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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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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 (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 meat sources 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 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 (Magothe 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 (Shepherd 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.

	Starter					Grower					Finisher				
Ingredient (%)	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	0.80	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	100.00	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. Furthermore, the intensification of soybean farming has resulted in environmental and social challenges, such as deforestation and land grabbing (Shepherd and Jackson, 2013).

Insect protein is a promising alternative since insects have the potential to sustainably produce biomass. The most promising insects, as a source of protein, include the black soldier fly (BSF) (*Hermetia illucens* L.), yellow mealworm (*Tenebrio molitor* L.), common housefly (*Musca domestica* L.), bluebottle (*Calliphora vomitoria* L.), and blowfly (*Chrysomya* spp.) (Charlton et al., 2015). These insect species have not only been used as feed ingredients but also as stimulants of immune responses (Chaklader et al., 2019; Abdel-Latif et al., 2021). Among these insects, black soldier fly is particularly promising because it upcycles low-quality material into high-quality protein (Xiao et al., 2018). Furthermore, it has been used to degrade organic waste from human systems (Choi et al., 2012) implying that feeding BSFLM is sustainable since this does not portend competition with humans for nutrients. The utilization of BSFLM in poultry nutrition has gained interest due to their availability, nutritional value coupled with their production sustainability (Lu et al., 2022).

Although the nutritional composition of insects is now well documented, information on the innate immune responses and growth factors associated with insect-based feeds for broiler chickens in Africa remains limited. Increasing evidence suggests that body parts of insects, such as black soldier fly larvae (BSFL) contain bioactive compounds capable of stimulating immune responses (Nishiguchi et al., 2024). For instance, Zarantoniello et al., (2021) reported increased levels of immunomodulators, including tumor necrosis factor-alpha (TNF-α), in zebrafish fed BSFLM-supplemented diets. These findings suggest that incorporating BSFL meal (BSFLM) into poultry diets could enhance both growth and immunological outcomes, although the underlying molecular mechanisms require further investigation. Addressing these knowledge gaps, this study investigated the effects of replacing soybean meal (SBM) and fishmeal (FM) with BSFLM in broiler chicken diets, focusing on growth performance and immune response gene expression. The findings provide evidence-based recommendations for the use of BSFLM as a sustainable poultry feed ingredient, supporting improved nutrient utilization, poultry health, and product quality, while contributing to several Sustainable Development Goals (SDGs), including SDG 1 (No Poverty), SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 12 (Responsible Consumption and Production) (Chia et al., 2019; Edrisi and Abhilash, 2021).

Materials and methods

Study area

The feeding trial and bird performance components of this study were conducted at the Jomo Kenyatta University of Agriculture and Technology (JKUAT). The gene expression aspects of the work was carried out at the International Centre of Insect Physiology and Ecology (*icipe*).

Ethical approval

All experimental procedures were conducted in accordance with ARRIVE guidelines and the ethical principles for animal research approved by the Institutional Animal Care and Use Committee (IACUC) of the Veterinary Science Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO; approval no. KALRO-VSRI/IACUC028/16032022). 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), with additional approval from the Institutional Scientific and Ethics Review Committee of JKUAT (JKU/ISERC/02317/1399).

Black soldier fly larvae meal preparation and diets 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 and Valdivié, 2021). 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 slaughter house wastes. The larvae were harvested at their fifth instar and sterilised in 83 °C water and later oven dried at 120 °C for 2 h. Feed ingredients and additives were sourced from 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 and their composition, protein, and energy properties are presented in Table 1.

Diets and protein ingredient analysis

The formulated diets and the protein ingredients were analyzed for their proximate composition before the start of feeding trial. The dry matter content of the diets was determined using an electric oven at 135 °C for two hours while the ash content was determined using a muffle furnace at 550 °C for two 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 contain 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).

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 each (n = 200 chicks total, 10 chicks per replicate, sex-balanced). This replicate number was selected based on facility constraints and aligns with similar broiler trials (Sumbule et al., 2021). After arrival, the chicks were weighed and randomly distributed in 15-square-foot and provided clean drinking water and feed were offered *ad libitum*. The experimental birds were exposed to 24 h lighting on the first three days, 20 h light:4 h darkness from the fourth day and 16 h: 8 h darkness on the last week of the experimental period with a relative humidity of 65 % ± 5 %. The temperature gradually decreased from 34 °C on the first day to 20 °C on the 47th day (Martínez and 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 day 7 and day 28 as well as against Gumboro on day 14 and 21 with the Cevac ND-IBD vaccine according to manufacturer protocol (Ceva Santé Animale, Libourne, France).

