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# Hematological and Biochemical Indices and Performance of Sasso $T_{44}$ Chicken Fed on Sweet Lupine-Based Formulated Feed

**Belayneh Bazie Tiruneh[1,2]*, Yeshambel Mekuriaw Chekol[1], Bimrew Asmare Limenih[1], and Semahegn Yilkal[1]**

[1]*College of Agriculture and Environmental Sciences, Department of Animal Sciences, Bahir Dar University, P.O.Box: 79, Bahir Dar, Ethiopia*
[2]*College of Agriculture, Department of Animal Science, Injibara University, P.O. Box: 40, Injibara, Ethiopia*

*Corresponding author's Email: bbazie21@gmail.com

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## ABSTRACT

Protein sources are essential components of poultry diets, with Soybean meal (SBM) being the most widely used due to its high protein content and balanced amino acid profile. However, its increasing cost and limited availability have encouraged the search for alternative plant protein sources such as sweet lupine (*lupinus angustifolius*). Dietary protein plays a crucial role in growth, performance, and blood profile parameters in determining the overall effectiveness of dietary treatments. Hematological and biochemical indices are valuable indicators of the physiological condition, metabolic activity, and health status of poultry. The current study aimed to evaluate the effect of replacing SBM with sweet vitabor lupine (SVL) in Sasso $T_{44}$ dual-purpose chicken diets on growth performance and blood hematological and biochemical indices. A total of 180 chickens with five dietary treatments (Ts) were used. SBM was replaced by SVL at 0% (T1, control), 15% (T2), 25% (T3), 30% (T4), and 50% (T5). Each treatment had three replicates of 12 chickens and was fed for 90 days. Blood hematological parameters and biochemical indices of experimental chickens were measured on day 90. Results revealed that increasing SVL inclusion significantly affected the hematological, biochemical, and growth performance of experimental chickens. Live weight, carcass weight, and dressing percentage were significantly improved in T5 chickens compared to T1, T2, T3, and T4, indicating enhanced production performance with SVL inclusion. However, feed conversion efficiency remained statistically unchanged across treatments. Compared with the control diet (T1), chickens fed the highest SVL level (T5) had higher red blood cell counts and hemoglobin concentrations; however, despite the hematocrit value (36.18%) remaining within the normal standard reference range (25-45%), it was significantly lower. Diets T4 and T5 also resulted in higher total protein and lower uric acid and creatinine levels relative to the control, while glucose and triglycerides were unaffected; however, cholesterol was higher in T5. Overall, the results of the present study suggest that SVL may serve as a potential alternative plant protein source to partially replace SBM in diets of Sasso $T_{44}$ dual-purpose chickens, with no observable negative effect on growth performance and almost all hematological and biochemical parameters.

**Keywords:** Biochemical indices, Hematological parameter, Growth performance, Sasso $T_{44}$ chicken, Soybean meal, Sweet lupine

## INTRODUCTION

Genetically improved chicken breeds have been selectively developed for either meat (broiler) or egg (layer) production, requiring intensive nutritional and health management to fully realize their genetic potential (FAO, 2014). On the other hand, dual-purpose breeds are designed to serve both functions; egg and meat production, although achieving this balance often involves nutritional and performance trade-offs (Wilson et al., 2023). Compared to specialized breeds, dual-purpose chickens generally exhibit moderate growth rates and egg production, making it challenging to formulate diets that adequately support both traits without compromising one. Therefore, appropriate nutritional strategies are essential to optimize productivity in such breeds (Wilson et al., 2023). Feed remains a critical factor in poultry production, accounting for approximately 65 to 75% of total production costs (Kim et al., 2022), while growth performance and hematological characteristics are influenced by both dietary composition (Das et al., 2025) and genetic factors (Tiemann et al., 2020).

The poultry industry relies heavily on conventional feed ingredients such as maize and soybean meal (SBM) as primary sources of energy and protein. However, constraints such as increasing cost, fluctuating supply, and limited availability of SBM driven by climatic variability, rising global demand, and disruptions in international trade pose significant challenges to sustainable poultry production (David et al., 2024; Jiang, 2025). Such constraints have intensified the search for cost-effective, locally available alternative protein sources.



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Non-oilseed grain legumes, including sweet lupine, are recognized as promising partial substitutes for soybean meal due to their substantial protein content, essential amino acids, and energy value. Advances in the development of low-alkaloid lupine cultivars have further enhanced their suitability as vegetable protein sources in animal feeding systems (Andrianova et al., 2019). In this context, sweet lupine (*Lupinus angustifolius*) has gained considerable attention due to its high protein content, balanced amino acid profile, and adaptability as a plant-based protein source in poultry diets (Ayeni et al., 2024; Tiruneh et al., 2025). Sweet lupine is particularly relevant in countries like Ethiopia, where the scarcity and high cost of conventional protein feed ingredients necessitate the evaluation of alternative locally available feed resources (Bachano, 2021).

Dietary composition plays a crucial role in maintaining the physiological and metabolic status of poultry. Hematological and biochemical parameters are widely used as reliable indicators of health, nutritional adequacy, and metabolic function of chicken, especially when evaluating novel feed ingredients (Toghyani et al., 2010); however, these values may vary depending on breed, age, sex, nutrition, environmental conditions, and laboratory methods (Thrall et al., 2012). Balanced diets support immune and antioxidant defenses, whereas nutrient imbalances or anti-nutritional factors may alter blood profiles, reflecting physiological stress or organ dysfunction (Etim et al., 2014). Sweet lupine, in particular, has been associated with improved immune response, antibody production, and disease resistance in poultry (Sufiriyanto et al., 2019). Therefore, the present study aimed to evaluate the potential of sweet lupine as an alternative protein source in the diets of Sasso $T_{44}$ dual-purpose chickens by assessing its effects on feed intake, growth performance, hematological parameters, and serum biochemical indices as indicators of nutritional adequacy and overall physiological health.

