019) was acquired before the beginning of the experiment. The birds were reared in pens (1 m x 1 m) housed in the poultry research unit, Non-Ruminant Research Institute of the Kenya Agricultural and Livestock Research Organization (KALRO) located in Naivasha, Kenya. The facility is located at an altitude of 1800 m above sea level (0°43'12.85" S, 36°25'42.71" E), and has ambient temperatures that range from 17 to 22 °C and an average annual rainfall of 1000 mm.

The pens were carefully designed inside a house with concrete floors and walls separated using wire mesh. A plastic feeder (73 cm length × 26 cm width × 48 cm height) with an open top and eight holes on the sides to allow the birds access feed. Three (3) liter plastic drinker was placed inside each pen. The conditions in the rearing room were maintained at 30±1°C and 70 ± 2% relative humidity (RH). Dark: light cycle was set as 12:12 h throughout the egg laying phase. The birds were vaccinated to prevent disease (bacteria and virus) from building up in the pens. The birds were provided vitamins through drinking water when a new batch of diet was being introduced following vaccination episodes.

The drinking containers were cleaned daily and filled with good quality water every day particularly during morning hours. Poultry diets were carefully introduced into clean plastic feeders every day at 08:30 am. The bedding of the birds consisted of sterilized wood shavings in each pen, which was changed after 3 weeks to reduce the build-up of ammonia and pathogenic bacteria. The birds were allowed *ad libitum* access to feed daily (Table 5). Five different types were used, which included: Diet₀ (100% FM + 0% BSFL meal) (Control diet), Diet₂₅ (75% FM + 25% BSFL meal), Diet₅₀ (50% FM + 50% BSFL meal), Diet₇₅ (25% FM + 75% BSFL meal) and Diet₁₀₀ (100% BSFL meal+ 0% FM). The final feed that was formulated was iso-carolic and iso-nitrogenous and this was achieved by following the National Research Council (NRC) (1994) poultry nutrient requirements and by the use of Winfeed software (version 2.8). Wheat pollard and Whole maize were used as energy sources. The layers were fed on the experimental diets for 80 weeks, during which eggs were collected daily and recorded. Fifteen eggs obtained from each treatment were sampled at 45 and 80 weeks for purposes of measurements of both internal and external egg characteristics.

Table 3.1: Amino Acid Composition of BSFLM and FM

Parameter	Content (% DM)	
	BSFLM	FM
CP	43.8	65.7
Methionine	0.6	1.7
Cystine	0.3	0.6
Met. + Cys ^a .	0.9	2.2
Lysine	1.9	4.6
Threonine	1.3	2.5
Arginine	1.7	3.6
Isoleucine	1.4	2.5
Leucine	2.4	4.4
Valine	2.0	2.8
Histidine	0.9	1.5
Phenylalanine	1.3	2.4
Glycine	1.8	4.1
Serine	1.4	2.4
Proline	2.1	2.7
Alanine	2.2	3.9
Aspartic acid	3.2	5.6
Glutamic acid	3.1	8.2

^aMet- Methionine, Cys- Cystine, CP- Crude protein and DM- Dry matter. Source: (Esther, 2021)

Table 3.2: Ingridients of Layers Mash (Esther, 2021)

Ingredient (Kg/100 Kg diet)	Diet₀ (control)	Diet₂₅	Diet₅₀	Diet₇₅	Diet₁₀₀
Maize germ	53.0	53.0	53.0	53.0	53.0
Pollard (wheat)	18.0	18.0	18.0	18.0	18.0
Soy bean meal	10.0	10.0	10.0	10.0	10.0
Fish meal	10.0	7.5	5.0	2.5	0
BSFLM	0.0	2.5	5.0	7.5	10
Lime	6.5	6.5	6.5	6.5	6.5
DCP	2.0	2.0	2.0	2.0	2.0
NaCl	0.3	0.3	0.3	0.3	0.3
Super layer premix ^a	0.2	0.2	0.2	0.2	0.2
Calculated nutrient composition					
Energy Kcal/kg	2900	2878	2895	2899	2901
CP %	16	16	16	16	16
CF %	3	3	3	3	3
Ca %	1	1	1	1	1

^a Super chicken layer premix per 2.5 kg: Vitamin A: 8,000,000 IU ; Vitamin D3: 2,000,000 IU ; Vitamin E: 3,000 mg ; Vitamin K3: 2,000 mg ; VitaminB2: 3,500 mg ; Pantothenic acid: 6,600 mg ; Niacin: 20,000 mg ; Folic acid: 550 mg ; Vitamin B12: 6 mg ; Choline chloride: 200000 mg ; Lysine: 350 mg ; Methionine: 120 mg ; Manganese: 63,000 mg ; Iron: 23,000 mg ; Zinc: 63,000 mg ; Copper: 14,000 mg ; Cobalt: 1,000 mg ; Iodine: 2,000 mg ; Selenium: 100 mg and BHT: 120000 mg. BSFL- Black soldier fly larvae ; Ca- Calcium ; DCP - Dicalcium phosphate ; FM – fish meal; BSFL meal- black soldier fly larval meal; CP - Crude protein ; CF - Crude fat

3.2 Physical Properties

Whole eggs from each treatment were weighed using a Radwag scale (Model WLC 1/A2). The eggshells were broken, and the yolk was carefully separated completely from its albumen. The shell from individual eggs were air dried and weighed (Bejaei

& Cheng, 2020) using a Radwag scale (Model WLC 1/A2). The egg shell thickness was measured using a micrometer screw gauge. Yolk diameter was determined using a vernier caliper, while the height of egg yolk was measured using a tripod micrometer. The Haugh unit, which describes egg quality was determined according to the formula below (Aderemi, Adenowo, & Oguntunji, 2012):

$$Hu = 100 \log H + 7.57 - 1.7W^{0.37}$$

Whereby, *Hu* stands for Haugh unit, *H* represents albumen height, and *W* indicates the weight of the egg in grams

Albumen index was calculated using the formulae by Bulbul et al. (2014).

$$\% \text{ Albumen index} = \frac{\text{Albumen height (mm)} \times 100}{[\text{Average Albumen length (mm)} + \text{Average Albumen width (mm)}] / 2}$$

The computation of the yolk index was undertaken following the formulae described by Tilki and Saatci (2004).

$$\text{Yolk index} = \frac{\text{Yolk height (mm)} \times 100}{\text{Yolk diameter (mm)}}$$

3.3 Proximate Content of Eggs

Comparative analysis of the proximate content of the eggs was estimated according to the techniques described by the AOAC (Association of Official Analytical Chemists, 2000). Moisture compositions of the samples were estimated through the hot air-drying techniques (Method 925.10), while protein was determined by the semimicro-Kjeldahl (Method 920.87). Total lipid was determined using Soxhlet extraction technique (Method 920.85). Nitrogen conversion introduced the 6.25

factor variable to calculate the crude protein value of each sample. Determination of crude ash was done by incinerating using a muffle furnace set at 550 °C (Method 923.03).

3.4 Fatty Acids Analysis in Egg Samples

Extraction of the lipids in eggs was conducted using chloroform/methanol (2:1 v/v). Using pentadecanoic acid standard, trans- methylation of the fatty acids was calculated through refluxing efforts in methanol: toluene: sulphuric acid (20:10:1, v/v/v). Quantification of fatty acids was carried out with a gas chromatography following injection of samples through CP9010 autosampler onto the capillary column (Marelli et al., 2020). Gas chromatography (GC) (Model: Shimadzu GC-14B, Yokohama, Japan) equipped with Flame Ionization Detector (FID) and fused capillary column (OmegawaxTM 5530) of 30 m by 0.53 mm by 0.5 µm was used to express the fatty acids methyl ester (FAME). Fatty acids identification was carried out by comparing the retention times of esterified samples and methyl ester standards. Fatty acid quantification was in percent of the total FAMEs and was obtained through calculating the percent peak area of each fatty acid.

3.5 Vitamin A and E Analysis in Egg Samples

Analysis of vitamin A and E was conducted using the protocols described and tested by Kondyli, Katsiari, & Voutsinas (2007) with some modification. Two grams of the yolk and albumen samples were added into 50 ml stoppered centrifuge tubes. Absolute ethanol (5 ml) containing ascorbic acid [0.1% (w/v)] was added, followed by 2 ml of 50% potassium hydroxide (KOH). The centrifugation tubes were closed and vortexed in a water tank at 80 °C for approximately 20 minutes. Complete fat digestion was enhanced by vortexing the tubes periodically. The centrifuge tubes were then cooled using running water after the saponification process. The centrifuge tubes were carefully placed in an icy cold-water bath. Thereafter, 20 ml of hexane with 0.01% butylated hydroxytoluene (BHT) were added before vortexing vigorously the tubes for 2 min to enhance complete mixing.

To each tube, 5 ml of cold water at 1°C was added and inverted ten times, and centrifugation was done for 10 minutes at 1000×g. Using a pipette, 10 ml of the supernatant was carefully transferred into a rotary flask and solvent evaporated at 40 °C. Thereafter, residues were instantly dissolved in 2 ml of methanol. Furthermore, the standards and processed egg samples were introduced into the High-Performance Liquid Chromatography (HPLC) (Yokohama, Japan) with the help of 20 µl Rheodyne injector loop (Yokohama, Japan) with a 250 mm by 4.6 mm by 0.5 µm stainless steel reversed phase HPLC column at a temperature of 30 °C; a quaternary solvent delivery system (LC 20 AD Yokohama, Japan); photodiode array detector at 325 nm wavelength and 285 nm wavelength for retinol and α-tocopherol, respectively. The conditions of the HPLC were as follows; mobile phase comprised of methanol: water (95:5, v/v) for α-tocopherol detection and methanol: water (90:10, v/v) for retinol detection. Using a flow rate with a mobile phase of 1.0 mL/minute, the injectable volume attained was 20 µl. Comparative analysis of the external standards and egg sample extracts was conducted to determine the concentration of the vitamins.

3.6 Determination of Cholesterol

Determination of cholesterol was determined using High-Pressure Liquid Chromatography (HPLC) (De Almeida, Perassolo, Camargo, Bragagnolo, & Gross, 2006). Saponification process was carried out by adding potassium chloride, 50% potassium hydroxide, 95% absolute ethanol and 5% distilled water to the sample. The sample was then heated in a water bath at 60 °C for 10 min. 5 ml distilled ice-cold water was then added after which the sample was cooled in an ice bath. Extraction of the nonsaponifiable fraction was carried out using 10 ml of hexane. Aliquots of hexane extracts was then dried under vacuum. The extract was dissolved in 3 ml of acetonitrile-isopropanol solution (70:30, v/v) and analysis done with reverse phase HPLC. The mobile phase consisted of acetonitrile and isopropanol (70:30, v/v). The column used was C18 ODS from Phenomenex® in reverse phase. Cholesterol identification was done by comparing sample retention times with standard retention times. Sample quantification was done using a standard curve.