Feeding and performance data collection

Daily feed intake was determined by the difference between the offered feed and the 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 calculated as shown in equation 1, 2, and 3 respectively. Two birds (sex balanced) per cage were randomly picked and sacrificed for tissue sampling and organ weight determination on the 21st, 35th and 42nd days. The birds were humanely euthanized in accordance with institutional Institutional Scientific and Ethics Review Committee (ISERC) via cervical dislocation resulting to 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.

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 in the calculation since all other cost, 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 inclusion amount in feed and summing up all ingredients. The prices were determined in Kenyan shillings (KES), which were then exchanged to United state dollar using an exchange rate of (1USD = 130.00 KES) The CBR was calculated as the ratio between the production revenue and the production cost as shown in equation 4. 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 in equation 5 (Onsongo et al., 2018; Sumbule et al., 2021; Maina et al., 2025).

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 min 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 OD_{260}/OD_{280}. 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 min. 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.

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 4) were used. Annealing temperatures were optimized by gradient PCR above and below the estimated melting temperatures (Tm). 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-µl reactions comprising 4 µl HOT FIREPoI

Table 2
Analysed nutrient composition of formulated diets.

Parameter (%)	Starter					Grower					Finisher				
	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)	5567.00	7781.00	8276.00	7522.00	7568.00	5191.00	5988.00	7069.00	6976.00	6900.00	4820.00	7419.00	7570.00	6665.00	7860.00
ME (kcal/kg)	2859.00	2890.00	2816.00	2886.00	2812.00	2983.00	2907.00	2883.00	2934.00	2900.00	3110.00	3187.00	3122.00	3127.00	3179.00

T1 - SBM, T2 - FM, T3 - FM +BSFLM, T4 – SBM+BSFLM T5 - BSFLM, BSFL - Black soldier fly larvae, CP - Crude protein, ADF-Acid detergent fibre, NDF-Neutral detergent fibre, ME-metabolizable energy.

EvaGreen qPCR Mix (Solis BioDyne, Tartu, Estonia), 2 µl cDNA template, 12 µl PCR grade water, 1 µl forward primer, and 1 µl reverse primer. The optimized cycling conditions included initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 55–60 °C for 20 s, and extension at 72 °C for 20 s. 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 $2^{-\Delta\Delta Ct}$ as described by Pfaffl, (2001) using 28S rRNA as the reference housekeeping gene.

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.

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}} \quad (1)$$

Table 3
Analysed nutrient composition of protein ingredients.

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

BSFLM- Black soldier larvae meal, FM- Fish meal, SBM- Soya bean meal

Table 4
Oligonugleotide sequence of target and reference gene primers.

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

IL-2- Interleukin 2, IL-6- Interleukin 6, TNF-α-Tumor necrosis factor alpha, IFN-γ- Interferon gamma, cGH- Chicken growth hormone, IGF-1- Insulin growth factor 1

Table 5
Effect of partial and full replacement of SBM and FM with BSFLM on mean (±SE) growth performance of broiler chiken.

Variables	SBM	FM	FM + BSFLM	SBM + BSFLM	BSFLM	P Values
Starter Phase d1-14						
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
Live weight at day 14 (g)	208.64 ± 7.30^{c}	213.98 ± 8.19^{bc}	234.00 ± 9.19^{ab}	236.14 ± 10.15^{a}	155.06 ± 3.41^{d}	0.001
BWG (g)	169.61 ± 7.43^{b}	177.00 ± 8.35^{ab}	197.12 ± 9.34^{a}	197.91 ± 10.28^{a}	115.81 ± 3.54^{c}	0.001
ADG (g/day)	12.12 ± 0.53^{b}	12.64 ± 0.59^{ab}	14.80 ± 0.67^{a}	14.14 ± 0.73^{a}	8.27 ± 0.25^{c}	0.001
ADFI (g/day)	25.21 ± 0.86^{ab}	23.02 ± 0.93^{b}	24.23 ± 1.07^{b}	27.02 ± 1.29^{a}	17.02 ± 0.72^{c}	0.001
FCR	1.93 ± 0.04^{a}	1.71 ± 0.04^{bc}	1.60 ± 0.04^{c}	1.78 ± 0.04^{abc}	1.90 ± 0.03^{ab}	0.025
Grower Phase d14-21						
Initial weight (g)	208.64 ± 7.30^{c}	213.98 ± 8.19^{bc}	234.00 ± 9.19^{ab}	236.14 ± 10.15^{a}	155.06 ± 3.41^{d}	0.001
Live weight at day 21 (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
BWG (g)	155.95 ± 5.10^{a}	136.14 ± 5.54^{ab}	148.83 ± 5.74^{ab}	164.20 ± 6.56^{a}	120.90 ± 5.33^{b}	0.038
ADG (g/day)	22.28 ± 0.73^{a}	19.45 ± 0.79^{ab}	21.27 ± 0.82^{ab}	23.46 ± 0.94^{a}	17.27 ± 0.76^{b}	0.038
ADFI (g/day)	57.39 ± 2.60^{b}	48.15 ± 2.87^{bc}	48.81 ± 3.23^{bc}	71.59 ± 3.65^{a}	44.71 ± 2.62^{c}	0.001
FCR	2.58 ± 0.08^{b}	2.53 ± 0.09^{b}	2.29 ± 0.09^{b}	3.07 ± 0.09^{a}	2.57 ± 0.09^{b}	0.028
Finisher Phase d21-42						
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
Live weight at day 42 (g)	1187.50 ± 80.02c	1386.10 ± 75.89c	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 ± 3.13c	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-Liveweight, BWG-Body weight gain,ADG-Average daily gain, ADFI-Average daily feed intake, FCR-feed conversion ratio, SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal

