

**BLACK SOLDIER FLY (*Hermetia illucens*) LARVAL
MEAL FOR BROILER CHICKEN FEED: EFFECT ON
GROWTH PERFORMANCE, PROFITABILITY, AND
GENE EXPRESSION**

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**Black Soldier Fly (*Hermetia illucens*) Larval Meal for Broiler
Chicken Feed: Effect on Growth Performance, Profitability, and
Gene Expression**

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the Degree of Master of Science in Animal Nutrition of the Jomo
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2026

DECLARATION

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

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DEDICATION

This work is dedicated to my lovely parents Mrs. Nora Rotich and late Mr. John Rotich for providing me support during my studies.

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ABBREVIATIONS AND ACRONYMS

ADFI	Average daily feed intake
ADG	Average daily gain
ANOVA	Analysis of variance
ARRIVE	Animal Research: Reporting of In Vivo Experiments
BSF	Black soldier fly
BSFL	Black soldier fly larvae
BWG	Body weight gain
cDNA	complementary deoxyribonucleic acid
CF	Crude fat
CP	Crude protein
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
ICIPE	International centre of insect physiology and ecology
IFN-γ	Interferon γ
IGF- I	Insulin growth factor I
IL-6	Interleukin-6
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LW	Live weight
PAMP	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
Q-PCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid

RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SBM	Soya bean meal
SDG	Sustainable development goals
TNF-α	Tumour necrosis factor alpha

ABSTRACT

The African poultry industry is characterized by high feed prices due to the limited availability of sufficient, high-quality protein. This has led to significant shortages, driving up the costs of poultry products and stagnating the sector's growth. Protein is the most expensive component of poultry feed, and its quality and availability are the limiting factors. This study investigated the effects of black soldier fly (*Hermetia illucens* L.) larvae meal (BSFLM) on growth performance, profitability and gene expression in 200 Ross 308 broiler chickens. Soybean meal (SBM) and fishmeal (FM) were substituted with varying levels of the BSFLM, resulting in five dietary treatments: SBM only, FM only, FM + BSFLM (1:1), SBM + BSFLM (1:1), or BSFLM only. Growth rates, organ weights, immune genes; interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrotic factor alpha (TNF- α), interferon-gamma (IFN- γ) and growth-related genes; chicken growth hormone (cGH), insulin growth factor 1 (IGF-1) expression were measured by quantitative Polymerase chain reaction (qPCR). Our results demonstrate that birds fed FM+BSFLM had significantly higher final body weight ($p < 0.001$), average daily weight gain ($p < 0.001$), and a low feed conversion ratio ($p < 0.001$). Birds fed FM+ BSFLM had significantly higher carcass weight ($p = 0.026$), while those fed SBM only yielded the lowest carcass weight. There were no significant differences in all the profitability parameters across the five treatments. The IL-2, IL-6, TNF- α , IFN- γ , IGF-1 and cGH gene expressions were significantly upregulated in the liver and spleen of birds fed diets containing BSFLM. Growth-related genes (cGH) positively correlated with live weight, body weight gain, and ADG. In conclusion, BSFLM at partial inclusion levels enhances broiler growth without significantly affecting profitability. Additionally, the inclusion of BSFLM in broiler diets upregulates the expression of genes associated with growth and immunity, leading to improved productivity in broiler production. It is recommended to use BSFLM as a partial replacement for soybean and fishmeal to improve growth and immune gene expression in broiler chickens.

Keywords: black soldier fly larvae meal, profitability, immune modulation, gene expression, and poultry nutrition.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

According to the United Nations, the global population is projected to reach approximately 9.7 billion persons by 2050, which will directly increase the consumption of animal protein (Attia et al., 2024; Bist et al., 2024). Correspondingly, the consumption of poultry and pork meat is expected to grow significantly between 2010 and 2050 (Erdaw, 2023). Poultry production is an important source of food for middle- and low-income countries (Carron et al., 2017). It is one of the most efficient feed-to-meat conversion industries (1.5-1.8) (Smil, 2002). Indigenous and exotic chicken breeds are reared in varying production systems. Broiler chicken production is one of the most economical, viable, and important sources of meat for human consumption (Govoni et al., 2021). Globally, populations of broiler and indigenous chicken have increased between 2006 and 2020, with the former dominating the sector. Though the indigenous chicken population is higher than the broiler chicken in Kenya (Magothe et al., 2012), there has been a continuous increase in commercial chicken production (broiler) in areas near urban centres. Such areas include Nairobi, Mombasa, Nakuru, Kisumu, and Nyeri, where there is a ready market, compared with rural areas, where indigenous chicken are the dominant species (Kugo, 2014). The prediction for poultry meat consumption in Kenya indicates a rise from 55 thousand metric tonnes in 2000 to 165 thousand metric tonnes in 2030 (Mutisya et al., 2021). The rise is attributed to factors such as urbanization, population growth, economic growth, and the continuing viability of current broiler chicken systems. To meet these expected demands, poultry production is expected to increase (Kleyn et al., 2021).

In Kenya, poultry production accounts for more than 30% of agricultural GDP and 8% of the country's total GDP. Poultry production is a significant source of income for rural populations and a major source of protein. Poultry feed is an important component of production, accounting for 80% of total production costs. This

highlights the important economic role of feed (Shumo et al., 2019). Demand for nutrients has been increasing with the growing human population, which underscores the food-feed competition for constituents that can be consumed by humans, such as soya bean and fishmeal. The competition is the main contributor to the increase in feed costs (Munguti et al., 2014), as well as to production disruptions caused by climate change. Broiler chicken production relies heavily on the availability of nutritious feed. Animals and plant sources are the major contributors to meeting the chicken's dietary needs. The increase in feed cost is attributed to higher protein costs, which are among the most costly ingredients in poultry feed and are conventionally sourced from soya bean and/or fishmeal (Onsongo et al., 2018). In most developing countries, soybeans are the primary protein source in poultry feed (Chianu et al., 2010). In Uganda and Kenya, the silver cyprinid fish (*Rostrineobola argentea*), commonly referred to as 'Omena', is also used as a protein source (Ojwang et al., 2014). Over the years, the marine fish supply has gradually declined due to overexploitation, which has increased demand for fish products and thereby compromised the availability of fishmeal as livestock feed. On the other hand, the intensification of soybean farming has resulted in negative social and environmental impacts, which include deforestation and land grabbing (Sheppard et al., 2013).

In light of rising conventional feed prices and the overexploitation of fish, there is an urgent need to consider non-conventional, bio-innovative, sustainable, beneficial, and economical protein sources. A good example of this is insect protein, which is considered a valuable protein source for monogastric animals, as they have a better feed conversion ratio, feed on a wide variety of materials, including waste, and thus reduce costs and wastage (Khusro et al., 2012). Most insects are cheap and readily available, are high in protein, minerals, and amino acids, and can complement most cereal-based foods consumed (Ifie et al., 2011). Palatability, digestibility, feed conversion rates, animal health issues, and the quality of the resulting products are of great importance in insect feeding, and, in this regard, some countries regulate the use of insect protein in animals intended for food purposes (Belluco et al., 2013). Many insects have the potential to produce biomass, but among the most promising ones include the black soldier fly (BSF) (*Hermetia illucens*), Yellow mealworm

(*Telebrio molitor*), common housefly (*Musca domestica*), Bluebottle (*Calliphora vomitoria*), and blowfly (*Chrysomya* spp) (Thrastardottir, 2021). The black soldier fly naturally occurs in poultry droppings and cow dung, while yellow mealworms can thrive in distillers' dry grain and vegetables. Among these insects, the BSF (*Hermetia illucens*) is particularly promising because it can accumulate tissue from materials not intended for human consumption, such as food-processing waste, industrial by-products, and spoiled feed. It has an amino acid profile that is documented to be slightly higher and more balanced than that of soybean (Veldkamp & Bosch, 2015).

Production of BSF larvae (BSFL) is an innovative and promising alternative protein feed source, as the species can utilize a wide variety of organic waste, thereby enabling sustainable production (Shumo et al., 2019; Van Huis & Tomberlin, 2018). The BSF has six feeding stages and a seventh non-feeding pre-pupal stage that metamorphoses into an adult BSF (Barros-Cordeiro et al., 2014). The dry weight of a BSFL contains approximately 43-50% crude protein, up to 35% lipids, and an amino acid profile similar to that of fishmeal. An adult fly can go for 1-2 weeks without food, depending on body fat and water (Tomberlin & Sheppard et al., 2002). The BSFL does not carry diseases, secretes a repellent infochemical that deters potential insect disease vectors, and helps reduce *E. coli* and *Salmonella enterica* in chicken droppings (Liu et al., 2017). There is variability in amino acid composition among different BSFL due to differences in organic substrate composition (Liland et al., 2017).

There is increasing recognition that certain dietary components affect the expression of specific gene profiles. Nutrigenomics seeks to study the influence of nutrients on the genome, in which nutrients serve as dietary signals detected by cellular sensory mechanisms and influence metabolite production, gene expression, and protein expression (Meiliana & Wijaya, 2020). The patterns of gene expression and metabolite production are considered dietary signatures, and nutrigenomics aims to study these signatures in animal tissues and cells and to determine how nutrition affects homeostasis. Nutrigenomics tools, such as transcriptomics and metabolomics, provide important insights into feed research and livestock nutrition. Reverse

transcription polymerase chain reaction and microarray technology are the main tools of transcriptomics, and they have facilitated research on the physiological effects of dietary protein and polyunsaturated fats, as well as the conditioning of colon cancer (Zduńczyk & Pareek, 2009).

Several studies have been carried out to determine the effects of incorporating BSFL in different livestock feeds; however, very few studies have been carried out to determine its impact on specific gene(s) expression in broiler chickens. This research, therefore, intended to study the effects of BSFL meal inclusion in broiler chicken diets on their growth, profitability, and gene expression. This will contribute to insights into the use of black soldier fly larvae (*Hermetia illucens*) as a potential feed resource to improve nutrient utilization and product quality in poultry production in Kenya and the tropics.

1.2 Problem Statement

In livestock, particularly poultry, protein is a very important nutrient and is essential for the maintenance and repair of body tissues, thereby facilitating the growth and development of the animal (Barszcz et al., 2024). Fish and soybean meals are important sources of locally available protein feed for non-ruminant livestock, in both subsistence and commercial production systems. The rising costs and scarcity of protein sources such as groundnut cake, fishmeal, and soybean meal are the main factors hindering large-scale poultry production (Adeniji et al., 2007). On the other hand, the steady decline in fish stocks due to overexploitation and competition for soybean-based feed has prompted consideration of insects as an alternative protein source for poultry (Melo et al., 2011).

With the constant increase in the human population, urbanization, and improved living standards, native agricultural lands near urban centres are being converted into housing estates and industrial parks, thereby making cropland for crop production inadequate. This has led to the feeding of poultry with rice and sorghum residues that are low in digestibility and intake, due to low levels of essential nutrients such as proteins, vitamins, and minerals, and to high lignification of structural carbohydrates.

Furthermore, challenges such as climate change are expected to worsen the vulnerable poultry production sector by impairing the supply of conventional poultry feed resources (Hidoso & Guyo, 2017). Moreover, the increasing livestock population due to the commercialization of livestock production has been a significant cause of inadequate feed resources, especially in areas with extensive poultry production. The lack of a proper, efficient solution to the depletion of conventional feed sources will lead to food insecurity, since poultry is one of the main sources of food. To alleviate the limited availability of high-value feed resources, gene expression patterns and improved production should be studied (Sawal et al., 2004).

The utilization of insects in poultry nutrition has gained interest due to their availability, potential nutritional value, and sustainability (Lu et al., 2022). There is a vast amount of information on the dietary components of BSFL and its promotional use as a feed ingredient. However, the physiological effects of their dietary components on gene expression in poultry remain poorly understood. The effects of BSFL on gene expression remain limited and are speculated to be influenced by the chitin content of larval exoskeletons (Komi et al., 2018). Understanding the effects of BSFL on immunological parameters and gene expression is crucial for evaluating their potential genetic and health benefits when fed to animals. Therefore, there is a need to investigate the impact of BSFL dietary components on the expression of immune- and growth-related genes in broiler chickens, and to elucidate the molecular mechanisms underlying the observed effects. Such knowledge will contribute to the development of evidence-based recommendations for incorporating BSFL in animal diets and provide insights into the potential implications for animal health and well-being. To address these knowledge gaps, this study investigated the effects of replacing soybean meal and fish meal with BSFLM in broiler chicken diets.

1.3 Objectives of the Study

1.3.1 General Objective

To determine the effect of Black Soldier Fly (*Hermetia illucens*) Larval Meal on Growth Performance, Profitability, and Gene Expression of broiler chicken.

1.3.2 Specific Objectives

- i. To determine the growth performance of broiler chicken fed on diets with varied levels of black soldier fly (*Hermetia illucens*) larvae meal.
- ii. To determine the effects of black soldier fly (*Hermetia illucens*) larvae meal inclusion in diets on the profitability of broiler chicken.
- iii. To determine the effect of dietary inclusion of black soldier fly (*Hermetia illucens*) larvae meal on immune and growth-related gene expression in broiler chicken.

1.4 Research Questions

- i. What are the growth performance changes of broiler chicken fed on diets with varied levels of black soldier fly (*Hermetia illucens*) larvae meal?
- ii. What are the effects of black soldier fly (*Hermetia illucens*) larvae meal inclusions in diets on the profitability of broiler chicken?
- iii. What are the effects of dietary inclusion of black soldier fly (*Hermetia illucens*) larvae meal on immune and growth-related gene expression in broiler chicken?

1.5 Research Hypotheses

- i. There is a significant effect on the growth performance of broiler chicken fed on diets with varied levels of black soldier fly (*Hermetia illucens*) larvae meal.
- ii. There are significant effects of black soldier fly (*Hermetia illucens*) larvae meal inclusions in diets on the profitability of broiler chicken.

- iii. There are significant effects of dietary inclusion of black soldier fly (*Hermetia illucens*) larvae meal on gene expression in broiler chicken.

1.6 Significance of the Study

The study is of scientific and economic importance, as the findings contribute to the development of sustainable and cost-effective feeding strategies for poultry in Kenya and in regions where BSF can thrive. Determination of the modification of immune genes by BSFL meal is of potential significance for poultry health and, hence, its direct impact on good health and well-being (Sustainable Development Goal; SDG 3). The findings reinforce the goals of the Government of Kenya under the agricultural transformation agenda, which seeks to increase livestock productivity, improve food and nutritional security, and promote climate-smart agricultural practices. Assessing the impact of BSFL meal on gene expression provides insight into the molecular mechanisms underlying any physiological effects. The findings lay the foundation for understanding the therapeutic, physiological, and nutritional properties of BSFL and provide leads for further research into gene-nutrient interactions. The outcomes, therefore, affect sustainability and nutraceutical development and provide a lead for more knowledge.

Conducting this study was worthwhile, as the knowledge gained contributes to our understanding of BSFL as a potential feed source and its implications for animal growth performance and health. Studying the impact of BSFL on gene expression in animals, specifically poultry, provides valuable insights into the development of high-nutritive feed supplements and their genetic implications. By adopting sustainable management practices and exploring the economic potential of BSFL, local communities can transform BSF into a valuable resource for their well-being. In conclusion, the study will have the potential to contribute to several SDGs, including SDG 2-Zero hunger, SDG 3-Good health and well-being, and SDG 12-Responsible consumption and production (Edrisi et al., 2020).

1.7 Scope and Limitation of the Study

The study focused on the black soldier fly larvae meal fed to a homogenous group of Ross 308® broiler chickens. The experimental units were grouped according to the test and control treatment diets. The study findings reported in this thesis were therefore limited to Ross 308® broiler chicken with the assumption that the results can be extrapolated to other poultry or monogastric stock. The findings are further limited to specific conditions, and the study's experimental design may not generalize to other conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Chicken (*Gallus gallus domesticus*) is believed to have been domesticated from the wild in South China, India, Indonesia, and the Philippines, and was introduced to Africa through Egypt from South-Western Asia (MacDonald, 1992). Between 1425 and 1123 BC, chickens were mainly kept for recreational activities such as cockfighting, and after 650 BC, the increase in the chicken population shifted their roles to cultural and spiritual ones, such as a timekeeping system and offering sacrifices during planting and harvesting. It was also during this time that the chicken became a significant source of food for the human population. Human migration to the south in 500 BC spread chicken to central Africa, and the subsequent migration of the Bantu from central Africa into Kenya led to the arrival of chicken in western Kenya. Furthermore, the migration of Nilotic communities from the north in 50AD led to an increase in chicken in Kenya (Magothe et al., 2012). Chicken gained economic importance in 100AD during the Greco-Roman east coast trade, which led to the introduction of Asiatic and game chickens on the Kenyan coast (Blench & MacDonald, 2006).