3.7 Determination of Minerals (Calcium, Zinc, Magnesium, Potassium, Sodium)

The quantification of Calcium, zinc, magnesium, potassium, sodium zinc and calcium was done by atomic absorption spectrometry (AAS) (Shimadzu AA-6200, Tokyo, Japan) following AOAC methods (AOAC, 1996). External standards (SigmaAldrich Chemie, Steinheim, Germany) were used for quantification.

3.8 Color Measurement

The yolk color was analyzed using the colorimeter (Model CR-200, Osaka, Japan) which applied the $L^*a^*b^*$ color space (Qiao, Fletcher, Smith, & Northcutt, 2001). The albumin and the yolk were separated after breaking the eggs and the yolk was placed in a transparent bottle made of glass for measurement using the colorimeter. The values for the $L^*a^*b^*$ for the yolk measurement were recorded from three different places on the bottom of the glass bottle.

3.9 Sensory Evaluation

Eggs for sensory evaluation were prepared as illustrated by Hayat, Cherian, Pasha, Khattak, and Jabbar (2010). Eggs for evaluation were obtained from 45 and 80 weeks old Isa Brown hens fed as follows; Diet 0, Diet 25, Diet 50, Diet 75 and Diet 100. Different panelists were used to do the sensory evaluation both at 45 and 80 weeks. The eggs were subjected to the sensory assessment within 24 h after collection. Fifty (50) eggs from every treatment were boiled in a stainless steel pot containing water, for 5 minutes, cooled in cold water at room temperature and maintained at room temperature. The egg samples were unshelled and cut into quarters which were placed into plates for onward testing.

For the difference test, eggs from diets Diet 25, Diet 50, Diet 75 and Diet 100 were coded with 3-digits, and one sample, Diet 0 was indicated as the control and presented to 50 trained panelists. The panelists were to evaluate the difference between the treatments and the control in terms of flavor, off-flavor, aroma, yolk color, and overall difference. Overall sensory parameter referred to integrated sensation that was based on flavor, off-flavor, and aroma. Panelists used a 9-point

intensity scale to rate the differences between the control (Diet 0) and the treated diet types containing insect-based meal. The scale included, Zero (0) = no difference; Three (3) = slightly different; Five (5) = moderately different; Seven (7) = largely different; Nine (9) = extremely different (Hayat et al., 2010).

For the consumer preference test, eggs from the five diets were coded with 3-digits and 20 untrained panelists were asked to rank the following attributes; aroma, flavor, overall acceptability and yolk color using a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely) based on the degree of liking and disliking (Al-Ajeeli et al., 2018). In both tests, fresh water was provided to rinse the palate after evaluating each sample. Panelists were given 10 min to indicate their observations and provided comments after each parameter.

3.10 Statistical Analyses

Data on physical properties, proximate analysis, fatty acids and vitamins of the egg samples were analyzed using two-way ANOVA (i.e., Analysis of Variance) to evaluate the influence of different diet types, chicken eggs harvesting date (i.e., at 45 and 80 weeks), and the interactions between the two factors. The sensory data on yolk color, flavor, off-flavor, aroma, and overall difference (i.e., the difference between the control diets and the rest of the treatments with added BSFLM) were analyzed using two-way ANOVA to estimate the impact of diets and age of the eggs at the time of harvest and their interactions. Choice-based conjoint analysis was used to determine consumers' preferences for eggs from layer chicken fed insect-based feeds. Shapiro test was used for the normality test and Bartlett test was used for testing homogeneity of variance. The Student–Newman–Keuls test (SNK) was applied to separate the mean values at $P < 0.05$ (i.e., 95% confidence interval). All analyses carried out in the present study were done using R-software packages (version 3.5.3).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Inclusion of Black Soldier Fly Larvae Meal at Different Inclusion Rates to the Layers Feeds on the Physical Quality of Eggs

4.1.1 The Physical Characteristics of Eggs

Table 4.1: Physical Properties of Eggs as Affected by the Incorporation of Insect Larval Meal in Diets

Diet	Birds age (wk)	Weight of eggs (g)	Haugh Unit	Yolk index	Albumen index	Yolk weight (g)	Shell weight (g)	Shell thickness (mm)
0	45	55.23±0.58	80.96±0.33	35.55±0.45	9.57±0.06	17.16±0.77	5.50±0.20	0.43±0.00
	80	56.25±0.63	76.71±0.18	32.40±0.84	8.65±0.22	16.29±0.35	6.45±0.07	0.44±0.00
25	45	55.93±0.79	81.26±0.48	34.30±0.35	9.78±0.03	16.78±0.60	5.54±0.40	0.44±0.00
	80	57.21±0.16	76.03±0.026	32.02±0.38	8.72±0.19	17.97±0.27	6.44±0.16	0.44±0.00
50	45	57.48±0.55	81.72±0.44	33.50±0.50	9.93±0.23	16.43±0.91	4.86±0.16	0.44±0.00
	80	56.49±0.56	76.45±0.29	31.43±0.25	8.91±0.07	17.91±0.35	6.18±0.13	0.45±0.00
75	45	58.10±0.81	80.97±0.15	33.88±1.62	9.63±0.09	18.20±0.39	5.47±0.18	0.45±0.00
	80	58.10±0.50	75.70±0.43	30.84±0.04	8.85±0.18	18.95±0.38	6.27±0.19	0.45±0.00
100	45	81.26±0.48	81.42±0.48	36.03±2.50	9.60±0.10	18.30±0.2	5.49±0.09	0.45±0.00
	80	61.27±0.52	75.13±0.24	30.90±0.18	8.52±0.07	19.84±0.09	6.54±0.26	0.46±0.00
Two-way ANOVA								
Diet	$F_{4,20}$	15.241	2.049	0.895	1.957	8.068	2.001	49.214
	P	< 0.001	0.126	0.485	0.140	< 0.001	0.133	< 0.001
Age	$F_{1,20}$	4.574	614.32	23.682	116.588	6.849	59.049	36.389
	P	0.045	< 0.001	< 0.001	< 0.001	0.017	< 0.001	< 0.001
Diet *	$F_{4,20}$	2.758	2.333	0.707	0.365	2.031	0.460	1.281
Age	P	0.056	0.091	0.597	0.830	0.129	0.764	0.311

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

Partial or complete replacement of the FM hen diet with BSFL meal did not show significant effects on the Haugh unit, yolk index, albumen index and shell weight, except egg weight ($F_{4, 20} = 15.241$, $P < 0.001$) and yolk weight ($F_{4, 20} = 8.068$, $P < 0.001$) (Table 4.1). The egg weight and yolk weight were observed to increase with increased inclusion levels of BSFLM in the laying hen diet. Laying age of the hen significantly affected the physical properties of the eggs harvested (Table 4.1). There were no significant interactions between age of hen and diets for any of the physical parameters of eggs (Table 4.1).

BSFLM based diets had significant effects on the egg weight with an upward trend from week 45 to week 80. The observed egg weight increase ranged from 55.23 ± 0.58 to 61.27 ± 0.52 g. Egg weight increase was contributed by the fact that BSFLM contains high fat and protein content that enhanced increased yolk deposition leading to increase in egg weight Zhao et al. (2022). The significant weight increase could also be attributed to improved utilization of the nutrients, specifically, use of BSFL has significantly increased the jejunum length in layers thus increased surface area for the absorption of amino acids (Secci Giulia et al., 2020).

Contrary to our research findings, Bejaei and Cheng (2020) reported a significant decrease in egg weight for Novogen Brown layer birds fed on diets which had varying inclusion ratios of chopped BSF larvae (sizes 2-3 mm) as opposed to a BSFM (powder) used in our study. Cullere et al. (2016) further confirmed these observations and reported that the chitin content of BSFLM (roughly 87 g/kg DM), was capable of causing negative effects on nutrient digestibility, particularly lipid and protein fractions. Therefore, the results from the current study confirms that use of BSFL in powdered form optimized digestibility leading to increased egg weights compared to the chopped BSFL. Major factors that influence the weight of the eggs are the yolk sizes that is affected by the hen's body weight and the dietary metabolizable energy. Diets administered in the trials were isoenergetic and therefore the changes in BSFL eggs weight can be attributed to the increased egg yolk sizes (Secci Giulia et al., 2020). Sulphur amino acid and methionine content have an

impact on the egg weights but this were however balanced in the administered diets (Secci Giulia et al., 2020).

In the present study, egg weight significantly increased with the bird's age. This is a natural pattern in which eggs produced in early stages are small and get bigger with age as a result of increased yolk proportion in comparison to the egg albumin as the layer hens ages (Suparman, Purwanti, & Nahariah, 2020). There was no significant interaction between time and diet suggesting that BSFL supports natural growth of the layers ensuring production of high quality eggs throughout the laying cycle. The present study suggest that inclusion of the BSF larvae in the diet has the potential to sustainably produce eggs without compromising on the egg weight (Tahamtani et al., 2021).

According to the present study, the egg shell thickness was significantly affected ($F_{4, 20} = 49.214$, $P < 0.001$) by the inclusion of BSFL in the layers diet. This results compares to those reported by Mwaniki et al. (2020) suggesting that layers fed on BSFM had an improvement on calcium absorption and metabolism contributing to development of stronger eggshells through calcium deposition as calcium carbonate (Jin et al., 2024). In another study conducted by Brah, Issa, and Houndonougbo (2017) the results showed that there was no significant difference in the thickness of the eggshell with an increase of grasshopper meal in the layers diet. The author suggested that this could be a result of the chitin present in grasshopper meals that could have inhibited the absorption of calcium which is essential for the formation of the eggshell. This discrepancy could be as a result of using different insects' species. According to Secci Giulia et al. (2020), egg shell percentage and thickness decreased with the increase in egg weight in BSFL eggs due to limited availability of calcium ($2.0\text{-}2.5 \text{ g Ca}^{2+}$) for the production of the shell irrespective of the weight of the egg. Egg shell thickness and weight are physical factors that influence egg strength and pathogenic challenges throughout the supply chain from production to the consumer.