$$\text{Individual feed intake} = \text{Offered feed} - \text{Remnant feed}\ldots \quad (2)$$

$$\text{BWG} = \text{Average weight gain at the end} - \text{Average weight at the beginning}\ldots \quad (3)$$

$$\text{CBR} = \frac{\text{Production revenue}}{\text{Production cost}} \quad (4)$$

$$\text{RoI} = \frac{\textit{Production revenue} - \textit{production cost}}{\textit{Production cost}} \times 100\%\ldots \quad (5)$$

Results and discussion

Effects of partial and full substitution SBM and FM with BSFLM on growth performance

The metabolizable energy attained the nutritive requirements of 2800 Kcal/kg, 2900 Kcal/kg, and 3100 Kcal/kg for the Ross 308 starter, grower, and finisher, respectively. The starter, grower, and finisher also attained a minimum of 22 %, 21 %, and 19 % of protein, respectively. The analyzed nutritional compositions of experimental diets and protein ingredients are presented in Tables 2 and 3, respectively. The slight variations in the analyzed chemical compositions of the diets has been documented (Vasilopoulos et al., 2024). This is attributed to several factors such as ingredient processing and origin (Lee et al., 2016).

At the starter phase of the experiment, the live weights (LW) taken on day 1 were not significantly different across 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 recorded in the starter phase ($p < 0.05$, Table 5). 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

Table 6
The effect of full and partial replacement of SBM and FM with BSFLM in broiler chicken diets on the mean weights of carcass, thigh, back, breast, wing and relative weights of internal organs.

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

SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal

the lowest FCR value at 1.60, while the SBM-only diets had the highest FCR value at 1.93. At this phase significant differences observed in live weight, BWG, and ADG can be attributed to variations in feed intake across 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 demonstrated the lowest ADFI (17.02 g/day), directly correlating with the 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. There was an observed significant reduction in feed intake when BSFLM was included at 8 % of the diet compared to 6 %. Heita et al., (2023) 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 %) compared to 8.5 % and 2.4 % in FM and SBM, respectively. The high fat content can be reduced by defatting of the black soldier fly larvae meal. The high fat content in the BSFLM treatment groups is a direct reflection of high-energy content of the resultant diets, which can subsequently result in low feed intake as reported by (Veldkamp et al., 2024).

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 resulted in the highest means of these parameters, while the BSFLM-only group had the lowest means. 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 were significantly different but pairwise comparisons across treatments were not always statistically significant. This can be attributed to individual bird variation, potential variations in feed digestibility or interactions between soybean meal and BSFLM components that influenced feed utilization. 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., 2018a). Findings from this study suggest that BSFLM can be a viable alternative 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). The BSFLM at 50 % can substitute fishmeal and soybean meal, aligning with studies by Fruci et al., (2023), who demonstrated that incoporating BSFLM at 12.5 %, 25 %, and 50 % led to higher body weights compared to 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.

During the finisher phase, the LW, BWG, ADFI, ADG, and FCR at day 42 were significantly different ($p < 0.001$) 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 5). Their respective FCR values significantly ($p < 0.001$) differed. For instance, the FM+BSFL treatment group realized the lowest FCR value at 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 respectively lowest in the SBM-only treatment group at 1187.50 g, 1148.47 g, and 27.34 g/day. 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 ($p = 0.001$). The low FCR in the finisher phase indicates very high growth efficiency in the finisher phase due to controlled experimental conditions. Generally, the FM+BSFLM-based experimental diets resulted in better performance across broiler growth phases. The results of the current study showed that FCR across all phases was lowest when FM was combined with BSFLM hence indicating a possible superior feed utilization efficiency. Consistent with previous research (Marono et al., 2017; Dabbou et al., 2018a), this study confirms that using BSFLM as the sole source of protein in chicken diets generally results in poorer performance compared to partial replacements (Brah et al., 2024). 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 color and less desirable flavor 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.