2.2 Chicken Production Systems in Kenya

In Kenya, village chicken production systems are mainly characterized by birds feeding on agro-processing by-products, grass verges, insects, and domestic wastes, and are occasionally supplemented with grains (Upton, 2009). In a study conducted in Western Kenya, the results showed that most farmers in suburban areas supplemented their poultry diets with crushed maize, whereas those in rural areas provided no supplementary feed (Olwande et al., 2010). In rural areas, indigenous poultry production is characterized by low production costs, since the initial investment is minimal, mainly because chickens are presented as gifts or loans. There are no housing costs since the birds are locked in living houses and the kitchen at night. This system requires low capital and is therefore suitable for low-income

and vulnerable households. Such low inputs usually yield very low outputs as compared to intensive systems. Indigenous chicken plays major economic and food security roles in Kenya and has been identified as the most valuable tool for combating rural poverty and household food insecurity (Magothe et al., 2012). Chicken production in Kenya is classified into free-range system (FRS), semi-intensive system (SIS), and intensive system (IS) (Menge et al., 2005). The aims of rearing chickens are subsistence (home consumption only), income-home consumption, and income only (Alebachew et al., 2022). The profitability of FRS is higher than that of SIS and IS systems. However, due to the constant reduction of land resources caused by population growth, intensive production is encouraged (Menge et al., 2005). The major phenotypes of chicken found in Kenya include the normal feathered, naked-neck, frizzle-feathered, dwarf, crested-head, feathered shanks, and rump-less (Magothe et al., 2012). The pictorial presentation of the types is shown in Figure 2.1.

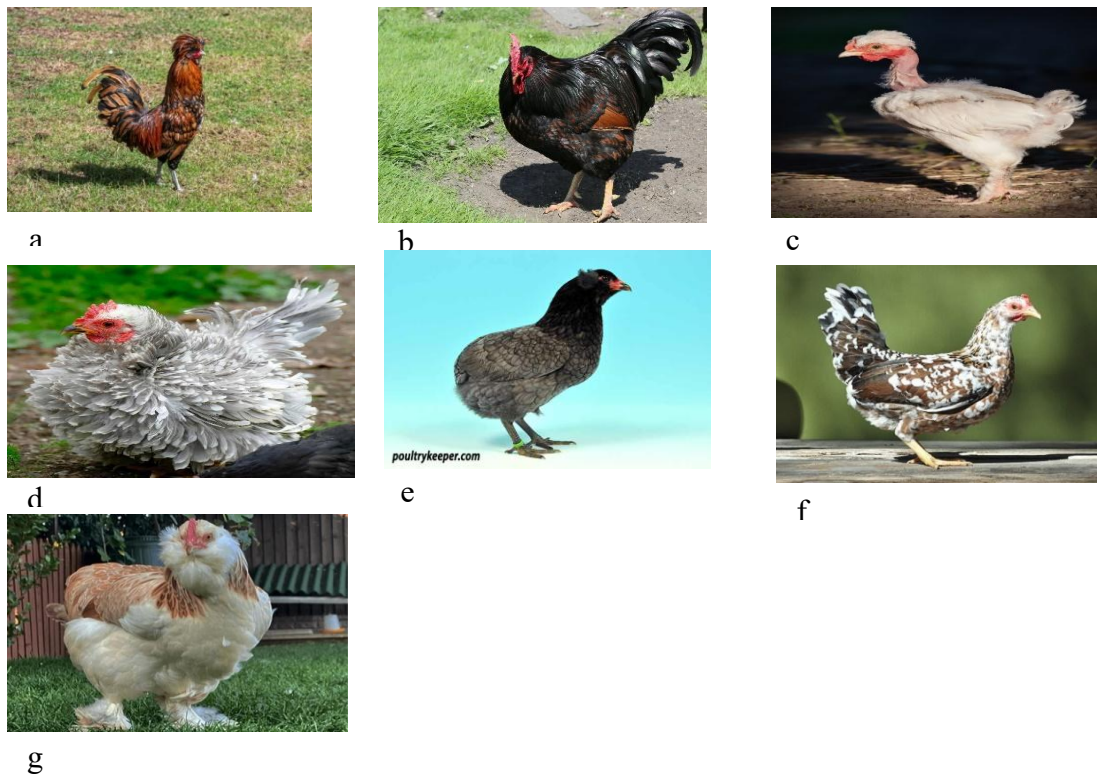


Figure 2.1: Indigenous Chicken breeds in Kenya: (a) Crested head, (b) Normal feathered, (c) Naked neck, (d) Frizzle feathered, (e) rump-less, (f) dwarf, (g) feathered shanks

There has been a rise in genetic improvements of indigenous chicken for better production and commercialization of the chicken sub-sector, and this has led to the introduction of broiler chicken breeds that achieve high productivity at an early age.

2.3 Current Commercial Broiler Chicken Status in Kenya

There is a continuous growth of commercial broiler production in Kenya as it is one of the popular animal husbandry sub-sectors (Mohamud et al., 2023). There has been a continuous increase in the broiler population in areas of Kiambu, Kajiado, Machakos, Thika, Mombasa, and Nairobi, where most of the farmers practice intensive commercial production (Omondi, 2019). Furthermore, reports have shown an annual increase in poultry meat consumption in Kenya from 54.8 metric tonnes in 2000 to 91.4 metric tonnes in 2010, and it is expected to undergo an exponential increase by 2030 (Carron et al., 2017). In Nairobi, the consumption is projected to rise by 24 thousand metric tonnes in 2030 (Rogers et al., 2011). Some of the main factors considered in the selection of commercial-intensive broiler chicken include high carcass yield, high growth rates, and efficiency in feed conversion (Nicol & Davies, 2013). Intensification of broiler chicken has been contributed to by an increase in demand for poultry meat, and this leads to keeping of broiler chicken in high densities in a deep litter system. Some of the commercial broiler breeds kept under intensive deep litter systems include Cobb 500, Ross 308, and Hubbard Flex (see Figure 2.2). The main aim of keeping the chicken in high densities is to maximize profits in the enterprise. However, stocking birds with more than 30kg per m² affects their growth rate due to stress and heat (Berg et al., 2012).

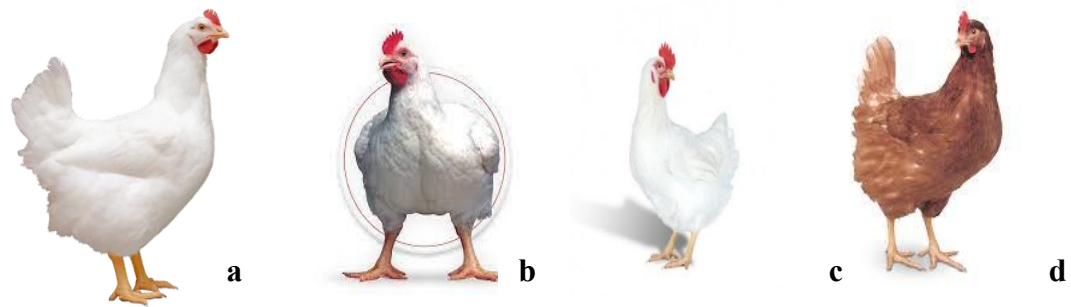


Figure 2.2: Exotic Chicken Breeds in Kenya- (a) Cobb 500, (b) Ross 308®, (c) Hubbard Flex, (d) Lohmann Brown

2.4 Commercial Broiler Feed and Protein Sources

In poultry production, feed costs account for 70% of total production costs. Low-quality, low-quantity, and high-cost feed are the major constraints in intensive commercial poultry production. This usually leads farmers to adopt a small number of birds. There is also underutilization of land, as farmers' stock numbers are small and manageable (Fosta et al., 2007). The continuous rise of feed costs in Kenya may hinder commercial production, which has the potential to lead to vulnerable farmers dropping out of the business (Barragan-Fonseca et al., 2023). Over the last decades, the cost of fish meal, soybean, and maize increased by 70%, 94%, and 65%, respectively (Abro et al., 2022). The high cost of materials that are also used to make animal feeds is mainly caused by increasing protein costs since it is the most expensive ingredient in the feed, and it impacts the farmers since it reduces the producer's profit (Abro et al., 2020). In Kenya, the protein component in conventional feed is mainly sourced from fish and soybean meals (Shumo, 2018).

There is a high demand for fish meal, which exceeds its supply and therefore initiates a search for alternative protein sources that can be used to substitute conventional protein sources (Ojewola, 2005). The poultry feed demand in Kenya can be determined using the following formulae;

$$\text{Broiler feed demand} = A \times C \times D$$

Where;

A= Number of chickens in millions, C= Optimol recommended feed (g/bird/day), and D = Life expectancy i.e. 49 days for broilers (Heita et al., 2023).

Some of the unconventional protein sources utilized in substitution include maggots, housefly pupa, fermented spent-sorghum mash, and chicken offal meals from evisceration plants (Babiker, 2012). The use of by-products from agro-industries has also gained recognition to replace conventional feedstuff and reduce feed costs. Sunflower seed meal from sunflower oil has also been used to replace high-cost soybean protein in broiler feed since sunflower is adaptive to a wide variety of soil (Ditta & King, 2017). Currently, insect protein is a potential protein alternative in broiler diets since it has a comparable or relatively high protein content as conventional feed.

2.5 Insects as a Source of Animal Feed

Entomophagy or insectivory is the practice of feeding on insects by man or livestock. Chickens in their normal outdoor feeding, pick insects at different stages of life and this indicates that they are adapted to insects as their natural diets. Therefore, it is essential to consider the incorporation of insects in commercial broiler feed manufacturing (Jozefiak & Engberg, 2015). The use of insects as animal feed supplements is a reliable solution to the challenges of high protein costs in the poultry sector (Chia et al., 2019). There is feasibility in feeding insects with different kinds of waste and feeding the same insects to poultry (Khusro et al., 2012). The practice of feeding insects to chickens requires an investigation of the rearing, feeding, and production methods of insects (Anand et al., 2008). This will be beneficial in sustainable agriculture, as it will reduce pollution through the utilization of a variety of organic waste materials.

Worldwide, over 2000 insect species are edible to man and livestock and it has been forecasted to reduce the cost of protein in food and feed supplementation (Ramos-Elorduy, 2008). Insects possess a high feed conversion efficiency, which is mainly attributed to their poikilothermic nature, that is, they do not feed to maintain body temperature. A good example is crickets which convert plants into biomass five times more than that of cattle (Nakagaki & Defoliart, 1991). Insects such as mealworms, BSFLs, silkworms, crickets, locusts, and grasshoppers have calorific value and high nutritive content in protein, fats, minerals, and vitamins. Several studies have shown they can be safely fed to poultry without necessarily altering the palatability and quality of their products (Khusro et al., 2012). The BSF, which is the subject of this study, has 38.5-62.7% crude protein, 14.0-39.2% fat, and 5282 Kcal/Kg of gross energy (Abro et al., 2020).

2.6 Black Soldier Fly Larvae (*Hermetia illucens*) as a Source of Poultry Feed

The black soldier fly (*Hermetia illucens*) is one of the promising insects in the animal feed industry because it can attain growth peaks on materials that are not intended for human nutrition e.g. waste from food processing, industrial by-products, and spoiled feed. BSFL is native to the South American savannah and has gradually spread across tropical, subtropical, and temperate areas with temperatures of 25°C and 30°C (Shumo et al., 2019). The fly cannot survive in regions with temperatures below 5°C and therefore their survival in North-western Europe is limited (Spranghers et al., 2017). BSF is one of the five genera of the subfamily Hermetiinae and order Diptera (Woodley, 2011). The other genera include *Patagiomysia*, *Chaetosargus*, *Notohermetia* and *Chaetohermatia*. *Hermetia illucens* is probably the most common and most distributed among the species (Singh & Kumari, 2019). This species is black with a slender body and three body parts i.e. head, thorax, and abdomen with tentacles (Ustuner et al., 2003). They are composed of five cycle stages egg, larvae, pupa, pre-adult, and adult, among which the larval and the pupae are the most nutritive stages and largely depend on a variety of feed (Liu et al., 2017). The adult fly can live for weeks without feed but relies on water and body fat (Tomberlin et al., 2005). The BSF has no attraction to humans and fresh food and therefore has no possibility of being a disease vector (Sheppard et al., 2002).

BSF are saprophytic and naturally occur in animal droppings but can also thrive well in waste products of plants, animals, and agricultural by-products such as coffee pulp, distillers dry grain (DDGs), and offal (Van Huis & Tomberlin, 2018). They add value by converting organic waste biomass to elevated protein biomass (Sheppard et al., 1994). In the event of degradation of organic waste, BSFL converts organic waste into important components such as oils, amino acids, chitin, peptides, vitamins, and proteins, a process that limits harmful microorganisms such as *E. coli* and *Salmonella* spp (Erickson et al., 2004). Through this conversion, they are perfect sources of diets for livestock and hence considered an alternative source of poultry feed to substitute soybean and fishmeal (Purba et al., 2025). The dry weight of BSF is constituted of up to 50% crude protein content, 35% lipid content, and a similar amino acid profile to that of fish (Musinguzi et al., 2024). Table 2.1 presents an analysis of the nutrient composition of BSFL according to Mutisya et al. (2021).

Table 2.1: Analysed percentage (%) nutrient composition of BSFL meal and other insects potential for feed

Constituent	BSFLM	Mealworm	Locust	Silkworm
Dry matter	95.9	85.9	91.7	91.4
Crude protein CP	43.8	46.9	50.6	50.9
Ether extracts	28.3	36.1	8.5	25.7
Crude fibre	20.9	12.9	8.5	3.9
Ash	12.9	3.1	6.6	5.8
Essential Amino acids				
Methionine	0.8	1.5	2.3	3.5
Lysine	2.9	5.4	4.7	6.7
Threonine	1.7	4.0	3.5	5.1
Arginine	2.2	4.8	5.6	5.6
Histidine	1.3	3.4	3.0	2.6
Leucine	2.8	6.6	5.8	7.0
Isoleucine	1.8	3.7	4.0	5.1
Valine	2.6	2.0	4.0	4.5
Phenylalanine	1.9	3.9	3.4	3.9

2.7 Social and Economic Effects of Using BSFL as a Source of Protein in Poultry

2.7.1 Economic Effects on the Producer and Consumer

The rise in technological advances in the animal feed sector, which include the use of BSFL, impacts producers through the reduction of production costs and increasing productivity. On the other hand, it affects the consumer indirectly by reducing the prices of products from the animal sector (Abro et al., 2020). The impacts of technological advances are represented by the k -shift parameter, which acts for the production cost per unit due to technology (Alston et al., 1995). The k -shift parameter is expressed by the following formula;

$$k_m = \left(\frac{ATT_{ym}}{\epsilon} + \frac{ATT_{cm}}{1 + ATT_{ym}} \right) \times r \quad \text{Equation 1}$$

(K-shift due to technological advancement of BSFL usage)

Where;

m = poultry meat products, ATT_{ym} = Change in meat productivity, ATT_{cm} = Change in poultry meat production due to technological innovation, ϵ = Elasticity of supply price for poultry meat, and r = rate of substitution of conventional feed with BSFL (Onsongo et al., 2018). The k -shift is calculated by the substitution rate (r) of conventional feeds with BSFL in poultry feed after which the economic surplus of the consumer and the producer of poultry meat can be determined by the following equations (Alston et al., 1995).

$$\Delta PS_m = P_m Q_m (K_m - Z_m) (1 + 0.5 Z_m \eta_m) \quad \text{Equation 2}$$

(Economic surplus of the producer due to BSFL usage)

$$\Delta CS_m = P_m Q_m Z_m (1 + 0.5 Z_m \eta_m) \quad \text{Equation 3}$$

(Economic surplus of the consumer due to BSFL usage)

Where;

P_m = Average producer price of poultry meat, Q_m = Average quantity of poultry meat production, Z_m = Relative change in price of poultry meat, and η_m is the absolute price elasticity of demand (Shibia et al., 2017). The totals of ΔCS_m and ΔPS_m denote the total economic change surplus due to BSFL usage.

2.7.2 Foreign Currency Savings Due to BSFL Usage

Adopting the usage of BSFL as poultry feed can save the country foreign currency compared to imported conventional feed such as soya and sunflower (Abro et al., 2020). The foreign currency saved can be determined by the following equation;

$$F = \sum j r \times M_{jq} \times P_{jq} \quad \text{Equation 4}$$

(Foreign currency saved due to BSFL usage)

Where;

F = Foreign currency saved by replacement of conventional feed with BSFL, j = Soybean, maize, and fishmeal index, r = Substitution rate of conventional feeds with BSFL, P_{jq} = Average price of conventional feed for specific period and M_{jq} = Countries average of imported conventional feed in tonnes.

2.7.3 Food Security and Benefits that could Result Due to the Replacement of 'Omena' with BSFL

The benefits that could potentially occur by replacement of Omena with BSFL can be determined by the equation

$$Q_{omena} = r \times O_{mena \text{ feed}} \quad \text{Equation 5}$$

(Volume of fishmeal intended for human consumption)

$$Nf = \frac{Q_{omena}}{Cf} \quad \text{Equation 6}$$

(Food security due to BSFL usage)

Where;

Omena feed = Harvested omena utilized as poultry feed, r = rate of replacement of omena with BSFL, Q_{omena} = Volume of omena intended for human consumption, N_f Potential number of people which could be fed on omena and C_f = Per-capita fish consumption in Kenya (Abro et al., 2020).

2.7.4 Employment Effects of BSFL Production

There is a significant rate of unemployment of youths in Kenya which stands at 12% of the total youth population between 15-34 years (KNBS, 2018a). The rate of unemployment is relatively high in the female gender as compared to the male gender. BSFL farming is a potential remedy for employment (Abro et al., 2020).

