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**EFFECT OF DIETARY INCLUSION LEVELS OF BLACK SOLDIER FLY
LARVAE MEAL ON BROILER GROWTH PERFORMANCE, CARCASS
CHARACTERISTICS, HEMATOLOGICAL PARAMETERS, CECAL
MICROBIAL LOAD, AND MEAT QUALITY**



MSc THESIS

**ADDIS ABABA UNIVERSTIY COLLEGE OF VETERINARY MEDICINE
AND AGRICULTURE DEPARTMENT OF MICROBIOLOGY,
PARASITOLOGY AND POULTRY HEALTH**

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JUNE, 2025

BISHOFTU, ETHIOPIA

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**A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis
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Master
of Veterinary Science in Poultry Health and Management**

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Effect of Dietary Inclusion Levels of Black Soldier Fly Larvae Meal on Broiler
Growth Performance, Carcass Characteristics, Hematological Parameters, Cecal
Microbial Load, and Meat Quality

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I confirm that the research titled "**Effect of Dietary Inclusion Levels of Black Soldier Fly Larvae Meal on Broiler Growth Performance, Carcass Characteristics, Hematological Parameters, Cecal Microbial Load, and Meat Quality**" is entirely my own work, and I have appropriately acknowledged all sources used in this study. This research is being submitted as part of the requirements for an advanced degree of Masters of Veterinary Science at Addis Ababa University, College of Veterinary Medicine and Agriculture, and will be made available in the University's library for borrowing according to library rules. I affirm that this research has not been submitted for the purpose of obtaining any academic degree, diploma, or certificate from any other institution.

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LIST OF ABBREVIATION

ABWG	Average Body Weight Gain
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
BSFL	Black Soldier Fly Larvvae
BSFLM	Black Soldier Fly Larvae Meal
BWG	Body Weight Gain
Ca	Calcium
CF	Crude Fiber
CP	Crude Protein
DM	Dry Matter
EDTA	Ethylenediamine Tetraacetic Acid
EE	Ether Extract
FBW	Final Body Weight
FCR	Feed Conversion Ratio
FI	Feed Intake
GIT	Gastrointestinal Tract
Hb	Hemoglobin
IBW	Initial Body Weight
ME	Metabolizable Energy
P	Phosphorus
PCV	Packed Cell Volume
SBM	Soy Bean Meal
TVC	Total Variable Cost

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Effect of Dietary Inclusion Levels of Black Soldier Fly Larvae Meal on Broiler Growth Performance, Carcass Characteristics, Hematological Parameters, Cecal Microbial Load, and Meat Quality

ABSTRACT

The introduction of Black soldier fly larvae meal (BSFLM) as a sustainable protein alternative in poultry diets is gaining attention for its potential to enhance broiler performance, health and economic efficiency while reducing dependence on conventional feed sources. This study aimed to investigate the effects of incorporating BSFLM at varying inclusion levels 0% (T1), 2.5% (T2), 5% (T3), and 7.5% (T4) on broiler chickens, focusing on parameters such as growth performance, carcass traits, hematological indices, ceecal microbiota composition, meat quality attributes, and economic viability.. The experimental design involved a total of 192 unsexed day-old Cobb500 broiler chicks, which were systematically allocated into four distinct treatment groups. Each treatment comprised 48 chicks, subdivided into four replicates, with each replicate containing 12 chicks, employing a completely randomized design. The analysis revealed that body weight gain, feed consumption, and carcass characteristics exhibited no statistically significant differences among the treatments ($p > 0.05$). Feed conversion ratio showed significant improvements ($p < 0.05$) in T3 and T4 during the grower phase and overall rearing period pointing to better feed efficiency. The control group (T1) had a higher gizzard weight ($p < 0.05$), while BSFLM inclusion did not affect other carcass parameters. Cost-wise, T3 brought in more net income and had a higher marginal rate of return showing it was more profitable and cost-effective. Blood tests revealed higher packed cell volume and hemoglobin levels in T3, without any negative effects on immune-related blood parameters. BSFLM did not change the total bacterial count, *E.coli*, or coliform populations in the ceecal. Meat pH and taste tests such as appearance, flavor, tenderness, and overall liking were similar across all treatments. These results suggest that adding BSFLM to broiler diets up to 7.5% is safe, with the 5% level (T3) giving the best performance and financial return without influencing health or meat quality.

Key words: *Black soldier fly larvae; Body weight; Broiler; Economic benefit; Hematology.*

1. INTRODUCTION

The world population will exceed 10 billion by 2050 (Statistics, 2019). By then, the increased population will consume twice as much animal protein as it does today; between 2010 and 2050, consumption of pork and poultry meat is expected to grow by 105% and 173%, respectively, which will create enormous challenges in the production of protein feed ingredients, estimated as being more than 1.3 billion tons of dry matter (McLeod, 2011). Poultry meat is the preferred meat around the world due to increasing meat demand, which is the primary factor supporting this growth. Poultry meat's availability, versatility, and nutritional value make it a popular choice among consumers (Garnaut, 2011). In order to satisfy the growing worldwide population, meat output must be increased by 40% by 2030 and 70% by 2050, respectively, in anticipation of the requirement for a secure global food supply. As a result, by 2030, chicken meat is predicted to overtake all other meats in consumption, accounting for 39% of total consumption of protein (Kleyn and Ciacciariello, 2021).

However, the need for livestock feed was directly impacted by the rising demand for animal protein, placing additional pressure on the already limited natural resources (Van Huis, 2013). The price of feedstuffs increased due to their high demand and limited supply. The cost of chicken feed is continuously rising due to the rising demand for quality feed ingredients and the increasing cost of soybean meal (SBM) (Makkar *et al.*, 2014). About 70% of production expenses go towards chicken feed, which increases the price of poultry products. Other possibly unconventional feed sources that are less common or inedible for human consumption must be looked afterwards. Additionally, the use of locally available or alternative feed ingredients has always been investigated by chicken nutritionists due to market volatility and rising pricing. Furthermore, there are disadvantages to increasing SBM, including greenhouse gas emissions, deforestation, and excessive water use (Hortenhuber *et al.*, 2011; Costa *et al.* 2021).

As an alternate source of protein, insect-based feed is one type of unconventional feed that has become more and more popular worldwide in recent years (Van Huis, 2013). The high-quality, sustainable supply of dietary fat and protein that terrestrial invertebrate insects provide has made them a viable feed component for chicken diets (Chobanova *et al.*, 2023). In addition to being high in fatty acids, minerals, and amino acids (AA), insects have been demonstrated to reproduce quickly on a range of waste organic materials. According to Khan (2018) and Dörper *et al.* (2021), the black soldier fly (*Hermetia illucens*) larvae (BSFL) are among the most promising insects to be used as feed in chicken diets. Black soldier fly larvae grow quickly, are good at converting organic waste, and produce meal with consistent amino acid profiles in a variety of substrates and different situations (Mwaniki *et al.*, 2020).

Black soldier fly (*Hermetia illucens*) larvae meal (BSFLM) is an inexpensive, nutrient-dense alternative protein source that is on level with or better than plant and fish meal (Sumbule *et al.*, 2021). The essential amino acid profile of BSFLM (Methionine: 0.7% to 0.9%; Valine: 2.3% to 2.8%; Lysine: 2.3% to 2.6%; Arginine: 1.8% to 2.0%), as well as its crude protein content (53%), crude fat content (58%), and calcium content (7%) on a DM basis, make it a desirable ingredient in mono-gastric animal feed (de Souza Vilela *et al.*, 2021). However, the nutritional composition of BSFL is known to be significantly determined by the nature of the organic substrate (Gobbi *et al.*, 2013; Liland *et al.*, 2017). Chitin from insects' exoskeletons contains polysaccharides that improve immune system performance. According to Malematja *et al.* (2023), insects are considered to be a rich source of protein with a good amino acid composition. They also include a variety of bioactive compounds, including chitin, lauric acid, and antimicrobials. These chemicals have been shown to have antibacterial and immunomodulatory effects, as well as hypolipidemic efficiency and growth promoters. Additionally, growing black soldier larvae on decomposing organic waste decreases the quantity of waste that enters the environment, is more efficient and eco-friendly, and promotes sustainable farming (Spranghers *et al.*, 2017).

In the starting and growth periods, the inclusion of 15% BSF to broiler chicken diets produced both linear and quadratic responses in terms of body weight and average daily gain. However, there was a linear decline in average daily growth in the finisher stage, which might have been caused by the adverse effects on gut morphology of feeding a high level (10%) of dietary BSF larvae meal (Dabbou *et al.*, 2018). A 15%–20% inclusion level affected immunologic parameters (de Souza Vilela *et al.*, 2021). The weight of the thighs and carcass was shown to be significantly affected by the inclusion of BSFLM by Heita *et al.* (2023). Additionally, they came to the conclusion that adding 5% BSFLM enhanced the growth performance, carcass production, and characteristics of broiler chickens. Additionally, adding 5% partially defatted black soldier fly larvae meal to broiler diets improved the meat quality and development performance, according to Schiavone *et al.* (2017) and Dabbou *et al.* (2018). Higher levels of BSFL were associated with decreased protein utilisation efficiency, despite its high fat content. This could be because of a decrease in overall calorie intake (Seyedalmoosavi *et al.*, 2022). The nutritional makeup of BSFLM produced on fruit waste and food leftover of resultant and hotel substrates is not explored, and its effects of inclusion in broilers are not evaluated, despite the fact that there are several studies on the use of BSFLM in poultry feed.

1.1. Statement of the Problem

The high cost of protein sources like fish and soybeans is driving vulnerable communities of poultry farmers out of business. Investigating affordable alternative protein sources is necessary for ensuring the chicken industry's viability and profitability (Chia, 2019). Insects, BSFL have shown great promise in waste management, biofuel generation, and protein supply (Kooienga *et al.*, 2020). Because of its versatility, BSFL can be added to chicken feed, which could reduce production expenses without losing nutritional content. The use of BSFL meal in broiler feed has not well-explored, despite the possible benefits. According to the literature now in release, more investigation is required to properly analyses the use of insects in animal nutrition and determine how they affect animal health (Tykałowski *et al.*, 2023). This study aims to feed broiler chickens diets with varying proportions of BSFL meal as an alternative protein source, evaluating its effects on their health and immune system. The research aims to unlock the potential of BSFL as a sustainable protein source,

addressing ecological concerns and emphasizing its nutritional advantages for broiler growth. Assessing its economic viability could lead to significant changes in industry practices. However, the limited understanding of the effects of BSFL larvae meal on broiler performance underscores the need for additional studies in this area.

1.2. General Objective

The aim of study is to investigate the impact of including graded levels of BSFL meal in the diets of broiler chickens on growth performances, carcass characteristics, cecal bacterial health, hematological parameters and meat quality.

1.3. Specific Objectives

- To evaluate the effects of dietary inclusion levels of BSFLM on broiler chickens' feed intake, body weight gain, feed conversion, and carcass characteristics;
- To determine the effects of dietary inclusion of BSFLM on meat pH and the meat sensory quality of broiler chickens;
- To determine the effects of dietary inclusion of BSFLM on hematology of broiler chickens;
- To assess the effects of dietary inclusion of BSFLM on gut microbial load of broiler chickens;
- To analyze the economic benefits of dietary inclusion levels of BSFLM on broiler chicken production.