The carcass and relative internal organ weights did not differ significantly at day 21 ($p > 0.05$). However, there was a general trend of the diets with partial replacement resulting in higher mean values of measured parameters when compared to those with full replacement as shown in Table 6. 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 ($p > 0.05$, Table 6). 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. This implied that at the early stages of broiler development, the FM+BSFLM treatment group led to superior muscle accretion, which was higher as compared to other groups, which could be explained by higher feed intake. 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 BSFLM on immunological hyperplasia and hypertrophy. This 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 and Park, 2022). Aprianto et al., (2023) noted that broilers commonly deposit muscles on the breast and thighs. The current study showed greater muscle deposition in the thighs, breasts, and back. Birds in the FM+BSFLM treatment group had heavier organ weights, although these differences were not statistically significant ($p \leq 0.05$). 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.

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 7). The absence of significant differences in CBR and RoI as economic indices indicates that BSFLM can be utilized as sustainable replacements 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.

Table 7
Economic impact of partial and full replacement of SBM and FM with BSFLM on production cost.

Variables	SBM	FM	FM + BSFLM	SBM + 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

SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal, CBR-Cost benefit ratio, ROI- Return on investment, Sale of birds – USD/bird based on Kg bodyweight.

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 change depicted several

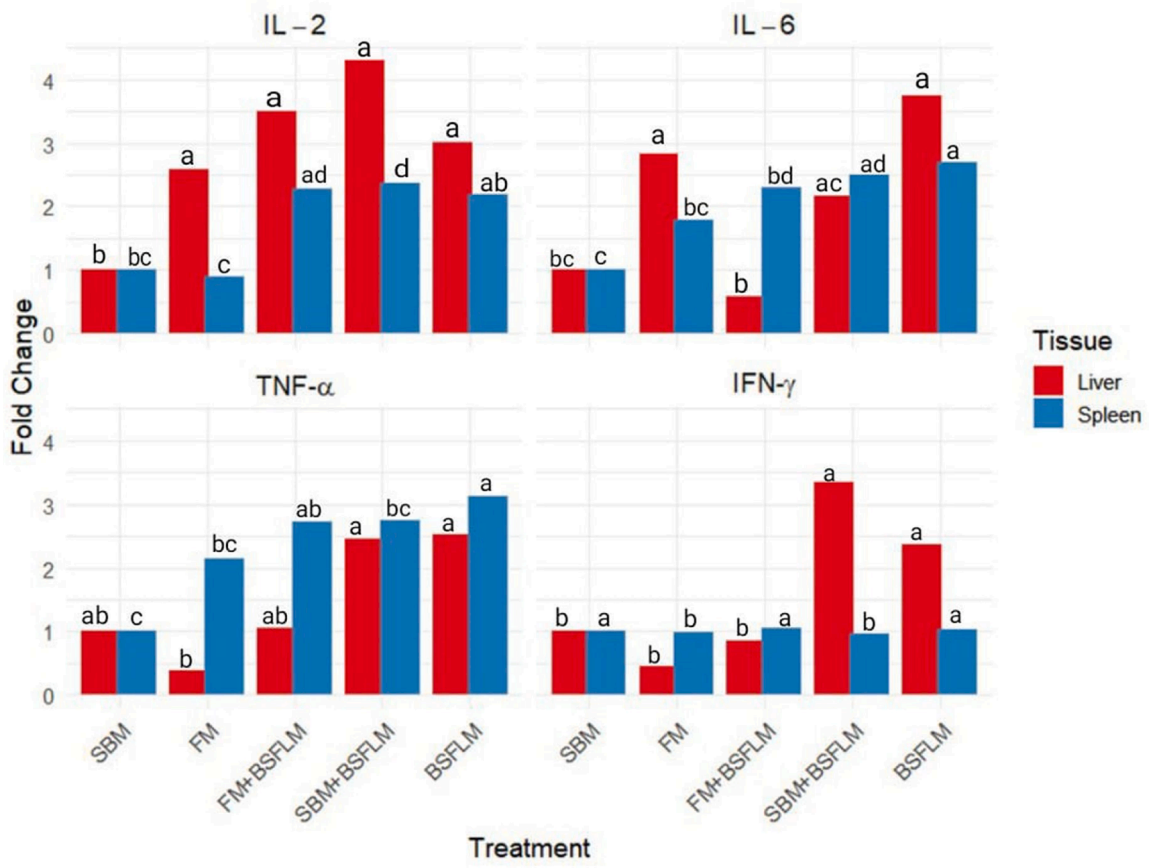


Fig. 1. Relative expression (Fold change) of immune-related genes in the liver and spleen tissues of Ross 308 broiler chicken fed diets enriched with black soldier fly larvae meal. SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal. IL-2-Interleukin 2, IL-6- Interleukin 6, TNF-α Tumor necrosis factor alpha, IFN-γ Interferon gamma.