Inclusion of BSFLM in layers diet at varying levels did not reveal significant effect on the Haugh unit values ($F_{4, 20} = 2.049$, $P = 0.126$). The Haugh units obtained in this study were above 72 thus classified as AA grade that is the best quality standard

(Suparman et al., 2020). These results are in agreement with findings by Al-Qazzaz, Ismail, Akit, and Idris (2016) and Bejaei and Cheng (2020) who observed that inclusion of BSFL in layers diet maintains the albumin integrity. In a similar study on quails eggs, Suparman et al. (2020) reported similar results and hypothesized that BSFLM inclusion in the diet had no impact since the content of the protein in each treatment was enough to meet the needs of the laying birds (Suparman et al., 2020). The stable Haugh unit values in the current study is as a result of balanced amino acid profile in the BSFL sufficient for the ovalbumin synthesis by the magnum (Chang, Wang, Zhang, Qiu, & Wu, 2024).

Albumen appearance has extensively been used to describe egg quality (Zotte, Singh, Michiels, & Cullere, 2019). Based on the findings by Mwaniki et al. (2018), soy bean meal replacement with 7.5% BSFLM did not affect the Haugh unit significantly. Also in a study by Mwaniki et al. (2020) and by Patterson et al. (2021), incorporation of the BSFLM in the layers diet had no significant effect on the Haugh units. Similar to our results, a study conducted by Cufadar et al. (2021) on the inclusion of mealworms larvae meal on layer quails diet did not have a significant effect on the Haugh units. However, contrary to our study, the results obtained by Brah et al. (2017) indicated that the Haugh units increased with the increase in grasshopper meal inclusion in the diet which was a result of the increase in the albumin height. This discrepancy could be as a result of variations in the basal diets utilized in both studies. The results obtained in the current study showed that the age of the hens had a significant effect ($F_{1, 20} = 614.32, P < 0.001$) on the Haugh unit. The Haugh unit was observed to decrease significantly as the hen's age increased, which directly led to a decrease in the albumen height of the eggs (Silversides & Scott, 2001). Decrease in albumin quality with layers age is associated with reduced magnum health, antioxidant capacity, and downregulation of genes involved in synthesis and secretion of proteins (Chang et al., 2024).

Statistical analysis showed that BSFLM inclusion in the diet had no significant effect on the yolk index ($F_{4, 20} = 0.895, P = 0.485$) and this agrees with the results obtained by Suparman et al. (2020) in quail eggs. Lack of significant effect on the yolk index suggest that inclusion of BSFLM provides enough nutrients that enhance

preservation of the vitelline membrane structural integrity. Yolk index is influenced by the vitelline membrane strength which is influenced by birds origin, feeding and birds age (Marzec et al., 2016). Further, for the current study, inclusion of BSFL meal in the layers diet had no significant effect on the albumin index ($F_{4, 20} = 1.957$, $P = 0.140$). High albumin quality is linked to high ovomucin levels which is a main component of albumin (Obianwuna et al., 2022). Inclusion of BSFL in the diet enhanced a favorable microbial environment in the gut thus enhancing protein synthesis which improved the albumin height and the Haugh unit (Obianwuna et al., 2022). The results obtained in the present study showed that the age of the birds had a significant effect on the yolk index ($F_{1, 20} = 23.682$, $P < 0.001$) and the albumin index ($F_{1, 20} = 116.588$, $P < 0.001$). While the birds age, the magnum degenerates characterized by reduced glandular tissue, mucosal folding, and epithelial thickness and these impairs the secretion of the albumin proteins leading to poor albumin quality (Chang et al., 2024).

Table 4.2: Egg Yolk Color (l*a*b*) as Affected by the Incorporation of Insect Larval Meal at Different Incorporation Rates in Diets and by the Age of the Birds (Weeks)

Diet	Age of the birds (weeks)	Average of L*	Average of a*	Average of b*
0	45	52.83±0.98 _c	-5.90±0.15 _a	14.17±0.87 _c
	80	54.33±0.57	-6.17±0.09	17.10±0.55
25	45	52.27±0.27 _c	-6.17±0.09 _b	15.63±0.29 _c
	80	53.83±0.41	-6.47±0.09	16.87±0.30
50	45	55.40±0.57 _b	-6.53±0.07 _b	15.20±0.00 _c
	80	54.33±0.03	-6.67±0.07	16.93±0.19
75	45	53.77±0.30 _b	-6.07±0.03 _b	22.77±0.61 _b
	80	55.43±0.19	-6.73±0.07	24.60±0.12
100	45	56.80±0.20 _a	-6.33±0.07 _b	24.73±0.09 _a
	80	56.53±0.42	-6.47±0.22	27.83±0.73

Two-way ANOVA

Diet	$F_{4,20}$	17.852	7.451	231.384
	P	<0.001	<0.001	<0.001
Age	$F_{1,20}$	5.314	19.853	53.890
	P	0.0320	<0.001	<0.001
Diet	$F_{4,20}$	3.665	2.108	1.509
*Age		P	0.021	0.118

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal

The results obtained from the current study showed that partial or complete replacement of the FM hen diet with BSFL meal significantly influenced Lightness (l^*) ($F_{4, 20} = 17.852, P < 0.001$), yellowness (b^*) ($F_{4, 20} = 231.384, P < 0.001$) and redness (a^*) ($F_{4, 20} = 7.451, P < 0.001$) with an increase in l^* and b^* (Table 4.2). These values are in line with those obtained by Heuel, Sandrock, et al. (2021) in a study conducted to completely replace soy bean oil and cake with BSFLM in layers diets in which the egg yolk color showed a tendency of shifting towards more yellow and red coloration for the BSFL based diet. According to Cufadar et al. (2021) the values of L^* , a^* , and b^* were reported to be significantly higher in layers quails fed with the mealworm meal in comparison to the control group.

Similar results were obtained by Patterson et al. (2021) in a study conducted on inclusion of BSFL meal and oil on layers diet in which dark yolk color was obtained with linear increase of the dietary BSFL. The author hypothesized that besides the plant derived pigments, insects specifically BSF have a history in synthesizing melanin pigment in all their life cycles. The melanin can be isolated in a complex nature with lipids and also in a pure form. In the larval stage, this complex includes the fatty acids especially the lauric acid that accounted for a higher percentage of above 80% (Patterson et al., 2021). These lipid melanin complexes are incorporated into the egg yolk creating a deeper yellow color.

Mwaniki et al. (2020) reported similar findings showing an increased yolk color intensity with BSFL meal inclusion in the diet. The author suggested that lutein and β -carotene are the major pigments that are responsible for orange yellow color in the yolks. Feeds contain pigments that are mainly derived from plant ingredients. However, it has been established that BSFL also contains tocopherols and carotenoids. The pigments are characterized by high affinity towards non-polar compounds for example lipids. The pigments absorb wavelengths that range between 400 to 550 nm and appearing as yellow, red or orange. The yolk has a high fat content which promotes the accumulation of the carotenoid thus modifying the color of the yolk (Secci Giulia et al., 2020). The results of a study conducted by Gernat (2001) on substituting soy bean meal with shrimp meal for the layers hen's diet indicated a significant improvement in the intensity of the yolk pigmentation. The

author hypothesized that this could have been a result of the shrimp meal containing a high amount of astaxanthin which is a carotenoid pigment.

Also Brah et al. (2017) conducted a study that involved replacing fish meal with grasshopper meal in layers' diet and the results showed a significant improvement in the yolk color. Grasshopper meal contains carotenoids that improve the color of the yolk and eventually enhance the egg's commercial quality (Brah et al., 2017). In a study conducted by Park et al. (2021) involving the replacement of soy bean meal with BSFL dried in a microwave, the results indicated an improvement in the yolk color intensity. The color alteration was due to the presence of carotenoids for example the lutein and β -carotene found in BSFL. According to Secci et al. (2018), lutein, β -carotene, and retinol are transferred against the concentration gradient to the egg yolk where they accumulate to a high amount.

Egg color is an essential characteristic of the yolk and it determines the internal quality of the egg (Secci Giulia et al., 2020). Consumers preference of the eggs is influenced by the yolk color and this is influenced by the coloring nature of the oxycarotenoids known as xanthophylls (Cufadar et al., 2021). According to Cufadar et al. (2021), majority of the consumers prefer egg yolk whose color is dark yellow for example orange, gold, and yellow yolk. The amount of the insects used in the layers diet formulation affects the level of alterations of the yolk color (Cufadar et al., 2021). However, other studies obtained contrary results and this could be as a result of the different feed rearing substrate used in different studies to feed the BSFL (Lokaewmanee et al., 2023).

4.2 Effect of Inclusion of Black Soldier Fly Larvae Meal at Different Inclusion Rates to the Layers Feeds on the Nutritional Quality of the Eggs

4.2.1 Proximate Composition

Table 4.3: Proximate Composition of Egg Yolk as Affected by the Inclusion of Insect Larval Meal at Different Incorporation Rates in Laying Hen Diet and Age (Weeks).

Diet	Age of the birds (weeks)	Moisture Content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
0	45	57.47±0.15	1.50±0.05	14.06±0.53	25.92±0.60	1.05±0.02
	80	57.32±0.61	1.52±0.08	14.30±0.61	25.82±1.22	1.04±0.02
25	45	57.31±0.52	1.60±0.13	13.62±0.38	26.41±0.65	1.04±0.03
	80	57.53±0.06	1.57±0.005	13.20±0.17	26.64±0.22	1.05±0.02
50	45	57.73±0.66	1.55±0.03	14.00±0.55	25.64±1.18	1.08±0.01
	80	56.47±0.78	1.63±0.05	13.25±0.55	27.61±1.27	1.05±0.01
75	45	56.77±0.41	1.64±0.02	13.80±0.31	26.75±0.58	1.04±0.02
	80	56.20±0.38	1.78±0.00	13.04±0.31	27.92±0.12	1.06±0.01
100	45	57.21±0.35	1.66±0.01	13.70±0.63	26.29±0.59	1.14±0.02
	80	55.91±0.03	1.79±0.00	13.61±0.17	27.63±0.18	1.06±0.02
Two-way ANOVA						
Diet	<i>F</i> _{4,20}	1.873	5.423	0.963	0.978	2.782
	<i>P</i>	0.155	0.004	0.449	0.442	0.055
Age	<i>F</i> _{1,20}	4.410	3.672	1.555	3.496	1.842
	<i>P</i>	0.049	0.070	0.227	0.076	0.190
Diet * Age	<i>F</i> _{4,20}	1.042	0.837	0.462	0.588	1.745
	<i>P</i>	0.410	0.518	0.763	0.675	0.180

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

The experimental results established that egg's yolk ash content was significantly influenced by diet type fed to the laying hen ($F_{4, 20} = 5.423$, $P = 0.004$), while there were no considerable differences observed for the moisture content, crude protein, fat and carbohydrate contents (Table 4.3). The significant increase in ash in the egg yolk ranged from 1.50 ± 0.05 for the eggs laid at 45 weeks to $1.79 \pm 0.00\%$ for the eggs obtained at 80 weeks. This increase in ash content could be attributed to the high mineral profile such as Mg, K, Ca, and P in BSFLM Zotte et al. (2019). Similarly to the egg yolk, BSFLM inclusion in the layers diet contributed to a significant improvement in the ash content of the egg albumin ($F_{4, 20} = 2.807$, $P = 0.041$). The values increased from 0.57 ± 0.32 to $0.76 \pm 0.42\%$ further confirming the hypothesis that BSFLM enhance deposition of minerals across different egg components. The moisture, proteins, and carbohydrates contents in the egg albumin were not significantly affected by diet change (Table 4.4). Contrary to the results reported by this study, Zotte et al. (2019) demonstrated a significant decrease in protein content of quail eggs following increased inclusion levels of BSFLM in the diets which was attributed to high chitin content causing low availability of proteins. Birds' age had no significant effect on the proximate composition of the eggs apart from the moisture content. Also, there were no significant interactions between diet type and age of the hen in the proximate composition of the eggs (Table 4.4).