2. LITERATURE REVIEW

2.1. Broiler Production in Ethiopia

Chickens are the most common bird in Ethiopia, and practically every rural family owns chickens. They are a great source of earnings and protein for the family. An estimated 56.5 million chickens live in the nation, of which 96.9% are native breeds, 0.54% are hybrids, and 2.56% are exotics. The most common chicken breeds raised in Ethiopia are local ecotypes, which vary greatly in terms of productivity, body position, plumage colour, and comb type. Due to numerous productions, reproduction, and infrastructure constraints, the sector's economic contribution is still out of proportion to the enormous number of chickens (Afras, 2018). The production of broilers is essential for maintaining food security for Ethiopia's fast growing population. Broiler chicken production is highly desirable in Ethiopia because of its short production cycle, excellent feed efficiency, and high biomass per unit of agricultural area (Smith, 2001). Within five to six weeks, broilers can reach a significant weight, but achieving a desirable level of production depends on the availability of high-quality feed and efficient disease prevention strategies (Tallentire *et al.*, 2016).

2.2. Poultry Feed Ingredients

The primary expense of raising poultry is feed, which accounts for as much as 70% of the total. Approximately 95% of the overall cost of feed is spent on meeting energy and protein needs, 3% to 4% on major, trace, and vitamin mineral requirements, and 1% to 2% on various feed additives. Cereal grains, cereal by-products, lipids, plant protein sources, animal by-products, vitamin and mineral supplements, crystalline amino acids, and feed additives are some of the elements used to produce poultry diets (Ravindran, 2013). These feedstuffs which include proteins (amino acids), carbs, lipids, minerals, and vitamins along with water give the bird the energy and nutrients it needs for development, reproduction, and overall health. According to Anjum *et al.* (2014), balanced meals are composed up of the proper ratios of different elements to provide a diet that contains all the nutrients needed for an animal's regular functioning.

2.3. Poultry Protein and Energy Feed Ingredients

Dietary protein serves as a source of amino acids for muscle growth, maintenance, and egg protein synthesis. Twenty amino acids are needed for the synthesis of muscle and egg proteins; these are all physiological necessities. Ten of these are classified as essential components of the diet because they are either not synthesized at all or are synthesized too slowly to satisfy the metabolic needs. They must be supplemented in the diet. Other amino acids, known as dietary non-essential elements, can be synthesized to provide the balance; feed formulations do not need to take this into account (Ravindran, 2013). The second most important part of a practical poultry diet is protein sources. In the mainstream commercial poultry industry, protein is supplied by a small number of ingredients that are generally more expensive than energy sources and have limited distribution. One of the best sources of protein for the poultry industry is soybean seed. Probably the second most significant source of protein is canola or rapeseed. The poultry industry uses the residue left over after oil extraction as its primary ingredient, rather than both seeds, which are rarely fed as whole seed meals (Iji *et al.*, 2017).

The soybean (*Glycine max*, L.) is a high-quality vegetable protein used in animal feed all over the world in addition to providing high-quality edible oil for human consumption. Its favorable qualities, including a relatively high protein content and an appropriate amino acid profile (apart from methionine), minimal nutrient content variation, year-round availability, and relative freedom from intractable anti-nutritive factors when processed properly, have contributed to its widespread acceptance in animal feed. The use of soybeans as an alternative protein source in animal diets has also drawn attention because of the fluctuating availability or permitted applications of animal proteins as well as their affordable price (Dei, 2011).

2.4. Insects as Poultry Feed

Since their protein content (40–60% DM) and fat (30–40% DM) are comparable to those of fish meal (FM) or soybean meal (SBM), insects are recommended as an alternative protein source for poultry feed. A switch to insect meals from traditional protein sources like SBM and FM could limit eutrophication of water environments (nutrient loss), reduce greenhouse gas emissions, and make better use of natural resources (Khan, 2018). When Black soldier fly larvae were fed instead of soymeal, the weight gain was comparable, but the feed intake was lower than the control, suggesting better feed conversion. Insects with high levels of chitin in their exoskeletons have detrimental effects on feed intake and disrupt protein utilization. The primary indigestible component of insects' exoskeleton, chitin (54 g/kg to 106 g/kg DM), is also present in BSFL larvae. Chitin-protein matrixes can have a detrimental effect on digestibility and nutrient utilization, especially protein digestibility (Dörper *et al.*, 2021). It has been demonstrated that chickens' proventriculus and hepatocytes produce chitinase, but chitin's digestibility appears to be restricted, especially in young birds. There is currently debate regarding whether chitin, like probiotics, may improve the microbial balance in the gastrointestinal tract by acting as a substrate for microbial fermentation (Khusro *et al.*, 2012).

2.5. Insects in Waste Management

It is often known that using insects, such as BSF, can help address problems related to the large amounts of organic waste that are dispersed around the globe. It has been used more and more to treat biological waste since it is seen to be a cost-effective and environmentally friendly method. In recent decades, increased emphasis has been paid to the vital function that BSF larvae (BSFL) play in recycling biological wastes. The ability of BSFL to recycle a wide range of waste materials, including human faeces, food waste, fruit and vegetable waste, and abattoir waste, was highlighted. BSF is naturally found in temperate tropical regions and belongs to the Diptera family of the order Stratiomyidae (Siddiqui *et al.*, 2022). BSFL was found to have a remarkable ability (75%) to recycle biological wastes, producing 800 g of larval biomass from 4 kg of garbage in a study by Ibadurrohman *et al.* (2020). BSFL needs to be kept in optimal environmental conditions, including those related to temperature, oxygen content,

physical characteristics, nutrient composition, and humidity, in order to maximize its bioconversion efficiency (Siddiqui *et al.*, 2022).

Additionally, the kind of food substrate is essential for BSFL's optimum bioconversion activity. For BSFL to grow, they require nutrition, just like every other living thing. Therefore, BSFL must eat organic wastes high in digestible nutrients in order to perform better during bioconversion. Furthermore, it was emphasized that BSFL can efficiently break down different kinds of organic waste if it has a sufficient quantity of carbohydrates and protein (Siddiqui *et al.*, 2022). Black soldier fly larvae (BSFL) *Hermetia illucens* L. not only stabilize garbage but also convert a lot of organic wastes into larval biomass, which is high in fat and protein and can be used as a source for animal feed (Salam *et al.*, 2023). The bioconversion procedure is one of the more appealing methods for treating food waste. Waste can be treated using living things, like insects, in accordance with the principles of bioconversion. One alternate approach that enables the management of waste and energy production at the same time is the employment of insect larvae for waste processing. Larvae of the Black soldier fly, *Hermetia illucens*, are one example. These larvae are distinguished by their high dry matter protein and fat content and quick biomass growth (Czekala *et al.*, 2020).

2.6. Black Soldier Fly

The black soldier fly, *Hermetia illucens*, belongs to the Stratiomyidae family of true flies (Diptera). Though it was originally indigenous to the Americas, it is now found in tropical and temperate regions all over the world, and its inability to withstand cold prevents it from invading non-native areas like Northern Europe. Adults only drink water, avoid getting close to people, don't bite or sting, and don't spread any particular illnesses. Black soldier fly larvae (BSFL) have been used for small-scale waste management by feeding on a wide range of organic materials, including manure, rice straw, food waste, distillers' grains, fecal sludge, animal offal, kitchen waste, and so forth (Wang and Shelomi, 2017).

2.6.1. Raring temperature of black soldier fly larvae

It has been established that temperature plays a crucial role in the growth and survival of insects. Furthermore, it is commonly known that BSF larvae are sensitive to their surroundings and that temperature affects both their survival and development. However, the interplay between temperature and nutrition influences important life-history characteristics in insects, including survival, reproduction, maturity, and development rate. Numerous investigations examined the effects of either temperature-controlled laboratory-reared diets or organic side streams as feeding substrates on the life-history characteristics of BSF larvae. As the temperature rose, BSF immatures raised on both substrates had shorter development times. At 25 °C and 30 °C, the BSF prepupae weighed the most, and their weights increased with temperature (Shumo *et al.*, 2019).

2.6.2. Black soldier fly life cycle

The four stages of the BSF life cycle are the egg, larva, pupa, and adult. The prepupae migrate to a suitable and dry pupation site during the unique final stage of the larval stage, where they transform into pupae. The adult flies do not spread disease or cause pests. They eat nothing but water later on, having survived on the fat they stored during their larval stage. The female BSF do not spread pathogens from the wastes because they only oviposit around the edges of the larval food source, not on the food itself. In addition to lowering the population of houseflies and dangerous bacteria, this insect has the ability to cut pollution from plant and animal waste by up to 50%. By recycling human feces, vegetable waste, dairy manure, swine manure, poultry manure, and human food waste, BSFL recycles wasted nutrition (Liu *et al.*, 2017).

There are two steps involved in producing BSF. The first step, which involves producing adults and eggs, is done in an insectarium, and the second step is done in a larvarium, where the larvae are developing (Caruso *et al.*, 2011). This species could be self-collected at the prepupal stage or used at the larval stage after manual harvesting or sieving for incorporation into feed formulation (Gougbedji *et al.*, 2021). Several factors have already been determined to be necessary for the successful breeding of *H. illucens*: the ambient temperature should be between 26 and 27 °C for adult

reproduction, the relative humidity should be between 60 and 70 percent, and the light intensity for oviposition should be between 135 and 200 $\mu\text{mol}/\text{m}^2/\text{s}$. (Barragan-Fonseca *et al.*, 2017). Regarding adult densities in breeding cages, the highest egg production occurs in a breeding unit with regulated environmental conditions at a density of 8500 individuals/ m^3 . A variety of goods, such as decomposing organic matter, poultry manure, or carrots, can be suggested as attractants for female oviposition; however, any other locally available co-products should be employed, taking into account any differences in attractiveness. Information about devices for raising larvae that correspond to the developmental cycle of *H. illucens* is currently available in the literature. Depending on the feeding substrates, one of two systems could be employed (Barragan-Fonseca *et al.*, 2017). When employing plant or animal co-products, like in this study, the first method relies on the prepupae's self-harvesting; when using high-quality items, such flour or cereal bran, in a drier climate, the second system relies on the last instar larvae sieving. In laboratory conditions, the average prepupa weight is 0.11 0.01 g, and the average larval growth time is 24.6 6.2 days (Barragan-Fonseca *et al.*, 2017).