patterns of upregulation and downregulation of the target genes in the liver and spleen tissues (Figs. 1 and 2). Kruskal-Wallis tests indicated significant differences ($p < 0.001$) in the fold change of the immune and growth-related genes among dietary treatments. Dunn's test with Bonferroni adjustment grouped dietary treatments as shown in (Table 8). Principal component analysis for both immune (Fig. 3) and growth-related genes (Fig. 4) showed that dietary treatments were well separated along PC1 and PC2 indicating a significant effect of the diets on gene expression. The Pearson correlation between the immune and growth-related gene expression and the growth traits are presented in Fig. 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 (0.65). However, the genes had a negative correlation with feed conversion ratio ($r = -0.24$, $r = -0.39$) respectively. 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 ($r < 0$) (Fig. 5).

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 since insect protein may have activated the innate immune response of 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 understood in relation to immunological balance rather than excessive inflammation. 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* infection by reducing lung inflammation. Notably, treated birds did not exhibit the upregulation of 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

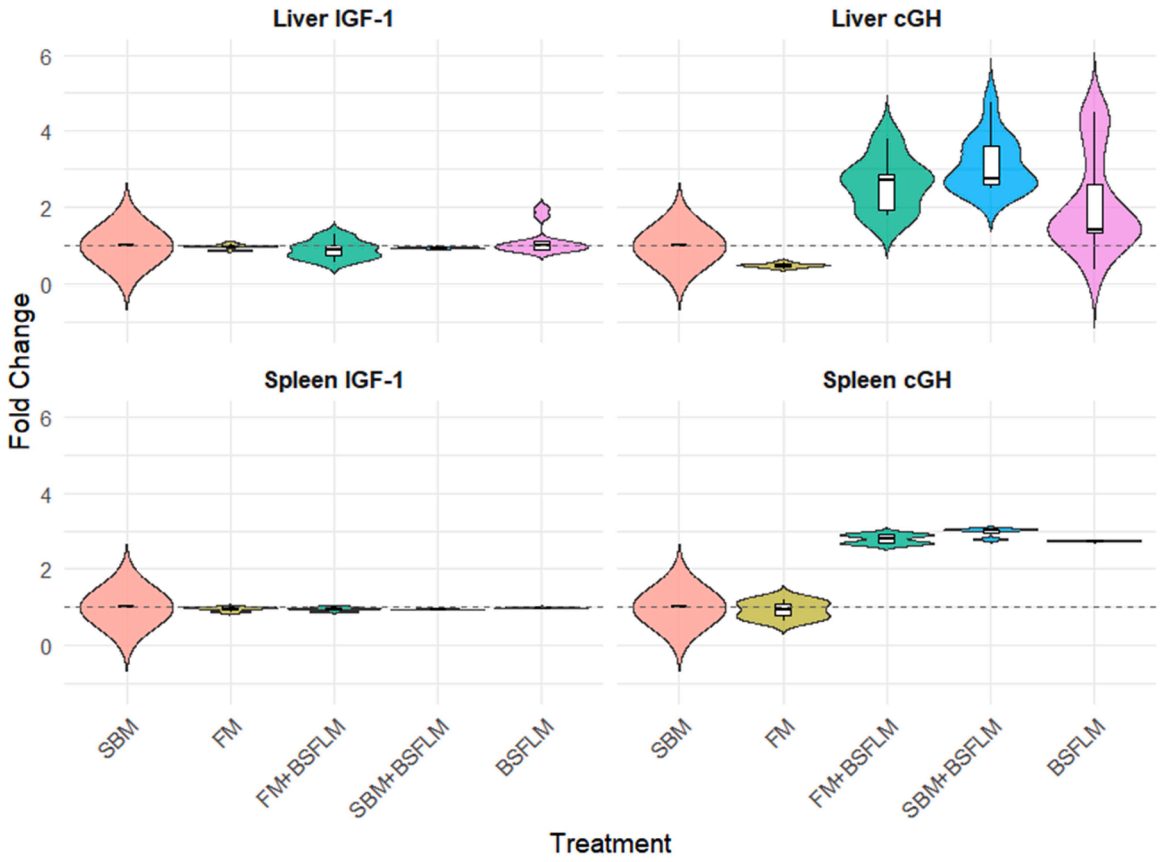


Fig. 2. Violin and box plot of distribution of growth-related gene expression in liver and spleen tissues of Ross 308 broiler chicken fed diets enriched with black soldier larvae meal. SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal, cGH- Chicken growth hormone, IGF-1- Insulin growth factor 1.