## MATERIALS AND METHODS

### Ethical approval

In the current study, chicken handling and experimental procedures were conducted in accordance with the ethical standards for the care and use of experimental animals approved by Bahir Dar University Institutional Review Board of Research Ethics Committee (BDUIRBREC /1207335), dated April 2, 2024, Ethiopia. Adequate measures were taken to minimize pain, stress, and discomfort to the experimental chickens throughout the study period.

### Experimental design, ingredient inclusion rate, and treatment layout

A completely randomized design (CRD) was used in the present study. A total of 180 Sasso $T_{44}$ dual-purpose chickens were randomly assigned to five dietary treatments, each with three replications. Each replication consisted of 12 chickens, resulting in 36 chickens per treatment group. Chickens were randomly allocated to the treatment groups using a simple randomization procedure to ensure equal probability of assignment to each treatment. Regarding the experimental treatments, rations of adequate quantity and appropriate quality were formulated and prepared, and the treatment groups were established according to the predetermined experimental layout.

### Feed ingredients preparation

Both macro and micro feed ingredients were collected from different sources, where they easily available and these include maize grain, wheat short, noug seed cake, soybean meal and finger millet, and the cultivar sweet vitabor lupine as macro ingredients, and limestone, meat and bone meal, vitamin premixes, and salt as micro ingredients, which bought from Mulie animal feed processing and manufacturing industry, Ethiopia. Maize grain, finger millet, and sweet lupine were slightly roasted in a flat griddle heated from 140 to 150 ($^{\circ}$C) by a burned wood flame for five to seven minutes until a part-brownish color was observed for maize grain and sweet lupine. However, the other ingredients, such as soybean meal (SBM), noug seed cake (NSC), and wheat shorts, were not roasted since they were already processed from feed processing industries. The macro ingredients were milled by a grinder (miller) to reduce the particle size, which in turn is thought to increase the palatability and digestibility of feed.

Feed ingredients were processed at Zenzelma Dairy and chicken feed processing plant, Bahir Dar University, Ethiopia. The macro-ingredients were first ground and subsequently mixed using a mechanical mixer with adjustable settings to ensure that the experimental diets were isocaloric and isonitrogenous. In contrast, micro-ingredients were incorporated manually in accordance with the recommended inclusion levels to achieve uniform distribution.

### Chemical analysis of feed ingredients and experimental treatments

Representative samples of each ingredient and experimental treatment were analyzed in triplicate for dry matter, ether extract, crude fiber, and crude ash following the procedures described by AOAC (1990). The nitrogen content of

the samples was measured using the Kjeldahl method, and crude protein (CP) was estimated by multiplying the nitrogen value by a factor of 6.25. The Metabolizable energy (ME) of the experimental diets was subsequently estimated using the following formula prediction equation proposed by Wiseman (1987).

$$ME\left(\frac{kcal}{kg}\text{Dry matter}\right) = 3951 + 54.4EE - 88.7CF - 40.8\ Ash \quad \text{(Formula 1)}$$

**Experimental diet formulation**

The diets have been formulated using linear programming through WinFeed (2.8 Demo version) feed formulation software to ensure that the experimental diets meet the intended nutrient requirements of experimental chickens and provided a more rigorous basis for comparison among treatments. Regarding the preparation of the experimental diets, both major and minor feed ingredients were procured from various readily accessible sources. The major ingredients included maize grain, wheat shorts, noug seed cake, soybean meal, finger millet, and the cultivar sweet vitabor lupine (SVL), while the minor ingredients consisted of limestone, meat and bone meal, vitamin premixes, and salt, which were purchased from a private company in Ethiopia called Mulie Animal Feed Processing and Manufacturing Industry.

**Experimental chickens and management**

A total of 180 day-old Sasso $T_{44}$ dual-purpose chickens were used in this experiment. The chickens were randomly assigned to five dietary treatment groups, each comprising three replicates (12 chickens per replicate). After the chickens arrived at the experimental site, different vaccinations supplied by Kemin Industries, Inc., Des Moines, Iowa, USA, were administered according to the standard poultry health schedule. Newcastle disease (HBI strain) at 7 days of age, Newcastle disease (Lasota strain) at 21 days, and fowl pox at 28 days. Furthermore, the chickens were routinely monitored for any signs of internal or external pathological conditions or other abnormalities, and appropriate prophylactic or therapeutic measures were undertaken in accordance with prevailing veterinary recommendations. Apart from the ingredients listed in the diet formulation, no additional medications, antibiotics, growth promoters, or other feed additives were included in the experimental diets. The chickens were fed *ad libitum* from day 1 to day 90. Cumulative body weight and weight gain were recorded to monitor growth performance, whereas blood hematology and serum chemical indices were recorded at the end of the feeding trial period (day 90).

The experimental chickens were reared under standard management conditions in a well-ventilated house. Each treatment group was allocated to pens measuring l.5 × $2m^2$, equipped with a separate feeder and drinker per pen. The ambient temperature of each pen was adjusted and maintained by hanging a 60-watt bulb during the first 3 weeks. Chickens were brooded under supplemental heat immediately after placement. The brooding temperature was maintained at approximately 30˚C during the first week of age and then gradually reduced by about 2 to 3˚C per week as the chickens grew and feather development progressed. Accordingly, the temperature was maintained at 28˚C in the second week, 26˚C in the third week, and 23˚C in the fourth week. During the experimental period, the chickens were managed under a controlled lighting program. Both natural daylight and supplemental artificial lighting were used to maintain the required photoperiod in the experimental house.

A photoperiod of 23 hours of light and 1 hour of darkness (23L: 1D) was provided during the first week of age to stimulate early feed and water intake and help chickens adapt to the rearing environment. After the brooding period, the lighting schedule was gradually adjusted to 20 hours of light and 4 hours of darkness (20L: 4D; Aviagen, 2018). Clean water and feed were provided *ad libitum*, and proper hygiene and biosecurity measures were strictly followed throughout the experimental period.

**Measurements of experimental variables**

The present study evaluated key response variables, including growth performance (feed intake, body weight gain, and feed conversion ratio), hematological and biochemical indices to assess physiological and metabolic status, and carcass characteristics to determine dressing percentage and relative organ weight. Dressing percentage (DP), calculated as the carcass weight divided by the live weight.