2.8 Impacts of Black Soldier Fly Larvae on Growth Performance

Several studies investigating the effect of replacing conventional protein sources with black soldier fly larvae in broiler diets have yielded varying results. Some reports showed improved growth performance parameters such as body weight gain and feed conversion rate (Cullere et al., 2019). Cobb500 broiler chicken supplied with a diet composed of 5% BSFL showed elevated feed efficiency, those supplied with 7.5% BSFL showed an improved thigh weight as well, and those supplied with 10% BSFL showed a better growth rate (Dahiru et al., 2016). Diets of BSFL supplied to Ross308 chicken showed improved meat quality due to an increase in lauric acid, and myristic acid and partly reducing PUFA concentration (Vilela et al., 2021). Similarly, diets containing 10% BSFL resulted in improved carcass and breast weight while 15% BSFL diets improved body weight, abdominal fat percentage, meat redness, protein meat percentage on monounsaturated fatty acid as well as reduction of PUFA (Schiavone et al., 2019). Black soldier fly larvae meal at different concentrations resulted in increased feed efficiency and breast meat yield in Ross 308 broiler chicken; however, it resulted in decreased weight gain (Sajjad et al., 2024).

In a study by (Barca et al., 2023) using a Zebrafish (*Danio reno*), there was a greater body weight gain of the experimental diet IM17 and IM50 supplemented with BSFL as compared to that of the control diet. However, others found no significant

differences compared to conventional diets (Gasco et al., 2019; Bruni et al., 2020). The inconsistency in outcomes may be attributed to differences in larvae processing techniques and inclusion levels (Henry et al., 2015). The growth performance of Turbot fed on BSF pre-pupae was high and it was affected by inclusion since high inclusions are attributed to lower feed intake because of excess chitin (Kroeckel et al., 2012). Excessive chitin in BSFL leads to reduced feed intake and therefore low growth rate (Caimi et al., 2020). Replacement of fishmeal with BSFL upregulates the expression of genes for appetite and chitinase activity (Zarantoniello et al., 2020). In a study on the inclusion of 16% or 60% BSFL in diets of *Oncorhynchus mykiss*, there was improved growth and a significant reduction of intestinal enteritis (Kumar et al., 2021). The elevated growth is attributed to the upregulation of genes related to hepatic growth hormones such as GH and IGF (Fischer et al., 2022).

2.9 Effects of Black Soldier Fly Larvae on Immunological Parameters

The use of Black Soldier Fly Larvae (*Hermetia illucens*) as a feed supplement for poultry has gained attention due to their high protein and fat content, as well as their potential to recycle organic waste. Some studies have explored the effects of incorporating BSFL into the diets of chickens and their impact on haematological parameters and blood plasma constituents. A study was performed to determine the effects of defatted black soldier fly larvae (Def-BSFL) meal as a protein source on the performance and blood plasma characteristics of broiler chickens. The study involved 360 one-day-old chicks divided into four diet groups: a control group with no BSFL (0% BSFL), T1 group with 4% BSFL, T2 group with 8% BSFL, and T3 group with 12% BSFL. This dietary arrangement lasted for six weeks. After the trial period, blood samples were taken from three birds in each group to examine plasma constituents. The results showed that the control group had the highest count of red blood cells with a mean standard deviation of 7.5 ± 0.34 , whereas the T2 group had the highest levels of haemoglobin. The T1, T2, and T3 groups showed elevated levels of monocytes compared to the control group. White blood cell counts, on the other hand, did not significantly differ across the groups. Additionally, the T2 group demonstrated higher blood urea nitrogen levels than the other groups (Mat et al., 2022).

In a different study performed to determine the effect of BSFL inclusions, some of the blood constituents that increased as a result of feeding BSFL included cholesterol ($P = 0.008$), HDL ($P = 0.015$), LDL ($P = 0.015$), NEFA concentration ($P = 0.027$), and immunological serum traits ($P = 0.039$). There was no significance in dietary treatments observed for IgM, IgY, and IL-2 ($P > 0.05$) (Kierończyk et al., 2020). In a study to determine the effect of 1%, 2%, and 3% BSFL on immunological parameters, the results showed modulation of immunological parameters due to elevation of CD3+, CD4+, and T lymphocyte percentage as shown in figure 2.3(a). The serum lysozyme across the three treatment diets was higher than that in the control group as shown in Figure 2.3(b) (Lee et al., 2018). The main reason for the elevation of immunological parameters is due to chitin which is composed of N-acetylglucosamine held by β -(1,4)-glycosidic bonds and hence exhibits a pathogen-associated molecular pattern (PAMP) which triggers innate and adaptive immune system to recognize pathogens and prevent the host against infections (Komi et al., 2018).

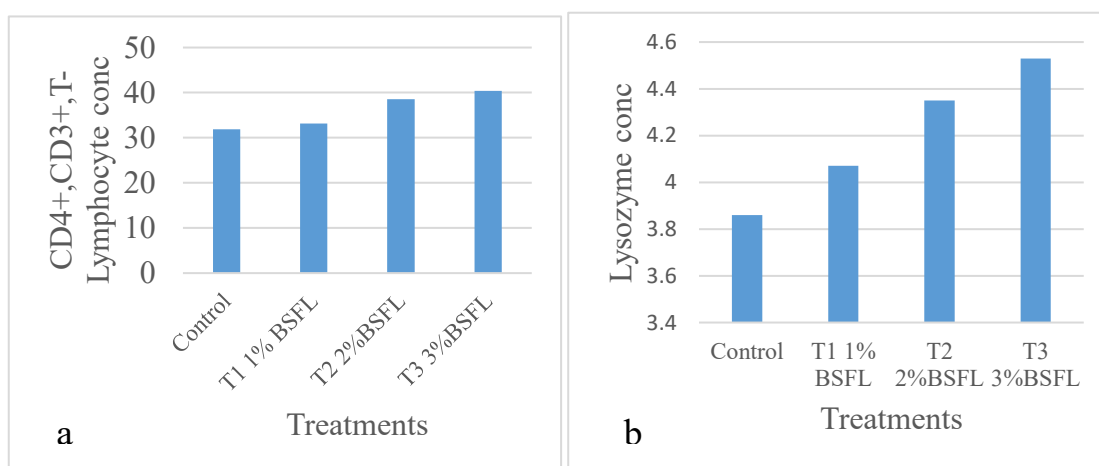


Figure 2.3: (a) CD4+, CD3+, T Lymphocyte, and (b) Lysozyme Concentrations under Different BSFL Inclusions

Studies performed in fish to determine the effect of BSFL on haematological parameters led to feeding fish on different BSFL inclusion levels (T1-0% BSFL, T2-6.5% BSFL, T3-13% BSFL, T4-19.4% BSFL and T5-24.5% BSFL). There was a significant difference ($P < 0.05$) in haematological results across the treatments where T3 recorded the highest white blood cell, lymphocyte, red blood cells, haematocrit

(HCT), and mean corpuscular volume (MCV). There were also significant variations identified for red cell distribution, granulocytosis (GRA), and platelet distribution (Kari et al., 2023).

2.10 Impacts of Black Soldier Fly Larvae on Gene Expression

Exploring the effects of black soldier fly larvae diets on gene expression patterns in broiler chickens is an emerging field. Initial investigations have shown alterations in gene expression related to growth, nutrient metabolism, immune responses, and stress tolerance (Hernandez-Baixauli et al., 2021). The specific genes affected can vary based on factors such as the inclusion levels of larvae and the duration of feeding (Barca et al., 2023). In a study by (Fischer et al., 2022; see Figures 2.4(a and b) and 2.5(a and b)), using diets D1 (0% BSFL), D2 (0% BSFL), D3 (11% BSFL), and D4 (9.83% BSFP), there was significant upregulation of growth hormone (GH) gene in D2 compared to D4 under different inclusions of BSFL. The study further depicted that subjecting fish to D2 initiated downregulation ($P < 0.05$) in hepatic IGF-I expression levels compared to D1, D2, and D4.

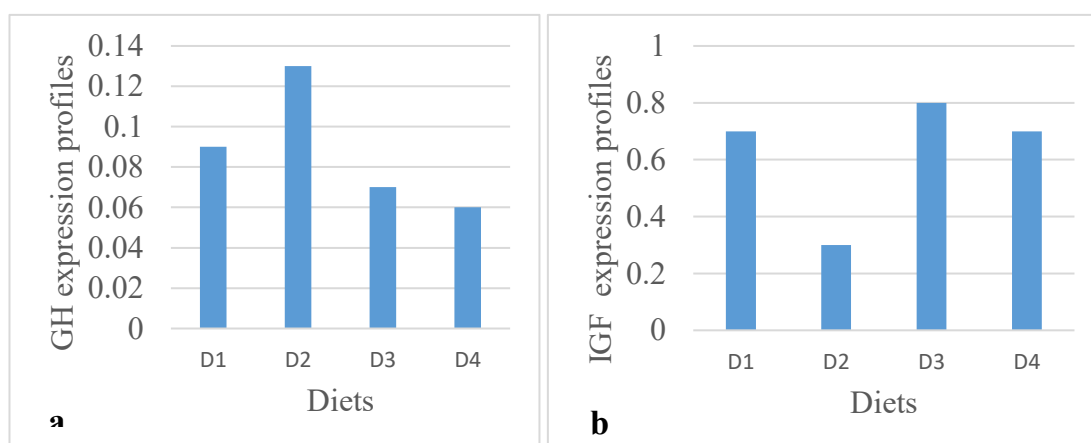
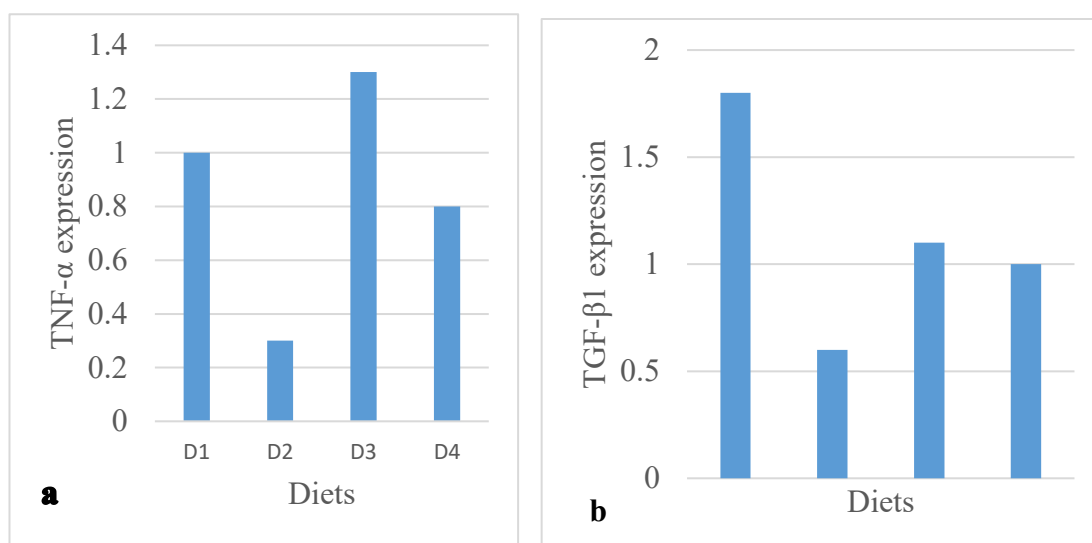


Figure 2.4: Expression Profiles of (a) GH and (b) IGF Genes under Different BSFLM Diet Levels

In the same study, animals supplied with D2 had low TNF- α expression profiles in the liver as compared with those supplied with D1, D3, and D4. Those supplied with D3 had the highest expression levels of TNF- α in their liver but it was not significantly different. Diets D2 and D4 led to downregulation of TGF- β 1 as compared to those supplied to D1. In the intestines, there were no significant differences between the expression profiles of TNF- α and TGF- β 1 levels (Fischer et



al., 2020).

Figure 2.5: Expression Profiles of (a) TNF- α and (b) TGF- β 1 Genes under Different BSFLM Diets

2.11 Gene-Nutrient Interactions

In the past decades, molecular biology has facilitated research on the participation of genes in a wide variety of diseases as well as the contribution of environment or nutrients on gene modifications (Jones and Laird, 1999). The existing research on gene-nutrient interactions depicts that several macro- and micronutrients are intermediate factors of the metabolic pathways leading to nucleic acid synthesis, and hence gene expression and deficiency of such nutrients will lead to alteration of DNA and disruption of genomic integrity within the tissues (Friso and Choi, 2005). The field of gene-nutrient interactions affects DNA methylation and hence becomes

a good concept to explain the effect of diet exposure at a molecular level although the specific magnitude of such interactions has not been specified.

A new era in nutritional sciences was introduced in the 21st century with the first publication of a human genome sequence draft and other related publications (Waterson et al., 2002; Venter et al., 2001). The study of how the gene interacts with dietary compounds to generate a phenotypic change is referred to as nutritional genomics or 'Nutrigenomics' (Kaput et al., 2004). In this field, many studies have been carried out in humans and mice with the aim that nutritional genomics will be a tool in the containment of several conditions such as obesity, cancer, coronary diseases, and common respiratory diseases (Chadwick, 2004). On the other hand, Nutrigenomics focuses on how structural genetic variation influences the diet effects of phenotype, and thousands of gene polymorphisms cause deviations in nutritional metabolism and hence a tool for personalized nutrition recommendations (Benitez et al., 2017).

2.12 Applications of Transcriptomic in Animal Feed Sciences

The primary aim of transcriptomic is to determine the whole or a set of expressed genes regarding the level of RNA in tissue samples. In humans, transcriptomic is concerned with the expression of 30,000 genes (Muller and Kersten, 2003). In repeated experiments mainly conducted in animals, the scope is limited to access the influence of dietary components on transcript levels mainly in targeted tissues and organs. The use of microarray probe technologies demonstrated that there was a change of more than 33% of the 8000 genes in the liver of rats fed on soya-based diets as compared to those fed on casein-based diets (Takamatsu et al., 2004). Significance difference was observed in genes related to anti-oxidation enzymes, transcription factors, energy metabolism, and lipid metabolism. In a related study by (Tachibana et al., 2005), a total of 120 genes related to lipid metabolism, anti-oxidation enzymes, and energy metabolism were altered by soya-based diets as compared to casein-based diets.

The utilization of black soldier fly larvae as an alternative protein source in broiler diets holds promise for improved growth performance and potential positive impacts on haematological parameters and gene expression. However, further comprehensive research is warranted to unravel the underlying mechanisms of these effects, including the influence of processing methods, inclusion levels, and potential interactions with other dietary components (Spranghers et al., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The feeding trial and bird performance components of this study were conducted at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja, 36 km northeast of Nairobi, along the Nairobi-Thika Superhighway, off Exit 15 [01° 05' 30"S and 37° 00' 41"E]. The farm is located at 1,527 metres above sea level and the climate is classified as warm and temperate with an average temperature of 18.8 °C and annual rainfall of 1014 millimetres. The gene expression laboratory analysis was carried out at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville, Kasarani Campus, Off the Nairobi – Thika Superhighway [1° 17' 11.0004" S and 36° 49' 2.0028" E; 1684 m above sea level.

3.2 Ethical Approval

All experimental procedures were conducted in accordance with The ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) and the ethical principles for animal research approved by the Institutional Animal Care and Use Committee (IACUC) of the Institutional Scientific and Ethics Review Committee of JKUAT (JKU/ISERC/02317/1399). The study was authorized under Kenyan animal welfare regulations by the National Commission for Science, Technology, and Innovation (NACOSTI; permit no. NACOSTI/P/25/418110).

3.2 Black Soldier Fly Larvae Meal Preparation and Diet Formulation

The isonitrogenous and isoenergetic diets were formulated using Brill software by replacing soybean meal (SBM) and fishmeal (FM) with BSFLM to meet the nutritional requirements of Ross 308 broiler chicken at different growth phases (Martínez & Valdiviá, 2021). To balance nutrients in the test diets, an analysis of the nutritional characteristics of SBM, FM, and BSFLM was conducted, and the results are presented in Table 3.1.

Table 3.1: Nutritional Properties of the SBM, FM, BSFLM used in Formulating the Test Diets

Parameter analysed (% DM basis)	BSFLM	FM	SBM
Dry matter	93.45	89.54	89.52
Crude protein	34.34	55.62	47.51
Ether extracts	20.60	8.70	2.30
Crude ash	10.60	29.60	6.40
Crude fibre	4.57	1.59	6.20
Phosphorous (mg/Kg)	5206	27953	5515
Metabolizable energy (Kcal/Kg)	3288	2603	2307
Essential amino acids			
Methionine	1.72	1.07	0.54
Cystine	0.82	0.28	0.48
Methionine + Cystine	2.33	1.33	1.03
Lysine	5.25	3.01	2.47
Tryptophan	1.37	0.34	0.55
Arginine	4.83	2.35	3.10
Isoleucine	4.01	1.68	1.83
Valine	5.58	1.89	1.88
Histidine	2.60	0.83	1.06
Phenylalanine	3.53	1.58	2.07
Non-Essential Amino acids			
Glycine	5.16	3.06	1.62
Serine	3.85	1.37	2.06
Proline	5.50	1.96	2.00
Alanine	5.81	2.83	1.69
Glutamic acid	10.68	5.48	7.44
Lauric acid	17.02	0.03	0.04

Treatment 1 (T1) consisted of SBM as the source of protein, treatment 2 (T2) had FM, treatment 3 (T3) consisted of FM + BSFLM, treatment 4 (T4) had SBM + BSFLM, and treatment 5 (T5) contained BSFLM only. The BSF larvae for the feed formulation were sourced from the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The larvae production conditions were a temperature of 27 °C and 60% humidity, reared under substrate from market and slaughterhouse wastes. The larvae were harvested at their fifth instar and sterilised in 83 °C water, and later oven dried at 120 °C for 2 hours. Feed ingredients and additives were sourced from a local feedstuff supplier. The dried larvae were hammer-milled and thoroughly mixed with other ingredients using an inclined auger

mixer (ABC Hansen, Pretoria, South Africa). Three broiler phases' diets, starter (0-14d), grower (14-21d), and finisher (22-42d) with partial and full replacement of SBM and FM with BSFLM across the treatments were prepared.