2.6.3. Black soldier fly larvae meal preparation

Given the possibility of producing bigger and more frequent batches of insects, it is necessary to comprehend possible stabilization methods for intermediate or finished insect products ideally with a cost-benefit analysis included. Dehydration is currently the method of insect preservation that has attracted the most interest from both the scientific and commercial communities. It is well known that removing water from a product can stop or drastically slow down the processes that cause microbiological and chemical deterioration. The stability of the chemicals and/or microbes when preserving dehydrated edible insects has been the focus of numerous research projects (Van Campenhout *et al.*, 2021). Among the drying technologies covered in the previously mentioned studies are oven, freeze, microwave, and solar drying. the production of meal from black soldier fly larvae (BSFL) and investigated how the chemical makeup and in vitro digestibility of the meal were affected by six different killing techniques, followed by oven drying and grinding. According to the previously mentioned research, drying has generally been shown to be a useful technique for extending the shelf life of

edible insects when the moisture content and water activity can be significantly decreased (Van Campenhout *et al.*, 2021).

2.7. Effect of Black Soldier Fly Larvae Meal on Growth Performance of Broiler Chicken

The potential advantages of using Black Soldier Fly Larvae (BSFL) meal as a poultry feed ingredient have drawn attention, especially in low-input agricultural systems and organic farming. BSFL meal might offer a sustainable and affordable source of protein in these environments with less feed processing, cheaper transportation, and the potential to incorporate insect production into the farming cycle. Utilizing locally accessible organic wastes as feed substrates, BSFL farming can be incorporated into production cycles. This method improves the sustainability of farming systems while lowering dependency on traditional feed sources (Nyakeri *et al.*, 2017).

The inclusion of BSFL meal in broiler diets has been shown in numerous studies to improve growth performance. As an illustration, Moula *et al.*(2018) found that the chickens fed a regular diet supplemented with 8% whole defrozed BSFL had a significantly higher body weight (BW) than the control group. Similarly, Ipema *et al.* (2020) and Bellezza *et al.* (2021) found that adding 5 and 10 percent live BSFL to broiler diets enhanced activity and overall performance without having any negative effect. According to these results, broiler performance, especially growth and activity, can be enhanced by using BSFL as a dietary supplement.

The dietary composition of BSFL, which is essential to the bird's energy intake and general performance, is what affects broiler growth. According to Latshaw (2008), broiler energy intake is directly influenced by the form and nutrient composition of their diet. They may additionally alter their feed intake (FI) in response to changes in energy level (Hu *et al.*, 2021), amino acid balance (Ferket and Gernat, 2006), fibre content (Jha and Mishra, 2021), and mineral balance (Delezie *et al.*, 2015). It has been demonstrated that adding up to 15% BSFL meal to broiler diets has a good effect on the growth performance of the birds by having a significant impact on body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) (Onsongo, 2017).

2.8. Effect of Black Soldier Fly Larvae Meal on Meat Quality of Broiler

The overall health, development, and meat quality of avian species are much enhanced when they are fed BSF larvae (Makokha *et al.*, 2023). BSFL and other insect fats have a different fatty acid profile than soybean oil. Compared to insect fat, like that from BSFL, which has been shown to contain 75% saturated fatty acids and 12.9% total polyunsaturated fatty acids, soybean oil contains a lower percentage of saturated fatty acids (16.2%) and a larger percentage of polyunsaturated fatty acids (57.5%) (De Souza Vilela, *et al.*, 2021). Up to 15% BSFL feed included in broiler chickens has an effect on the cooked breast meat's sensory qualities (Onsongo, 2017). The effects of supplementing medium-growing chickens with live black soldier fly larvae (BSFL) on the quality of their meat were examined. They found that adding BSFL, even up to 20% of the weekly feed intake, had little to no impact on final pH values of the thigh and breast muscles. This implies that post-mortem muscle acidification is not negatively impacted by BSFL supplementation, preserving the pH stability necessary for meat quality characteristics including tenderness, shelf life, and water-holding capacity (Tognoli *et al.* in 2025).

2.9. Effect of Black Soldier Fly Larvae Meal Hematology of Broilers

Because hematological measures are important indicators of poultry health and immunological state, the potential effects of adding black soldier fly larvae (BSFL) meal to broiler diets have drawn more attention. Broilers fed diets containing BSFL meal showed noticeably increased levels of hemoglobin, packed cell volume, and red blood cells than those on control diets, according to research by Mohammed *et al.* (2020). Furthermore, broilers fed BSFL diets showed an increase in monocyte counts, which may indicate an immune-stimulating impact related to the presence of bioactive substances like chitin and lauric acid in the larvae. These results suggest that adding BSFL to the diet can have a beneficial impact on hematological health by providing nutritional value and possible health advantages without causing negative side effects.

2.10. Effect of Black Soldier Fly Larvae Meal on Gut Microbial Load

Diet-dependent genes encoding putative antimicrobial peptides in the BSFL have defensive power against a wide range of bacteria, and the BSFL is also known to contain a variety of microbiota modulating substances, including chitin, lauric acid, and up to 57 different antimicrobial peptides. The amount of chitin depends on the BSFL life stage and the rearing substrate (de Souza Vilela *et al.*, 2023). Depending on the rearing substrate, the dry matter percentage of chitin has been found to range from 5.6% to 6.7%. On a dry matter basis, the BSFL lauric acid content ranges from 13.4% to 51.8%, dependent on the rearing substrate (e.g., bread and ensiled mussels, respectively) (Ewald *et al.*, 2020). Nevertheless, the BSFL's growing conditions may be closely regulated and observed, even though these nutrient levels may vary. This offers a significant benefit over conventional feed ingredients like grains, whose nutrient levels frequently vary depending on the weather, environment, harvesting circumstances, and other factors. Poultry's gastrointestinal tract (GIT) microbiota has connections to nutritional status, brain function, behaviour, immunity, performance, and welfare. The GIT microbiome is essential to the productivity of production animals, like broiler chickens, because it can boost the host's energy metabolism, modify the immune system either directly or indirectly, determine the effects of different pathogens, and have a direct impact on farmers' financial success (de Souza Vilela *et al.*, 2023).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was carried out at the experimental farm of the International Livestock Research Institute (ILRI). It is situated in Addis Ababa, more precisely around Bole, close to the Gurd Shola, along ILRI Campus Road. The elevation above sea level is approximately 2,355 meters (7,726 feet). The city's average annual rainfall is about 1,200 millimeters, and its geographic coordinates are roughly 9.02°N latitude and 38.74°E longitude and average temperature 15.5 °C and 18.8 °C (Kabite *et al.*, 2012).

3.2. Sources and Production of Black Soldier Fly Larvae Meal

Black soldier fly larvae (BSFL) were raised at the International Livestock Research Institute (ILRI) in Ethiopia, with temperatures between 25 and 32 °C and relative humidity levels between 60 and 70 percent following the procedure described by Son *et al.* (2023). The smells of manure and fruit waste were used as attractants to get adult female flies to lay eggs (Zim *et al.*, 2023). Using the steps described by Julita *et al.* (2020), a greenhouse-managed breeding environment was created to maximize the success of mating. The eggs were moved to an appropriate hatching unit after being laid. After hatching, the larvae were raised for 25 days on a substrate made of leftover food and fruit waste that was gathered from the International Livestock Research Institute Zebu Club. During this stage of rearing, the larvae's growth and development were closely monitored, and a steady supply of organic waste or food scraps was provided to support healthy development and optimal nutrition. The black soldier fly (BSF) life cycle consists of five primary stages: eggs, larvae, pupae, prepupae, and adult flies. The cuticles of a mature BSF have a high lipid and protein content of 38-86% and 40-96%, respectively, and a chitin content of up to 40% (Tran *et al.*, 2022). When the larvae were fully grown, they were collected prior to pupation using effective harvesting methods such mechanical separation and separating. Boiling water (50-70°C) was then used to kill the collected larvae, so halting their life cycle and acting as a sterilizing method (Spranghers, 2017). After that, drying was done using the sun and

air, which is a crucial technique for maintaining nutritional value and avoiding spoiling (Larouche, 2019).

3.3. Experimental Animals and Their Management

A total of 192 day-old unsexed Cobb-500 broiler chicks purchased from Alema hatchery were randomly distributed to four groups each with four replications. Each replication consisted of 12-chickens made a total of 48 chickens reared per treatment. The study conducted for 49 days. Wire mesh was used to prepare and partition the experimental pens. The experimental chicks were housed in deep litter houses with sufficient space for flooring in each pen, measuring 1m x1.5m. A 200–300 watt bulb that was placed at the ideal height for the chicks to ensure they received enough heat was used to brood the chicks in each pen. For 23 hours, the lighting program was adjusted for the first week and the lighting was gradually reduced with the bird age. At late grower and finisher the light became 12 hour light during the day. *Eragrostis tef* straw was utilized to bed the deep litter housing system, and before the chickens entered, hydrogen peroxide was used to clean and disinfect every single pen. Each experimental pen's feeding and watering troughs were carefully cleaned and set up. In accordance with the Cobb-500 feeding standard, the experimental diet was given to the group on an as-needed basis, and water was always available during the trial. The Newcastle (Iasota) vaccine, sourced from the National Veterinary Institute, was administered to the chickens. The recommended vaccine dosage was provided through eye drops on the 10th and 20th days of the chicken's life. Following the vaccination, vitamin supplements vita stress were provided.

3.4. Experimental Diets

Samples 500 g BSFLM was taken, and analyses was performed for calcium, crude protein, crude fat, DM, and gross energy. Four experimental diets with four inclusion levels of full-fat BSFLM were then formulated using the nutrient composition data for each stage of the broiler chicken's growth: starter (0-14 days), grower (15-28 days) and finisher (29-49 days) (Melkamet *al*, 2023). The experiment was designed to meet the broiler chickens' nutritional requirements during every stage. Using Coob-500's

production guidelines, the experimental diets were formulated to be iso-nitrogenous and iso-caloric, meeting its nutrient requirements for starter (CP=22%, ME=3000 kcal/kg DM), grower (CP=20%, ME=3100 DM), and finisher (CP = 19%, ME=3200 DM) (Cobb-Vantress, 2017).

Table 1: Experimental feed proportion for different Broilers growth stage

Ingredients	Ingredient proportion of the experimental diets (%)											
	Starter				Grower				Finisher			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Maize	45.1	44.0	43.4	43.3	51.49	51.2	50.0	50.0	53.1	56.7	55.5	55.3
SBM	20.0	18.4	17.8	17.3	16.0	15.5	15.3	14.1	12.0	13.0	13.0	12.0
BSFLM	0.0	2.5	5.0	7.5	0.0	2.5	5.0	7.5	0.0	2.5	5.0	7.5
Noug Seed Cake	13.3	12.8	12.8	12.6	15.0	12.0	10.8	12.0	15.5	12.0	12.0	12.3
Meat And Bone Meal	6.0	6.0	4.8	4.0	2.5	3.0	2.5	2.0	2.5	2.5	1.5	0.8
Wheat Short	10.8	12.0	12.2	11.8	12.0	11.0	11.0	10.5	12.2	9.5	9.5	9.2
Limestone	0.6	0.3	0.2	0.1	0.9	0.7	0.5	0.2	1.0	0.5	0.3	0.2
Di-calcium Phosphate	0.3	0.3	0.6	0.7	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
L-Lysine	0.6	0.7	0.7	0.7	0.8	0.8	0.8	0.8	0.6	0.6	0.7	0.7
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2
Soybean Oil	2.0	1.7	1.5	1.0	2.5	2.0	1.8	1.5	2.0	1.5	1.3	0.8
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3
Total	100	100	100	100	100	100	100	100	100	100	100	100

T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion,

3.5. Experimental Design And Treatments

The chickens were randomly assigned in to four treatments each with four replicates. The design is a completely randomized design (CRD), with 12 chickens in each replication. The experiment had three phases of feed, such as Starter (0-14days), Grower (15-28days) and Finisher (29-49 days). Accordingly, the experiment had four different inclusion levels (0% = T1), (2.5% = T2), (5%= T3), and (7.5% = T4).