Table 8
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 Tumor necrosis factor alpha, IFN-γ Interferon gamma, ε^2-effect size

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 expression of IL-2 and IL-6 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 (Nam et al., 2005; Fischer et al., 2022). 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

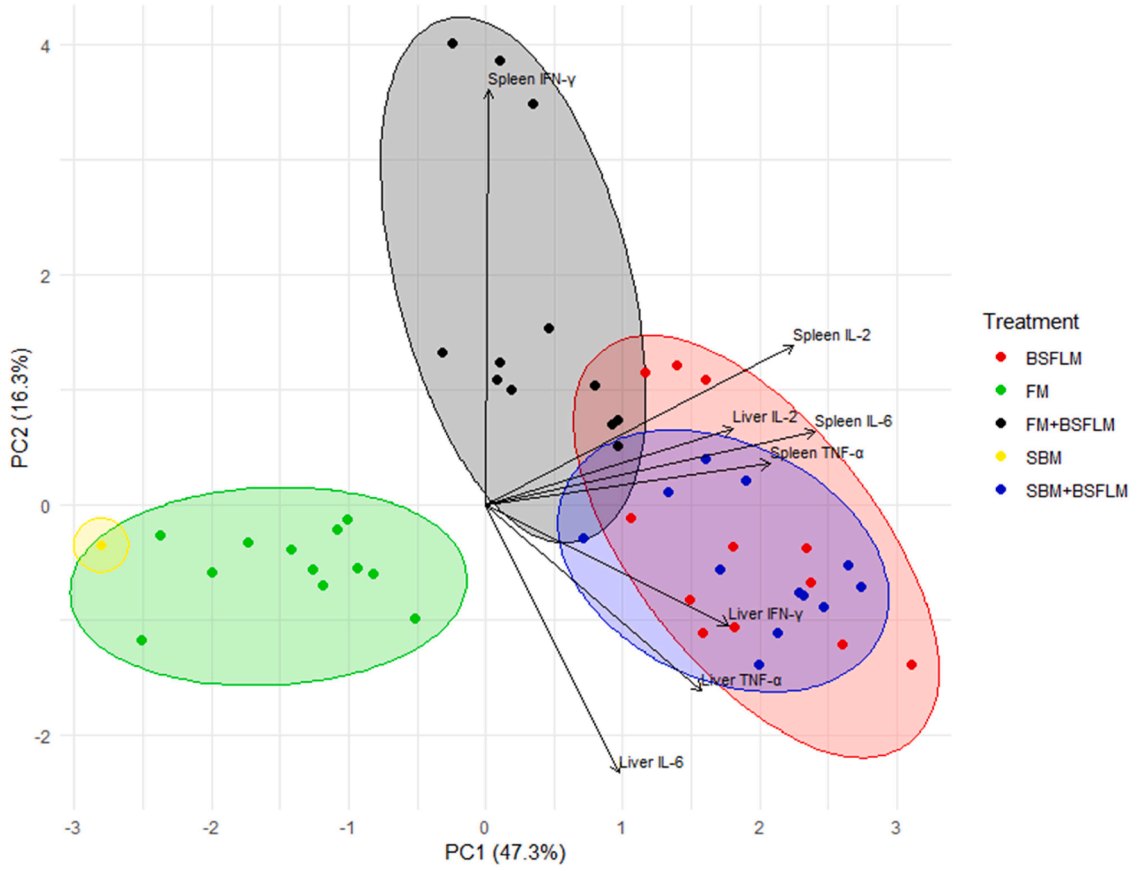


Fig. 3. Principal component analysis (PCA) biplot of immune-relate gene expression in liver and spleen tissues of Ross 308 broiler chicken fed diets enriched with black soldier larvae meal. SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal, IL-2, Interleukin 2, IL-6- Interleukin 6, TNF-α Tumor necrosis factor alpha, IFN-γ Interferon gamma.