3.4 Diets and Protein Ingredient Analyses

The formulated diets and protein ingredients were analyzed for proximate composition prior to the start of the feeding trial. The dry matter content of the diets was determined in an electric oven at 135 °C for 2 hours, while the ash content was determined in a muffle furnace at 550 °C for 2 hours, according to Thiex et al. (2012). The protein ingredients were further analyzed to determine crude protein, crude fibre, and metabolizable energy using the respective Official Methods of Analysis (AOAC, 2023). The crude protein values of the BSFLM recorded were obtained using the nitrogen- protein conversion factor for edible insect of 4.76 since the total nitrogen in BSFLM contains nitrogen originating from both protein and non-protein compounds such as chitin (Janssen et al., 2017). The amino acid profiles were determined by conventional methods of AMINONIR Evonik East Africa Laboratories (Nairobi, Kenya) (Sumbule et al., 2021; Wamai et al., 2024). The results are presented in Tables 3.1 and 3.3.

Table 3.2: The Composition of Experimental Feeds Fed to Broiler Chickens

Ingredient (%)	Starter					Grower					Finisher				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Maize	39.10	55.73	49.52	39.10	39.10	39.10	56.28	48.56	39.10	39.10	39.10	56.28	48.56	39.10	39.10
Pollard	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Maize germ	19.55	10.00	10.00	14.06	23.36	15.40	10.00	10.72	11.29	20.43	19.93	10.00	10.72	16.53	20.34
Soybean	31.33	0.00	0.00	18.23	0.00	32.52	0.00	0.00	18.23	0.00	28.00	0.00	0.00	15.00	0.00
Fishmeal	0.00	20.55	13.18	0.00	0.00	0.00	20.00	13.00	0.00	0.00	0.00	20.00	13.00	0.00	0.00
Wheat bran	1.00	4.00	4.00	1.00	1.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
BSFL	0.00	0.00	13.58	18.59	27.43	0.00	0.00	14.00	18.40	27.50	0.00	0.00	14.00	16.40	27.59
Salt	0.30	0.30	0.30	0.30	0.30	0.26	0.30	0.30	0.26	0.25	0.25	0.30	0.30	0.25	0.25
DCP	1.20	1.40	1.40	1.20	1.20	1.20	1.40	1.40	1.20	1.20	1.20	1.40	1.40	1.20	1.20
Lime	0.50	0.80	0.80	0.50	0.50	0.50	0.80	0.80	0.50	0.50	0.50	0.80	0.80	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.40	0.60	0.60	0.40	0.49	0.40	0.60	0.60	0.40	0.40	0.40	0.60	0.60	0.40	0.40
Toxin binder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Methionine	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ME (Kcal/Kg)	2999.5					3100					3200				
CP (%)	23.0					21.5					20.0				

T1 - 100% Soybean meal, T2 - 100% Fishmeal, T3 - 50% Fishmeal + 50% Black soldier fly larvae meal T4 - 50 % Soybean meal + 50 % Black soldier larvae meal
T5 - 100% Black soldier fly larvae meal, BSFL - Black soldier fly larvae, DCP - Dicalcium phosphate, CP - Crude protein.

Table 3.3: Analysed Nutrient Composition of Formulated Diets

Ingredient (%)	Starter					Grower					Finisher				
Parameter	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Dry matter	90.49	90.21	90.39	90.59	90.36	90.24	89.97	90.13	90.4	90.50	90.30	90.47	90.45	90.45	90.58
CP	21.90	22.00	21.80	21.60	21.80	21.94	20.93	20.75	20.80	20.30	19.50	19.80	19.10	19.70	19.73
Ether extract	3.90	4.80	6.50	7.91	8.30	3.70	4.10	6.30	5.80	5.30	3.70	4.00	6.30	6.60	9.50
Crude Fibre	4.50	3.00	2.30	2.70	2.20	4.10	2.70	2.40	3.10	3.90	4.50	2.90	2.80	3.10	2.90
Crude Ash	7.00	6.60	7.10	7.50	7.60	6.60	6.10	6.80	7.70	7.10	6.70	7.30	7.00	7.30	7.50
Starch	33.00	41.00	39.50	35.80	38.00	36.20	46.80	43.30	34.90	40.30	36.90	40.20	40.90	38.40	36.70
ADF	6.90	4.20	3.70	4.30	4.00	6.30	3.90	3.70	5.20	5.00	6.80	5.00	4.20	4.70	4.10
NDF	14.60	14.80	14.60	13.50	14.10	13.60	13.20	13.00	13.70	13.80	14.80	15.30	15.40	14.50	15.50
Sugar	3.90	2.10	2.20	2.80	2.00	3.70	1.90	1.80	3.30	3.40	3.20	2.20	1.70	2.40	2.10
Phosphorus (mg/kg)	5567	7781	8276	7522	7568	5191	5988	7069	6976	6900	4820	7419	7570	666	786
Gross energy (MJ/Kg)	16.21	16.29	16.68	16.86	16.95	16.10	16.03	16.56	16.49	16.76	16.04	16.58	16.59	16.63	17.25
Gross energy (Kcal/Kg)	3871	3892	3985	4027	4049	3845	3831	3956	3940	3934	3833	3897	3970	3971	4117
Apparent ME (MJ/Kg)	10.29	11.26	11.79	11.663	11.77	10.39	11.43	12.07	11.02	11.89	10.386	11.02	11.82	11.61	12.47
Apparent ME (kcal/kg)	2859	2890	2816	2886	2812	2983	2907	2883	2934	2900	3110	3187	3122	3127	3179

BSFLM: Black soldier fly larvae meal (*Hermetia illucens* Linnaeus), FM: Fish meal (*Rastrineobola argentea*), SBM: Soybean meal (*Glycine max* L. Mer

3.5 Experimental Approach and Flock Management

Two hundred pre-vaccinated one-day-old Ross 308 broiler chicks (mixed sex) were obtained from a commercial hatchery (Kenchic Limited, Kenya). The study used a completely randomized design with five treatment groups and four replicates per group ($n = 200$ chicks total; 10 chicks per replicate; sex-balanced). This replicate number was selected based on facility constraints and aligns with those used in similar broiler trials (Sumbule et al., 2021). Upon arrival, the chicks were weighed and randomly distributed into 15-square-foot, disinfected pens, and clean drinking water and feed were offered *ad libitum*. The experimental birds were exposed to 24-hour lighting on the first three days, 20 hours light: 4 hours darkness from the fourth day, and 16 hours light: 8 hours darkness in the last week of the experimental period, with a relative humidity of $65\% \pm 5\%$. The temperature gradually decreased from 34°C on the first day to 20°C on the 47th day (Martínez & Valdivié, 2021). Two humidity and temperature data loggers (Hazari Tech Connect, Mumbai, India) were placed in the middle and two at the ends of the poultry house to monitor environmental conditions. The cages were housed in a well-ventilated room, with the cage floors covered in softwood shavings. The birds were vaccinated against Newcastle on days 7 and 28, and against Gumboro on days 14 and 21, with the Cevac ND-IBD vaccine according to the manufacturer's protocol (Ceva Santé Animale, Libourne, France).

3.6 Feeding and Performance Data Collection

Daily feed intake was calculated as the difference between the feed offered and the feed remains (Wamai et al., 2024). The intake was determined for each dietary treatment group. It was estimated each morning by subtracting the feed remaining in troughs from the amount of feed offered to the birds. The birds' growth performance data were obtained by individually weighing each bird at the start of the experiment and once per week for each dietary group up to the last week of the experiment using an electronic balance (Shimadzu TXB222L, Shimadzu Corporation, Kyoto, Japan). Body weight gain was calculated as the difference between the initial and final bird

weight. The feed conversion ratio (FCR), feed intake, and body weight gain per treatment group were respectively calculated as shown:

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}} \quad (a)$$

$$\text{Individual feed intake} = \text{Offered feed} - \text{Remnant feed} \quad (b)$$

$$\text{BWG} = \text{Average weight gain at the end} - \text{Average weight at the beginning} \quad (c)$$

Two birds per cage were randomly picked and sacrificed for tissue sampling and organ weight determination on the 35th and 42nd days. The birds were humanely euthanized in accordance with the Institutional Scientific and Ethics Review Committee (ISERC) via cervical dislocation, resulting in immediate loss of consciousness as reported in other studies (Fruci et al., 2023a; Tsementzis et al., 2025). Absence of both response to stimuli and rhythmic breathing confirmed unconsciousness, which was then followed by exsanguination by severing the jugular vein.

3.7 Economic Impacts of Partial and Full Replacement of SBM and FM with BSFLM

To determine the economic effects of partial and full replacement of SBM and FM with BSFLM, cost-benefit ratio (CBR) and return on investment (RoI) were calculated as the main indices. Only the feed cost was factored into the calculation since all other costs, which include labour, drugs, water, housing, feeders, drinkers, and electricity, were considered constant across the treatments. The cost of feed was calculated by multiplying the unit price of each ingredient by the inclusion amount in the feed and summing up all ingredients. The prices were determined in Kenyan shillings (KES), which were then exchanged to United States dollars using an exchange rate of (1USD = 130.00 KES). The CBR was calculated as the ratio between the production revenue and the production cost. The RoI was determined as the difference in production revenue and production cost divided by total production

cost and expressed as a percentage and expressed in percentages as shown below (Onsongo et al., 2018; Sumbule et al., 2021; Maina et al., 2025).

$$CBR = \frac{\text{Production revenue}}{\text{Production cost}}$$

$$RoI = \frac{\text{Production revenue} - \text{production cost}}{\text{Production cost}} \times 100\%$$

3.8 RNA Extraction and cDNA Synthesis

Liver and spleen tissues were collected from two chicken per cage in 100-mg aliquots, half of which were stored in liquid nitrogen and frozen at negative 80 °C, while the other half was stored in RNAlater solution (Ambion, Tokyo, Japan) at 4 °C for 20 minutes ready for disruption and homogenization. The 50 mg tissue was placed in a 1.5 ml RNase- free microcentrifuge tube with tissue lyser and stainless beads. A bead mill homogenizer (Qiagen, Hilden, Germany) was used to homogenize the tissue until a uniform solution was achieved after which, the homogenates was divided to duplicates. Total RNA was extracted from the liver and spleen samples using the ISOLATE II RNA Mini kit with DNase I treatment according to the manufacturer's protocol (Meridian Bioscience, USA). The extracted RNA concentrations and purity were determined using a Nanodrop spectrophotometer (Thermofisher Scientific, USA) at OD260/OD280. RNA integrity was assessed by 2% agarose gel electrophoresis with ethidium bromide staining. Any DNA contamination was treated with DNase I through incubation at 37 °C for 50 minutes. The RNA was reverse transcribed using the FIREScript RT cDNA synthesis kit (SOLIS BIODYNE, Tartu, Estonia) in a Proflex thermocycler (Applied Biosystems, Foster City, USA) with both No-template controls (NTC) and no-reverse transcriptase (–RT) controls. The 20-μl reverse transcription mixtures contained 1 μl FIREScript reverse transcriptase, 0.5 μl RiboGrip RNase inhibitor, 2 μl 10X RT reaction buffer, 0.5 μl dNTP mix, 10 μl nuclease-free water, 5 μl RNA template, and 1 μl oligo dt primers.

3.9 Gene Expression Profiling of Growth and Immune-Related Genes by Quantitative Polymerase Chain Reaction (qPCR)

Gene expression profiles for growth (chicken growth hormone (cGH), and insulin growth factor 1 (IGF-1)), and immune-related (interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrotic factor (TNF- α), interferon-gamma (IFN- γ)) genes in the liver and spleen cDNA by qPCR were also assessed. The standard primers for these gene targets as well as 28S rRNA as a reference housekeeping gene (Table 3.4) were used. Annealing temperatures were optimized by gradient PCR above and below the estimated melting temperatures (T_m). The temperatures that gave a sharp and distinct band under gel electrophoresis were set as the annealing temperature of the primers. We then quantified target and reference genes by qPCR using a Quantstudio3 (Applied Biosystems, Foster City, USA) in 20- μ l reactions comprising 4 μ l HOT FIREPoI EvaGreen qPCR Mix (Solis BioDyne, Tartu, Estonia), 2 μ l cDNA template, 12 μ l PCR grade water, 1 μ l forward primer, and 1 μ l reverse primer. The optimized cycling conditions included an initial denaturation at 95 °C for 12 minutes, followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 55-60 °C for 20 seconds, and extension at 72 °C for 20 seconds. During cycling, amplification curves were obtained in real time, with those crossing the threshold being recorded as cycle threshold (Ct) values. The data were exported into Microsoft Excel. Fold change (FC) was calculated by $2^{-\Delta\Delta C_t}$ as described by Pfaffl (2001) using 28S rRNA as the reference housekeeping gene.

Table 3.4: Oligonucleotide Sequence of Target and Reference Gene Primers

Gene	Primer sequence (5' – 3')	Annealing temperature (°C)	Reference
IL-2	F-ATCTTTGGCTGTATTTTCGGTAG	58	(Wang et al., 2020)
	R-CTGGGTCTCAGTTGGTGTGTAG		
IL-6	F-GCGAGAACAGCATGGAGATG	58	(Al-Zghoul et al., 2019)
	R-GTAGGTCTGAAAGGCCGAACAG		
TNF- α	F-AGACCAGATGGGAAGGGAATGAA	55	(Bhanja et al., 2015)
	R-GAAGAGGCCACCACACGACAG		
IFN- γ	F-ACTAGATCTCATACTGCAAGTAGTCTAAATR- ACTAAGCTTTTAGCAATTGCATCTCCTCTG	60	(Lowenthal et al., 2009)
cGH	F-CACCACAGCTAGAGACCCACATC	58	(Bhanja et al., 2015)
	R-CCCACCGGCTCAAACCTGC		
IGF-1	F-GGTGCTGAGCTGGTTGATGC	58	(Bhanja et al., 2015)
	R-CGTACAGAGCGTGCAGATTTAGGT		
28s RNA	F-CAGGTGCAGATCTTGGTGGTAGTA	58	(Bhanja et al., 2015)
	R-GCTCCCGCTGGCTTCTCC		

IL-2- Interleukin 2, IL-6-Interleukin 6, TNF- α - Tumor necrotic factor alpha, IFN- γ - Interferon-gamma, cGH- Chicken growth hormone, IGF-I- Insulin-like growth factor 1, 28S rRNA- 28S ribosomal RNA.

3.10 Calculations and Statistical Analysis

The data were recorded and organized using Microsoft Excel software and then exported to R Studio (version 4.2.2) for analysis. A retrospective power analysis using R-package (“pwr”) for a select significant means for FCR in starter, grower, finisher and overall phases were 0.80, 0.88, 0.79, and 0.80, respectively. Further, power analysis on fold change data was more than 0.85 for each measured gene. Normality and variance homogeneity were tested using the Shapiro-Wilk test and Bartlett's test, respectively. With the pen as the experimental unit, the statistical model had the experimental diet as the only source of variation. Data on growth performance and economic analysis were analyzed using analysis of variance (ANOVA), and means that showed significant differences were subjected to a *post hoc* test using the least significant differences (LSD) method at $p < 0.05$ to identify significant differences between means. Permutational multivariate analysis of

variance (PERMANOVA) and principal component analysis (PCA) were performed for the immune and growth genes. Additionally, Pearson correlation were performed between the gene expression and different growth traits. Within pen, the averages of gene expression data were analyzed using the Kruskal–Wallis test for differences among dietary treatments ($p < 0.05$). Dunn’s *post hoc* test with Bonferroni correction was used for multiple pairwise comparisons and treatments with the same letters were not significantly different.

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

$$\text{Individual feed intake} = \text{Offered feed} - \text{Remnant feed}$$

$$BWG = \text{Average weight gain at the end} - \text{Average weight at the beginning}$$

$$CBR = \frac{\text{Production revenue}}{\text{Production cost}}$$

$$RoI = \frac{\text{Production revenue} - \text{production cost}}{\text{Production cost}} \times 100\%$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effects of Partial and Full Substitution of SBM and FM with BSFLM on Growth Performance

The metabolizable energy of the starter, grower and finisher phase diets ranged from 2812-2890 Kcal/kg, 2883-2983 kcal/kg and 3050-3150kcal/kg respectively. The crude protein in starter, grower, and finisher phase diets ranges from 21.60-22.00%, 20.30-21.94%, and 19.10-19.80%, respectively. All these metabolizable energy values were within the nutritive requirements of 2800 Kcal/kg, 2900 Kcal/kg, and 3100 Kcal/kg for the Ross 308 starter, grower, and finisher, respectively. Similarly, the crude protein for starter, grower, and finisher attained minimum levels of 22.00%, 21.00%, and 19.00%, respectively. The analyzed nutritional compositions of experimental diets and protein ingredients are presented in Tables 3.3 and 3.1, respectively.