3.6. Measurements And Observations

3.6.1 Chemical analysis of feed ingredients and experimental deits

Proximate analysis, including crude protein, dry matter, crude fiber, crude fat and calcium were performed according to the standard methods proposed by the Association of Official Analytical Chemists (AOAC, 2012). After calculating the sample's moisture content, the dry matter was calculated as follows: %moisture = [(Initial sample weight - sample weight after drying)/sample weight] x 100%, and %DM = 100 - %moisture. Using the Kjeldahl method, the sample's nitrogen concentration was determined, and the crude protein content (% CP) was calculated as % N x 6.25. The crude protein was done at international livestock research institute (ILRI) nutrition laboratory, Addis Ababa. The total metabolizable energy content was estimated by using the formula of Wiseman (1987): ME (Kcal/kg DM) = 3951 + 54.4 EE - 88.7 CF - 40.8 Ash. The proximate chemical analyses of BSFL dry matter, crude fiber, crude fat and calcium was done at national veterinary institute, Bishoftu city.

Table 2 Nutritional composition feed ingredients

Ingredients	Nutritional Composition of Major Ingredients					
	DM%	CP%	ME(K/cal)	CF%	EE%	Ca%
BSFL	95.3	36.34	4000	12.8	42.8	5.246
Maize	90	8.7	3340	2.1	3.6	0.04
SBM	90.2	43	2120	6.5	1.8	0.3
Meat and Bone Meal	92	52.4	2830	6.5	12	7.08
Nuge Cake	92.4	29	3574	5.83	8.11	0.58
Wheat Meddling	90	15.6	1980	9.2	3.6	0.11

Table 3: Nutritional composition of the experimental diets

Nutrient (%)	Broiler chickens Growth Stage											
	starter				grower				finisher			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
DM%	90.3	90.4	90.8	91.0	90.5	90.5	90.6	90.8	90.4	90.3	90.4	90.6
CP%	21.9	22.2	22.1b;	22.3	19.5	19.8	20.0	20.3	18.2	18.4	18.8	18.9
ME(K/cal)	301.0	301.9	303.5	304.2	309.5	310.8	311.2	314.5	312.5	314.2	316.1	317.7
CF%	4.4	4.7	4.9	5.1	4.2	4.3	4.5	4.7	4.1	4.1	4.3	4.5
EE%	7.2	7.9	8.5	8.9	7.4	7.8	8.4	9.1	6.8	7.2	7.8	8.3
Ca%	1.0	1.0	1.0	1.1	0.8	0.8	0.9	0.9	0.8	0.8	0.7	0.8
P%	0.9	0.8	0.8	0.8	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5

T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion; DM=Dry Matter; CP=Crude Protein; CF=Crude Fiber; EE=Ether Extract; Ca=Calcium; P = phosphorus; ME: Metabolize energy (Kcal/kg DM).

3.6.2 Feed intake

The experimental ration is given twice a day at 4:00 PM and 8:00 AM. Each pen's feed refusal is collected the following morning before another feed is administered. Each replication's feed offer and refusal are recorded daily. Feed intake is calculated as the difference between the feed that was offered and that was refused.

$$\text{Feed intake(g/bird)} = \frac{\text{Daily feed offered} - \text{Daily feed refusal}}{\text{Number of chickens in pen}}$$

3.6.3 Body weight gain

At the start and then every week, the body weight of the chickens was measured in groups using a sensitive balance. Each replicate's average body weight gain or loss is calculated by deducting the initial weight from the final weight, and then dividing that result by the number of experimental days within consecutive measurements.

$$\text{Body weight gain(g/bird)} = \text{Final body weight} - \text{Initial body weight}$$

$$\text{Average Daily weight gain (ADG)(g/bird)} = \frac{\text{Body weight gain(g)}}{\text{Number of experimental days}}$$

3.6.4 Feed conversion ratio

The average daily feed intake divided by the average daily body weight gain gives the feed conversion ratio (FCR).

$$\text{Feed conversion ratio(FCR)} = \frac{\text{Average daily feed intake (g)}}{\text{Average daily body weight gain(g)}}$$

3.6.5 Mortality rate

The chickens were observed closely both during the day and at night. During the course of the experiment, the number of deaths was documented, and the mortality rate was computed as follows:

$$\begin{aligned} \text{Mortality rate\%} \\ = \frac{\text{Number of dead chickens}}{\text{Total number of chickens at the beginning of the experiment}} \times 100 \end{aligned}$$

3.6.6 Carcass characteristics

On the 49th day of the experimental period, two chicks from each pen were randomly chosen and isolated. They were then deprived of feed for 12 hours before being weighed right before slaughter. The chicks were manually slaughtered by cutting the jugular vein, allowing them to bleed out completely, and then weighed to determine their post-bleeding weight. Following slaughter, the birds were de-feathered through manual plucking. Once de-feathered, all inedible parts (including visceral, head and shank) were removed and weighted separately. The carcass was measured for weight and the percentage was calculated. Each part and organ was weighed using a digital balance and recorded. Information on the pre-slaughter live weight, weight after bleeding, and the weights of the back, neck, breast, drumstick, thigh, wing, gizzard, and all visceral organs (including the heart, kidneys, spleen, lungs) was recorded, along with the shank, head, and abdominal fat. The total non-edible carcass (TNE), head, shank, and abdominal fat were noted separately (Mekonnen *et al.*, 2023). Finally, the dressing percentage calculated following the method of FAO (2001) as follows:

$$\text{Dressing percentage (\%)} = \frac{\text{Carcass weight}}{\text{Live body weight}} \times 100$$

3.7. Hematological Parameters

Blood was collected from the wing vein using sterile syringes and placed into vacutainer tubes with EDTA as an anticoagulant to avoid clotting. The samples were then immediately transported under refrigeration to the laboratory of the College of Veterinary Medicine at Addis Ababa University. The packed cell volume (PCV), was determined using the microhematocrit method. Approximately 50 μ L of blood was placed into a capillary tube, sealed at one end, and centrifuged at 12,000 rpm for 5 minutes. The PCV was calculated by measuring the height of the red blood cell column in relation to the total blood volume. Hemoglobin concentration was measured using the acid hematin method. For the differential leukocyte count, a peripheral blood smear was prepared by placing a small drop of blood onto a glass slide and spreading it using another slide at 45° angle. After air-drying, the smear was stained using Giemsa staining dye and allowed for 30 minutes. Examined by using oil immersion objective (100x). A total of 100 white blood cells were counted and categorized into neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Washington *et al.*, 2012).

3.8. Caecal Bacterial Load Determination

After slaughter, the caecal contents were carefully collected into sterile bacterial transport media to prevent contamination and transport to Addis Abeba University, college of veterinary medicine laboratory. For uniformity the caecal samples which were taken were homogenized. To make serial dilutions of the caecal content, 10 grams of the sample were mixed with 90 mL of sterile saline (0.85% NaCl) to make a 10^{-1} dilution. 1ml of the 10^{-1} dilution was added to 9 ml of saline to make additional dilutions, which were repeated until a 10^{-5} dilution was obtained. On Brilliance *E. coli*/coliform selective medium, a 100 μ L aliquot of the 10^{-5} dilution was plated to help in the differentiation and selection of *E. coli* and coliforms (Harrigan, 1998; Wohlsen, 2011; Andrews *et al.*, 2018). To allow for bacterial growth, the plated samples were incubated for 24 to 48 hours at 37°C. Following the incubation period, the plates were examined for colonies that were characteristic of *E. coli* and coliforms. The number of colony-forming units (CFU) per gram of caecal material was calculated manually using colony counts. The bacterial load and microbial contamination in the caecal samples have been determined using these counts.

3.9. Measurement of Meat pH

After carcass evaluation chicken meat parts were used to assess the pH of different Measurements. pH were made from the drumstick, thigh, and breast muscles. Standard saline solution was used to calibrate a portable digital pH meter before each usage. pH readings were taken at two different times: right after slaughter and 24 hours later after being stored at 4°C. The pH probe was placed straight into the tissue of each muscle, and in order to ensure accuracy, readings were made three times (Glamoclija *et al.*, 2015).

3.10. Sensory Evaluation

A sample of 500g chicken breast was randomly selected from two birds per each treatment and transferred to bishoftu agricultural research center to conducted sensory test. Chicken breast cooked using a consistent method roasting at a temperature of 160-165°C (325-330°F) until the internal temperature reached 74°C (165°F) for 20-30 minutes (Lawrie and Ledward, 2006) A random panel of 24 customers was given uniformly sized quantities of the roasted breast meat to evaluate their preferences for a variety of sensory qualities, such as appearance, color, aroma, taste, juiciness, tenderness, and overall acceptance. To reduce bias, every panelist was given one sample at a time, and the samples were presented to them in a randomized order. The evaluation was conducted using a 7-point hedonic scale, with 1 indicating "dislike extremely" and 7 indicating "like extremely." Between samples, water was supplied for palate washing in order to reduce flavor transfer (Inusa *et al.*, 2020).

3.11. Partial Budget Analysis

A partial budget was calculated as the difference between the overall cost, which included the total variable cost per bird during the trial period, and the sale of birds in order to determine the profitability of feeding BSFL meal. Total variable costs (TVC) were subtracted from total return (TR) to determine net income (NI).

$$TR - TVC = NI$$

Where, TVC = total variable cost

The marginal rate of return (MRR) which measures the increase in net income (c NI (NI from diets with BSFL minus NI from control diets)) associated with each additional unit of expenditure (c TVC (TVC from diets with BSFL minus TVC from control diets)) was computed as:

$$MRR = \frac{cNI}{cTVC} \text{ (Miles and Jacob, 2000).}$$

3.12. Statistical Analysis

The data collected were analyzed using one way ANOVA procedures (SAS 9.0). The differences between the treatment means were determined using Duncan's multiple range test, and a significance level of $p < 0.05$ was used to take to be considered (Duncan, 1955).

The model used was:

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

Where: Y_{ij} = the j^{th} observation with treatment i

μ = overall mean

α_i = the i^{th} treatment effect (BSFL Meal inclusion level)

ϵ_{ij} = the random error term

4. RESULTS

4.1. Growth Performance

The effect of different levels of black soldier fly larvae meal (BSFLM) included in the diet on the body weight gain of the broilers over the starter, grower, and finisher phases is shown in Table 4. It was observed that there were no significant differences ($p > 0.05$) in the initial body weight (IBW), final body weight (FBW), average body weight gain (ABWG) and daily weight gain (DWG) throughout the phases of growth. However, T2 and T4 had higher weight gain relative to the control (T1) during the starter phase, during the grower phase, broilers fed T3 had numerically the best ABWG and DWG, indicating a better growth potential at this level of inclusion.