Table 4.4: Proximate Composition of Egg Albumin as Affected by the Inclusion of Insect Larval Meal at Different Incorporation Rates in Laying Hen Diet and Age (Weeks)

Diet	Age of the birds (weeks)	Moisture Content (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
0	45	84.24±0.39	0.57±0.32	14.00±0.41	0.25±0.06	0.93±0.07
	80	84.22±0.55	0.62±0.36	14.10±0.26	0.17±0.02	0.88±0.01
25	45	84.46±0.21	0.75±0.43	13.60±0.21	0.33±0.04	0.86±0.01
	80	84.22±0.19	0.74±0.42	13.04±0.22	0.27±0.03	0.71±0.09
50	45	83.89±0.51	0.60±0.03	14.10±0.60	0.50±0.04	0.82±0.16
	80	84.66±0.60	0.61±0.34	13.53±0.60	0.43±0.05	0.77±0.09
75	45	85.46±0.35	0.63±0.36	12.56±0.42	0.52±0.05	0.83±0.06
	80	83.87±0.36	0.73±0.42	13.99±0.43	0.54±0.02	0.86±0.08
100	45	83.72±0.68	0.74±0.43	14.05±0.66	0.69±0.05	0.81±0.14
	80	84.12±0.61	0.76±0.42	13.62±0.67	0.79±0.06	0.71±0.04
Two-way ANOVA						
Diet	$F_{4,20}$	1.225	2.807	0.774	28.523	2.623
	P	0.332	0.041	0.555	0.068	0.066
Age	$F_{1,20}$	0.444	0.831	0.243	0.197	3.784
	P	0.513	0.373	0.627	0.662	0.066
Diet * Age	$F_{4,20}$	1.301	0.226	0.843	1.057	0.863
	P	1.303	0.921	0.514	0.403	0.5031

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFLM: Black soldier fly larvae meal.

4.2.2 Comparative Analysis of Vitamin A and E in Egg Yolk and Whole Eggs

Results obtained from this study revealed that inclusion of BSFLM in the layers diet significantly influenced the concentration of vitamin A content in whole egg ($F_{4, 20} = 4.636$, $P = 0.008$) and the egg yolk ($F_{4, 20} = 44.648$, $P = < 0.001$). The vitamin A levels

recorded in the egg yolk and whole egg was observed to decrease with increasing inclusion levels of BSFLM in layer diet (Table 4.5). The values obtained in this study for vitamin A in the yolk ranged between 0.32 ± 0.01 and 0.63 ± 0.00 mg/100 g while vitamin A content for the whole eggs ranged between 0.02 ± 0.00 and 0.06 ± 0.01 mg/100 g. The vitamin E levels recorded in the egg yolk ($F_{4, 20} = 0.988$, $P = 0.437$) and whole egg ($F_{4, 20} = 1.001$, $P = 0.430$) was observed to decrease with increasing inclusion levels of BSFLM in layer diet (Table 4.5) though not statistically significant.

Studies have demonstrated that the levels of vitamins A and E in layer diets can significantly affect the content of tocopherol and retinol in the egg yolk (Mori, Mendonça, Almeida, & Pita, 2003). This implies that vitamin A and E content in eggs largely depends on dietary supply. In general Kouřimská and Adámková (2016), reported that insects contains low amounts of preformed vitamin A and also identified that the vitamins content of insects is determined by the feed used to grow them. According to Mwaniki et al. (2018), egg yolk from hen fed on diets containing BSFLM showed significantly elevated concentrations of carotenoids compared to egg yolk obtained from hens subjected to diet enriched with soy bean meal. Aro, Larmo, Bäckman, Kallio, and Tahvonen (2005) found that vitamin A activity in eggs was based on carotenoids and retinoids that may undergo conversion into retinol. Secci et al. (2018) conducted a study on fat-soluble vitamins in BSFLM and revealed that the amount of α -tocopherol was 38.18 mg/kg. These authors also identified that the total carotenoid value was 2.15 mg/kg, though no retinol was identified in the BSFLM. Vitamin E can help to prevent the formation of free radicals, especially from PUFAs that are implicated in heart health, boosting of the immune system and utilization by cell membranes in the brain (Bourre & Galea, 2006). Vitamin E is a nutrient that is fat soluble and occurs naturally and helps in preventing chronic diseases that include cancer and cardiovascular diseases due to its anti-inflammatory and antioxidant activities (Kim, Ferruzzi, & Campbell, 2016). The dietary benefit of vitamin A include enhanced immune maintenance function, bone health, healthy vision, and cell integrity (Cherian, 2009).

The age at which the eggs were harvested had a significant impact on vitamin A content in whole egg ($F_{1, 20} = 9.807$, $P < 0.005$) but no significant difference was noted in the egg yolk ($F_{1, 20} = 3.996$, $P < 0.059$) (Table 4.5). The age at which the eggs were harvested had a significant effect on the Vitamin E content on both the egg yolk ($F_{1, 20} = 13.869$, $P < 0.001$) and the whole eggs ($F_{1, 20} = 6.238$, $P < 0.021$). No interactive effects of the diet and age of the birds was observed on vitamin A and E content of the eggs harvested for both the egg yolk and whole eggs.

Table 4.5: Vitamins A and E Contents of Egg Yolk and Whole Eggs as Affected by the Insect Larval Meal Inclusion at Different Rates in the Layer Diet and Age (Weeks)

Diet	Age of the birds (weeks)	Vitamin A		Vitamin E	
		Yolk (mg/100g)	Whole egg (mg/100g)	Yolk (mg/100g)	Whole egg (mg/100g)
0	45	0.63±0.00 a	0.06±0.02 a	1.97±0.39 a	1.09±0.07 a
	80	0.59±0.05 a	0.06±0.01 a	1.13±0.25 a	1.01±0.09 a
25	45	0.61±0.03 a	0.05±0.01 ab	1.65±0.01 a	1.03±0.05 a
	80	0.56±0.03 a	0.03±0.00 a	1.45±0.18 a	0.91±0.05 a
50	45	0.42±0.05 a	0.07±0.01 abc	1.91±0.34 a	1.06±0.09 a
	80	0.31±0.03 a	0.03±0.00 abc	1.20±0.13 a	0.97±0.06 a
75	45	0.33±0.00 b	0.07±0.01 bc	1.56±0.03 a	1.02±0.17 a
	80	0.33±0.03 b	0.03±0.00 bc	1.18±0.11 a	0.87±0.04 a
100	45	0.32±0.01 b	0.03±0.00 c	1.37±0.12 a	1.00±0.04 a
	80	0.32±0.00 b	0.02±0.00 c	1.10±0.08 a	0.81±0.05 a
Two-way ANOVA					
Diet	$F_{4,20}$	44.648	4.636	0.988	1.001
	P	< 0.001	0.008	0.437	0.430
Age	$F_{1,20}$	3.996	9.807	13.869	6.238
	P	0.059	0.005	0.001	0.021
Diet * Age	$F_{4,20}$	0.991	2.050	0.925	0.158
	P	0.435	0.126	0.469	0.957

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

4.2.3 Comparative Analysis of Fatty Acids in Eggs

Data on fatty acid content of egg yolk is presented in Table 4.6. Diet type had a significant impact on the monounsaturated fatty acids ($F_{4, 20} = 41.054$, $P < 0.001$), palmitoleic ($F_{4, 20} = 13.346$, $P < 0.001$), palmitic ($F_{4, 20} = 8.154$, $P < 0.001$), oleic ($F_{4, 20} = 35.813$, $P < 0.001$) and Docosahexaenoic acid (DHA) ($F_{4, 20} = 33.238$, $P < 0.001$). The age of the laying hen also showed a significant impact on palmitoleic ($F_{1, 20} = 6.863$, $P < 0.016$), palmitic ($F_{1, 20} = 9.801$, $P < 0.001$), linoleic ($F_{1, 20} = 26.779$, $P < 0.001$), PUFA ($F_{1, 20} = 20.472$, $P < 0.001$) and n6/n3 ratio ($F_{1, 20} = 36.146$, $P < 0.001$). This study revealed significant interactions between the diet types and age of laying hen on butyric, capric, arachidic and palmitoleic fatty acids (Table 4.6).