At the start of the experiment, the live weights (LW) measured on day 1 did not differ significantly among the experimental groups ($p = 0.684$). The dietary treatments significantly affected overall body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), and weights during the starter phase ($p < 0.05$; Table 4.1). The SBM+BSFLM treatment group had the highest means, while the BSFLM-only group had the lowest means of the measured parameters. Similarly, in this phase, there was a significant difference ($p = 0.0248$) in the feed conversion ratio (FCR). The FM+BSFLM treatment group had the lowest FCR (1.60), while the SBM-only diets had the highest (1.93). Conversely, the BSFLM treatment group demonstrated the lowest ADFI (17.02 g/day), which directly correlated with significantly lower live weight, BWG, and ADG.

There were significant differences with respect to LW ($p < 0.001$), BWG ($p = 0.038$), ADG ($p = 0.038$), and ADFI ($p = 0.001$) during the grower phase. The SBM+BSFLM group had the highest means for these parameters, while the BSFLM-only group had the lowest. Significant differences ($p = 0.028$) were also detected in

FCR values with birds fed on the FM+BSFLM realizing the lowest values, while those fed on the SBM+BSFLM diet resulted in the highest values. The overall growth performance differed significantly, but pairwise comparisons among treatments were not always statistically significant.

During the finisher phase, LW, BWG, ADFI, ADG, and FCR on day 42 differed significantly ($p < 0.001$) among dietary treatments. Birds fed diets with partial replacement of the protein sources realized significantly higher means compared to those fed on wholly SBM, FM, and BSFLM diets (Table 4.1). Their respective FCR values differed significantly ($p < 0.001$). For instance, the FM+BSFL treatment group achieved the lowest FCR of 0.97. In the entire experimental period, there were significant differences ($p < 0.001$) in LW, BWG, ADG, ADFI, and FCR. The LW (2071.60 g), BWG (2034.70 g), and ADG (48.44 g/d) were highest in the FM+BSFLM treatment group. These parameter values were lowest in the SBM-only treatment group, at 1187.50 g, 1148.47 g, and 27.34 g/day, respectively. The SBM+BSFLM treatment group had the highest ADFI at 69.38 g/d while the BSFLM-only treatment group had the lowest intake at 53.68 g/d. The FCR was significantly lower ($p = 0.001$) in the FM+BSFLM treatment group at 1.13, while it was highest (2.09) in the SBM-only treatment group ($p = 0.001$).

Table 4.1: Effect of Partial and Full Replacement of SBM and FM with BSFLM on the Mean ($\pm$ SE) Growth Performance of Broiler chicken

Variables	100% SBM	100% FM	50% FM + 50% BSFLM	50% SBM + 50% BSFLM	100% BSFLM	P Values
Starter Phase d1-14						
Initial weight (g)	39.02 $\pm$ 0.62	36.98 $\pm$ 0.64	36.88 $\pm$ 0.69	38.23 $\pm$ 0.64	39.25 $\pm$ 0.37	0.684
Live weight at day 14 (g)	208.64 $\pm$ 7.30 ^c	213.98 $\pm$ 8.19 ^{bc}	234.00 $\pm$ 9.19 ^{ab}	236.14 $\pm$ 10.15 ^a	155.06 $\pm$ 3.41 ^d	0.001
BWG (g)	169.61 $\pm$ 7.43 ^b	177.00 $\pm$ 8.35 ^{ab}	197.12 $\pm$ 9.34 ^a	197.91 $\pm$ 10.28 ^a	115.81 $\pm$ 3.54 ^c	0.001
ADG (g/day)	12.12 $\pm$ 0.53 ^b	12.64 $\pm$ 0.59 ^{ab}	14.80 $\pm$ 0.67 ^a	14.14 $\pm$ 0.73 ^a	8.27 $\pm$ 0.25 ^c	0.001
ADFI (g/day)	25.21 $\pm$ 0.86 ^{ab}	23.02 $\pm$ 0.93 ^b	24.23 $\pm$ 1.07 ^b	27.02 $\pm$ 1.29 ^a	17.02 $\pm$ 0.72 ^c	0.001
FCR	1.93 $\pm$ 0.04 ^a	1.71 $\pm$ 0.04 ^{bc}	1.60 $\pm$ 0.04 ^c	1.78 $\pm$ 0.04 ^{abc}	1.90 $\pm$ 0.03 ^{ab}	0.025
Grower Phase d14-21						
Initial weight (g)	208.64 $\pm$ 7.30 ^c	213.98 $\pm$ 8.19 ^{bc}	234.00 $\pm$ 9.19 ^{ab}	236.14 $\pm$ 10.15 ^a	155.06 $\pm$ 3.41 ^d	0.001
Liveweight at day 21 (g)	364.59 $\pm$ 11.17 ^{ab}	350.11 $\pm$ 12.49 ^b	382.83 $\pm$ 14.05 ^{ab}	400.34 $\pm$ 16.14 ^a	275.96 $\pm$ 7.98 ^c	0.001
BWG (g)	155.95 $\pm$ 5.10 ^a	136.14 $\pm$ 5.54 ^{ab}	148.83 $\pm$ 5.74 ^{ab}	164.20 $\pm$ 6.56 ^a	120.90 $\pm$ 5.33 ^b	0.038
ADG (g/day)	22.28 $\pm$ 0.73 ^a	19.45 $\pm$ 0.79 ^{ab}	21.27 $\pm$ 0.82 ^{ab}	23.46 $\pm$ 0.94 ^a	17.27 $\pm$ 0.76 ^b	0.038
ADFI (g/day)	57.39 $\pm$ 2.60 ^b	48.15 $\pm$ 2.87 ^{bc}	48.81 $\pm$ 3.23 ^{bc}	71.59 $\pm$ 3.65 ^a	44.71 $\pm$ 2.62 ^c	0.001
FCR	2.58 $\pm$ 0.08 ^b	2.53 $\pm$ 0.09 ^b	2.29 $\pm$ 0.09 ^b	3.07 $\pm$ 0.09 ^a	2.57 $\pm$ 0.09 ^b	0.028
Finisher Phase d21-42						

Variables	100% SBM	100% FM	50% FM + 50% BSFLM	50% SBM + 50% BSFLM	100% BSFLM	P Values
Initial weight(g)	364.59 ± 11.17 ^{ab}	350.11 ± 12.49 ^b	382.83 ± 14.05 ^{ab}	400.34 ± 16.14 ^a	275.96 ± 7.98 ^c	0.001
Liveweight at day 42 (g)	1187.50 ± 80.02 ^c	1386.10 ± 75.89 ^c	2071.60 ± 77.75 ^a	1784.50 ± 54.60 ^b	1350.50 ± 10.55 ^c	0.001
BWG (g)	822.90 ± 75.40 ^d	1036.00 ± 68.79 ^{cd}	1688.80 ± 67.90 ^a	1384.20 ± 41.96 ^b	1074.60 ± 16.19 ^c	0.001
ADG (g/day)	39.19 ± 3.59 ^d	49.33 ± 3.28 ^{cd}	80.42 ± 3.23 ^a	65.92 ± 2.00 ^b	51.17 ± 0.77 ^c	0.001
ADFI (g/day)	75.82 ± 2.81 ^{bc}	65.57 ± 3.13 ^c	74.44 ± 2.93 ^{bc}	95.91 ± 2.49 ^a	80.14 ± 2.37 ^b	0.002
FCR	2.04 ± 0.09 ^a	1.41 ± 0.07 ^b	0.97 ± 0.08 ^c	1.53 ± 0.05 ^b	1.66 ± 0.07 ^b	0.001
Entire Phase d1-42						
Initial weight (g)	39.02 ± 0.62	36.98 ± 0.64	36.88 ± 0.69	38.23 ± 0.64	39.25 ± 0.37	0.684
Final LW (g)	1187.50 ± 80.02 ^c	1386.10 ± 75.89 ^c	2071.60 ± 77.75 ^a	1784.50 ± 54.60 ^b	1350.50 ± 10.55 ^c	0.001
BWG (g)	1148.47 ± 80.18 ^c	1349.18 ± 76.00 ^c	2034.70 ± 78.00 ^a	1746.28 ± 54.83 ^b	1311.27 ± 10.39 ^c	0.001
ADG (g/day)	27.34 ± 1.91 ^c	32.12 ± 1.81 ^c	48.44 ± 1.86 ^a	41.57 ± 1.31 ^b	31.22 ± 0.26 ^c	0.001
ADFI (g/day)	56.25 ± 0.43 ^b	48.78 ± 0.40 ^b	53.80 ± 0.42 ^b	69.38 ± 0.29 ^a	53.68 ± 0.06 ^b	0.001
FCR	2.09 ± 0.08 ^a	1.54 ± 0.07 ^b	1.13 ± 0.08 ^c	1.70 ± 0.05 ^b	1.76 ± 0.06 ^b	0.001

LW- Live weight, BWG-Body weight gain, ADG-Average daily gain, ADFI-Average daily feed intake, FCR-Feed conversion ratio, SBM- Soya bean meal (*Glycine max* L. Merr), FM- Fish meal (*Rastrineobola argentea*), BSFLM- Black soldier fly larvae meal (*Hermetia illucens*), FM + BSFLM - Fish meal and Black soldier fly larvae meal, SBM + BSFLM-Soybean meal and Black soldier fly larvae meal. Means followed by different letters (a, b, c, d) are significantly different by multiple

The carcass and relative internal organ weights did not differ significantly at day 21 ($p > 0.05$). However, there was a general trend whereby diets with partial replacement yielded higher mean values for the measured parameters than those with full replacement, as shown in Table 4.2. The results further show no significant differences in carcass weight, thigh weight, breast weight, back weight, and the relative weights of the liver and bursa at day 35. There were significant differences in carcass weight ($p = 0.026$), with a high of 1,528.60 g in the FM+BSFLM treatment group and a low of 918.20 g in the SBM treatment group on day 42 of the experiment. However, the weights of the body parts and the internal organs did not differ significantly ($p > 0.05$, see Table 4.2).

Table 4.2: The Effects of Full and Partial Replacement of SBM and FM with BSFLM in Broiler Chicken Diets on the Mean ($\pm$ SE) Weights of Carcass, Thigh, Back, Breast, Wing, and Relative Weights of Internal Organs

Variables	100% SBM	100% FM	50% FM + 50% BSFLM	50% SBM + 50% BSFLM	100% BSFLM	P-values
Day 21						
Carcass weight (g)	359.90 $\pm$ 34.74	353.73 $\pm$ 34.74	393.05 $\pm$ 34.74	372.65 $\pm$ 34.74	305.58 $\pm$ 34.74	0.419
Relative weight: Liver (%)	3.04 $\pm$ 0.20	4.14 $\pm$ 0.20	4.37 $\pm$ 0.21	4.30 $\pm$ 0.20	4.23 $\pm$ 0.08	0.067
Relative weight: Gizzard (%)	3.53 $\pm$ 0.18	4.38 $\pm$ 0.19	4.25 $\pm$ 0.19	3.12 $\pm$ 0.11	3.96 $\pm$ 0.086	0.753
Relative weight: Heart (%)	0.86 $\pm$ 0.04	0.88 $\pm$ 0.04	0.88 $\pm$ 0.03	0.94 $\pm$ 0.03	0.91 $\pm$ 0.03	0.580
Relative weight: Spleen (%)	0.06 $\pm$ 0.02	0.03 $\pm$ 0.02	0.05 $\pm$ 0.02	0.19 $\pm$ 0.02	0.07 $\pm$ 0.01	0.121
Relative weight: Bursa (%)	0.17 $\pm$ 0.02	0.19 $\pm$ 0.02	0.19 $\pm$ 0.02	0.27 $\pm$ 0.02	0.18 $\pm$ 0.01	0.489
Day 35						
Carcass weight (g)	756.38 $\pm$ 22.32	656.00 $\pm$ 22.62	649.50 $\pm$ 20.31	755.13 $\pm$ 21.77	598.63 $\pm$ 10.41	0.089
Weight: Thigh (g)	133.88 $\pm$ 4.69	120.00 $\pm$ 4.93	115.75 $\pm$ 4.48	129.00 $\pm$ 4.99	98.38 $\pm$ 2.56	0.137
Weight: Breast (g)	151.00 $\pm$ 7.49	124.00 $\pm$ 7.87	127.88 $\pm$ 7.36	149.25 $\pm$ 7.72	106.25 $\pm$ 4.29	0.293
Weight: Wing (g)	88.00 $\pm$ 2.90 ^a	65.25 $\pm$ 2.24 ^b	53.25 $\pm$ 1.92 ^b	65.88 $\pm$ 2.09 ^b	53.00 $\pm$ 1.45 ^b	0.001
Weight: Back (g)	149.50 $\pm$ 15.97	143.88 $\pm$ 17.76	149.75 $\pm$ 20.32	209.00 $\pm$ 24.75	109.13 $\pm$ 2.00	0.414
Weight: Liver (g)	19.55 $\pm$ 0.83 ^b	19.13 $\pm$ 0.89 ^b	19.36 $\pm$ 0.95 ^b	25.89 $\pm$ 1.00 ^a	17.74 $\pm$ 0.48 ^b	0.012
Weight: Gizzard (g)	24.00 $\pm$ 0.78	23.21 $\pm$ 0.76	20.76 $\pm$ 0.70	20.03 $\pm$ 0.71	18.15 $\pm$ 0.14	0.105
Weight: Heart (g)	4.93 $\pm$ 0.25 ^b	5.40 $\pm$ 0.25 ^b	5.51 $\pm$ 0.24 ^b	7.20 $\pm$ 0.27 ^a	5.35 $\pm$ 0.18 ^b	0.027
Weight: Spleen (g)	0.59 $\pm$ 0.12 ^b	0.25 $\pm$ 0.13 ^b	0.29 $\pm$ 0.14 ^b	1.63 $\pm$ 0.16 ^a	0.51 $\pm$ 0.09 ^b	0.001
Weight: Bursa (g)	1.91 $\pm$ 0.09	1.39 $\pm$ 0.09	1.79 $\pm$ 0.09	1.89 $\pm$ 0.08	1.36 $\pm$ 0.07	0.090
Relative weight: Liver (%)	2.62 $\pm$ 0.10	2.97 $\pm$ 0.10	3.02 $\pm$ 0.11	3.49 $\pm$ 0.12	2.97 $\pm$ 0.06	0.087
Relative weight: Gizzard (%)	3.17 $\pm$ 0.09 ^{ab}	3.62 $\pm$ 0.09 ^a	3.21 $\pm$ 0.08 ^{ab}	2.64 $\pm$ 0.06 ^c	3.01 $\pm$ 0.07 ^{bc}	0.001
Relative weight: Heart (%)	0.64 $\pm$ 0.03 ^b	0.82 $\pm$ 0.03 ^a	0.85 $\pm$ 0.03 ^a	0.98 $\pm$ 0.03 ^a	0.90 $\pm$ 0.03 ^a	0.001
Relative weight: Spleen (%)	0.07 $\pm$ 0.02 ^b	0.032 $\pm$ 0.02 ^b	0.04 $\pm$ 0.02 ^b	0.22 $\pm$ 0.02 ^a	0.09 $\pm$ 0.02 ^b	0.001

Variables	100% SBM	100% FM	50% FM + 50% BSFLM	50% SBM + 50% BSFLM	100% BSFLM	P-values
Relative weight: Bursa (%)	0.26 ± 0.01	0.22 ± 0.02	0.29 ± 0.02	0.26 ± 0.02	0.23 ± 0.02	0.538
Day 42						
Carcass weight (g)	918.20 ± 65.36 ^b	1306.10 ± 56.72 ^a	1528.60 ± 49.40 ^a	1377.03 ± 31.80 ^a	1258.20 ± 29.96 ^{ab}	0.026
Weight: Thigh (g)	183.50 ± 11.44	241.50 ± 10.53	246.30 ± 9.88	254.80 ± 7.35	226.00 ± 2.29	0.317
Weight: Breast (g)	255.80 ± 18.90	313.00 ± 17.81	381.80 ± 17.96	348.80 ± 9.15	333.30 ± 11.06	0.311
Weight: Wing (g)	90.75 ± 4.15	95.25 ± 4.24	98.25 ± 4.33	111.00 ± 2.67	104.00 ± 1.80	0.624
Weight: Back (g)	170.80 ± 15.84	286.00 ± 15.98	271.80 ± 10.58	203.80 ± 5.89	218.50 ± 5.72	0.100
Weight: Liver (g)	38.75 ± 2.40	37.60 ± 2.53	43.63 ± 2.28	38.29 ± 1.63	38.75 ± 2.22	0.225
Weight: Gizzard (g)	25.33 ± 2.00	30.40 ± 2.14	38.10 ± 2.14	29.43 ± 1.23	23.45 ± 1.00	0.158
Weight: Heart (g)	7.70 ± 0.54	10.18 ± 0.51	10.60 ± 0.56	11.93 ± 0.68	9.45 ± 0.14	0.137
Weight: Spleen (g)	2.38 ± 0.21	1.43 ± 0.10	1.45 ± 0.09	1.25 ± 0.08	1.00 ± 0.10	0.335
Weight: Bursa (g)	2.13 ± 0.22	2.23 ± 0.23	2.48 ± 0.20	1.65 ± 0.18	1.35 ± 0.22	0.518
Relative weight: Liver (%)	3.16 ± 0.14	2.80 ± 0.11	2.87 ± 0.12	2.34 ± 0.11	2.24 ± 0.13	0.179
Relative weight: Gizzard (%)	2.77 ± 0.13	2.34 ± 0.12	2.52 ± 0.12	2.12 ± 0.06	1.87 ± 0.07	0.209
Relative weight: Heart (%)	0.85 ± 0.04	0.79 ± 0.04	0.71 ± 0.05	0.88 ± 0.05	0.76 ± 0.02	0.724
Relative weight: Spleen (%)	0.27 ± 0.03	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.113
Relative weight: Bursa (%)	0.23 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.10 ± 0.02	0.149

100% SBM-100% Soybean meal (*Glycine max* L. Merr), 100% FM- 100% Fish meal (*Rastrineobola argentea*), 100% BSFLM- Black soldier fly larvae meal (*Hermetia illucens*), 50% FM+50% BSFLM-50% Fish meal and 50% Black soldier fly larvae meal, 50%SBM+50%BSFLM-50% Soybean meal and 50% Black soldier fly larvae meal. Means followed by different letters (a, b, c, d) are significantly different by multiple comparisons test (P value < 0.05).