The result in Table 4 indicate that the various treatment groups (T1, T2, T3, and T4) did not have any significant effect on feed intake across all the growth stages because ($p > 0.05$). In the starter phase, feed intake was comparable in the treatment groups, with T3 and T4 having somewhat higher figures relative to T1 but not statistically significant ($p > 0.05$). Under the growing period, treatment group T1 had the greatest feed intake, with treatment groups T2, T3, and T4 having somewhat lesser values; however, the differences among groups were not statistically significant ($p > 0.05$). Under the finishing period, the measurements for feed intake did not differ significantly for all treatment groups, and there were no significant differences ($p > 0.05$). Throughout the entire duration of the study, total feed intake followed the same trend across the various treatments, and the differences that existed were still statistically insignificant ($p > 0.05$). This shows that the treatments did not have a significant influence on the feed intake of the broilers ($p > 0.05$).

The inclusion of black soldier fly larvae meal (BSFLM) in broiler diets had a significant effect on the feed conversion ratio (FCR), especially at the higher levels of inclusion (T3 and T4) in the starter phase, as presented in Table 4. Conversely, in the grower phase, no significant differences were observed in FCR between the treatments ($p > 0.05$), meaning that BSFLM did not have any detectable influence on feed efficiency at this starting phase. However, at the grower stage, broilers fed diets T3 and T4 exhibited

better feed efficiency significantly compared to T1 and T2, with better FCR significantly ($p < 0.05$). This suggests that BSFLM may improve nutrient utilization or metabolic efficiency at this stage. During the finisher phase, statistics revealed no significance in feed conversion ratio (FCR), although the best feed efficiency was in birds fed the T3 diet, and the least efficient feed conversion was seen in the T1 group. However, when the whole rearing period is taken into consideration, FCR significant differences were noted ($p < 0.05$), with T3 and T4 being better than T1.

Table 4: Effect of the meal dietary inclusion level of Black Soldier Fly Black Meal Dietary Inclusion Level on Broiler Growth Performance

Growth phase	Parameters	Treatments				p-value	sig.
		T1	T2	T3	T4		
Starter	IBW(g /bird)	44.17	43.99	44.61	44.08	0.81	ns
	FBW(g/bird)	215.62	219.32	209.81	217.51	0.75	ns
	ABWG(g/bird)	171.45	175.32	165.2	173.43	0.69	ns
	DWG(g/bird/day)	12.25	12.52	11.8	12.39	0.69	ns
	TFI (g/bird)	330.63	333.23	339.38	336.56	0.66	ns
	ADFI(g/bird/day)	23.62	23.80	24.24	24.04	0.66	ns
	FCR	1.94	1.91	2.06	1.94	0.26	ns
Grower	IBW(g/bird)	215.62	219.32	209.81	217.51	0.75	ns
	FBW(g/bird)	659.83	666.64	694.79	672.57	0.64	ns
	ABWG(g/bird)	444.21	447.32	484.99	455.06	0.27	ns
	DWG(g/bird/day)	31.73	31.95	34.64	32.5	0.27	ns
	TFI(g/bird)	1034.42	1026.55	964.71	927.17	0.15	ns
	ADFI(g/bird/day)	73.89	73.33	68.91	66.23	0.15	ns
	FCR	2.34 ^a	2.30 ^a	1.99 ^b	2.04 ^b	0.01	**
Finisher	IBW(g/bird)	659.83	666.64	694.79	672.57	0.64	ns
	FBW(g/bird)	1708.92	1727.68	1844.41	1813.66	0.29	ns
	ABWG(g/bird)	1049.09	1061.04	1149.61	1141.1	0.31	ns
	DWG(g/bird/day)	49.96	50.53	54.74	54.34	0.31	ns
	TFI (g/bird)	2499.08	2299.82	2338.76	2362.09	0.44	ns
	ADFI(g/bird/day)	119.00	109.52	111.37	112.48	0.44	ns
	FCR	2.39	2.18	2.05	2.07	0.14	ns
Entire period	ABWG (g/bird)	1664.75	1683.69	1799.8	1769.58	0.29	Ns
	TFI (g/bird)	3864.13	3659.59	3642.86	3625.82	0.39	ns
	FCR	2.33 ^a	2.18 ^{ab}	2.03 ^b	2.05 ^b	0.05	*

$P < 0.05$; * = significant; ^{ab} Means within a row with different superscripts differ significantly ($P < 0.05$);

T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion;

IBW = initial body weight; FBW = final body weight; ABWG =average body weight gain; DWG =daily body weight gain, FI = Feed intake; FCR = Feed conversion ratio.

4.2. Mortality

In this study, 11 chicks were culled due to leg deformities and severe leg weakness. The mortality rate during the starter phase was 2.08 %. During the grower and finisher phases, the mortality rates were 1.56% and 1.04%, respectively. Overall, the total mortality rate throughout the entire experimental period was 4.68%.

4.3. Carcass Characteristics

Inclusion of black soldier fly larvae meal (BSFLM) in broiler ration influence the carcass traits that is presented in table 5. All the carcass parameters and other internal were not influenced by different BSFLM level inclusion in broiler ration of different age groups ($p > 0.05$). Specifically, there were no significant differences found between the treatment groups for dressing percentage, live weight, after-bleeding weight, carcass weight, muscle weights (breast, thigh, drumstick, and wing), visceral organ weight, neck weight, back weight, shank weight, head weight, and abdominal fat. However, an exception was found in gizzard weight, which was significantly higher ($P < 0.05$) in T1 (control group) compared to the groups that received BSFLM (T2–T4).

Table 5: Effect of dietary inclusion level of BSFLM on broiler carcass characteristics

Carcass parameters	Treatment group				p-value	sig.
	(LSmeans)					
	T1	T2	T3	T4		
Dressing (%)	67.58	68.27	69.75	68.29	0.4692	ns
Live weight(g/bird)	1981.3	1800	1978.1	1903.9	0.6394	ns
Weight after bleeding(g/bird)	1843.8	1650.1	1852.5	1781.3	0.5349	ns
Carcass weight(g/bird)	1346.9	1236.3	1382.5	1302.5	0.6888	ns
Breast muscle weight(g/bird)	390.63	383.13	441.88	390	0.6954	ns
Thigh muscle weight	242.5	214.38	248.75	223.75	0.4456	ns
Drumstick weight(g/bird)	195	168.75	188.13	190	0.4349	ns
Wing weight(g/bird)	151.25	148.13	160.63	158.13	0.7509	ns
Gizzard weight(g/bird)	58.75 ^a	44.375 ^b	44.75 ^b	46.25 ^b	0.0106	*
Visceral(liver, intestine, heart)(g/bird)	178.75	173.75	163.13	164.38	0.5712	ns
Neck(g/bird)	51.875	45.625	54.375	49.375	0.3618	ns
Back (espinal cord, ribs)	251.88	218.75	246.88	233.13	0.5184	ns
Shank(g/bird)	83.75	73.13	82.5	81.88	0.4434	ns
Head(g/bird)	54.375	51.25	54.38	53.12	0.8583	ns
Abdominal fat(g/bird)	51.875	38.75	40.63	41.88	0.4522	ns

P<0.05; * = significant; ^{ab} Means within a row with different superscripts differ significantly (*P*<0.05); T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion; g=gram.

4.4. Hematological Parameters and Cecal Bacterial Count

The effect of Black Soldier Fly Larvae Meal (BSFLM) on selected hematological parameters of broiler chickens revealed that the dietary treatments significantly impact ($p < 0.05$) blood parameters, particularly packed cell volume and hemoglobin concentration (table 6). Treatment T3 induced a notable increase in both packed cell volume and hemoglobin levels, outperforming treatments T1, T2, and T4 which did not show similar improvements. Specifically, the values for packed cell volume and hemoglobin concentration were lower in those treatments. Regarding leukocyte cells, basophils were significantly more prevalent in T2 compared to the other treatments, but no significant differences were observed in the counts of heterophils, lymphocytes, eosinophils, or monocytes across the treatments ($p > 0.05$).

The effect of black soldier fly larvae Meal (BSFLM) on microbial load in the ceecal is presented in table 6. Cecal microbial population analysis indicated that no significant differences ($p > 0.05$) existed between the various dietary treatments for *E. coli* or coliform levels. The absence of statistical significance indicates that the dietary alterations made in the study did not result in a measurable effect on the microbial composition in the ceecal of the broiler chickens. Although minor numerical differences in the levels of microbes were noted in the various treatments, the differences were not sufficient to attain statistical significance.

Table 6: Effect of dietary inclusion level on broiler blood parameters and ceecal microbial population

Variables	Treatment (LSmean)				p-value	sig.
	T1	T2	T3	T4		
Blood parameters(%)						
PCV	29.25 ^b	29.63 ^b	31.38 ^a	29.25 ^b	0.0102	*
Hb	9.5 ^b	9.313 ^b	10.28 ^a	9.44 ^b	0.0184	*
Heterophil	58.25	45.63	50.63	43.75	0.2482	ns
Basophil	0 ^b	0.5 ^a	0 ^b	0 ^b	0.0012	*
Eosinophil	6.25	3.625	4.25	3.38	0.5378	ns
Lymphocyte	32.875	47.375	42.375	50	0.1955	ns
Mono	2.63	2.88	2.88	2.88	0.9771	ns
Cecal Microbial population(log CFU/g)						
E.coli	6.03	6.11	6.24	6.04	0.9816	ns
Coliform	5.19	5.93	5.21	5.66	0.4308	ns

*ns = non-significant, * = significant at $P < 0.05$; ^{ab} Means within a row with different superscripts differ significantly ($P < 0.05$); T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion; PCV= Packed cell volume; HB%= hemoglobin, TBC = Total bacterial count*

4.5. Sensory Quality and pH of Meat

The pH analysis of the breast, drumstick, and thigh muscles, both in their fresh state and after 24 hours, revealed slight differences between the four treatments (T1, T2, T3, and T4); however, none of these differences were statistically significant ($p > 0.05$), indicated on table 7. In the case of the breast muscle, T1 recorded the highest pH in its fresh state, while T2 had slightly lower values; however, these differences did not reach statistical significance. During drumstick analysis, T3 exhibited the highest pH fresh, while T4 had a slightly higher pH after 24 hours; no differences were, however, observed ($P > 0.05$). In contrast, the thigh muscle experienced little pH changes, with T3

exhibiting the highest fresh pH and T2 exhibiting relatively lower values. The pH readings for all the treatments were, however, almost undistinguishable after 24 hours.

The inclusion of BSFLM in broiler diets does not affect ($p > 0.05$) the meat sensory quality (Table 7). Sensory evaluation results showed that no significant differences among the four treatments (T1, T2, T3, and T4) were observed for all the sensory attributes that had been measured, such as appearance, color, aroma, taste, juiciness, tenderness, and overall acceptance since all p-values were above 0.05. While T1 gained slightly higher mean scores in some of the attributes like appearance, color, aroma, and tenderness, there were no statistical differences ($p > 0.05$). T2, nonetheless, had a slight better in taste and juiciness while T1 scored the best result in overall acceptance. This implied that the inclusion of BSFLM up to 7.5% in broiler diets has no negative effect on the sensory quality of the meat.