**Growth performance**

The growth performance of experimental chickens was evaluated through measurements of feed intake, body weight gain, and feed conversion ratio (FCR) throughout the experimental period. Feed intake was recorded on a pen basis. A known quantity of feed was offered, and refusals were measured on an as-fed basis to each pen daily, while feed intake was subsequently calculated and expressed on a dry matter basis to eliminate variation associated with moisture content among experimental diets. Mean daily dry matter intake per chicken was then computed by Formula 2.

$$\text{Mean dry matter}\frac{\text{Intake}}{\text{Chicken}} = \frac{\text{Feed offered-residue}}{\text{Number of chickens}} \quad \text{(Formula 2)}$$

Feed intake was recorded on a pen basis throughout the experimental period. The amount of feed offered to each pen was weighed daily, and the refusals were collected and weighed the following day. Daily feed intake for each pen was calculated as the difference between the amount of feed offered and the refusals. Mean daily dry matter intake per chicken was then determined by dividing the total pen feed intake by the number of chickens present in the pen during the respective period.

In the present study, body weight measurements were taken on a pen (group) basis rather than individually. All chickens within each pen were weighed collectively using a digital weighing scale, and the average body weight per chicken was then calculated by dividing the total pen weight by the number of chickens in the pen. Body weight measurements were taken using a digital weighing balance with appropriate precision. All chickens were weighed at the beginning of the experiment and subsequently at weekly intervals until the end of the experimental period. Chickens were weighed in the morning before feeding to minimize variation due to gut fill. The average body weight gain was calculated by the following formula.

$$\text{Average body weight gain (ABWG)} = \frac{\text{Final body weight-Initial body weight}}{\text{Number of experimental days}} \quad \text{(Formula 3)}$$

Feed conversion ratio (FCR) was used as an indicator of feed utilization efficiency during the experimental period. It was calculated on a pen basis by dividing the total dry matter feed intake by the corresponding body weight gain of the chickens over the same period. Thus, FCR was computed using formula 4.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry matter feed intake (g)}}{\text{Total body weight gain (g)}} \quad \text{(Formula 4)}$$

**Sample collection for hematological and biochemical analysis**

After the feeding experiment (day 90), six chickens per treatment were utilized for hematological and biochemical analysis. Blood samples were collected through the wing vein using sterile disposable 5 mL syringes fitted with 23-gauge needles, following standard blood collection procedures as described by Parasuraman et al. (2010). Approximately 3 mL of blood was collected from each chicken. Of this, 1.5 mL was transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for hematological analysis, while the remaining 1.5 mL was placed into plain tubes without anticoagulant to allow serum separation for biochemical analysis. Samples were kept on ice and transported immediately to the laboratory. Serum was separated after centrifugation at 3,000 rpm for 10 minutes (Hettich, Tuttlingen, Germany) and stored at -20˚C until analysis.

Hematological measurements, including hemoglobin concentration, packed cell volume (PCV), and counts of red and white blood cells (RBC and WBC), were performed using a BC-2800Vet Automated Hematology Analyzer (Mindray, Shenzhen, China). Serum biochemical parameters were analyzed using commercial diagnostic kits (Randox Laboratories, Crumlin, UK), and absorbance was read with a UV-Visible Spectrophotometer (Shimadzu, Kyoto, Japan). Blood smears were prepared immediately after collection from the EDTA-anticoagulated samples. A small drop of blood was placed on a clean glass slide and spread thinly using the edge of another slide at an angle of approximately 30-45˚. The smears were then air-dried at room temperature, fixed in methanol for 2-3 minutes, and subsequently stained using Wright's stain following standard hematological procedures. The stained smears were examined under a light microscope at 1000 times magnification with oil immersion to evaluate erythrocyte morphology, leukocyte differential counts, and other hematological characteristics.

**Carcass evaluation**

At day 90, the completion of the feeding trial, six chickens per treatment (n = 6) with a total of 30 chickens used for hematological and biochemical analysis were also used for carcass evaluation. Each chicken was weighed, then fasted for 12 hours before being humanely slaughtered by severing the jugular vein. Prior to exsanguination, the chickens were humanely handled and manually restrained, but no electrical or mechanical stunning was applied. The method of slaughter was performed in accordance with standard poultry handling procedures, ensuring minimal stress to the chickens during the terminal procedure. The carcasses were briefly immersed in hot water boiled at 100˚C for approximately one minute to facilitate feather removal. Following slaughter, the carcasses were allowed to cool at room temperature for a short period (approximately 30 minutes) before dissection. The cooling period was intended to allow the muscles to firm up prior to weighing and cutting, thereby minimizing immediate postmortem changes that could affect carcass and cut weights. Subsequently, slaughter weight, carcass weight, dressing percentage, and the weights of major cuts, including thigh, drumstick, breast, back, wings, neck, and skin, were recorded.

**Statistical analysis**

Data for the key experimental parameters were analyzed using a one-way analysis of variance (ANOVA) with SAS software (version 9.4). Differences between treatment means were separated using the Tukey range test at a 5% significance level. Prior to statistical analysis, the assumptions of normality and homogeneity of variance were verified. The normality of residuals was assessed using the Shapiro-Wilk test, and homogeneity of variances among treatment groups was evaluated using Levene's test. As these assumptions were met ($p > 0.05$), the data were subsequently analyzed using the following model. The model used for the experiment was as follows.

$$Yi = u + Ti + ei$$

Where Yi is the observed response variable of chickens in the $i^{th}$ treatment, $\mu$ is the overall mean, $T_i$ represents the fixed effect of the $i^{th}$ treatment, and $e_{ie}$ is the random error associated with the observation.