4.2 Economic Impacts of Partial and Full Replacement of SBM and FM with BSFLM

On analyzing the economic impacts of partial and full replacements of SBM and FM with BSFLM, the cost (USD/Kg) of formulated feed did not vary significantly ($p > 0.05$). However, the 50% SBM + 50% BSFLM group had the highest cost (0.50 USD/kg), while the 100% FM group had the lowest cost (0.44 USD/kg) in the starter and grower phases. There was a significant difference ($p = 0.025$) in the cumulative feed intake, with a high of 2.40 kg/bird in the SBM treatment group and a low of 2.08 kg/bird in the 100% FM treatment group. Although there were no significant differences ($p = 0.306$) in the cost of feed consumed, feeding birds with SBM+BSFLM resulted in a higher cost of 1.46 USD per bird compared to 1.03 USD per bird in the 100% BSFLM treatment group. The highest sale price (6.33 USD), profit (5.26 USD), and return on investment (ROI) (496.64%) were obtained from the FM+BSFLM treatment group, while the SBM-only group had the lowest sale price (3.63 USD/bird), profit (2.492 USD/bird), and ROI (219.89%) (Table 4.3).

Table 4.3: Economic Impact of Partial and Full Replacement of SBM and FM with BSFLM on Production Costs

Variables	100% SBM	100% FM	50% FM + 50% BSFLM	50% SBM + 50% BSFLM	100% BSFLM	P- values
Cost of feed (USD/kg)						
Starter feed	0.48 ± 0.01	0.44 ± 0.01	0.46 ± 0.01	0.51 ± 0.01	0.45 ± 0.01	0.714
Grower feed	0.48 ± 0.01	0.41 ± 0.01	0.46 ± 0.01	0.51 ± 0.01	0.45 ± 0.01	0.958
Finisher feed	0.46 ± 0.003	0.44 ± 0.03	0.46 ± 0.01	0.47 ± 0.01	0.45 ± 0.01	0.340
Feed intake (kg/bird)						
Starter phase	0.33 ± 0.01 ^a	0.30 ± 0.01 ^a	0.32 ± 0.01 ^a	0.35 ± 0.02 ^a	0.22 ± 0.01 ^b	0.037
Grower phase	0.40 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.50 ± 0.03	0.31 ± 0.02	0.920
Finisher phase	1.67 ± 0.06	1.44 ± 0.07	1.64 ± 0.06	2.11 ± 0.06	1.80 ± 0.05	0.046
Entire feed intake	2.40 ± 0.08 ^b	2.08 ± 0.09 ^b	2.29 ± 0.09 ^b	2.29 ± 0.09 ^b	2.30 ± 0.07 ^b	0.025
Cost of feed consumed (USD/bird)						
Starter phase	0.16 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.10 ± 0.01	0.136
Grower phase	0.19 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.25 ± 0.01	0.14 ± 0.01	0.975
Finisher phase	0.76 ± 0.03	0.62 ± 0.04	0.76 ± 0.03	0.99 ± 0.03	0.79 ± 0.02	0.056
Cumulative cost consumed (C)	1.13 ± 0.05	0.91 ± 0.05	1.07 ± 0.05	1.46 ± 0.06	1.03 ± 0.03	0.306
Sale of birds USD (S)	3.63 ± 0.24	4.23 ± 0.23	6.33 ± 0.24	5.45 ± 0.17	4.12 ± 0.03	0.209
Profit (P)	2.49 ± 0.23	3.27 ± 0.22	5.26 ± 0.23	3.99 ± 0.12	3.09 ± 0.06	0.269
Cost-benefit ratio (CBR)	3.20 ± 0.24	4.69 ± 0.24	5.97 ± 0.25	3.73 ± 0.12	4.07 ± 0.15	0.656
Return on investment (ROI)	219.89 ± 23.70	368.97 ± 22.19	496.64 ± 25.12	273.26 ± 11.53	306.54 ± 15.06	0.656

USD- United States dollar, C- cost of feed consumed, P- Profit, S - Sale price of birds, CBR- Cost benefit ratio, ROI- Return on investment. Means followed by different letters (a, b) are significantly different by multiple comparisons test (P value < 0.05).

4.3 Effects of Partial and Full Replacement of SBM and FM with BSFLM on Immune and Growth-Related Gene Expression

Using SBM as the control group, the fold changes showed several patterns of upregulation and downregulation of the target genes in the liver and spleen tissues (Figures 4.1 and 4.2). Kruskal-Wallis tests indicated significant differences ($p < 0.001$) in the fold changes of immune- and growth-related genes among dietary treatments. Dunn's test with Bonferroni adjustment grouped dietary treatments as shown in Table 4.4. Principal component analysis for both immune (Figure 4.3) and growth-related genes (Figure 4.4) showed that dietary treatments were well separated along PC1 and PC2, indicating a significant effect of the diets on gene expression. The Pearson correlations between immune- and growth-related gene expression and growth traits are presented in Figure 4.5. Liver chicken growth hormone (cGH) had a positive correlation with LW ($r = 0.56$), BWG ($r = 0.56$), and ADG ($r = 0.52$). Similarly, Spleen cGH had a strong positive correlation with the LW ($r = 0.65$), BWG ($r = 0.65$), and ADG ($r = 0.65$). However, the genes showed negative correlations with feed conversion ratio ($r = -0.24$ and $r = -0.39$). All the genes had a positive correlation with ADFI except for liver IL-6 and IGF-1, Spleen IFN- γ , and IGF-1 which had a negative correlation ($r < 0$) (Figure 4.5).

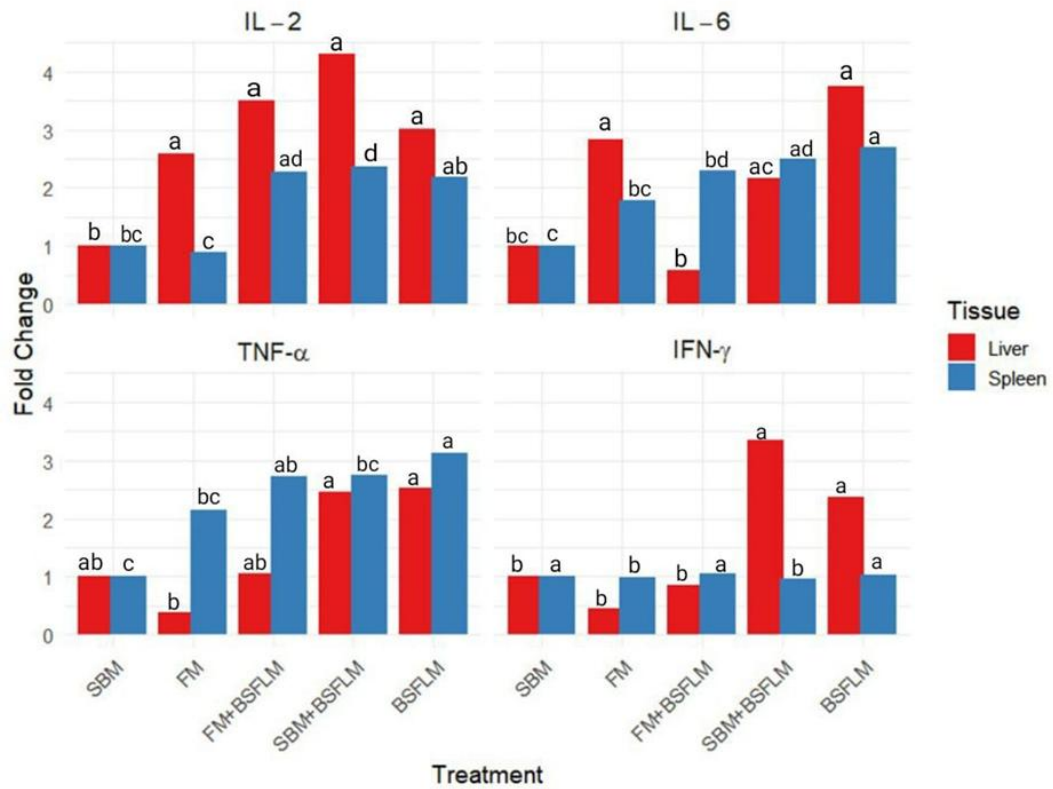


Figure 4.1: Relative Expression (Fold change $\pm$) of Immune-Related Genes in the Liver and Spleen Tissues of Ross 308 Broiler Chicken Fed Diets Enriched with Black Soldier Fly Larvae Meal. SBM-Soya Bean Meal, FM-Fishmeal, BSFLM- Black Soldier Larvae Meal. IL-2, Interleukin 2, IL-6- Interleukin 6, TNF- Tumour Necrosis Factor, IFN- Interferon

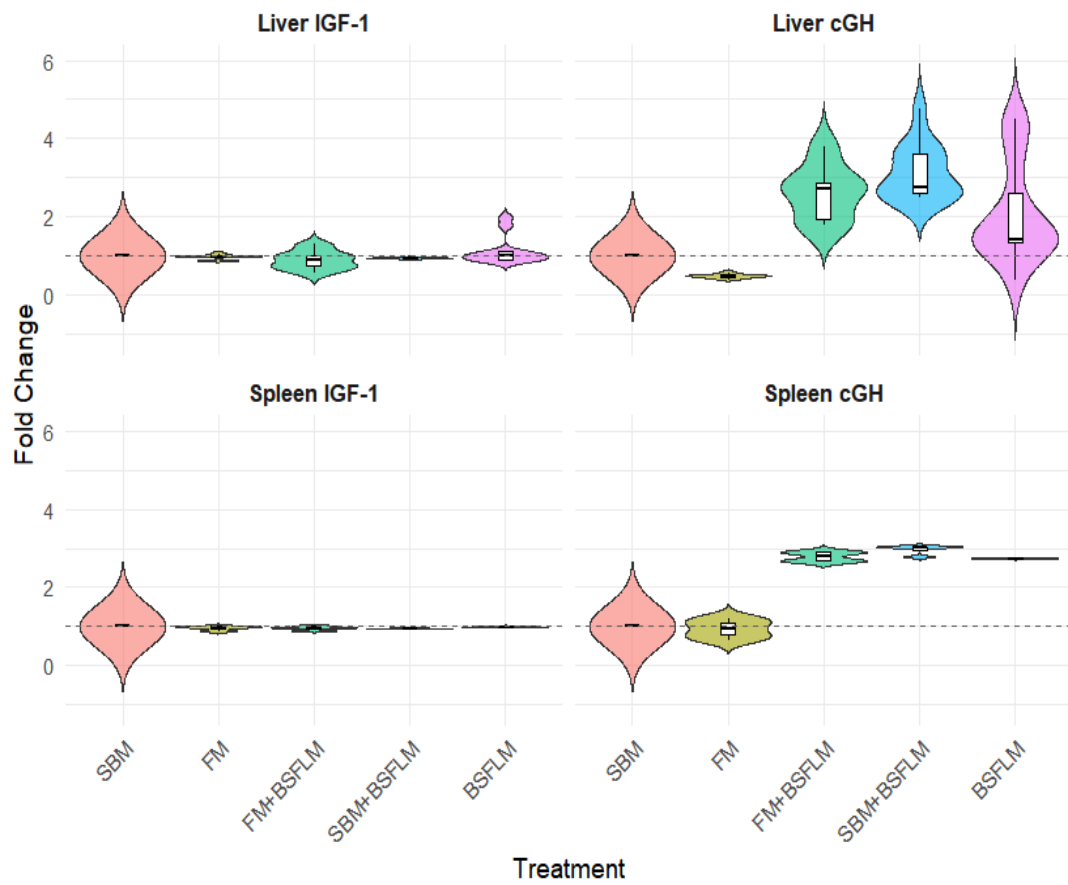


Figure 4.2: Violin and Box Plot of Distribution of Growth-Related Gene Expression in Liver and Spleen Tissues of Ross 308 Broiler Chicken Fed Diets Enriched with Black Soldier Larvae Meal. SBM-Soya Bean Meal, FM-Fishmeal, BSFLM- Black Soldier Larvae Meal, cGH- Chicken Growth Hormone, IGF-1- Insulin Growth Factor 1

The results showed that birds in the FM treatment group had significantly lower expression levels of TNF- α and IFN- γ in liver tissues, as well as IL-2, and IFN- γ in spleen tissues

Table 4.4: Kruskal-Wallis Test Results and Post Hoc Groupings (Dunn's Test) for Fold Change across Dietary Treatments. Treatments with the Same Superscript Letters are not Significantly Different

Gene	χ^2	df	SBM	FM	FM+BSFLM	SBM+BSFLM	BSFLM	ϵ^2	P-Value
Liver IL-2	46.61	4	1.00 ^b	2.59 ^a	3.51 ^a	4.31 ^a	3.01 ^a	0.57	<0.001
Spleen IL-2	76.49	4	1.00 ^{bc}	0.89 ^c	2.29 ^{ad}	2.38 ^d	2.19 ^{ab}	0.96	<0.001
Liver IL-6	61.03	4	1.00 ^{bc}	2.83 ^a	0.58 ^b	2.17 ^{ac}	3.75 ^a	0.76	<0.001
Spleen IL-6	61.39	4	1.00 ^c	1.80 ^{bc}	2.30 ^{bd}	2.50 ^{ad}	2.70 ^a	0.77	<0.001
Liver TNF- α	39.22	4	1.00 ^{ab}	0.38 ^b	1.06 ^{ab}	2.45 ^a	2.52 ^a	0.47	<0.001
Spleen TNF- α	76.49	4	1.00 ^c	2.15 ^{bc}	2.73 ^{ab}	2.75 ^{bc}	3.13 ^a	0.96	<0.001
Liver IFN- γ	60.68	4	1.00 ^b	0.46 ^b	0.84 ^b	3.36 ^a	2.38 ^a	0.76	<0.001
Spleen IFN- γ	75.26	4	1.00 ^a	0.99 ^b	1.06 ^a	0.97 ^b	1.03 ^a	0.95	<0.001
Liver IGF-1	72.43	4	1.00 ^a	0.98 ^{ab}	0.90 ^{ab}	0.95 ^b	1.14 ^{ab}	0.91	<0.001
Spleen IGF-1	76.54	4	1.00 ^a	0.96 ^{ab}	0.96 ^{ab}	0.95 ^b	1.00 ^a	0.96	<0.001
Liver cGH	60.90	4	1.00 ^{bc}	0.49 ^c	2.63 ^a	3.14 ^a	2.10 ^{ab}	0.76	<0.001
Spleen cGH	61.35	4	1.00 ^b	0.94 ^b	2.79 ^a	2.97 ^a	2.74 ^a	0.76	<0.001

SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal. IL-2-Interleukin 2, IL-6- Interleukin 6, TNF- α Tumour necrosis factor alpha, IFN- γ Interferon gamma. Means followed by different letters (a, b, c, d) are significantly different by multiple comparisons test (P value < 0.05).

Permutational multivariate analysis of variance (PERMANOVA) and principal component analysis (PCA) were performed for the immune and growth genes, and the results indicated that the dietary treatments had a significant effect on immune-related gene expression ($R^2 = 0.661$, $p < 0.001$). The PC1 and PC2 showed very significant differences ($p < 0.001$) among the dietary treatments, and this indicated the effect of insect-based diets on gene expression as shown in Figure 4.3.