Table 7: Effect of dietary inclusion level of BSFLM on broiler sensory quality and pH

Variables	Treatment (LSmean)				P-Value	sig.
	T1	T2	T3	T4		
Sensory Quality						
Appearance	5.17	4.88	4.58	4.83	0.773	ns
Color	5.54	5.42	5.25	5.13	0.508	ns
Aroma	5.42	5.33	5	5.21	0.697	ns
Taste	5.42	5.46	5.25	5.17	0.832	ns
Juiciness	5.33	5.42	5.13	5.17	0.784	ns
Tenderness	5.58	5.46	5.21	5.5	0.784	ns
Overall acceptance	4.88	4.79	4.42	4.54	0.745	ns
pH Measurements						
Breast muscle fresh	6.18	6.18	6.06	6.16	0.713	ns
Breast muscle after 24 hrs	5.85	5.80	5.74	5.78	0.642	ns
Drumstick fresh	6.33	6.26	6.4	6.39	0.606	ns
Drumstick after 24 hrs	5.87	5.99	6.06	6.07	0.189	ns
Thigh fresh	6.25	6.17	6.30	6.29	0.411	ns
Thigh after 24 hrs	5.97	5.99	6.03	6.05	0.723	ns

T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion; ns = non-significant; hrs= hours.

4.6. Partial Budget

The economic effects of including different amounts of BSFLM in broiler diets are presented in the table 8. Although fixed costs such as chick purchase, labor, and vaccines were the same for all of the groups, apparent differences were observed in feed cost, average growth performance although not significantly different ($p > 0.05$), and total profitability shows significant difference ($p < 0.05$). The highest total feed cost (TFC) and total variable cost (TVC) was recorded by the T1 control group, while feed cost was lower in BSFLM fed groups, especially in T4, suggesting better cost-effectiveness. Total return (TR) was maximized at T3, which was also at its highest growth rate. This led to substantially greater net income (NI) for both T3 and T4 than

the control. The resulting alterations in net income (cNI) and total variable cost (cTVC) further complement the economic advantage of BSFLM application, with the maximum marginal rate of return (MRR) being achieved by T3 followed by T4 and T2.

Table 8: Economic efficiency of dietary inclusion level BSFLM on broiler

Description	Treatment mean			
	T1	T2	T3	T4
Chick purchase(birr/bird)	125.00	125.00	125.00	125.00
Av. FBW (kg/bird)	1.71	1.73	1.84	1.81
Broiler sale (birr/kg)	350.00	350.00	350.00	350.00
Labor cost (Birr/bird)	26.04	26.04	26.04	26.04
Vaccine and vitamin cost (birr/bird?)	7.00	7.00	7.00	7.00
TFC (birr/bird)	196.75 ^a	173.50 ^b	170.39 ^b	164.63 ^b
TVC (birr/bird)	354.79 ^a	331.54 ^b	328.43 ^b	322.67 ^b
Total return(TR)	598.12	604.69	645.54	634.78
Net income	243.34 ^b	273.15 ^{ab}	317.12 ^a	312.11 ^a
Cni	0.00	29.81	73.78	68.77
Ctvc	0.00	-23.24	-26.36	-32.12
MRR(cNI/cTVC)	-	1.28	2.80	2.14

P<0.05; * = significant; ^{ab} Means within a row with different superscripts differ significantly (*P*<0.05); T1=0%BSFL inclusion; T2= 2.5% BSFL inclusion; T3=5% BSFL inclusion; T4=7.5% BSFL inclusion; NI= net income; TFC =total feed cost; cTVC=total variable cost; cNI = change in net income; ΔTVC = change variable cost; Av. FBW = average final body weight; MRR=Marginal rate of return

5. DISCUSSION

The BSFL proximate chemical analysis result obtained was 95.3% DM%, 36.3% CP, 42.8% EE, 4000 Kcal/kg ME, 12.8% CF and 5.246% Ca. BSFL's nutrient-dense profile in this study's proximate composition indicates that it has the potential to be used as an alternative feed ingredient. The 95.3% dry matter content indicates a very concentrated nutrition source with low moisture content, which is compatible with the works of Barragán-Fonseca *et al.* (2017), who determined that BSFL dry matter content varied from 90 to 95% in general. Rachmawati *et al.* (2010) report that dry matter content in BLSFM varies with the developmental stage of larvae and nutritional feed because it contains more DM in instars. The crude protein content measured falls within the range of 30-46%, as indicated in previous studies (Liland *et al.*, 2017; Spranghers *et al.*, 2017; Meneguz *et al.*, 2018). The content of crude protein in the BSF larvae meal that was analyzed was lower than the following reported content: 48.5% (Njoki *et al.*, 2025), 39.4% (Kirimi *et al.*, 2023), 43.9% (Onsongo *et al.*, 2018), 52.3% (Tschirner and Simon, 2015), and 51.17% (Palma *et al.*, 2020). Yet, De Marco *et al.* (2015) also recorded a close value of 36.9%. For example, Schiavone *et al.* (2017) protein content ranged from 37% to 43%, depending on age in the larvae, as well as on the rearing substrate.

This study demonstrated a higher ether extract (EE) value, which is in line with the findings of Spranghers *et al.* (2017), who reported that depending on rearing conditions, BSFL's EE values ranged from 34% to over 40%. The percentage of ether extract in BSFL meal was lower (23.5%) according to Njoki *et al.* (2025) while it was higher (44.84%) according to Kirimi *et al.* (2023). According to Koly *et al.* (2023), the black soldier fly had similar fat levels (40%) as well. Because of its notable protein and fat content, BSLFM can be used as a component of animal feed. Oonincx *et al.* (2015) observed that BSFL has a greater metabolisable energy (ME) in the range of 3500–4500 kcal/kg. The proportion of crude fiber in this study falls between 10 to 15 percent, as stated by De Marco *et al.* (2015). Njoki *et al.* (2025) found a higher content (16.2% CF). This BSFL meal's high calcium content (5.246%) is particularly noteworthy because it exceeds the typical range of 1.2–3.5% found in previous studies (Finke, 2013; Diener *et al.*, 2011). Because of its high calcium content, BSFL is a beneficial

mineral supplement that helps the broiler's skeleton develops. High calcium content like this may reduce the need for further mineral supplements, making BSFL a beneficial complement to mineral nutrition in addition to being a source of protein and fat. Variations in the raising substrate and harvesting stage of larvae may be the cause of the variance in the nutritional content of the BSF larvae meal.

The study evaluated the effect of BSFLM inclusion on broiler growth performance at different production phases did not influence most parameters except feed conversion ratio. The non-significant effect of BSFLM on body weight gain is in line with a previous study report (de Souza *et al.*, 2021) that showed no significant impact on BWG when 5% BSFLM inclusion was used. Similarly, in a large meta-analysis, Martínez Marín *et al.* (2023) found that BSFLM may be added to broiler diets at up to 15% without affecting growth performance, indicating that BSFLM can be used as a viable alternative for conventional protein sources. However, Mat *et al.* (2022) found that 4% BSFLM significantly improved growth, suggesting that lower inclusion levels may have more significant impacts. Higher amounts of BSFLM (such as 5% and 7.5%) could provide cumulative benefits, particularly in the later production phases, even if this study did not find any significant difference. This suggests that the performance of chickens was enhanced when BSFLM was included in higher levels. This can be attributed to the high nutrient density of BSFLM, which is rich in vital proteins, lipids, and amino acids all of which are crucial during the grower phase, when broilers undergo fast muscle development and growth. The idea was strengthened by Koly *et al.* (2023), who noted that animals' growth can be supported by the high protein and fat content of black soldier fly larvae.

Furthermore, BSFLM's healthy fats, like omega-3 and omega-6 fatty acids, could improve feed conversion efficiency and energy utilisation, which would boost growth further (Lu *et al.*, 2022). According to Njoki *et al.* (2025), the industry recommendation of 50g for the economic feasibility of broiler production was achieved by the ADBG value in this investigation at the finisher stage (50.53-54.74 g). The current study's ADBG was lower than 79.60g at a 15% inclusion level at 49 days of slaughter (Onsongo *et al.*, 2018) and 65.4 g at a 25% inclusion level at 42 days of slaughter (Kirimi *et al.*, 2023). The age at which the trial ended (49 days) and the level of BSFL meal inclusion could be the cause of the discrepancy in the ADG in this particular investigation. In

contrast, the findings by Onsongo (2017) who observed that BSFLM inclusion did not affect body weight. However, due to the high chitin content a particular kind of fiber present on the BSFL's exoskeleton a higher inclusion level could decrease the diet's efficacy. When chitin concentration is high, it may affect digestibility and nutrient utilization (Hossain and Blair, 2007; Schiavone *et al.*, 2018; El-Hack *et al.*, 2020), which is wherefore a drop in final live weight was noted.

In the present study, the inclusion of BSFLM did not significantly influence feed intake during the starter, grower, finisher, or overall production periods ($p > 0.05$). These findings suggest that BSFLM, within the tested inclusion levels, maintains good palatability and does not negatively affect voluntary feed consumption in broilers. This observation aligns with the findings of Cullere *et al.* (2016), who discovered that feed intake was consistent across broiler groups fed diets that contained up to 10% BSFLM. Likewise, De Marco *et al.* (2015) found no noticeable differences in feed intake when BSFLM was added at different levels, suggesting that even at modest inclusion rates, the larvae meal does not discourage feed consumption. Further confirming the consistency of this conclusion, Martínez Marín *et al.* (2023) found in their meta-analysis that BSFLM inclusion, on average, at 5.7%, had no negative impacts on broiler feed consumption across multiple studies. When Njoki *et al.* (2025) compared chickens that were fed 15% and 22.5% inclusion levels of BSF larvae meal to the control and 30% BSF larvae meal-based diet, they found a slight numerical change in the average daily feed intake and total feed, but no statistical difference.

In contrast, Hayat *et al.* (2024) observed a decrease in feed consumption at higher inclusion levels ($>10\%$), which they attributed to either the meal's altered texture or its higher chitin content. The current trial, however, limited BSFLM inclusion to 7.5%, therefore these negative effects were probably prevented. The control group consumed a bit more feed overall at the finisher stage than those who were fed the diet containing BSFL meal. This is in line with the findings of Kirimi *et al.* (2023), who found that the average daily feed consumption of the birds significantly dropped as the amount of larvae meal in the diets increased over time. This might be because there was more fat, which was caused by more BSF larvae diet. Feed intake was decreased as a result of the study's meal's high fat content, which increased dietary energy density.