## RESULTS AND DISCUSSION

**Chemical compositions of feed ingredients and experimental treatments**

The proximate compositions of the feed ingredients used for diet formulation (Table 1) were analyzed for DM (Dry matter), CP, CF (Crude fiber), EE (Ether extract), and crude ash. The CP, which was highest in SBM (42.6%) followed by SVL (35.9%) and NSC (31.9%), while the lowest CP was observed in Finger millet (9.5%) and Maize (12.2%). The EE was notably higher in SVL (10.2%) compared to other ingredients, whereas ME was highest in Maize (3.760 kcal/kg) and lowest in NSC (2,258 kcal/kg). The CF was highest in NSC (17.2%) and lowest in Maize and Finger millet (4.2%), and ash content was greatest in SBM (9.9%) and lowest in maize (2.6%).

**Table 1.** Chemical compositions of feed ingredients used for diet formulation

| Feed ingredients / Chemical composition (%) | Maize grain | Wheat short | Finger millet | SBM | NSC | SVL |
|---|---|---|---|---|---|---|
| DM | 90.73 | 91.2 | 92.2 | 91.3 | 92.9 | 91.2 |
| CP | 12.2 | 15.3 | 9.5 | 42.6 | 31.9 | 35.9 |
| CF | 4.2 | 8.7 | 4.2 | 9.8 | 17.2 | 9.6 |
| EE | 5.35 | 3.2 | 4.4 | 2.7 | 2.9 | 10.2 |
| Ash | 2.6 | 4.2 | 3.5 | 9.9 | 7.9 | 6.3 |
| OM | 97.4 | 95.8 | 96.5 | 90.1 | 92.1 | 93.7 |
| NFE | 66.3 | 59.8 | 70.5 | 26.2 | 32.9 | 29.2 |
| ME (kcal/kg) | 3760.1 | 3179.5 | 3674.8 | 2822.3 | 2257.7 | 3399.1 |

DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, OM: Organic matter, NFE: Nitrogen-free extract, ME: Metabolizable energy, NSC: Noug seed cake, SBM: Soya bean meal, SVL: Sweet vitabor lupine

The differences observed in the proximate composition of the evaluated feed ingredients, particularly in DM, CP, CF, EE, ash, and nitrogen-free extract, highlight the nutritional variability among conventional and alternative feed resources. Such variability has important implications for diet formulation, as the substitution of conventional protein sources like SBM with alternative ingredients such as sweet lupine or NSC can substantially influence the overall dietary balance of protein, energy, and fiber. The current proximate analyses are consistent with previous reports indicating that alternative plant protein sources differ markedly in their nutrient profiles and digestibility, thereby affecting the nutritional adequacy of compounded diets (El-Deek et al., 2020). Consequently, careful consideration of these compositional differences is essential to ensure that formulated diets remain balanced and isonitrogenous, thereby supporting optimal animal performance and feed efficiency. Overall, the observed variation underscores the nutritional diversity of the ingredients and provides the basis for designing diets that meet specific protein and energy targets in chicken feeding trials.

Table 2 presents the ingredient composition and nutrient profile of the finisher diet formulated using WinFeed (2.8 Demo version) feed formulation software for a 500 kg batch. The diet consisted primarily of maize grain, finger millet, and wheat short as energy sources, while soybean meal (SBM), sweet Vitabor lupine (SVL), noug seed cake (NSC), and bone and meat meal (BMM) served as the major protein sources.

**Table 2.** Ingredient's inclusion rate to make 500 kg chicken finisher diet

| Ingredients | Proportion/Rounded | Ingredients (%) |
|---|---|---|
| Maize grain | 107.14 | 21.43 |
| Wheat short | 91.03 | 18.21 |
| Finger millet | 109.31 | 21.86 |
| SBM | 43.87 | 8.77 |
| NSC | 48.51 | 9.7 |
| SVL | 65.14 | 13.03 |
| BMM | 25 | 5 |
| Vitamin premix | 2.5 | 0.5 |
| Limestone | 5 | 1 |
| Salt | 2.5 | 0.5 |
| Total | 500 kg | 100% |

| Nutrients | Nutrient requirements | | Analysed result |
|---|---|---|---|
| | Minimum | Maximum | |
| CP | 18 | 19 | 19 |
| ME | 3100 | 3200 | 3100 |

CP: Crude protein, ME: Metabolizable energy, NSC: Noug seed cake, SBM: Soya bean meal, SVL: Sweet vitabor lupine, BMM: bone and meat meal. The diet is formulated by WinFeed (2.8 Demo version) feed formulation Software.

**Table 3.** Treatment replacement levels and layout for the present study by replacing soybean meal with sweet vitabor lupine

| Experimental treatments | Level of replacement |
|---|---|
| $T_1$ | 0% SVL + 100% SBM based |
| $T_2$ | 15% SVL + 85% SBM based |
| $T_3$ | 25% SVL + 75% SBM based |
| $T_4$ | 30% SVL + 70% SBM based |
| $T_5$ | 50% SVL + 50% SBM based |

SVL: Sweet vitabor lupine, SBM: Soybean meal. Other ingredients (Table 2) were kept constant in type for all experimental treatments, and these included: Maize grain, wheat shorts, finger millet, and noug seed cake.

Table 3 presents the experimental treatment structure used to evaluate the effects of replacing soybean meal (SBM) with Sweet Vitabor lupine (SVL) in Sasso $T_{44}$ chicken diets. Five dietary treatments were formulated with increasing levels of SVL substitution, ranging from 0% (control) to 50% replacement of SBM. To ensure that any observed differences in performance, carcass characteristics, hematological, and biochemical parameters were attributable primarily to the varying levels of SVL inclusion, all other feed ingredients were maintained constant across treatments.

On the other hand, the proximate composition of the experimental diets revealed numerical variations across treatments T1 to T5 (Table 4). The DM remained relatively stable, ranging from 91.90% in T2 to 92.43% in T5, whereas the CP decreased progressively from 21.67% in T1 to 20.40% in T5, corresponding to the stepwise replacement of SBM with SVL, which contains lower protein. Concurrently, the EE increased from 4.63% in T1 to 6.70% in T5, while CF decreased from 8.03% to 6.40%, reflecting the higher lipid and lower fiber contribution of SVL compared to SBM.