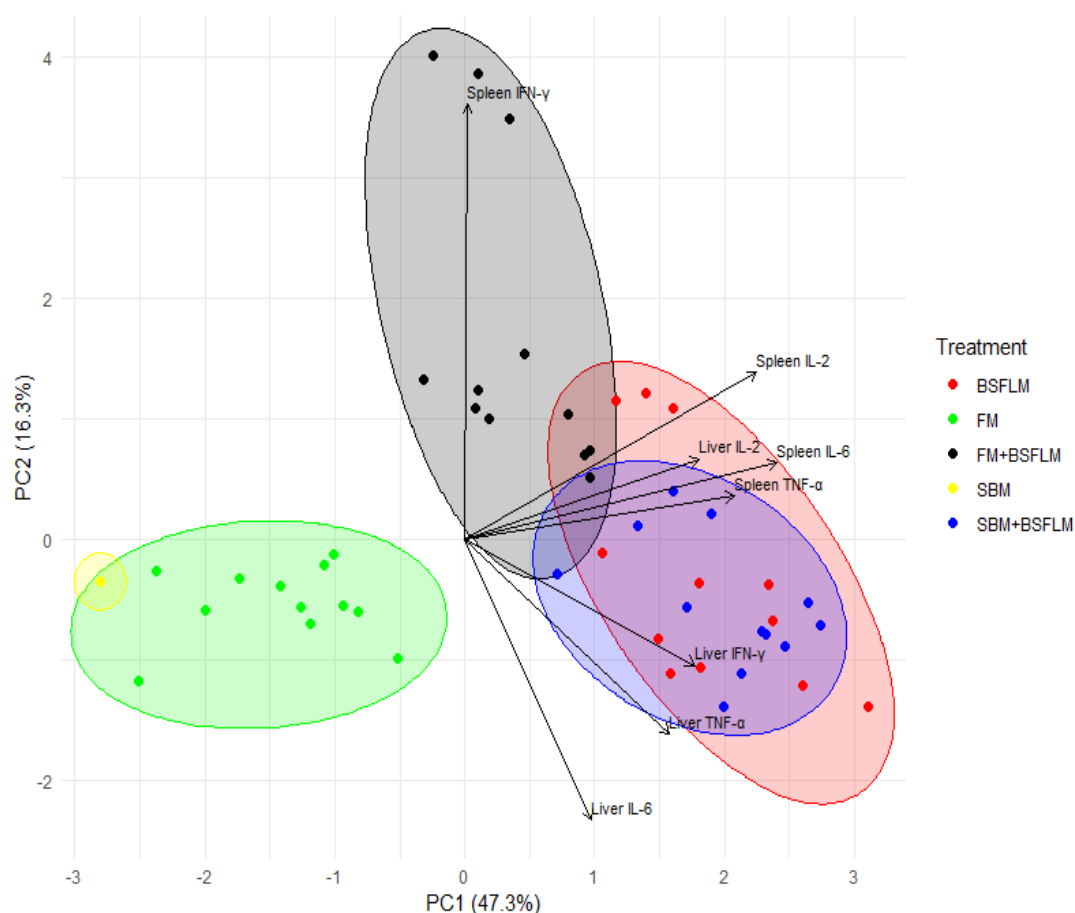


Figure 4.3: Principal Component Analysis (PCA) Biplot of Immune-Related Gene Expression in Liver and Spleen Tissues of Ross 308 Broiler Chicken fed diets enriched with black soldier larvae meal. SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal, IL-2, Interleukin 2, IL-6-Interleukin 6, TNF- α Tumour necrosis factor, IFN- γ Interferon.

Similarly, the PERMANOVA on growth-related gene expression indicated that the dietary treatments were the cause of variation ($R^2=0.531$, $p < 0.001$). However, the explained variation was lower than that of immune-related genes ($R^2=0.661$). PC1

and PC2 showed significant differences ($p < 0.001$) among the dietary treatments, as shown in Figure 4.4.

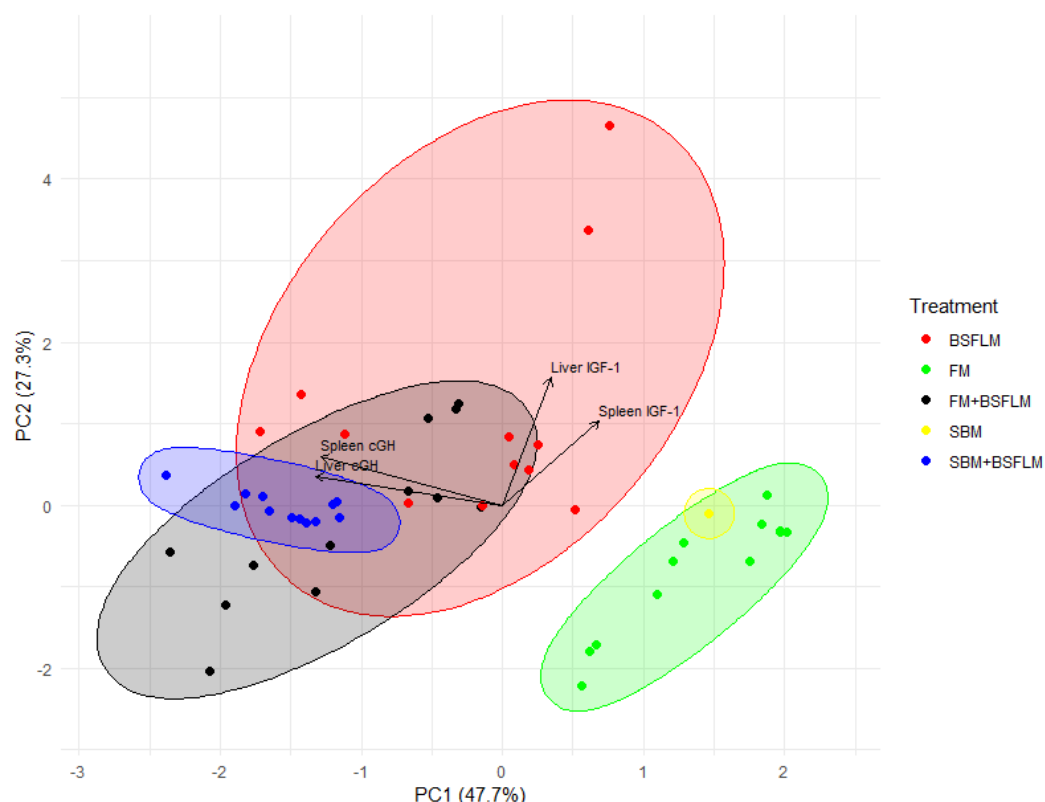


Figure 4.4: Principal Component Analysis (PCA) Biplot of Growth-Related Gene Expression in Liver and Spleen Tissues of Ross 308 Broiler Chicken Fed Diets Enriched with Black Soldier Fly Larvae Meal. SBM-Soya Bean Meal, FM-Fishmeal, BSFLM- Black Soldier Larvae Meal, Chicken Growth Hormone (cGH), Insulin Growth Factor 1 (IGF-1).

The Pearson correlation between the immune and growth-related gene expression and the growth traits are presented in Figure 4.5. Liver and spleen chicken growth hormone (CGH) had a stronger positive correlation ($r > 0.50$) with live weight (LW), body weight gain (BWG), and ADG. However, the genes had a negative correlation with feed conversion ratio ($r = -0.59$, $r = -0.50$) respectively. All the other genes had a positive correlation ($r > 0$) with LW, BWG, and ADG. All the genes had a positive correlation with ADFI except for liver and spleen IL-6, which had a negative correlation ($r = -0.07$, $r = -0.10$), respectively. All the genes had a negative correlation with FCR except for Liver IL-6, IFN- γ and spleen IL-2 as shown in Figure 4.5.

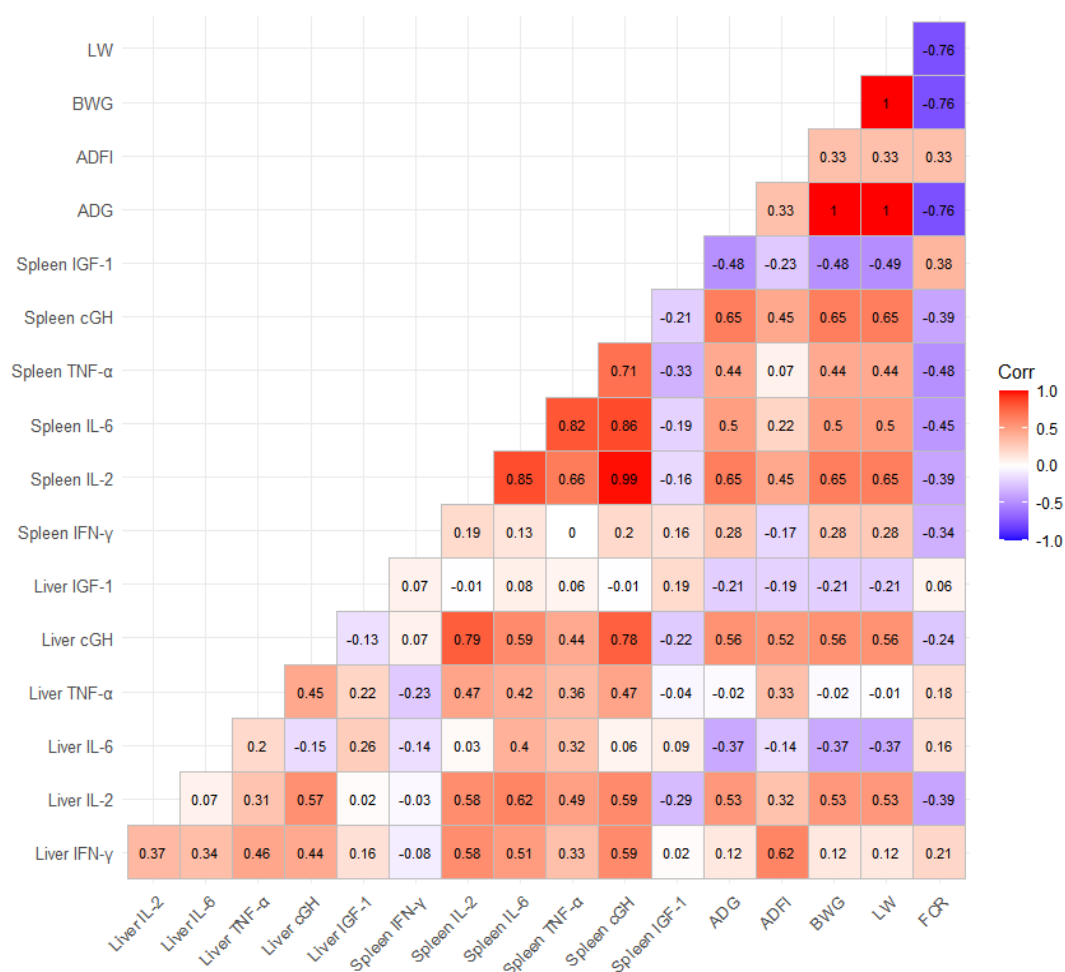


Figure 4.5: Heatmap of Pearson Correlation Matrix between Immune/Growth-Related Gene Expression and Growth Performance Traits of Ross 308 Broiler Chicken Fed Diets Enriched with Black Soldier Fly Larvae Meal. LW-Live Weight, BWG- Body Weight Gain, ADG- Average Daily Gain, ADFI- Average Daily Feed intake, FCR- Feed Conversion Ratio, IL-2, Interleukin 2, IL-6- Interleukin 6, TNF- Tumour Necrosis Factor, IFN- Interferon, cGH- Chicken Growth Hormone, IGF-1 - Insulin Growth Factor 1.

4.4 Discussion

This study investigated the effects of partial and full replacement of SBM and FM with the BSFLM on growth performance and immune gene expression in broiler chickens. Our results indicate that the inclusion of BSFLM, as both a partial and a full substitute for SBM and FM, significantly influenced growth and immune-related

gene expression. These findings suggest that BSFLM is a viable alternative protein source, with potential benefits for poultry production.

During the starter phase of the experiment, the significant differences observed in live weight, BWG, and ADG can be attributed to variations in feed intake among the treatment groups. The SBM+BSFLM treatment group showed the highest ADFI, which contributed to the improved growth performance, including higher live weight, BWG, and ADG. Conversely, the BSFLM treatment group had the lowest ADFI, which was directly correlated with significantly lower live weight, BWG, and ADG. These findings align with previous research by Heita et al. (2023), who reported higher feed intake at lower BSFLM replacement levels but a decline at higher inclusions. Similarly, Attivi et al. (2020) observed a significant reduction in feed intake when BSFLM was included at 8% of the diet compared to 6%. Mutisya et al. (2022) reported comparable trends in growth and feed intake during the initial phase of feeding trials, supporting the findings of this study. The results are, however, contrary to those reported by Onsongo et al. (2018), whose study determined no significant difference in LW, BWG, ADG, and ADFI on birds fed diets with different replacement levels of BSFLM at the starter phase. Low feed intake in the starter phase is attributed to the high-fat content of the feed, as depicted by the high-fat content of BSFLM ingredients (21.0%) compared to 8.5% and 2.4% in FM and SBM, respectively. The high fat content in BSFLM treatment groups can be attributed to the high-energy content of the resultant diets, which subsequently resulted in low feed intake as reported by Veldkamp et al. (2024).

The grower phase is considered the most important in the broiler industry as it is the period where the fastest biomass accumulation is realized (Dabbou et al., 2018). Findings from this study suggest that BSFLM can be a viable alternative to FM and SBM in broiler diets, as evidenced by the comparatively higher values realized in the LW, BWG, ADG, and ADFI, alongside a lower FCR. These results are consistent with the findings of Sumbule et al. (2021). BSFLM partially replaced other protein sources, aligning with studies by Fruci et al. (2023), who demonstrated that partial replacement of diets with BSFLM (12.5%, 25%, and 50%) resulted in higher body weights than diets containing 100% BSFLM. Similarly, Facey et al. (2023) reported

lower feed intake, body weight gain, and ADG, along with higher FCR, in birds-fed diets with full BSFLM replacement compared to partial replacements.

The FM+BSFLM achieved the lowest FCR across all phases, indicating superior feed utilization efficiency. Consistent with previous research (Marono et al., 2017; Dabbou et al., 2018; Brah et al., 2024), this study confirms that using BSFLM as the sole protein source in chicken diets generally results in poorer performance than partial replacements. Although the exact cause of the low feed intake in the BSFLM-only diets is not yet understood, Marono et al. (2017) attribute it to the dark colour and less desirable flavour of BSFLM compared to SBM. Cutrignelli et al. (2018) further suggest that high chitin levels in BSFLM may interfere with protein digestibility, leading to reduced nutrient utilization and slower growth. Additionally, there was low feed intake in T5 at the starter phase as compared to the grower and finisher phase due to limited digestive capacity in starter phase as compared to the other phase. The FM+BSFLM performed better than SBM+BSFLM because fishmeal generally has a more balanced amino acid profile and higher levels of essential amino acids such as lysine and methionine than soybean meal.

The carcass, organ weights, and relative weights of the internal organs determined the effect of partial and full replacement of BSFLM in poultry diets on muscle deposition in specific body organs. The results at day 21 of the study showed no significant difference in carcass weight and the relative weight of body organs. Although the measured parameters did not differ significantly, birds in the FM+BSFLM treatment group had higher carcass and liver weights. This implied that at the early stages of broiler development, the FM+BSFLM treatment group led to superior muscle accretion, which was higher compared to other groups, which could be explained by higher feed intake and digestibility. Chicken fed in the SBM+BSFLM treatment group had comparatively high relative weights of heart, spleen, and bursa, although it was not significantly different, and this is an indication of the effect of BSFLM on immunological hyperplasia and hypertrophy (Mohamed et al., 2017). Birds fed on the FM+BSFLM diet had the highest carcass weight at day 42, different from the other treatment groups, which is attributable to more efficient feed utilization in muscle deposition. The carcass weight corresponds to the live

weight as there exists a strong proportionate relationship between the parameters (Seo & Park, 2022). Aprianto et al. (2023) noted that broilers commonly deposit muscle on the breast and thighs. The current study showed greater muscle deposition in the thighs, breasts, and back. The findings align with those of Cullere et al. (2016), who reported that partial or full replacement of conventional protein sources with BSFLM had no significant effects on carcass traits. The absence of significant differences in CBR and RoI as economic indices indicates that BSFLM can be utilized as a sustainable replacement of FM and SBM as protein alternatives. These results are comparable with those reported by Wamai et al. (2024), where there were no significant differences in the CBR and RoI of chicks and growers under partial and full replacement of BSFLM.

With regard to gene expression, the results showed that birds in the FM treatment group had significantly lower expression levels of TNF- α and IFN- γ in liver tissues, as well as IL-2, and IFN- γ in spleen tissues. This downregulation is likely associated with higher levels of omega-3 fatty acids in FM, which are known to exert anti-inflammatory effects. The genes TNF- α , IL-2, and IFN- γ are key cytokines involved in mediating immune responses. Supporting this finding, Magee et al. (2012) demonstrated that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) modulate the synthesis of these cytokines by competing with arachidonic acid, altering membrane structure, and promoting the production of anti-inflammatory eicosanoids. In contrast, the FM+BSFLM, SBM+BSFLM, and BSFLM-only treatment groups showed upregulated cytokine expression. Instead of pathological inflammation, this elevation indicates increased immunological reactivity, as insect protein may have activated the innate immune response, including TNF- α , through its chitin prebiotic-like effects and high lauric acid content (Cardinaletti et al., 2019). Furthermore, a bioactive polysaccharide derived from BSFLM has been shown to initiate immune responses by activating pathogen-associated molecular pattern (PAMP) pathways in macrophage RAW264.7 cells (Nishiguchi et al., 2024). Importantly, this immune modulation has also been reported to exhibit no significant toxicity in livestock and aquatic animals (Wang et al., 2024). The results are also consistent with those of Zarantoniello et al. (2021), who found that diets containing BSFLM significantly upregulated TNF- α expression

in zebrafish fed 75% and 100% BSFLM diets, compared to those fed a control diet with FM. The SBM+BSFLM treatment group showed upregulated immune cytokines, which may be a result of combining both plant and insect proteins. Components of soybeans, such as Lunasin-enriched soybean extracts (LES), have been reported to exhibit immune modulation factors (Paterson et al., 2023). Findings from this study highlight the potential inherent in BSFLM to boost the immunity of broiler chickens.

Additionally, immune-related gene overexpression should be interpreted in the context of immunological balance rather than excessive inflammation, which was not observed phenotypically. Mateos-Hernández et al. (2020) showed that oral administration of an *E. coli* strain with high α -Gal content protected turkeys and chickens against *Aspergillus* spp infection by reducing lung inflammation. Notably, treated birds did not exhibit upregulation of the pro-inflammatory cytokines IL-2, IL-6, and IFN- γ in the lungs. In other words, the probiotic bacteria helped the birds tolerate infection by dampening IL-2/IL-6 mediated inflammatory damage in lung tissue, suggesting that controlling these cytokine responses can mitigate tissue damage during infection, which is highly relevant for maintaining poultry health and production. Similarly, Alizadeh et al. (2021) reported that the in-ovo and oral administration of a cocktail of beneficial *Lactobacillus* bacteria enhanced the immune development of newly hatched chicks. Treated chicks exhibited higher antibody levels, more activated T cells, and upregulated IL-2 and IL-6 expression in lymphoid organs. These results demonstrate that, depending on the tissue type and physiological situation, both cytokine overexpression and downregulation might have beneficial effects.