The inclusion of BSFLM in broiler diets demonstrated varying effects on feed conversion ratio (FCR) across different growth phases, suggesting a stage-specific response to BSFLM included feed. In the starter phase, the absence of significant differences in FCR ($p > 0.05$) among treatments indicates that younger broilers may have limited ability to utilize BSFLM efficiently. This observation aligns with findings by Dabbou *et al.* (2018), who reported limited improvements in early-stage broilers, likely due to the chitin content and immature digestive systems. A notable improvement in FCR was observed in the grower phase ($p < 0.05$), particularly in birds receiving 5% (T3) and 7.5% (T4) BSFLM. This suggests enhanced nutrient utilization during this phase of rapid growth. Kim *et al.* (2020), in their study also demonstrated better feed efficiency and weight gain in broilers fed diets with insect-based proteins. Furthermore, Kierończyk *et al.*, (2020) found that BSFLM inclusion improved feed conversion due to better nutrient digestibility and enhanced gut morphology. In the finisher phase, although numerical differences were observed with T3 having the most efficient FCR these differences were statistically non-significant ($p > 0.05$).

Similar results were found in the study by Cullere *et al.* (2016) which indicated performance trends favoring BSFLM but not always reaching statistical significance, possibly due to individual variability in the final growth phase. For the effective production of broiler chickens, the industry recommended for FCR value is ≤ 1.85 (Butcher and Nilipour, 2009). However, in this study the FCR at all growth stage somewhat exceeded this expectation. The FCR in this study is greater than the values reported by Choct (2015; 1.41) and Uushona (2015; 1.5), but at starter stage comparable value (1.9-2.0) is reported by Onsongo (2018). However, at 15% BSF larvae meal inclusion level the scored FCR was 1.88 over the total trial period in the study reported by Njoki *et al.* (2025). The chickens fed the control diet not well performed, scoring 2.33 FCR, whereas the chicks fed the diet containing BSF larvae meal scored 2.03 to 2.18. When compared to the control (T1) during the finisher stage, the BSF larvae meal diets had higher FCR score because they were nearly at the industrial requirement, indicating that their digestibility was good and could sustain broiler chickens' good growth performance. The chitin found in BSF larvae meal, which is indigestible by monogastric animals (Sánchez-Muros *et al.*, 2014) and can adversely affect protein digestibility, may be the cause of the reduced feed efficiency seen in the broiler chickens for the entire period in the current research.

The study indicated that the inclusion of BSFLM up to 7.5% in broiler diets did not significantly affect most carcass traits ($p > 0.05$) except gizzard weight, which was significantly lower in BSFLM treatments compared to the control (T1). This finding is consistent with research by Brah *et al.* (2024) and de Souza *et al.* (2021), which found no significant improvements in carcass yield when BSFLM was added to broiler diets. Furthermore, Njoki *et al.* (2025) found that the inclusion amounts of BSF larvae meal had no noticeable impact on dressing percent carcass yield ($P > 0.05$). Uushona (2015) found no significant variations in the dressing percentage and live weight, parameters at the inclusion levels of 5, 10, and 15%, which is consistent with the current investigations. Broiler chicken fed BSF larvae food during the growth and finishing stages showed no change in carcass weight or carcass % in response to the inclusion of BSF meal (Schivavone *et al.*, 2018). Both the dressed weight of carcasses and the relative weight of internal organs (liver, heart, spleen, gizzard, and intestines) were not impacted significantly by the addition of BSF meals to the broiler diet for all treatments (Mutisya *et al.*, 2021). The present study found that the broiler chicken in the BSF meal inclusion group did not impact most internal organs, with the exception of the gizzard, which is consistent with their findings. As proposed by Brah *et al.* (2024), the fiber content of BSFLM may be responsible for the observed decrease in gizzard weight. Because of the chitin in the BSFL exoskeleton, the carcass weight of broilers by BSF feed did not differ considerably. It was demonstrated that chitin negatively affects the digestion of proteins the high chitin content of BSF meal may prevent the digestion of proteins since it is indigestible. The indigestible chitin may be the cause of these reduced carcass characteristics. With BSF meal inclusion levels of 5, 10, and 15%, the carcass weight decreased linearly; at a high inclusion level of 15%, the weight decreased considerably (Nayohan *et al.*, 2022).

The study's overall mortality rate was 4.68%, with the starter phase accounting for the largest losses (2.08%), and followed by the grower phase (1.56%) and finisher phase (1.04%). Eleven chicks were culled early because of severe leg weakness and leg deformity. Further than the birds that were culled, no further health issues were noted as a result of the test diet effect or poor management techniques, such as biosecurity precautions. The current result was medium when we saw to Abera *et al.*, (2017) found that a 7.8% mortality rate in Cobb 500 broilers. The on-station study's reported mortality rate of 1% (Arega *et al.*, 2020).

The inclusion of BSFLM in broiler diets was found to positively influence specific hematological traits (Table 6). Packed Cell Volume (PCV) levels ranged from 29.25% in the control group (T1) to a peak of 31.375% in the group fed 5% BSFLM (T3), indicating improved red blood cell efficiency and oxygen transport capacity. The red blood cell transport oxygen from the lungs to the tissue and help in the removal of CO₂ from the tissue to the lungs through the hemoglobin (Anthony *et al.*, 2024). These values suggest that higher BSFLM levels may promote enhanced erythropoiesis and overall blood health. Importantly, all measured blood parameters remained within the normal reference ranges for chickens which have been stated as PCV (25-45%), Hemoglobin (7- 13g/dl) (Edeh *et al.*, 2023), heterophils %(15-50), eosinophils %(1.5-6), basophil %(0-1 rare) (Baudouin *et al.*, 2021), lymphocyte %(48.33 and 61.33), and monocyte %(2.00-3.67) (Asaniyan and Akinduro, 2020). This aligns with findings from Mat *et al.* (2021) and Dabbou *et al.* (2018), who also reported improvements in hematological parameters with the dietary inclusion of insect meals. Likewise, hemoglobin (Hb) concentrations were significantly elevated in T3 (10.275 g/dL), mirroring the PCV results and indicating enhanced red blood cell function. These levels also fall within the standard range for healthy broilers (7–13 g/dL), suggesting improved oxygen delivery and metabolic performance. This finding is supported by Mat *et al.* (2021), who noted that insect-based protein sources contribute positively to blood health and overall physiological function. Meanwhile, white blood cell (WBC) counts, including heterophils, eosinophils, lymphocytes, and monocytes, showed no significant differences among treatments. This stability suggests that BSFLM inclusion did not impair immune function. These results correspond with those of Wallace *et al.* (2017), who also found no negative impacts on immune parameters when broilers were fed insect-based diets.

The study revealed non-significant impact ($p > 0.05$) of varying inclusion levels of BSFLM on the cecal microbial population of broilers (Table 6). This finding suggests that BSFLM does not disrupt the overall microbial ecosystem of the gut. Similarly, *E. coli* counts remained unaffected by BSFLM inclusion. Benzertiha *et al.* (2019) and Józefiak *et al.* (2020) reported that feeding insect-based diets to 35-day-old female Ross 308 broilers raised in floor pens did not result in any significant changes in the alpha diversity of cecal microbiota compared to those fed a standard control diet. In contrast, Saidani *et al.* (2025) noticed that BSFLM inclusion had positive effect and reduced

E.coli in ceecal. In addition, coliform counts across treatments did not significantly differ, suggesting that BSFLM does not create an intestinal environment conducive to coliform overgrowth. Overall, the findings suggest that dietary BSFLM up to a 7.5% inclusion level does not disrupt the balance of the ceecal microbiota, confirming its microbiological safety in broiler nutrition similar to the study (de Souza *et al.*, 2013). Chinivasagam *et al.* (2016) reported that coliforms including *E. coli* in broiler ceca typically range from 10^6 to 10^8 CFU/g (6 logCFU/g and 8 log CFU/g) as a stable level throughout their lifespan. This stability is crucial for maintaining gut health, which is closely linked to overall performance, immunity, and nutrient utilization in poultry. Recently, BSFL becomes feasible to apply energy and nutrient matrices to formulate more precise diet formulations. Consequently, the benefits of BSF larvae meal may extend beyond its protein contribution to include functional effects attributed to its content of antimicrobial peptides, fatty acids, and chitin (Dong *et al.*, 2019).

The pH values after slaughter and safter 24 hrs for broiler breast muscle, drumstick, and thigh were not affected by the inclusion of BSF meal in the broiler diet, indicating a positive effect in the study (Table 7). In the same way, de Souza Vilela *et al.* (2021) observed that there were no significant differences in the pH values of broiler meat at any level of Black Soldier Fly Larvae Meal inclusion, either 24 hours after slaughter samples or not. The pH levels were kept within the typical range for high-quality broiler meat. According to Murawska *et al.* (2021), the most typical range is between 5.7 and 6.2. This implies that BSFL can be included to broiler diets even at higher levels without affecting the overall quality or freshness of the meat. The study found that the 24-hour pH values of the breast meat were within the usual range of 5.7 to 5.8 for all treatment groups. However, the group that consumed full-fat BSF meal had considerably lower pH values, indicating that the meat from BSFL included groups was more acidic. Anything made from BSF larvae would have significant amounts of lauric acid because larvae are known to be rich in this fatty acid. The lower pH levels seen in the broiler meat may be due to the higher lauric acid concentration in the full-fat BSF meal, however the findings have been inconsistent. Although this impact seems to vary depending on the broiler strain, specific studies have shown that the breast muscles of broilers fed lauric acid had noticeably lower pH levels (Popova *et al.*, 2020).

The sensory evaluation of broiler meat in this study showed that dietary inclusion of Black Soldier Fly Larvae Meal (BSFLM) up to 7.5% had no significant effect ($p > 0.05$) on key sensory attributes including appearance, color, aroma, taste, juiciness, tenderness, and overall acceptance. These findings suggest that the incorporation of BSFLM up to 7.5% does not negatively affect the organoleptic qualities of broiler meat as perceived by consumers. This result is consistent with research by Cullere *et al.* (2019), who looked into the effects of using fat from *Hermetia illucens* larvae (instead of meal) as a partial substitute for conventional fat sources in broiler diets. Additionally, the study found that the cooked meat's flavour, juiciness, and tenderness did not significantly change. Despite this, the paper focused on larval fat instead of protein diet. In contrast According to Ellawidana *et al.* (2022), the birds fed diets with 7.5% BSFL full fat meal and 10% BSFL defatted meal had the greatest mean score for meat softness among sensory criteria. Maximum juiciness was achieved in the meat of the birds given 10% BSFL on a full fat basis. Interestingly, for every other sensory property, the birds who were fed a diet that included 5% BSFL full fat meal scored the highest. Additionally, the BSFL meal, which has a particular flavour, might be utilised as an alternative for current understanding of fat sources. Overall, the results showed that including BSFL meal to the diet up to a 5% inclusion level did not impact the meat's sensory profile, which is technically feasible, and that the product is acceptable when compared to a conventional diet (Ellawidana *et al.*, 2022).