**Table 4.** The average proximate composition values of the treatments fed to the experimental chicken

| Treatment | DM (%) | CP (%) | CF (%) | EE (%) | Ash (%) | OM (%) | NFE (%) | ME (kcal/kg) |
|---|---|---|---|---|---|---|---|---|
| T1 | 92.30 | 21.67 | 8.03 | 4.63 | 4.50 | 95.50 | 53.43 | 3128.53 |
| T2 | 91.90 | 21.43 | 7.67 | 4.77 | 4.37 | 95.73 | 53.67 | 3148.74 |
| T3 | 92.07 | 21.20 | 7.17 | 5.40 | 4.10 | 95.77 | 54.17 | 3162.20 |
| T4 | 92.30 | 20.83 | 6.67 | 5.73 | 3.27 | 96.73 | 55.73 | 3168.94 |
| T5 | 92.43 | 20.40 | 6.40 | 6.70 | 2.97 | 97.07 | 55.97 | 3195.88 |

T1: 0% SVL + 100% SBM based, T2: 15% SVL + 85% SBM based, T3: 25% SVL + 75% SBM based, T4: 30% SVL + 70% SBM based, T5: 50% SVL + 50% SBM based, DM: Dry matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, OM: Organic matter, NFE: Nitrogen free extract, ME: Metabolizable energy.

Ash content declined, resulting in an increase in organic matter from 95.50% to 97.07%, and nitrogen-free extract increased from 53.43% to 55.97% in T1 and T5, respectively. The ME of diets also increased progressively from 3,128.53 kcal/kg in T1 to 3,195.88 kcal/kg in T5. The chemical compositional changes of experimental diets demonstrate that incremental replacement of SBM with SVL altered the dietary protein, energy, and fiber profiles, which is critical for interpreting subsequent growth performance, feed utilization, and nutrient metabolism in the experimental chickens.

The inverse relationship between dietary energy and CP content is consistent with findings by Musigwa et al. (2024), who reported that reducing dietary protein while maintaining appropriate energy and amino acid balance can optimize broiler performance without compromising growth. The CF content decreased from 8.03% in T1 to 6.40% in T5, in which T5 revealed better performance of chickens in the current study, and this was aligned with report of Zhang et al. (2023) who reported that high fiber diets can negatively impact nutrient digestibility by diluting nutrient concentration and increasing the bulk of the diet, which can lead to reduced feed intake and growth performance in broilers.

Ash content declined progressively from 4.50% in T1 to 2.97% in T5, while the organic matter content increased correspondingly from 95.50% to 97.07%. The reduction in ash percentage may reflect a lower inclusion of mineral-rich feed components, which may enhance the digestibility of the feed (Salo et al., 2025), and a diet rich in organic matter can lead to better nutrient absorption and overall health, potentially resulting in improved growth rates and productivity (Maruthamuthu et al., 2024). Likewise, the nitrogen-free extract increased from 53.43% in T1 to 55.97% in T5, and the ME values increased from 3,128.53 kcal/kg to 3,195.88 kcal/kg, indicating progressive enrichment of available carbohydrates and fats in the diets. The progressive increase in ME recorded in the present study agreed with the observations of Infante-Rodríguez et al. (2016), who also reported enhanced dietary energy levels when higher-energy feed ingredients were incorporated into poultry diets. Overall, the observed nutrient variations among treatments suggest that the formulated diets were progressively adjusted to achieve an optimal balance between protein and energy sources. The nutrient adjustment aligns with the modern poultry feeding strategy that emphasizes the optimization of net energy and digestible amino acid profiles rather than increasing CP levels alone (Musigwa et al., 2024).

**Formulated feeds**

For the experimental feed formulation (Table 2), the formulated diets were adjusted to contain a minimum of 3,100 to 3,200 kcal/kg ME and to contain CP from 19 to 20 percent to satisfy the experimental chicken's requirement for finishers. According to National Research Council (NRC, 1994), with the standard specifications for broiler and dual purpose grower and finisher feed requirements, chicken diets were formulated to contain a 3,050 to 3,100 Kcal/kg ME and 210 to 220g CP/kg for the growers whereas, 3,100 to 3,200 Kcal ME and 180g CP/kg to 190 g/kg for the finisher to satisfy their requirements. Both the ME and CP were approximately obtained to meet the requirement.

**Feed intake**

The influence of the experimental diets on average dry matter intake, body weight, body weight gain, and feed conversion of Sasso $T_{44}$ dual-purpose chickens throughout the feeding trial is observed in Table 5. Statistical analysis indicated significant ($p < 0.05$) differences among dietary treatments for all measured parameters, with the exception of feed conversion ratio. Both daily and cumulative dry matter intake revealed a significant ($p < 0.05$) increase as the replacement level of sweet lupine in the diets increased. Chickens fed the diet T5 recorded the highest mean daily (62.4 g/chicken) and total dry matter intake (5,611.8 g/chicken), whereas those fed the control diet (T1) had the lowest values (54.8 g/chicken and 4,933.1 g/chicken, respectively).

**Table 5**. The average dry matter intake, body weight, body weight gain, and feed conversion ratio of the chicken during the 90-day feeding trial

| Parameters \ Treatments | T1 | T2 | T3 | T4 | T5 | SEM | P -value |
|---|---|---|---|---|---|---|---|
| Mean daily DMI (g/chicken) | $54.8^{c}$ | $56.6^{bc}$ | $57.2^{bc}$ | $59.3^{b}$ | $62.4^{a}$ | 1.25 | 0.0001 |
| Mean total DMI (g/chicken) | $4,933.1^{e}$ | $5,091.5^{d}$ | $5,148.3^{c}$ | $5,335.9^{b}$ | $5611.8^{a}$ | 3.35 | 0.0001 |
| Mean final body weight (g/chicken) | $1,559.0^{e}$ | $1,631.5^{d}$ | $1,654.0^{c}$ | $1,849.5^{b}$ | $1,921.5^{a}$ | 2.92 | 0.0001 |
| Mean daily BWG (g/chicken) | $17.0^{c}$ | $18.5^{bc}$ | $18.4^{bc}$ | $20.1^{ab}$ | $21.5^{a}$ | 0.57 | 0.0002 |
| Mean weekly BWG (g/chicken) | $127.7^{d}$ | $138.8^{c}$ | $139.8^{c}$ | $150.4^{b}$ | $161.2^{a}$ | 1.88 | 0.0001 |
| Mean total BWG (g/chicken) | $1,532.3^{e}$ | $1,665.6^{d}$ | $1,678.1^{c}$ | $1,807.0^{b}$ | $1,935.1^{a}$ | 5.31 | 0.0001 |
| FCR (g DMI/g gain) | $3.2^{a}$ | $3.1^{a}$ | $3.1^{a}$ | $2.9^{a}$ | $2.9^{a}$ | 0.06 | 0.5962 |