Table 4.6: Fatty Acid Composition (% of Total FAMES) of Egg Yolk as Affected by the Inclusion of Insect Larval Meal at Different Incorporation Rates in Layers Diet and Age (Weeks)

Diet	Age of the birds (weeks)	C12:0	C14:0	C16:0	C18:0	C20:0	ΣSFA	C16:1 (n-7)	C18:1 (n-9)	ΣMUF A	C18:2 (n-6)	C20:4 (n-6)	C20:5 (n-3)	C22:6 (n-3)	ΣPUFA	N6/N3
		0	45	0.38±0.07	0.74±0.06	24.51±0.19	7.18±0.22	0.44±0.07	34.44±0.46	1.88±0.07	41.41±0.26	43.30±0.31	18.95±0.24	0.31±0.04	0.14±0.01	1.47±0.02
	80	0.40±0.03	0.72±0.07	23.84±0.40	7.09±0.02	0.59±0.03	33.87±0.90	1.78±0.06	41.27±0.63	43.01±0.69	19.28±0.25	0.27±0.04	0.10±0.02	1.44±0.01	21.72±0.35	9.09±0.45
25	45	0.38±0.02	0.80±0.03	24.81±0.31	7.62±0.17	0.42±0.06	35.04±0.2	1.81±0.09	42.87±0.76	44.68±0.75	17.44±0.84	0.34±0.01	0.14±0.00	1.45±0.03	20.00±0.88	8.00±0.27
	80	0.40±0.01	0.81±0.03	23.93±0.39	7.35±0.17	0.33±0.10	33.92±0.52	1.80±0.11	42.80±0.04	44.66±0.09	18.23±0.88	0.37±0.06	0.15±0.02	1.44±0.06	20.60±0.87	9.34±0.70
50	45	0.42±0.02	0.76±0.03	24.96±0.47	7.49±0.22	0.47±0.20	35.66±0.10	1.69±0.26	43.72±0.18	45.41±0.22	16.32±0.20	0.34±0.12	0.16±0.02	1.43±0.02	18.81±0.25	7.82±0.43
	80	0.47±0.06	0.73±0.01	24.28±0.09	7.42±0.15	0.10±0.03	34.01±0.05	1.95±0.02	43.28±0.30	45.25±0.28	17.54±0.13	0.28±0.04	0.12±0.02	1.34±0.03	19.65±0.15	9.73±0.25
75	45	0.41±0.01	0.80±0.05	25.46±0.22	7.79±0.15	0.92±0.01	37.29±0.26	1.58±0.22	45.43±0.15	47.02±0.27	13.18±0.06	0.37±0.03	0.12±0.01	1.33±0.02	15.35±0.07	7.54±0.16
	80	0.37±0.05	0.79±0.02	25.12±0.17	7.34±0.19	0.09±0.05	37.08±0.39	2.28±0.06	44.55±0.24	46.85±0.28	15.50±0.12	0.36±0.08	0.14±0.03	1.32±0.01	17.75±0.12	8.41±0.23
100	45	0.40±0.04	0.86±0.03	25.58±0.20	9.10±0.15	0.48±0.05	37.97±0.40	2.46±0.03	45.46±0.24	47.94±0.22	11.45±0.30	0.33±0.03	0.12±0.03	1.18±0.04	13.39±0.25	7.37±0.38
	80	0.48±0.02	0.83±0.04	25.22±0.06	8.43±0.17	0.17±0.01	37.71±0.14	2.62±0.05	44.89±0.33	47.53±0.37	14.16±0.53	0.30±0.09	0.12±0.05	1.12±0.04	15.99±0.45	9.50±0.40

Two-way ANOVA																
<i>Diet</i>	<i>F</i> _{4,20}	1.083	2.777	8.154	5.946	3.504	12.526	13.346	35.813	41.054	65.845	0.536	0.306	33.238	76.441	1.438
	<i>P</i>	0.391	0.055	< 0.001	0.003	0.03	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.711	0.870	< 0.001	< 0.001	0.258
<i>Age</i>	<i>F</i> _{1,20}	1.185	0.325	9.801	1.797	32.854	31.692	6.863	3.097	0.623	26.779	0.316	0.410	3.637	20.472	36.146
	<i>P</i>	0.289	0.575	< 0.001	0.195	< 0.001	< 0.001	0.016	0.09	0.439	< 0.001	0.580	0.529	0.061	< 0.001	< 0.001
<i>Diet * Age</i>	<i>F</i> _{4,20}	0.692	0.110	0.376	0.243	10.127	1.368	3.269	0.386	0.054	2.538	0.177	0.491	0.565	2.985	1.206
	<i>P</i>	0.606	0.978	0.823	0.910	< 0.001	0.281	0.03	0.816	0.994	0.072	0.947	0.742	0.691	0.04	0.339

Means having no superscripts in common in the same column are significantly different $P < 0.05$, SFA= saturated fatty acids (C4:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0), C24:0). MUFA = monounsaturated fatty acids (C16:1n7, C18:1n9). PUFA = polyunsaturated fatty acids (C18:2n6), (C22:6n3), (C20:5n3) (C20:4n6)

Inclusion of BSFLM in the layers diet significantly increased the monounsaturated fatty acids ($F_{4, 20} = 41.054$, $P < 0.001$) in the egg yolk. Liu et al. (2021), reported that egg yolk from hens fed with BSFLM contained high levels of MUFA of up to 47% which compares to the MUFA content obtained in this study that ranged from 43.30 ± 0.31 to $47.94 \pm 0.22\%$. This results compares to those reported by Romero, Cenalmor, Chamorro, and Redondo (2024) where by MUFA content significantly increased with BSFL inclusion due to a significant increase in oleic acid and palmitoleic acid in the egg yolks. The primary fatty acids of MUFA in the BSFLM is C18:1n-9 (Nyberg, 2017; Walczak, Bocian, Kowalkowski, Trziszka, & Buszewski, 2017). Park et al. (2021) obtained similar results on eggs produced by hens fed on microwaved BSFL meal. Positive research findings have also been reported by Zotte et al. (2019) who reported increased MUFA content in eggs with higher inclusion levels of BSFLM in hen diet, which can be attributed to desaturation and elongation of various fatty acids [C16:0, C14:0, and C12:0] by the laying quails to C17:1n-10 (cis-10-heptadecenoic), C14:1n-9 (myristoleic), and C16:1n-9 (palmitoleic) acids. However, our results differ with those obtained by Secci et al. (2018), whereby BSFLM inclusion to replace soya bean meal in Lohmann Brown-Classic hen diets did not compromise the quality of MUFA content in the eggs. This discrepancy could have resulted from the use of different formulations of the basal diet used in both studies.

Results from the present study revealed that, PUFA content reduced significantly ($F_{4, 20} = 76.441$ $P < 0.001$) with increase in BSFL in the layers diet. The percentage of PUFA in the BSFL meal has been shown to be less than 15% (Barragan-Fonseca et al., 2017; Zotte et al., 2019) and the primary fatty acids of PUFA is C18:2n-6 (Nyberg, 2017; Walczak et al., 2017). Liu et al. (2021) reported that the egg yolk from hens fed with BSFLM contained PUFA content of up to 19% which compares to the PUFA content reported in the current study that ranged from 13.39 ± 0.25 to $21.61 \pm 0.25\%$. The significant reduction of PUFA in eggs obtained from BSFLM fed layers was contributed by a reduction in omega 6 fatty acid in the egg yolk specifically linoleic acid. Similarly to the present study, Park et al. (2021) and Zhou, Liu, Ji, and Yu (2018) revealed that the total PUFA content (C22:6n-3, C18:2n-6 and C20:5n-3) of the egg yolk significantly reduced with inclusion of BSFL meal in the

layers feeds. DHA significantly reduced in the present study ($F_{4, 20} = 33.238$, $P < 0.001$) and Park et al. (2021) hypothesized that this could be as a result of the reduction of both the linoleic and alpha linolenic acid that are precursors of n-6 and n-3 fatty acids. The Eicosapentaenoic acid (EPA) content in the present study was not significantly affected. The higher amount of DHA compared to EPA in the egg yolks is a clear indication that most of the EPA were efficiently converted to DHA by the birds (Nyberg, 2017; Walczak et al., 2017).

The presence of arachidonic acid (AA) in the eggs might be attributed to *in vivo* acid biosynthesis from linoleic acid (LA) (Nyberg, 2017; Walczak et al., 2017). The amount of AA in the present study was not significantly affected by the inclusion of BSFL meal in the hen's diet. According to a study conducted by Ceylan, Ciftci, Mizrak, Kahraman, and Efil (2011), on influence of different oil sources on fatty acid composition of layers hens egg yolk, the AA significantly reduced and this was linked to an increase in the DHA content leading to a decrease in n-6 fatty acids and increase in n-3 fatty acids. Ceylan et al. (2011) associated this phenomenon with less utilization of (D)-6 desaturase enzyme in the n-6 pathway compared to the n-3 pathway that had greater utilization of the enzyme.

The ratio of n6/n3 values in the diets with BSFLM remained largely unaffected. According to Zaheer (2015), the PUFAs linoleic acid (n-6) and alpha-linolenic (n-3) are essential to the health of humans. Several studies have pointed out that there is a relationship between n6/n3 uptake and their benefits in controlling or preventing chronic illnesses such as cardiovascular (especially obstructive ischemia) and inflammatory diseases (Gómez Candela, Bermejo López, & Loria Kohen, 2011). A well-balanced intake of n6/n3 fatty acids has been proven to delay the onset of several disorders or minimizes their impact in causing diseases. Optimal ratios of n6/n3 ranged between 4:1 - 5:1, with an upper limit of 10:1 (Gómez Candela et al., 2011). According to de Souza Vilela et al. (2021), many studies have highlighted the significance of maintaining the omega 6 / omega 3 ratio at a level below 5 to ensure a good health status of the meat products. EPA and DHA are essential phospholipids components that gives flexibility to the cell walls and decrease the levels of plasma cholesterol (Zaheer, 2015). DHA and EPA have also been proven to decrease the risk

of central nervous system, mental health, and cardiovascular diseases, immune infections and inflammation and in addition they have therapeutic and preventive roles for other different chronic diseases (De Souza Vilela et al., 2021; Zaheer, 2015).

Therefore, future studies should focus on supplementing BSFL meal with diet rich in PUFA. An example is α -linolenic fatty acids and linoleic acids that are essential for human nutrition (Cattaneo et al., 2025). They are only obtained from dietary sources and the body cannot synthesize them. The essential fatty acids play a significant role to maintain the functional and structural integrity for the retina and the central nervous system (Cattaneo et al., 2025). Feeding layers hens with feeds containing different fats has previously been reported to modify yolk fatty acids (Ceylan et al., 2011). Hens also have the ability to deposit dietary lipids in the yolk and thus making the eggs to have the potential to provide PUFAs (Ceylan et al., 2011).

Results from the present study revealed that, SFA content increased significantly ($F_{4, 20} = 12.526$ $P < 0.001$) with increase in BSFL in the layers diet. Liu et al. (2021), reported that egg yolk from hens fed with BSFLM contained high levels of SFA of up to 37% which compares to the SFA content obtained in this study that ranged from 33.87 ± 0.90 to 37.97 ± 0.40 %. Similar results were reported by Bejaei and Cheng, 2020, Cattaneo et al. (2025), Heuel, Kreuzer, et al. (2021) and Zhou, Liu, Ji, and Yu (2018) whereby SFA increased in the egg yolks with increase in BSFL in the diet as a result of increase in the proportions of palmitic acid, myristic acids and lauric acids with inclusion of BSFLM in the layers diet. Transfer of the palmitic acid from the insects diet to the eggs have been reported to be approximately 100% as compared to lauric acid in which the transfer to the eggs has been reported to be below 1 % (Zawisza et al., 2023).