The findings of the partial budget analysis clearly indicate the cost benefits of including Black Soldier Fly Larvae Meal (BSFLM) in broiler feeding programs (Table 8). Although fixed costs, including chick procurement, labor, and veterinary costs, were equal in all treatments, there were significant differences in feed costs. The control treatment (T1), where BSFLM was not included, had the most expensive total feed cost (TFC) and the highest total variable cost (TVC). Conversely, T3 (5% BSFLM) and T4 (7.5% BSFLM) groups treated with BSFLM indicated decreased feed expenses, indicating better cost-effectiveness. While treatment differences in final body weight (FBW) were not significant ($p > 0.05$), T3 recorded the highest mean weight and therefore the highest total return (TR) and net income (NI). Treatment groups T3 and T4 earned significantly more net incomes than the control group ($p < 0.05$), reflecting economic advantages of integrating BSFLM. Marginal rate of return (MRR) also confirms the result, in which T3 was the most excellent MRR followed by T4 and T2.

These results align with previous studies, such as those by Abro *et al.*, (2022) and Makkar *et al.* (2014), which reported reduced feed costs and maintained performance with moderate BSFLM inclusion. Overall, the economic analysis suggests that an inclusion level offers the most optimal balance between cost reduction and profitability, making it a viable strategy for improving the economic sustainability.

6. CONCLUSION AND RECOMMENDATIONS

The results of this study showed that Black Soldier Fly Larvae Meal (BSFLM) can be included in broiler diets without any adverse effects on growth performance, feed consumption, carcass traits, meat pH, or sensory attributes. Even though the majority of the performance characteristics were not significant statistically, trends showed enhanced feed efficiency and economic returns. Hematological analysis also revealed heightened physiological status, better packed cell volume and hemoglobin content, towards the health-promoting value of BSFLM. Microbial analysis demonstrated no significant difference in ceecal bacterial counts, affirming its safety from a gut health standpoint. Based on this study, Black Soldier Fly Larvae Meal (BSFLM) is a cost-effective alternative protein source for broiler diets. The inclusion of BSFLM, particularly at 5% and 7.5%, had comparable growth performance to that of commercial feed ingredients but at reduced feed cost. Because feed constitutes a large proportion of broiler production cost, the inclusion of BSFLM could help poultry farmers reduce their overall feed cost without compromising bird performance and health. Furthermore, the trial noted beneficial impacts on hematological parameters, characterized by elevated packed cell volume (PCV) and hemoglobin (Hb) concentration, which indicates that BSFLM can have a potential in improving health and immune status. Such health-promoting advantages and enhanced feed conversion efficiency justify the consideration of BSFLM as an economical and ecologically friendly feed ingredient.

Based on the above conclusions the following recommendations are forwarded:

- ✓ Poultry producers adopt BSFLM to improve both production efficiency and bird health, especially in light of rising soybean meal prices.
- ✓ Further research into optimal inclusion levels and long-term health impacts will be beneficial, but the current results indicate that BSFLM is a viable option for improving broiler performance and health, while enhancing cost efficiency.

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8. APPENDICES

Appendix 1: Analysis of variance of growth performance

1. Analysis of Variance of initial body weight (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	0.91	0.30	0.32	0.81
error	12	11.41	0.95		
corrected total	15	12.32			

2. Analysis of Variance of body final body weight (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	51643.42	17214.47	1.4	0.2903
error	12	147407.9	12283.99		
corrected total	15	199051.3			

3. Analysis of Variance of dressing percentage (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	19.88459	6.628197	0.87	0.4692
error	28	213.7513	7.633974		
corrected total	31	233.6359			

4. Analysis of Variance of hot carcass weight(g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	95308.59	31769.53	0.49	0.6888
error	28	1797884	64210.16		
corrected total	31	1893193			

5. Analysis of Variance of hemoglobin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	4.56625	1.522083	3.94	0.0184
error	28	10.8225	0.386518		
corrected total	31	15.38875			

6. Analysis of Variance of E.coli count

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
model	3	0.109723	0.036574	0.06	0.9816
error	12	7.809867	0.650822		
corrected total	15	7.91959			

Appendix 2: Materials and procedure to conduct Differential white blood cell count

Materials and reagents:

- ✓ Un-coagulated whole blood
- ✓ Microscope
- ✓ Glass slides
- ✓ Immersion oil,
- ✓ Giemsa stain
- ✓ Xylene
- ✓ Methanol alcohol,
- ✓ a piece of gauze,
- ✓ Distilled water and Staining rack

Procedure:

- A drop of blood is placed near to the one end of a clean glass slide.
- A second slide is held at 45°, attached to the drop of blood, then pushed along the surface of the first in forward direction to form a thin smear. The smear is dried in the air.
- The smear is fixed in methanol for 3-5 minutes.
- The smear is stained with giemsa staining dye and allowed for 30 minutes.
- The slide is rinsed with water and dried in air.
- The stained smear is put on the microscope and one drop of oil is added on the smear. Using oil-immersion objective (100x), 100 WBCs are counted by Battleship method.
- Each type of leukocyte is identified, and results are recorded as % of the total leukocyte count then changed to absolute for more accuracy.

Appendix 3: Pictures that shows Black soldier fly larvae meal preparation and rearing period of chickens



mixing feed ingredients by mixer machine

live black soldier fly larva



chickens during arrival and during the rearing period



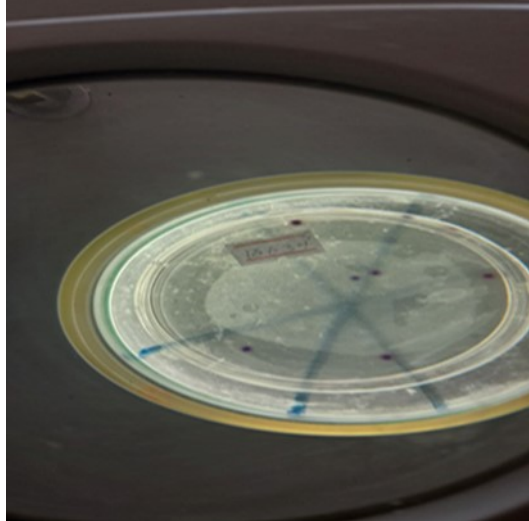
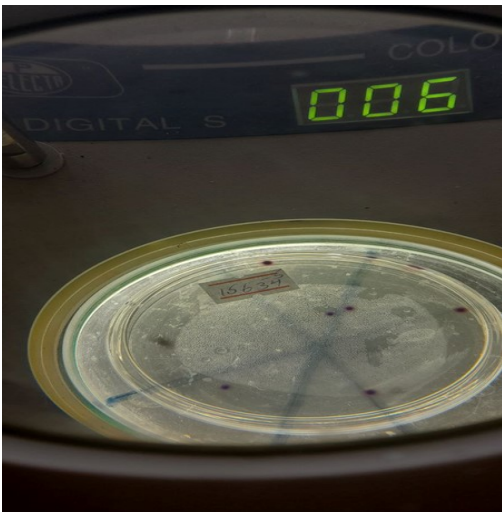
Appendix 4: pictures that shows slaughtering process and measuring carcass parts



measuring pH main carcass cut parts Sensory panelists and sensory evaluation

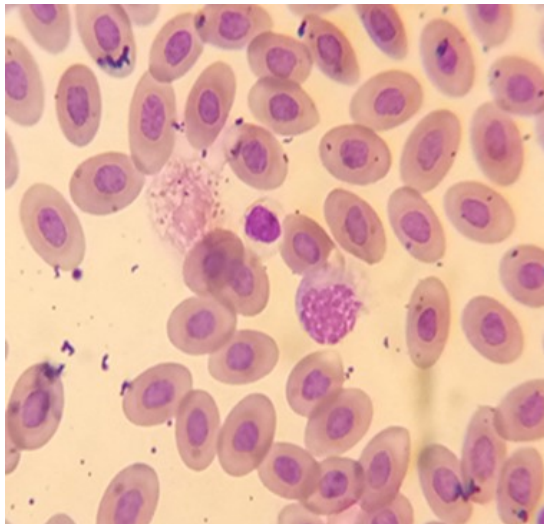
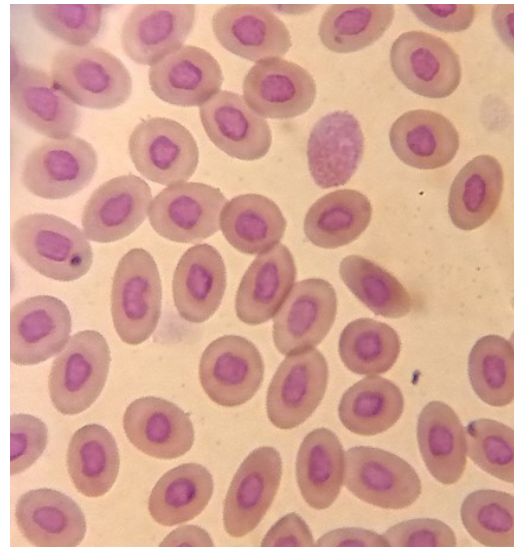


Appendix 5 picture that shows ceecal bacterial count, hematology Medias and equipment.

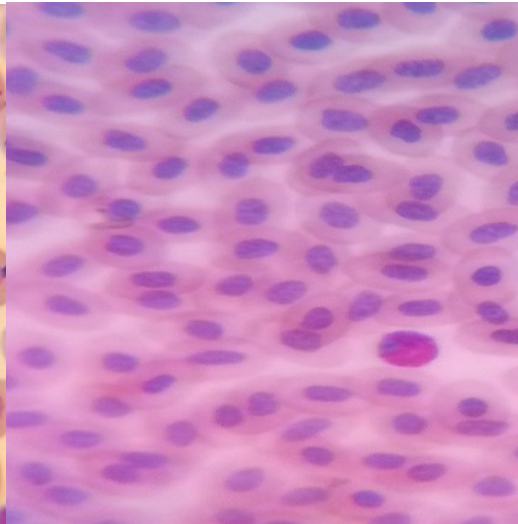


Differential leucocytes

A. Lymphocyte



b. eosinophil and Heterophil



c. monocyte

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
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Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/52/16/2024

Name of Applicant: **Ebenzer Mohammed (BSc, MSc student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Effect of the meal dietary inclusion level of black-soldier fly larvae on broiler growth performance, carcass characteristics, hematological parameters, ceecal bacterial load and meat quality*

Date of application: **April, 2024**
Nature of the project: **Experimental study**
Target animal species: **Broiler chicken**
Number of animals involved: **192**
Study area: **ILRI-Addis Ababa, Ethiopia**

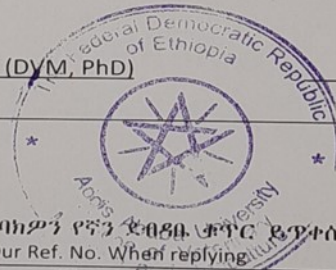
Minutes No. and date of review: **VM/ERC/04/16/024, 26/03/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Ebenzer Mohammed.

Professor Getachew Terefe (DVM, PhD)

Chairman

Signature



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EFFECT OF THE MEAL DIETARY INCLUSION LEVEL OF BLACK SOLDIER FLY LARVAE ON BROILER GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, HEMATOLOGICAL PARAMETERS, CEACAL BACTERIAL LOAD AND MEAT QUALITY

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