[a,b,c] Different superscript letters in a row indicate significantly different ($p < 0.05$). g: Gram, MDMI: Mean dry matter intake, BW: Body weight, IBW: Initial body weight, BWG: Body weight gain, FCR: Feed conversion ratio, SEM: Standard error of the mean, T1: 0% SVL + 100% SBM based, T2: 15% SVL + 85% SBM based, T3: 25% SVL + 75% SBM based, T4: 30% SVL + 70% SBM based, T5: 50% SVL + 50% SBM based.

The observed increase in feed intake with higher levels of SVL could be attributed to improved palatability and the balanced nutrient composition of sweet lupine-based diets. Comparable results have been documented by Al-Sagan et al. (2020), who reported a linear rise in voluntary feed intake in broiler chickens offered diets containing blue lupine (*Lupinus angustifolius*) seed meal. In a similar study, Dida and Melesse, (2024) found that partially replacing soybean meal with heat-treated sweet lupine seed meal improved feed consumption and palatability in broilers without negatively affecting growth performance. Moreover, David et al. (2024) emphasized that modern lupine varieties possess lower levels of alkaloids and improved amino acid profiles, which enhance diet palatability and digestibility in poultry.

**Body weight and feed conversion ratio**

Body weight and feed conversion ratio are presented in Table 5. The average final body weight and total body weight gain exhibited trends comparable to those observed for feed intake. Chickens in treatment T5 recorded the greatest final body weight (1,921.5 g/chicken) and total body weight gain (1,935.1 g/chicken), whereas chickens in T1 indicated the lowest values (1,559.0 g/chicken and 1,532.3 g/chicken, respectively). Mean daily and weekly weight gains also increased progressively with sweet lupine inclusion. Brand et al. (2019) reported that a 30% inclusion of lupine in the diet resulted in improved average daily gains and final body weights. The current study suggested that, the increased performance in the mean daily and weekly weight gains could be attributed to improved nutrient utilization efficiency and the favorable amino acid balance of sweet lupine, which may have enhanced protein synthesis and growth potential and this is consistent with Gresta et al. (2023) study**,** who reported that white lupine (*Lupinus albus*) provided a high-quality protein source capable of replacing soybean meal in poultry diets when appropriately processed. Furthermore, Straková et al. (2024) found that dehulled lupine seed inclusion improved muscle protein quality and growth parameters in broilers, reinforcing its potential as an alternative plant protein. The positive growth response in the present study, therefore aligned with earlier reports suggesting that lupine inclusion up to moderate levels supports optimal growth without compromising nutrient digestibility (Al-Sagan et al., 2020; David et al., 2024; Dida and Melese, 2024).

Although there was a numerical improvement in feed conversion ratio values with increasing dietary inclusion of sweet lupine, the differences among treatments were not statistically significant. Chickens fed T4 and T5 diets exhibited the lowest feed conversion ratio values (2.9 g DM intake/g gain), indicating more efficient feed utilization compared to T1 (3.2 g DM intake/g gain). The lack of statistical significance suggested that while sweet lupine inclusion improved intake and growth, the feed-to-gain efficiency remained relatively stable across treatments. The lack of statistical significance in feed conversion ratio implies that the diets were adequately balanced in energy and protein to support efficient utilization, consistent with the findings of Al-Sagan et al. (2020) and Dida and Melese (2022), who reported similar non-significant effects of blue lupine inclusion on feed conversion ratio despite improved growth rates.

**Carcass traits and organ weights**

The carcass performance of the experimental chickens, including carcass weight and the weights of major cut portions obtained from chickens sampled for blood hematological and serum chemistry evaluation, is presented in Table 6 for treatments T1 through T5. Significant ($p < 0.05$) treatment effects were observed for all evaluated traits.

**Table 6**. Carcass traits and organ weights of chickens during the 90-day feeding trial

| Parameters \ Treatments | T1 | T2 | T3 | T4 | T5 | SEM | P -value |
|---|---|---|---|---|---|---|---|
| Live weight (g) | 1,401.8$^{e}$ | 1,478.3$^{d}$ | 1,512.2$^{c}$ | 1,858.0$^{b}$ | 1,946.2$^{a}$ | 2.026 | 0.001 |
| Slaughter weight (g) | 1,347.4$^{e}$ | 1,418.0$^{d}$ | 1,471.5$^{c}$ | 1,812.6$^{b}$ | 1,912.5$^{a}$ | 0.86 | 0.001 |
| Carcass weight (g) | 935.5$^{e}$ | 1,048.2$^{d}$ | 1,166.5$^{c}$ | 1,458.0$^{b}$ | 1,580.2$^{a}$ | 2.035 | 0.001 |
| DP (%) | 66.7$^{d}$ | 72.8$^{c}$ | 76.1$^{b}$ | 77.5$^{b}$ | 80.8$^{a}$ | 1.50 | 0.001 |
| Breast weight (g) | 236.5$^{e}$ | 280.6$^{c}$ | 285.5$^{d}$ | 353.3$^{b}$ | 381.2$^{a}$ | 1.84 | 0.001 |
| Thigh weight (g) | 145.3$^{e}$ | 170.9$^{d}$ | 174.9$^{c}$ | 211.5$^{b}$ | 230.2$^{a}$ | 1.5 | 0.001 |
| Drumstick weight (g) | 192.7$^{b}$ | 140.8$^{e}$ | 150.9$^{d}$ | 189.3$^{c}$ | 219.6$^{a}$ | 0.34 | 0.001 |
| Wings weight (g) | 121.8$^{e}$ | 151.6$^{c}$ | 152.0$^{d}$ | 174.9$^{ba}$ | 185.5$^{a}$ | 0.17 | 0.001 |
| Neck weight (g) | 57.6$^{e}$ | 62.8$^{c}$ | 63.6$^{d}$ | 93.5$^{a}$ | 89.6$^{b}$ | 0.12 | 0.001 |
| Rib back weight (g) | 33.8$^{e}$ | 44.2$^{d}$ | 44.5$^{c}$ | 52.8$^{b}$ | 75.7$^{a}$ | 0.47 | 0.001 |
| Tail back weight (g) | 68.5$^{e}$ | 93.7$^{c}$ | 85.2$^{d}$ | 110.5$^{a}$ | 108.2$^{b}$ | 0.23 | 0.001 |
| Skin weight (g) | 78.3$^{d}$ | 84.3$^{d}$ | 98.0$^{c}$ | 123.6$^{b}$ | 130.5$^{a}$ | 0.22 | 0.001 |