Previous studies have demonstrated that the fatty acid status of BSFLM is mainly characterized by high SFA content accounting for over 75% (Barragan-Fonseca et al., 2017; Zotte et al., 2019). Dominant fatty acids include C12:0, C14:0, and C16:0 (Barragan-Fonseca et al., 2017; Cattaneo et al., 2025; Zotte et al., 2019). Also the study by Romero et al. (2024) reported an increasing trend in the content of the SFA

in the egg yolks even though this did not reach a significant level. Other studies that focused on the fatty acid, records of broiler breast meat when fed diet with BSFLM where the fatty acids content was highly influenced by the insect protein-based diets, whereby the SFA content increased (Schiavone, Cullere, et al., 2017).

According to de Souza Vilela et al. (2021), dietary fatty acid from BSFL meal modulates the fatty acid quality in animal tissues. Previous studies have reported that BSFLM have modified fatty acid composition in different animals for example by increasing lauric acid and total SFA in fish fillets, rabbit leg meat and broiler breast meat (de Souza Vilela et al., 2021).

Remarkably, the SFAs of the processed eggs were affected by the age of the layers, which compares to report by Lesic, Kresic, Cvetnic, Petrovic, and Pleadin (2017). The author revealed a significantly lower content of SFA in the egg yolk when eggs were harvested from 55-weeks old birds compared 21 weeks old birds (Lesic et al., 2017). According to the European Food Safety Authority, the recommended SFA intake should be very limited since they are positively correlated to raising of the blood LDL, cholesterol and occurrence of cardiovascular diseases (De Souza Vilela et al., 2021). However, other previous studies have reported contrary data in which SFA intake have not been associated with occurrence of cardiovascular diseases (De Souza Vilela et al., 2021).

4.2.4 Mineral Composition

Tables 4.7, 4.8 and 4.9 show the mineral composition of the egg yolk, albumin and whole egg respectively. Inclusion of BSFLM in the diet had a significant effect on calcium in albumin ($F_{4, 20} = 16.34$, $P = < 0.001$), yolk ($F_{4, 20} = 16.52$, $P = < 0.001$) and whole egg ($F_{4, 20} = 76.64$, $P = < 0.001$). The inclusion also had a significant effect on sodium in albumin ($F_{4, 20} = 8.07$, $P = < 0.001$), yolk ($F_{4, 20} = 3.18$, $P = 0.04$) and whole egg ($F_{4, 20} = 9.42$, $P = < 0.001$) and a significant effect on potassium ($F_{4, 20} = 22.64$, $P = < 0.001$) in both albumin and whole egg ($F_{4, 20} = 3.34$, $P = 0.03$) but was not significant in egg yolk. Age of the laying hen showed a significant effect in calcium, magnesium, potassium and sodium in albumin. Further, age also had a significant effect on calcium in whole egg and magnesium and potassium in egg yolk. There

were significant interactions between diet type and age of the hen on calcium and magnesium in egg yolk, potassium in albumin and calcium in whole egg.

Table 4.7: Mineral Composition of Egg Yolk as Affected by the Incorporation of Insect Larval Meal in Diets and Age of the Birds (Weeks)

Diet	Birds age (weeks)	Ca (mg/100g)	Zn (mg/100g)	Mg (mg/100g)	K (mg/100g)	Na (mg/100g)
0	45	128.14±1.69 c	2.80±0.03 a	12.71±0.58 a	107.71±0.82 a	48.90±0.43 b
	80	132.40±0.33	2.65±0.04	8.96±0.33	105.86±0.60	48.57±0.79
25	45	127.02±0.64 c	2.49±0.11 a	11.66±0.77 a	106.64±0.80 a	48.56±0.74 b
	80	133.68±1.71 c	2.79±0.07	12.28±1.36	104.85±0.33	48.53±0.57
50	45	134.28±1.80 b	2.78±0.09 a	10.90±0.47 a	107.13±0.58 a	48.60±0.85 ab
	80	135.85±1.13	3.44±0.64	12.03±0.22	105.10±0.56	49.40±0.71
75	45	138.41±0.79 ab	2.80±0.10 a	13.69±0.52 a	107.55±0.32 a	50.50±0.78 a
	80	135.44±0.83	3.99±0.50	11.57±0.61	104.76±1.82	51.10±0.87
100	45	139.04±2.51 a	2.72±0.10 a	14.26±0.85 a	108.59±0.74 a	49.24±0.71 ab
	80	140.59±1.64	3.89±0.74	11.03±0.26	107.43±0.37	50.01±0.67
Two-way ANOVA						
A						
Diet	$F_{4,20}$	16.515	1.857	2.624	2.440	3.175
	P	< 0.001	0.158	0.065	0.0804	0.036
Age	$F_{1,20}$	5.804	8.094	11.843	14.213	0.624
	P	0.026	0.010	0.003	0.001	0.439
Diet * Age	$F_{4,20}$	3.065	1.345	$F_{5,20}$	$F_{4,20}$ 0.262	0.253
	P	0.040	0.288	0.004	0.899	0.904

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

Table 4.8: Mineral Composition of Egg Albumin as Affected by the Incorporation of Insect Larval Meal in Diets and Age of the Birds (Weeks)

Diet	Birds (weeks)	Age	Ca (mg/100g)	Mg (mg/100g)	K (mg/100g)	Na (mg/100g)
0	45		3.80±0.07 ^c	13.81±0.04 ^a	112.14±0.58 ^b	143.65±0.64 ^b
	85		6.83±1.05	11.79±0.16	108.93±0.73	144.21±0.76
25	45		4.04±0.26 ^{bc}	12.88±0.33 ^a	112.45±1.20 ^a	142.56±0.50 ^b
	85		7.56±0.36	11.54±0.24	117.87±0.39	145.48±0.63
50	45		3.85±0.32 ^c	12.28±1.11 ^a	112.70±1.10 ^a	143.75±0.75 ^b
	85		7.05±0.63	11.12±0.91	119.74±0.98	145.53±0.29
75	45		5.33±0.13 ^b	12.66±0.82 ^a	113.85±0.49 ^a	145.67±0.27 ^a
	85		8.05±0.35	11.28±0.19	118.53±0.46	146.21±0.08
100	45		7.93±0.20 ^a	12.90±1.54 ^a	113.37±0.44 ^a	145.63±0.32 ^a
	85		8.86±0.06	11.77±0.10	119.35±0.44	146.51±0.40
Two-way ANOVA						
Diet	$F_{4,20}$		16.34	0.636	22.64	8.070
	P		< 0.001	0.642	< 0.001	< 0.001
Age	$F_{1,20}$		90.43	9.317	72.66	17.004
	P		< 0.001	0.006	< 0.001	< 0.001
Diet * Age	$F_{4,20}$		2.625	0.973	15.52	1.977
	P		0.065	0.973	< 0.001	0.137

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

Table 4.9: Mineral Composition of Whole Egg as Affected by the Incorporation of Insect Larval Meal in Diets and Age of the Birds (Weeks).

Diet	Birds age (weeks)	Ca (mg/100g)	Zn (mg/100g)	Mg (mg/100g)	K (mg/100g)	Na (mg/100g)
0	45	44.66±0.04 ^b	1.24±0.04 ^a	12.04±0.19 ^a	126.58±0.65 ^a	148.91±0.22 ^b
	85	67.37±0.33 ^b	1.25±0.05 ^a	12.00±0.36 ^a	126.21±0.51 ^a	148.92±0.09 ^b
25	45	42.82±0.59 ^a	1.30±0.02 ^a	11.96±0.17 ^a	125.52±0.60 ^a	148.86±0.61 ^b
	85	60.95±0.51 ^a	1.25±0.04 ^a	12.66±1.17 ^a	126.26±0.65 ^a	149.33±0.68 ^b
50	45	38.79±0.28 ^d	1.29±0.03 ^a	12.26±0.22 ^a	126.44±0.31 ^a	149.33±0.69 ^b
	85	61.41±1.02 ^d	1.24±0.03 ^a	12.40±0.10 ^a	125.60±0.68 ^a	149.41±0.40 ^b
75	45	41.74±0.06 ^c	1.31±0.02 ^a	13.39±0.19 ^a	127.58±0.27 ^b	150.17±0.71 ^a
	85	62.09±0.48 ^c	1.30±0.00 ^a	12.84±0.53 ^a	127.54±0.83 ^b	151.37±0.44 ^a
100	45	50.05±0.90 ^a	1.30±0.02 ^a	11.16±0.89 ^a	127.04±0.52 ^a	150.70±0.62 ^a
	85	67.33±0.61 ^a	1.29±0.01 ^a	12.01±0.24 ^a	127.23±0.30 ^a	152.49±0.57 ^a
Two-way ANOVA						
Diet	<i>F</i> _{4,20}	76.640	1.692	2.251	3.340	9.416
	<i>P</i>	< 0.001	0.191	0.100	0.030	< 0.001
Age	<i>F</i> _{1,20}	52.886	1.450	0.425	0.036	4.337
	<i>P</i>	2e-16	0.243	0.5218	0.850	0.05033
Diet * Age	* <i>F</i> _{4,20}	9.647	0.425	0.576	0.560	1.012
	<i>P</i>	< 0.001	0.789	0.683	0.694	0.425

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, $P < 0.05$). BSFL meal: Black soldier fly larval meal.

In a study conducted by Wallace et al. (2018), the chemical composition of the diet showed that BSFLM contained high ash content as compared to the fish meal diets. The calcium content in the BSFLM diet was also found in higher contents as

compared to the fish meal. Quality of feeds administered to the hens have the potential to modify mineral composition of the eggs (Kiczorowska et al., 2015). Dietary inclusion of BSFLM showed significant impact on the calcium and sodium content of egg yolk, albumin and whole egg. According to Zaheer (2015), the hen absorbs and deposits calcium from the feed that is contained in the hen's intestines. Calcium helps in regulating muscle contraction that is required by fetuses, infants and children for teeth and bone development (Bashir, Ossai, Shittu, Abubakar, & Caleb, 2015). The current study show that high amount of calcium was contained in the egg yolk. Marono et al. (2017) reported that the level of serum calcium was significantly higher in layers fed with BSFL than in the control even though the daily intake levels of calcium were 5.33 g and 5.36 g respectively for soy bean group and BSFL group. The recommended dietary allowance of calcium ranges between 600-1400 mg/kg thus implying that the high concentration in the egg yolk has the potential to contribute to the dietary calcium. Sodium has a recommended value of 1100-3300 mg/kg thus implying that its concentration in the egg albumin and the whole egg can contribute to the dietary sodium. Inclusion of BSFLM in the diet had a significant effect on potassium in albumin and whole egg.

4.2.5 Cholesterol Content

Table 15 show the cholesterol content of the egg yolk, albumin and whole egg. Inclusion of BSFLM in the diet did not significantly affect the cholesterol content in the egg yolk, albumin and whole egg. Age of the laying hen showed a significant effect on the cholesterol content in the egg yolk. There were no significant interactions between diet type and age of the hen on cholesterol content of the egg yolk, albumin and whole egg.