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) (Fig. 5). Additionally, cGH expression was negatively correlated with feed conversion ratio (FCR), suggesting its role 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) demonstrated moderate cytokine upregulation alongside superior growth performance, the 100 % BSFLM group (T5) exhibited a distinctly different 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. 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 a sustained upregulation of immune-related genes, including TNF-α, IFN-γ, IL-2, and IL-6, alongside 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 through enhanced disease resistance and nutrient partitioning, whereas excessive upregulation in complete replacement

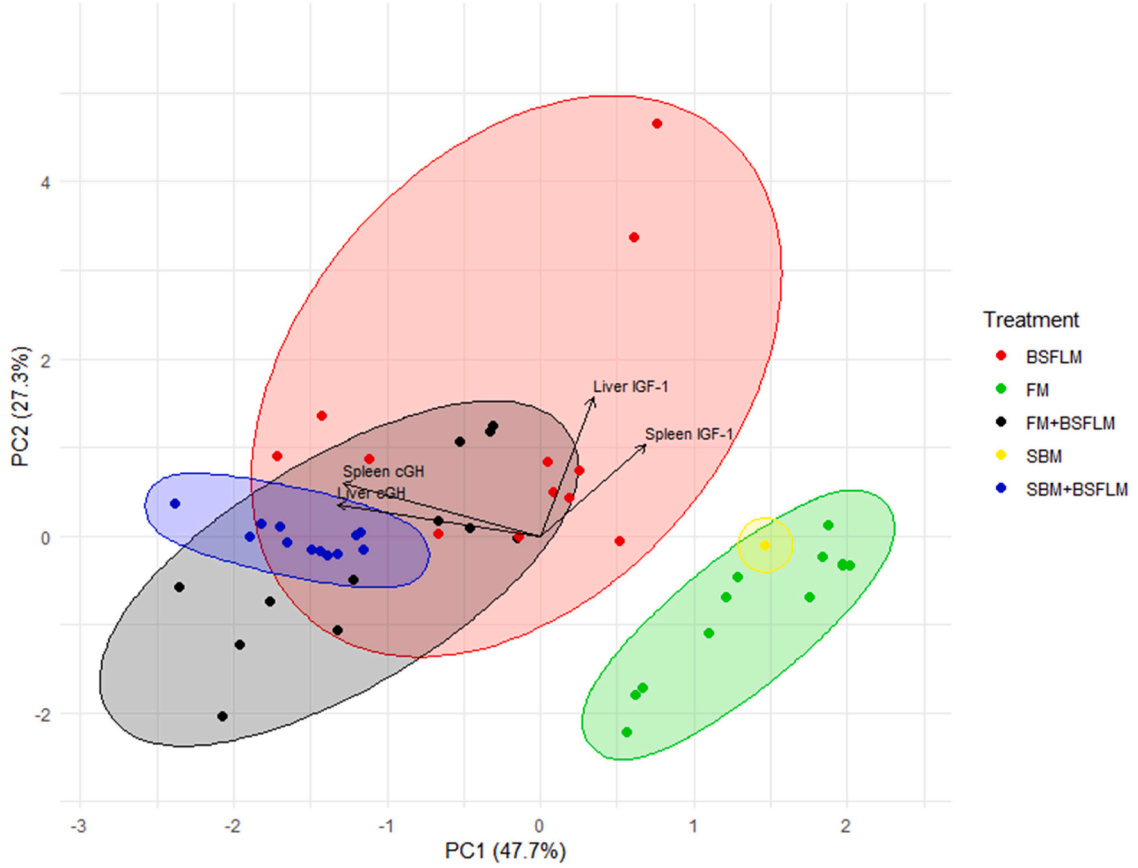


Fig. 4. Principal component anlysis (PCA) biplot of growth-related gene expression in liver and spleen tissues of Ross 308 broiler chicken fed diets enriched with black soldier fly larvae meal. SBM-Soya bean meal, FM-Fishmeal, BSFLM- Black soldier larvae meal, cGH- Chicken growth hormone, Insulin growth factor 1.

groups appears to impose metabolic costs that override 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 might be a sign of 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 further investigate the threshold at which BSFLM inclusion transitions 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 nutritional signaling role for BSFLM 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).

Conclusions and applications

1. 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), correlating with improved production metrics in 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