$^{a,b,c,d,e}$ Different superscript letters in a row indicate significantly different ($p < 0.05$). g: Gram, DP: Dressing percentage, SEM: Standard error of the mean, T1: 0% SVL + 100% SBM based, T2: 15% SVL + 85% SBM based, T3: 25% SVL + 75% SBM based, T4: 30% SVL + 70% SBM based, T5: 50% SVL + 50% SBM based.

Live body weight increased progressively across the treatments, with the lowest value recorded in T1 (1,401.8 g) and the highest in T5 (1,946.2 g). Likewise, the slaughter weight also indicated an upward trend, with T5 exhibiting the highest weight (1,912.5 g). In terms of carcass weight, the results reflected a similar pattern, with T1 having the lowest weight (935.5 g) and T5 the highest (1,580.2 g), whereas the carcass weight of chickens fed T2, T3, and T4 was between the interval values of T1 and T5. T1 had the lowest DP at 66.7%, while T5 demonstrated the highest DP at 80.8%. The present result for the DP is aligned with the studies, which reported that the carcass weight of chickens fed diets with sweet lupine was generally similar to that on traditional soybean diets (Suchý et al., 2010; Nuriev et al., 2021).

When examining individual cut parts, the breast weight followed a significant ($p < 0.05$) increasing trend, with T1 indicating the lowest weight (236.5 g) and T5 the highest (381.2 g). Similarly, the thigh weight increased from 145.3 g in T1 to 230.2 g in T5. The drumstick weight also varied significantly ($p < 0.05$) between treatments, with T5 having the heaviest drumsticks (219.6 g), while T1 had the lightest (140.8 g). The wing weight was significantly ($p < 0.05$) higher from 121.8 g (T1) to 185.5 g (T5). The neck weight followed a significantly ($p < 0.05$) higher trend, with T1 having the lowest at 57.6 g and T5 the highest at 93.5 g. A consistent upward trend was observed in the weights of rib back, tail back, and skin across the dietary treatments, increasing from 33.8 g to 75.7 g, 68.5 g to 108.2 g, and 78.3 g to 130.5 g, respectively, from T1 to T5. Overall, the values for all parameters indicated that as the levels of sweet lupine replaced soybean meal from T1 (0%) to T5 (50%), there was a consistent increase in live weight, slaughter weight, carcass weight, dressing percentage, and individual cut-up part weights, with significant ($p < 0.05$) differences observed across all parameters, suggesting a positive correlation between treatment groups and overall body mass and distribution of parts chickens.

In the findings of the present study, improved performance was observed at higher levels of sweet lupine substitution. The improved crude protein content and lower anti-nutritional factors of sweet lupine could be attributed to chickens' improved performance, and this aligns with the study by Sedláková et al. (2016), who stated that 33-40% crude protein content and lower anti-nutritional factors make it a valuable protein source and promote its use in animal feed.

Another study conducted by Suchý et al. (2010) demonstrated that substituting one-third to two-thirds of soybean meal with sweet lupine in broiler diets significantly influenced live weight during the fattening period. Evaluation of the dressed carcasses of slaughtered chickens also revealed significant variations in the weights of different cut-up parts. Chickens in treatment five exhibited the highest carcass weight and dressing percentage, whereas those in treatment one revealed the lowest values. The findings for carcass weight and dressing percentage are consistent with the report of Pietras et al. (2021), who indicated that replacing 60% of soybean meal with low-alkaloid lupine did not adversely affect the growth performance or dressing percentage of broiler chickens. However, the average weights of individual carcass cuts indicated irregular variation among treatments. The present results for carcass, thigh, and breast weights are also in close agreement with the observations of Dida and Melesse (2024), who evaluated Cobb 500 broilers fed diets containing sweet lupine. Additionally, Olver (2009) reported that incorporating sweet lupine at levels of up to 40% per kilogram of diet positively affected growth performance, feed efficiency, and carcass composition of broilers from hatch to 8 weeks of age.

**Hematological parameters**

Table 7 indicates that the hematological parameters measured in the present study, including white blood cell count (WBC), lymphocytes, mid-sized cells (MID), granulocytes, red blood cells (RBC), hemoglobin, and hematocrit, differed significantly ($p < 0.05$) among the treatments. The highest WBC value ($30.42 \times 10^9$/L) was recorded in chickens under T1, whereas the lowest value ($24.13 \times 10^9$/L) was observed in T5. Similarly, variation was also observed in granulocyte percentages, with the highest value in T5 (65.38%) and the lowest in T2 (30.38%). Changes in WBC and lymphocyte counts may be associated with differences in the immune status of chickens, as these parameters are often used as indicators of immune function (Suman and Prasad, 2020). Likewise, granulocytes are commonly linked with physiological responses to stress or inflammation (Ribeiro et al., 2024). Therefore, the differences observed among treatments in the present study may suggest possible variations in the physiological and immune responses of the chickens under the different dietary treatments.