Table 4.10: Cholesterol Content of Whole Eggs, Albumin and Egg Yolk as Affected by the Incorporation of Insect Larval Meal in Diets and the Age of the Birds (in Weeks)

Diet	Birds age (weeks)	Yolk (mg/g)	Whole (mg/g)	Albumin (mg/g)
0	45	10.53±0.34 ^a	3.84±0.06 ^a	0.34±0.02 ^a
	80	10.07±0.08	3.65±0.30	0.34±0.05
25	45	11.86±0.19 ^a	4.29±0.05 ^a	0.49±0.10 ^a
	80	9.88±0.39	3.68±0.43	0.40±0.02
50	45	10.35±0.24 ^a	3.89±0.39 ^a	0.48±0.13 ^a
	80	9.97±0.60	3.85±0.52	0.47±0.03
75	45	11.71±0.46 ^a	4.28±0.39 ^a	0.39±0.10 ^a
	80	10.38±0.79	3.80±0.36	0.30±0.04
100	45	11.83±0.27 ^a	4.38±0.22 ^a	0.36±0.10 ^a
	80	10.51±0.34	4.21±0.24	0.38±0.07
Two-way ANOVA				
Diet	<i>F</i> _{4,20}	2.335	0.793	1.286
	<i>P</i>	0.091	0.544	0.309
Age	<i>F</i> _{4,20}	17.182	20.209	0.447
	<i>P</i>	< 0.001	0.170	0.511
Diet * Age	<i>F</i> _{4,20}	1.280	0.267	0.229
	<i>P</i>	0.311	0.896	0.919

Entries are means ± standard errors. Diet data bearing different letters within a column are significantly different (SNK-test, *P* < 0.05). BSFL meal: Black soldier fly larval meal.

Inclusion of BSFLM in the layers diet showed no significant effect on the cholesterol content in the egg yolk (*F*_{4, 20} = 2.335, *P* = 0.091) and the results obtained ranged between 987.89±38.96 mg/100 g -1182.97±26.58 mg/100 g. Inclusion of BSFLM in the layers diet showed no significant effect on the cholesterol content in the egg albumin (*F*_{4, 20} = 1.286, *P* = 0.309) and the results ranged between 34.10±4.87-48.89±10.19 mg/100 g. Also, BSFLM inclusion in the layers diet had no significant effect on the cholesterol content in the whole eggs (*F*_{4, 20} = 0.793, *P* = 0.544) and the values ranged between 365.17±30.38 mg/100 g - 438.49±21.72

mg/100 g. Similar results were observed by Millet et al. (2006) in a study in which diet enriched with EPA and DHA had no significant effect on the cholesterol content of eggs. Also similar results were obtained by Kim et al. (2022) on a study conducted to replace soy bean oil with BSFL oil in layers' diet.

The results obtained in the present study agrees with the results obtained by Zotte et al. (2019) on eggs produced by laying quails fed on diet formulated with BSFLM. Zotte et al. (2019) hypothesized that cholesterol content in eggs is rarely dependent on diet. Cholesterol content in the eggs is also dependent on the different species (Zotte et al., 2019). In a study conducted by Cullere et al. (2018) on the cholesterol content of breast meat obtained from broiler quails fed with BSFL meal, the results agreed with the results of the present study in which the cholesterol content of the insects fed birds was comparable to those fed with conventional diets. .

The results obtained in this study for the whole eggs are consistent to the ones revealed by Beynen (2004) who reported ranges between 363 mg/100 g - 399 mg/100 g. Our results are further supported by Aydin and Dogan (2010) who also documented a cholesterol values ranging between 951 mg/100 g and 1045 mg/100 g. It is worth noting that the inclusion of BSFLM in the layer chicken diet did not show any significant effect on the cholesterol content in the egg yolk, whole egg and egg albumin. Similar results were presented by Millet et al. (2006), who demonstrated that diet had no significant effect on the cholesterol content of eggs. Additionally, Secci Giulia, Moniello, Gasco, Bovera, and Parisi (2018) also demonstrated that the cholesterol content of the Barbary partridge meat quality was not affected by insect meal inclusion in the feeds.

Previous findings by Vercese et al. (2013), showed that there was a correlation between the dietary energy balance and the yolk cholesterol content. This implies that excessive energy intake increase cholesterol synthesis and body weight (Vercese et al., 2013). Hossain & Blair (2007), conducted a study on the utilization of chitin by broilers and identified that chitin has a significant effect on reducing the content of triglycerides and serum cholesterol observed in the chitin treatment groups with the lowest level noted at chitin inclusion level of 50 g/kg. According to Marono et al.

(2017), the reduction of triglycerides and cholesterol can be explained by the presence of positive charges in chitin that have the capability to attract the negatively charged free fatty acids and the bile acids.

Park et al. (2021) identified that the cholesterol content of the eggs significantly reduced with the increase of BSFL meal in the diet of laying hens. Park et al. (2021) hypothesized that the decrease was associated with the decreased amount of serum cholesterol in hens that were fed on BSFLM, besides chitin binding to the fatty acids and lipids thus leading to a reduced lipid absorption. According to Ceylan et al. (2011), reduction of egg cholesterol through diet formulation has been unsuccessful owing to the physiological control mechanism of layer hen that primarily causes termination of egg production in the event of inadequate deposition of egg yolk cholesterol for the survival of the embryo.

Whole egg is a well-known food source of dietary cholesterol but their consumption is generally discouraged due to the high cholesterol content in egg yolk (Clayton, Fusco, & Kern, 2017; Hu et al., 1999; Song & Kerver, 2000). However, inconsistent research findings exist about the effect of dietary cholesterol from whole egg on the risk of cardiovascular diseases risk and favorable changes in blood lipids and lipoproteins after whole egg consumption are suggested (DiMarco et al., 2017; Li, Zhou, Zhou, & Li, 2013; Rong et al., 2013; Shin, Xun, Nakamura, & He, 2013)

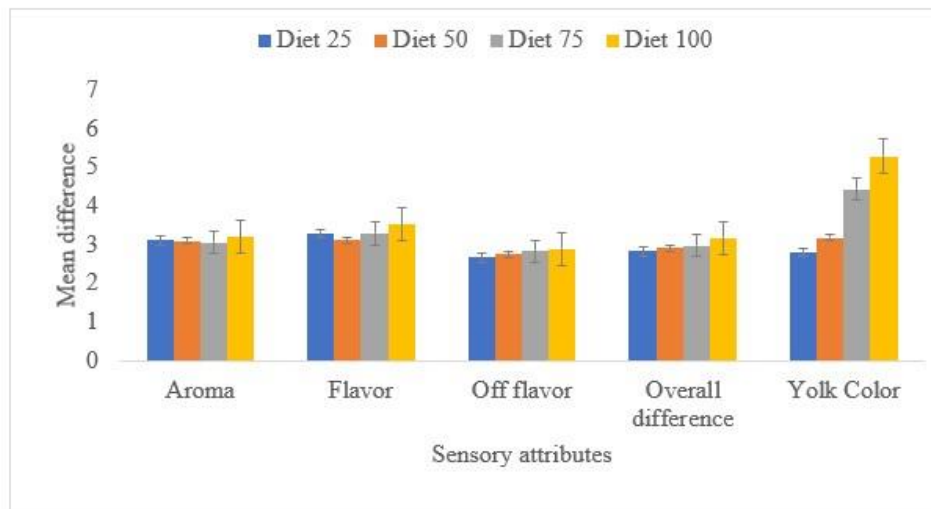
Cholesterol content in egg yolk has become a critical issue among consumers due to the risk of chronic diseases such as coronary heart diseases. Therefore, the perceived recommended daily cholesterol intake for healthy people should not surpass 300 mg/day in the United States, while European countries do not have an upper limit for dietary cholesterol (Secci Giulia et al., 2018; Tomaszewska et al., 2021).

4.3 Effect of Inclusion of Black Soldier Fly Larvae Meal at Different Inclusion Rates to the Layers Feeds on the Sensory Characteristics of Eggs

4.3.1 Sensory Attributes and Consumer Preference

According to the respondents surveyed, there was a significant overall difference ($F_{3, 272} = 3.662$, $P = 0.013$) and yolk color ($F_{3, 272} = 156.959$, $P < 0.001$) between the eggs obtained from birds fed diets on varying inclusion levels of BSFLM when compared to the control diet with 100% FM (Figure 6). There was a consistent increase in the yolk color intensity as perceived by the respondents for eggs harvested from bird fed diets with increasing inclusion levels of BSFLM in the diet (Figure 4.1).

A



B

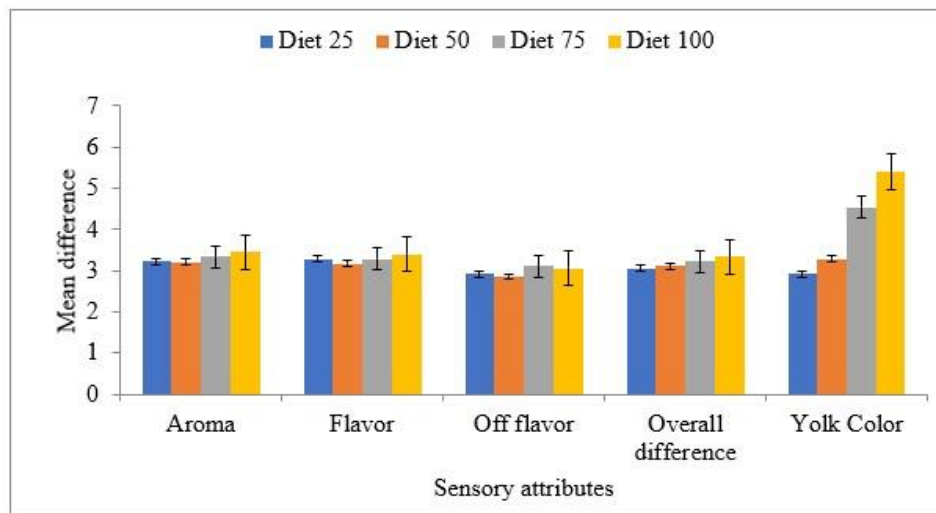


Figure 4.1: Illustration of the Differences Observed for the Sensory Attribute Scores of the Respondents for Boiled Eggs Obtained from Birds at the Age of 45 Week [A] and 80 Weeks [B] Fed the Control Diet (Diet 0) with 100% FM Compared to the Treated Diets (Diet 25, Diet 50, Diet 75 and Diet 100) with Varying Inclusion Levels of BSFLM. The scores attributed were: 0 = no difference, 3 = slightly different, 5 = moderately different, 7 = largely different and 9 = extremely different.