The elevation of IL-2, IL-6, TNF- α , and IFN- γ in samples obtained from birds fed BSFLM only, BSFLM+FM or BSFLM+SBM diets points to an increase in immune (IL-2, IL-6, TNF- α , IFN- γ) and growth (cGH) markers. The IL-2 and IFN- γ upregulation in birds fed BSFLM+SBM diets suggests a possible synergy of BSFLM and SBM in immunomodulation. The gene expression results may not always directly align with the observed phenotypic outcomes due to the time lag between transcription, translation, and the manifestation of measurable traits (Craig et al.,

2007; Nawata et al., 2010; Sinha et al., 2015; Zheng et al., 2016) as well as variations in receptor sensitivity (Fischer et al., 2022; Nam et al., 2005). Although we cannot completely distinguish the effects of insect meal from decreased consumption, the observed increase of immunological and stress-related genes in T5 may be due to a combination of feed intake and composition. Nevertheless, the upregulation of cGH in chickens fed BSFLM-based diets indicates the suitability of BSFLM as a feed ingredient and its positive impact on growth performance. This is supported by Pearson correlation analysis, which showed a strong positive correlation between cGH expression in the liver and spleen and growth traits such as live weight (LW), body weight gain (BWG), and average daily gain (ADG) (Figure 4.5). Additionally, cGH expression was negatively correlated with feed conversion ratio (FCR), suggesting a role for cGH in enhancing feed utilization efficiency.

Importantly, the relationship between cytokine upregulation and production outcomes appears to be dose-dependent. While partial replacement groups (FM+BSFLM and SBM+BSFLM) showed moderate cytokine upregulation alongside superior growth performance, the 100% BSFLM group (T5) exhibited a distinct pattern. Despite showing the highest upregulation of several cytokines, including liver IL-6 (3.75-fold change compared to 0.58 for FM+BSFLM), birds fed with 100% BSFLM had significantly lower final live weight (1350.50 g versus 2071.60 g for FM+BSFLM), reduced feed intake, and poor feed conversion efficiency. This inverse relationship between cytokine expression and growth performance in T5 suggests that the elevated immune gene expression in this group may represent chronic immune activation or subclinical inflammation induced by nutritional imbalance, rather than beneficial immunomodulation. The high chitin content in 100% BSFLM diets may impair protein digestibility (Cutrignelli et al., 2018), while sustained exposure to high concentrations of insect-derived bioactive compounds could trigger prolonged PAMP-mediated immune stimulation. Collectively, these factors may divert metabolic resources from growth toward immune function, explaining the poor production outcomes despite, or indeed because of, elevated cytokine expression. These findings underscore that the immunomodulatory benefits of BSFLM are optimized at partial inclusion levels, where immune priming occurs without the metabolic costs associated with chronic activation.

Additionally, the black soldier fly (BSF) meal was supplied in a whole, full-fat form; its relatively high chitin content likely acted as an anti-nutritional factor (Schiavone et al., 2017). Chitin is poorly digested by broiler chickens and has been shown to impair nutrient digestibility and availability, particularly protein and energy utilization, at higher inclusion levels of insect-derived feeds (Dabbou et al., 2018; Seyedalmoosavi et al., 2022; Fruci et al., 2023). This reduction in nutrient availability provides a primary nutritional constraint on growth performance when whole BSF is included at elevated levels. In parallel, birds fed diets containing more than 50% whole BSF exhibited sustained upregulation of immune-related genes, including TNF- α , IFN- γ , IL-2, and IL-6, as well as cGH expression in liver and spleen tissues. This gene expression profile indicates concurrent activation of immune and growth-related pathways, suggesting that BSF inclusion can stimulate host defense mechanisms. While such immune activation may be beneficial at moderate levels, prolonged or excessive cytokine expression is often associated with increased maintenance energy and amino acid requirements, as nutrients are preferentially redirected toward immune function rather than somatic growth (Martínez Marín et al., 2023; Peinado-Izaguerri et al., 2024). This metabolic reallocation can limit muscle protein accretion and overall growth efficiency (Maina et al., 2024).

Notably, while cGH expression showed strong positive correlations with growth traits across the dataset, the correlations between pro-inflammatory cytokines (IL-2, IL-6, TNF- α , IFN- γ) and growth performance were weaker and more variable. This pattern is consistent with a nonlinear relationship between immune activation and productivity: moderate cytokine upregulation in partial replacement groups may support growth by enhancing disease resistance and nutrient partitioning, whereas excessive upregulation in complete replacement groups appears to impose metabolic costs that outweigh any immunological benefits. The negative correlation observed between liver IL-6 and certain growth parameters further supports this interpretation, as IL-6, while essential for acute immune responses, can promote catabolic processes when chronically elevated (Fischer et al., 2022).

Although moderate immune activation may improve host defense, overexpression or prolonged expression may indicate subclinical inflammation with associated metabolic costs (Silva et al., 2019). The divergent outcomes observed between partial and complete BSFLM replacement groups in this study support this interpretation. While slaughtered birds across all treatments showed no gross pathological signs of inflammation, the absence of visible lesions does not preclude subclinical or chronic low-grade immune activation, which may manifest primarily through reduced growth efficiency rather than overt tissue damage. The poor growth performance of the 100% BSFLM group, despite elevated immune gene expression, likely reflects this phenomenon—a state where persistent immune stimulation diverts nutrients and energy from anabolic processes. In contrast, the partial replacement groups achieved an apparent balance where immune gene upregulation enhanced host defense capacity without compromising, and possibly supporting, optimal growth. Future research should investigate the threshold at which BSFLM inclusion shifts from beneficial immunomodulation to counterproductive immune stress, potentially through dose-response studies examining both gene expression and metabolic indicators. Phase-feeding techniques, enzymatic treatment of BSFLM to reduce chitin content, or optimized inclusion rates may help maximize immune benefits while avoiding chronic activation. Collectively, the combined effects of reduced nutrient digestibility due to chitin and nutrient diversion toward immune activation offer a biologically plausible explanation for the neutral or negative growth responses reported in broilers fed whole BSF diets, particularly at high inclusion levels (Fruci et al., 2023; Tsementzis et al., 2025).

A central objective in poultry nutrition research is to explain nutrient-responsive mechanistic pathways that can be strategically modulated through macro- and micronutrients, feed additives, and supplements to enhance performance metrics (Sneha et al., 2026). In the present study, the modest upregulation of IGF-1 expression in the liver and spleen of birds fed the BSFLM-only diet suggests that BSFLM has the capacity to stimulate key growth-associated endocrine signals. This observation aligns with findings reported by Zarantoniello et al. (2021), who observed elevated IGF-1 expression in BSFLM-fed groups relative to fishmeal-based controls. Comparable responses have also been reported in fish, where Cardinaletti et

al. (2019) demonstrated significant increases in IGF-1 expression in animals fed diets containing 25% or 50% BSFLM. Although species-specific regulatory differences exist, the consistency of IGF-1 responsiveness across taxa supports a conserved role for BSFLM in nutritional signaling, as shown in the current study. Collectively, these findings suggest that the upregulation of hepatic growth- and immune-related genes may be linked to the physicochemical and immunomodulatory properties of insect-derived feed ingredients (Lee et al., 2023)

CHAPTER FIVE

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Summary

This study has shown that partial replacement of conventional protein sources with BSFLM upregulates genes associated with growth (cGH, IGF-1) and adaptive immunity (IL-2, IL-6, TNF- α , IFN- γ), and correlates with improved production metrics in the FM+BSFLM and SBM+BSFLM treatment groups. However, complete replacement with BSFLM induced elevated pro-inflammatory cytokine expression coinciding with reduced growth, suggesting a dose-dependent threshold beyond which immune activation reflects nutritional stress rather than beneficial immunomodulation.

5.2 Conclusion

In conclusion, partial dietary replacement with BSFLM (combined with FM or SBM) also improves growth performance and feed utilization efficiency of broiler chicken. Adoption of BSFLM at appropriate inclusion rates would improve economic sustainability in the poultry industry, as farmers would use similar financial inputs at no extra cost. BSFLM inclusion also enhances the expression of growth and immune genes, leading to improved productivity and overall well-being of the birds

5.3 Recommendations

Based on the study, it is recommended that farmers, animal production industries, and nutritionists adopt black soldier fly meal as a sustainable alternative to commercial proteins. It is also recommended that partial inclusion be considered over full replacement due to their high chitin content, which may affect feed digestibility, especially during the early growth phases. The government and policy regulators are recommended to promote the use of black soldier fly larvae, as the findings show they can replace conventional feed at no extra cost. Adoption of BSFLM is therefore recommended to modulate gene expression and lead to immune responses in livestock.

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APPENDICES

Appendix I: Approval of Research Proposal and Appointment of Supervisors



**JOMO KENYATTA UNIVERSITY
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16TH OCTOBER, 2023

ROTICH KIPLANGAT VICTOR
C/o SONRAS
JKUAT

Dear Rotich

**RE: APPROVAL OF RESEARCH PROPOSAL AND APPOINTMENT OF
SUPERVISORS**

Kindly note that your MSc. research proposal entitled: **"BLACK SOLDIER FLY (HERMATIA ILLUCENS) LARVAL MEAL FOR BROILER CHICKEN FED: EFFECT ON GROWTH PERFORMANCE, HEALTH AND GENE EXPRESSION."** has been approved. The following are your approved supervisors:-

1. Dr. Isaac M. Osuga
2. Dr. Mathew G. Gicheka
3. Dr. Chrysantus M. Tanga
4. Dr. Jandouwe Villinger

Please be advised that you are expected to publish your research outputs in quality and indexed journals.

Yours sincerely

PROF. LOSENGE TUROOP
DIRECTOR, GRADUATE SCHOOL

Copy to: Dean, SONRAS/cao



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Appendix II: NACOSTI Research Permit License

 REPUBLIC OF KENYA Ref No: 205676	 NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION Date of Issue: 14/April/2025
RESEARCH LICENSE	
	
This is to Certify that Mr. VICTOR ROTICH KIPLANGAT of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kiambu on the topic: Black Soldier Fly (Hermetia illucens) Larval Meal for Broiler Chicken Feed: Effect on Growth Performance, Profitability and Gene Expression for the period ending : 14/April/2026.	
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Appendix III: Publication

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Research Report

Partial insect-based meal inclusion in the diets of Ross® 308 broilers upregulates growth and immune related genes

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ARTICLE INFO

Keywords:
Alternative feed ingredients
Gene expression
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SUMMARY

This work investigated the effects of black soldier fly (*Hermetia illucens* L.) larvae meal (BSFLM) on growth- and immune-related gene expression in Ross 308 broilers. Birds were fed diets containing soybean meal (SBM), fishmeal (FM), combinations with BSFLM, or BSFLM alone. Growth, organ weights, immune genes [interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrotic factor alpha (TNF-α), interferon-gamma (IFN-γ)] and growth-related genes [chicken growth hormone (cGH), insulin growth factor 1 (IGF-1)] expression were measured. Our results demonstrate that birds fed FM+BSFLM had significantly higher final liveweight, average daily weight gain (ADG), and low feed conversion ratio. Birds fed FM+BSFLM showed highest carcass weight, while the SBM-only group yielded the lowest. Average daily feed intake (ADFI) differed significantly, and birds fed SBM+BSFLM had the highest. The IL-2, IL-6, TNF-α, IFN-γ, IGF-1 and cGH gene expressions were significantly upregulated in the liver and spleen of birds fed diets containing BSFLM. Growth-related genes (cGH) positively correlated with liveweight, body weight gain, and ADG. Insect-based diet upregulates the expression of genes associated with protein synthesis, muscle hypertrophy, better feed efficiency, cell proliferation and adaptive immunity, leading to improved productivity in partial replacement groups, while complete replacement showed patterns suggesting nutritional stress.

Description of problem

According to the United Nations, the global population is projected to reach approximately 9.7 billion persons by 2050, which will directly increase the consumption of animal protein (Attia et al., 2024; Bist et al., 2024). Correspondingly, the consumption of poultry and pork meat is expected to grow significantly between 2010 and 2050 (Erdos, 2023). Poultry production is an important source of food for middle- and low-income countries (Carron et al., 2017). It is one of the most efficient feed-to-meat conversion industries (1.5–1.8) (Smil, 2002). Indigenous and exotic chicken breeds are reared in varying production systems. Broiler chicken production is one of the most economical, viable, and important meat sources for human consumption (Giovanni et al., 2021). Globally, populations of broiler and indigenous chicken have increased

between 2006 and 2020, with the former dominating the industry (Bennett et al., 2018).

There has been a continued increase in commercial broiler chicken production in urban and peri-urban regions of Kenya, despite the predominance of indigenous chicken populations. This growth is attributed to the superior ability of broilers to accumulate edible biomass more rapidly (Muganyizi et al., 2012). The production of broiler chicken is constrained by high-priced quality feed, leading to significant shortages and subsequent high costs of poultry products besides stagnation in the sector's expansion. Over the years, protein used in making poultry feeds has been sourced from marine fish whose supply has gradually declined due to overexploitation and increase in prices, which limits the availability of fishmeal as a livestock feed ingredient (Shophord and Jackson, 2013). In light of the increasing cost of conventional feed and

Primary Audience: Poultry farmer; scientist and researchers; industry professionals; policymakers, feed millers and consumers.

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Table 1
The composition of experimental feed fed to broiler chicken.

Ingredient (%)	Starter					Grower					Finisher				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Maize	39.10	55.73	49.52	39.10	39.10	39.10	56.28	48.56	39.10	39.10	39.10	56.28	48.56	39.10	39.10
Pollard	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Maize germ	19.55	10.00	10.00	14.06	23.36	15.40	10.00	10.72	11.29	20.43	19.93	10.00	10.72	16.53	20.34
Soybean	31.33	0.00	0.00	18.23	0.00	32.52	0.00	0.00	18.23	0.00	28.00	0.00	0.00	15.00	0.00
Fishmeal	0.00	20.55	13.18	0.00	0.00	0.00	20.00	13.00	0.00	0.00	0.00	20.00	13.00	0.00	0.00
Wheat bran	1.00	4.00	4.00	1.00	1.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
BSFLM	0.00	0.00	13.58	18.59	27.43	0.00	0.00	14.00	18.40	27.50	0.00	0.00	14.00	16.40	27.59
Salt	0.30	0.30	0.30	0.30	0.30	0.26	0.30	0.30	0.26	0.25	0.25	0.30	0.30	0.25	0.25
DCP	1.20	1.40	1.40	1.20	1.20	1.20	1.40	1.40	1.20	1.20	1.20	1.40	1.40	1.20	1.20
Lime	0.50	0.80	0.80	0.50	0.50	0.50	0.80	0.50	0.50	0.50	0.50	0.80	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.40	0.60	0.60	0.40	0.49	0.40	0.60	0.40	0.40	0.40	0.40	0.60	0.40	0.40	0.40
Toxin binder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Methionine	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
ME (Kcal/Kg)		2999.50				3100.00					3200.00				
CP (%)		23.00				21.50					20.00				

T1 - SBM, T2 - FM, T3 - FM+BSFLM, T4 - SBM+BSFLM, T5 - BSFLM, BSFL - Black soldier fly larvae meal, DCP - Dicalcium phosphate, CP - Crude protein

overexploitation of captured fish resources, there is an urgent need to consider non-conventional, bio-innovative, and sustainable protein sources that are both economically viable and environmentally friendly.

(Chia et al., 2019; Edrisi and Alshilash, 2021).

Materials and methods

Appendix IV: ISERC Ethical Approval



JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
P.O BOX 62000(00200) NAIROBI, Tel: (067) 58700001-4
(Office of the Deputy Vice Chancellor, Research Production and Extension Division)
JKUAT INSTITUTIONAL SCIENTIFIC AND ETHICS REVIEW COMMITTEE

REF: JKU/2/4/896B

Date: 9th October, 2024

ROTICH KIPLAGAT VICTOR
DEPARTMENT OF ANIMAL SCIENCE, JKUAT

Dear Mr. Rotich,

**BLACK SOLDIER FLY (*HERMATIA ILLUCENS*) LARVAL MEAL FOR BROILER CHICKEN FEED:
EFFECTS ON GROWTH PERFORMANCE, HEALTH AND GENE EXPRESSION**

This is to inform you that JKUAT Institutional Scientific and Ethical Review Committee has reviewed and approved your above research proposal. Your application approval number is JKU/ISERC/02317/1399. The approval period is 9th October, 2024 to 8th October, 2025. This approval is subject to compliance with the following requirements:

- i. Only approved documents including (informed consents, study instruments, Material Transfer Agreement (MTA)) will be used.
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JKUAT ISERC.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to JKUAT ISERC within 72 hours of notification.
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to JKUAT ISERC within 72 hours.
- v. Clearance for export of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to JKUAT ISERC.

Prior to commencing your study, you will be expected to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely,


Dr. Amos Mbugua
CHAIR, JKUAT ISERC



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Appendix V: Experimental Birds