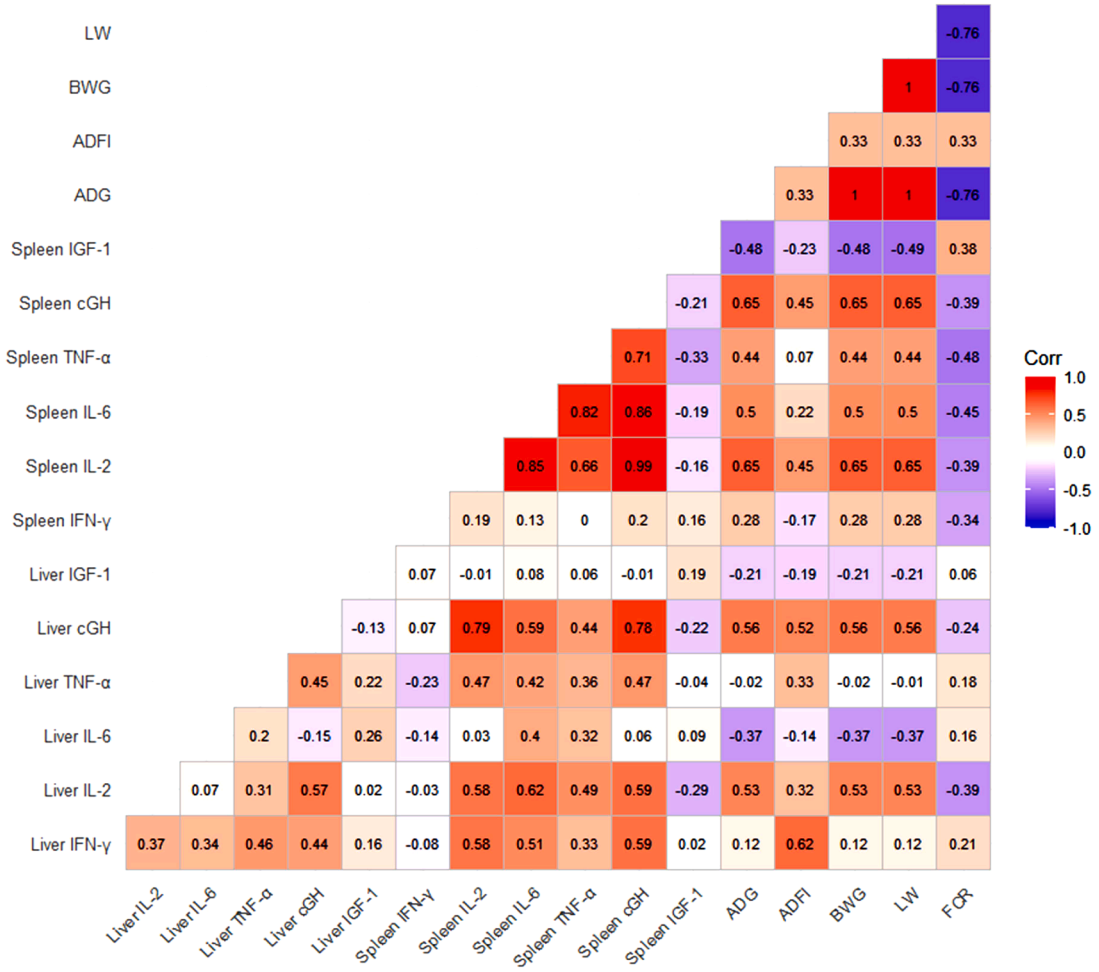


Fig. 5. Heatmap of Pearson correlation matrix between immune/growth-related gene expression and growth performance traits of Ross 308 broiler chicken fed diets enriched with black soldier fly larvae meal. LW-liveweight, BWG- Body weight gain, ADG- Average daily gain, ADFI- Average daily feed intake, FCR- Feed conversion ratio, IL-2- Interleukin 2, IL-6- Interleukin 6, TNF-α Tumor necrosis factor, IFN-γ Interferon gamma, cGH- Chicken growth hormone, Insulin growth factor 1.

which immune activation reflects nutritional stress rather than beneficial immunomodulation.
2. Partial dietary replacement with BSFLM (combined with FM or SBM) improves growth, feed utilization efficiency, and immune gene expression in poultry, while complete replacement compromises growth performance despite activating immune pathways.
3. Insect-based poultry feed at optimized partial inclusion levels should be considered as a promising alternative source that balances growth promotion with immune enhancement.
4. Adoption of insect-based feed innovation at appropriate inclusion rates would improve economic performance in the poultry industry and livelihoods of smallholder farmers in rural and peri-urban settings.

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Data availability statement

All data supporting the findings of this study have been deposited in an open-access repository and are available at the following link: https://data.mendeley.com/datasets/dzzycb38nm/2.

CRediT authorship contribution statement

Victor K. Rotich: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Isaac M. Osuga:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Mathew G. Gicheha:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Shaphan Y.**

Chia: Writing – review & editing, Visualization, Validation, Supervision, Methodology, Data curation. **Jandouwe Villinger:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Anderson N. Maina:** Writing – review & editing, Visualization, Validation. **Jinhua Xiao:** Writing – review & editing, Visualization, Validation. **Dennis Beesigamukama:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision. **Chrysantus M. Tanga:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

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