Figure 4.2 shows the results of consumer preference for boiled eggs harvested from laying hens at the age of week 45 and week 80. Aroma, flavor and yolk color significantly influenced consumer preference, especially eggs from hens fed diet on 100% inclusion level of BSFLM. According to the data obtained, there was a significant overall preference ($F_{4, 190} = 155.544, P < 0.001$) and yolk color ($F_{4, 190} = 138.487, P < 0.001$) on eggs obtained from birds fed diets with 100% BSFLM diet when compared to the control diet with 100% FM. There was also a consistent increase in the flavor and aroma intensity for eggs harvested from bird fed on diets containing increasing inclusion levels of BSFLM in the diet.

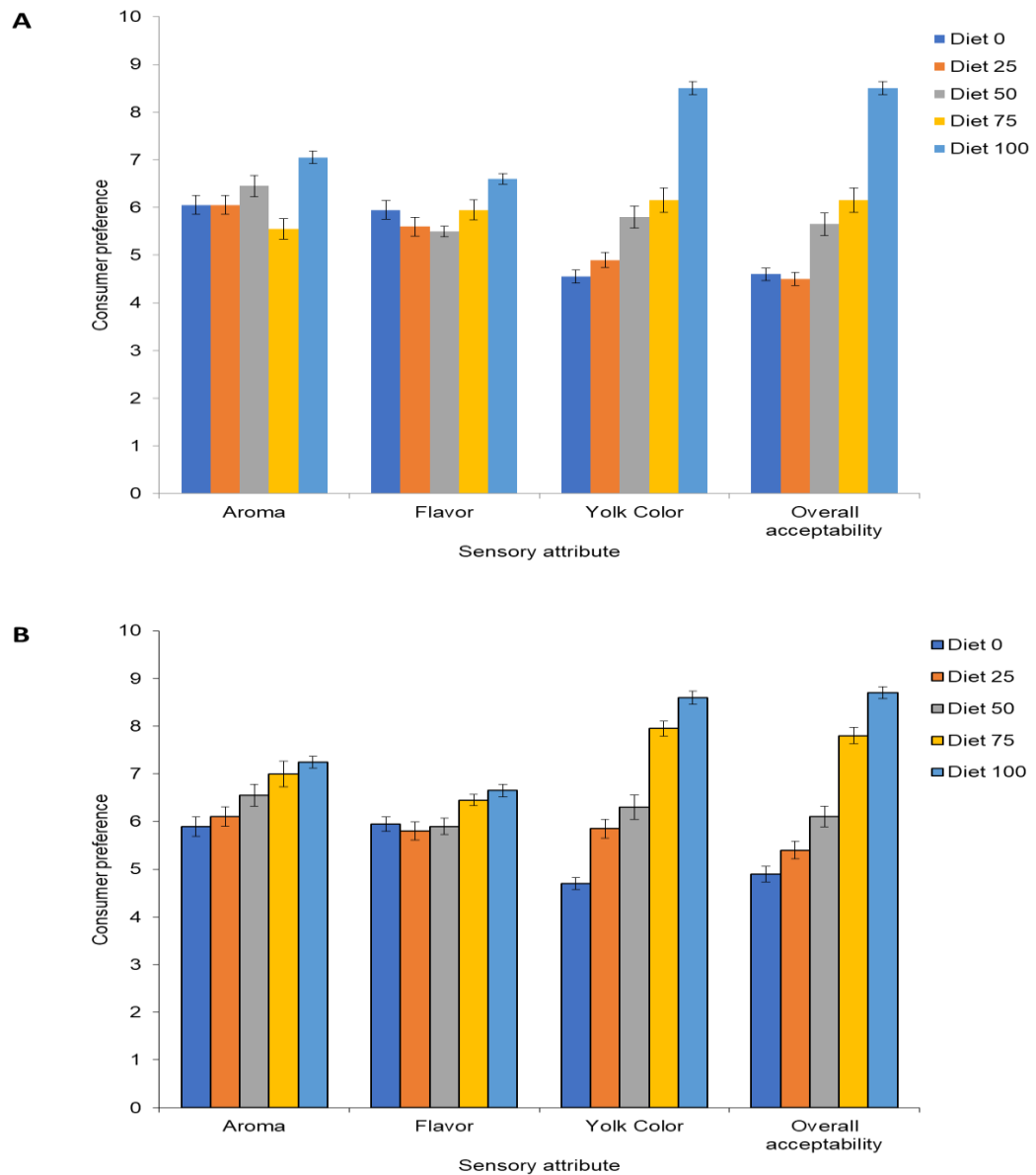


Figure 4.2: Consumer Preference for Boiled Eggs Harvested at Week 45 [A] and Week 80 [B] from Hens Fed Diets with Increasing Inclusion Levels of BSFL Meal. The scores attributed were: (1 = dislike extremely; 9 = like extremely) on a 9-point hedonic scale

Results obtained in this study were comparable to the results reported by Wallace et al. (2018) on the flavor and acceptability of meat from guinea fowls that were fed with different BSFLM levels. The author reported that meat flavor has a major influence on the level of the acceptability (Wallace et al., 2018). Flavor perception is influenced by the combination of juiciness, aroma, taste, and mouth feel. The fat

content and volatiles that come from lipids greatly influence the palatability and flavor of the meat. Flavor can be significantly altered by oxidized fatty acids even in their small amounts (Wallace et al., 2018). Different poultry feeds formulation have been reported to have an impact of the organoleptic and physicochemical characteristics of the meat including fatty acid profile and the forming of short chain fatty acids and thus can be utilized in improving the flavor of poultry meat (Wallace et al., 2018).

The principal sensory characteristics of food products of animal origin, such as flavor have been shown to be significantly influenced by the animals' diet (Réhault-Godbert, Guyot, & Nys, 2019). Results from previous studies by Bejaei and Cheng (2020) indicated that the fatty acid profile of eggs was influenced by dietary oil sources with direct consequences on sensory attributes. The flavor and aroma of the eggs varied considerably with the panelists showing higher preference for those harvested from 45 and 80 weeks old birds fed on diets with high inclusion levels of BSFLM. Similar observations have been reported for eggs produced by hens fed on flax seeds (Hayat et al., 2010). Sensory improvement for eggs collected from birds fed on diets containing BSFLM has been linked to the presence of high glutamic acid present in insect meal which is associated with formation of flavour compounds (Al-Qazzaz et al., 2016; Zotte et al., 2019). The sensory attribute (flavor and aroma) scores for boiled eggs obtained from birds fed on the control diet and diets with various inclusion levels of BSFLM showed significant variation. Also, there was a significantly improved yolk color in eggs from birds fed on diet with increased inclusion levels of BSFLM, which might have been one of the critical factors that could have significantly enhanced consumer acceptance of eggs from birds provided diet with BSFLM.

According to previous studies, many consumers believe that eggs from layers with access to insects are healthier and of better quality than those fed conventional fish meal diets which is in line with the current findings (Laudadio, Ceci, Lastella, Introna, & Tufarelli, 2014; Samli, Agma, & Senkoylu, 2005).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The current study has demonstrated that BSFLM is a more efficient and sustainable alternative compared to fish meal contributing to consumer acceptability and physical quality of eggs. The significant contribution of this study is the evidence that inclusion of BSFLM in layers diet produces eggs with superior yolk color, improved egg shell thickness, and increased egg weight directly addressing sensory and economic requirements in the poultry industry. Eggshell thickness and weight are physical factors that influence egg strength and pathogenic challenges throughout the supply chain from production to the consumer.

In addition, the findings indicate significant trade-off in the fatty acid profile. While the MUFA content increased with BSF inclusion, leading to improvement of the yolk's oxidative stability, long chain fatty acids such as DHA significantly reduced. This implies that BSFLM alters the lipid profile while effective in production of eggs with increased weights. In summary, Total or partial replacement of FM with BSFLM offers a sustainable solution for production of eggs that are highly marketable without compromising important nutritional parameters such as minerals and crude protein.

5.2 Recommendation

This study establishes that in order to maximize on yolk color and egg weights, replacement of FM of up to 75-100% BSFLM is recommended. This study also establishes the critical nutritional and sensory blueprint necessary for the industrial formulation and commercial launch of complete, ready-to-use BSF-branded Layer Mash.

5.3 Future Work

Further studies on mitigation of PUFA trade off specifically DHA should be conducted through supplementing BSFLM layers diet with sources rich in omega- 3 fatty acids. Further studies on Shelf-life, such as oxidative stability of the specific fatty acid profiles in BSFL-fed eggs, should be conducted.

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APPENDICES

Appendix I: Difference Test

Sensory Evaluation Instructions

- You are provided with 4 different coded samples and a control.
- Evaluate each egg sample and compare with the control and tick the box that best describes the difference between the various sensory attributes (aroma, flavor, off flavor, color of the yolk, and the overall difference).
- Cleanse the palate between samples with water provided.
- For each attribute tested, rate the difference between each sample and the control using the intensity scale where Zero (0) = no difference; Three (3) = slightly different; Five (5) = moderately different; Seven (7) = largely different; Nine (9) = extremely different.
- In addition, comment on each attribute regarding major differences observed in the samples provided.

Sensory Evaluation Table

Sensory Attribute	Sample Code	No Difference (0)	Slightly Different (3)	Largely Different (5)	Moderately Different (7)	Extremely Different (9)
Aroma	504					
	302					
	111					
	218					
Flavor	504					
	302					
	111					
	218					
Off-Flavor	504					

	302					
	111					
	218					
Color of the Yolk	504					
	302					
	111					
	218					
Overall Difference	504					
	302					
	111					
	218					

Appendix II: Consumer Preference Test

Sensory Evaluation Instructions

- You are provided with 5 different coded samples.
- Evaluate each egg sample and tick the box that best describes the sensory attributes (aroma, flavor, yolk color, and the overall preference).
- Cleanse the palate between samples with water provided.
- For each attribute tested, rate the degree of liking using a 9-point Hedonic scale whereby; 1 = Dislike Extremely
 2 = Dislike Very Much
 3 = Dislike Moderately
 4 = Dislike Slightly
 5 = Neither Like nor Dislike
 6 = Like Slightly
 7 = Like Moderately
 8 = Like Very Much
 9 = Like Extremely

Attribute	Sample Code	1	2	5	4	5	6	7	8	9
Aroma	504									
	302									
	111									
	218									
	415									
Flavor	504									
	302									
	111									
	218									
	415									
Yolk color	504									
	302									

	111									
	218									
	415									
Overall Acceptability	504									
	302									
	111									
	218									
	415									