Higher inclusion levels of sweet lupine in poultry diets have been reported to affect granulocyte profiles, which may be associated with changes in immune or inflammatory responses. It is supported by the Scientific Committee on Animal Nutrition (SCAN, 2024) as replacing soybean meal with lupine meal has been associated with improved health outcomes in poultry, including enhanced fat quality in meat and eggs, which may indirectly support immune function. In the present study, the proportion of MID varied significantly ($p < 0.05$) among treatments. The highest MID value was recorded in T1 (18.38%), followed by T2 (15.56%), whereas the lowest value occurred in T4 (10.60%). Despite such

variations, all hematological values remained within the normal physiological ranges reported by Ritchie et al. (1998), and these findings are consistent with the report of Olver (2009), who indicated that sweet lupine can be incorporated into broiler diets at levels of up to 40% without detrimental effects on hematological parameters. Similarly, Al-Sagan et al. (2020) found that replacing soybean meal with up to 30% blue lupine seed in broiler diets can serve as a cost-effective protein source without compromising growth performance, while also contributing to improvements in immune organ development.

**Table 7**. Hematological parameters of the chickens during the 90-day feeding trial

| Hematological parameters \ Treatments | T1 | T2 | T3 | T4 | T5 | SEM | P -value |
|---|---|---|---|---|---|---|---|
| WBC (×$10^9$/L) | 30.42$^{a}$ | 28.95$^{ab}$ | 27.76$^{b}$ | 25.19$^{c}$ | 24.13$^{c}$ | 2.775 | 0.01 |
| Lymph (%) | 42.78$^{b}$ | 38.47$^{bc}$ | 36.28$^{c}$ | 58.38$^{a}$ | 45.56$^{b}$ | 3.465 | 0.01 |
| MID (%) | 18.38$^{a}$ | 15.56$^{ab}$ | 11.71$^{bc}$ | 10.60$^{c}$ | 12.24$^{bc}$ | 0.763 | 0.02 |
| GRA (%) | 40.28$^{b}$ | 30.38$^{c}$ | 42.28$^{b}$ | 46.46$^{b}$ | 65.38$^{a}$ | 2.741 | 0.01 |
| RBC (×$10^{12}$/L) | 2.54$^{b}$ | 2.83$^{b}$ | 2.90$^{b}$ | 3.14$^{ab}$ | 3.56$^{a}$ | 0.095 | 0.016 |
| Hemoglobin (g/dL) | 10.92$^{b}$ | 10.59$^{b}$ | 10.92$^{b}$ | 11.19$^{b}$ | 13.38$^{a}$ | 0.263 | 0.01 |
| MCHC (g/dL) | 28.74$^{a}$ | 29.59$^{a}$ | 29.67$^{a}$ | 28.26$^{a}$ | 29.93$^{a}$ | 0.223 | 0.07 |
| MCH (pg) | 37.35$^{a}$ | 38.27$^{a}$ | 37.78$^{a}$ | 35.0$^{a}$ | 38.72$^{a}$ | 0.488 | 0.11 |
| MCV (fL) | 129.59$^{a}$ | 129.85$^{a}$ | 129.83$^{a}$ | 124.37$^{a}$ | 129.08$^{a}$ | 0.773 | 0.09 |
| Hematocrit (%) | 45.45$^{a}$ | 37.94$^{b}$ | 36.88$^{b}$ | 38.18$^{b}$ | 36.18$^{b}$ | 0.948 | 0.02 |
| Platelet (×$10^9$/L) | 317.83$^{a}$ | 263.98$^{a}$ | 274.59$^{a}$ | 228.17$^{a}$ | 275.53$^{a}$ | 5.476 | 0.17 |
| Plateletcrit (%) | 0.27$^{a}$ | 0.24$^{a}$ | 0.26$^{a}$ | 0.24$^{a}$ | 0.25$^{a}$ | 0.005 | 0.21 |

$^{a,b,c}$ Different superscript letters in a row indicate significantly different ($p < 0.05$). WBC: White blood cells, MID: Middle cells, GRA: Granulocytes, RBC: Red blood cells, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, pg: Pictograms, T1: Treatment one, T2: Treatment two, T3: Treatment three, T4: Treatment four, T5: Treatment five.

The RBC counts and hemoglobin concentrations increased with higher SVL inclusion, with the highest values observed in T5 (RBC: 3.56 × $10^{12}$/L; Hb: 13.38 g/dL), indicating enhanced erythropoiesis and oxygen-carrying capacity. The RBC counts and hemoglobin concentrations are consistent with reports by Suchý et al. (2010) and Lim and Choi (2023), who demonstrated that sweet lupine inclusion in broiler diets supports hematological stability and overall health without compromising erythrocyte integrity. Similarly, Sedláková et al. (2016) reported that higher dietary inclusion of sweet lupine may promote erythropoiesis, likely due to improved nutrient availability.

In contrast to T5, the lower RBC count observed in T1 suggests that exclusive reliance on soybean meal may not optimally support red blood cell production, which aligns with the report by David et al. (2034) that replacing soybean meal with the Zulika variety of lupine seed meal had no adverse effects on performance and was associated with improved health in fattening chickens. Conversely, hematocrit values decreased in T5 (36.18%), possibly reflecting a dilution effect associated with increased plasma volume. Similar erythropoietic-hematocrit trade-offs in lupine-based diets have been reported by Lim and Choi (2023). However, the hematocrit results observed in the present study differ from the findings of Lim and Choi (2023), who reported that partially replacing soybean meal with sweet lupine did not adversely affect hematocrit values or overall performance.

Regarding hemoglobin concentration, as a measure of the blood's oxygen-carrying capacity, T5 has the highest hemoglobin level (13.38 g/dL), significantly ($p < 0.05$) higher than all other treatments, while T1 has the lowest (10.92 g/dL). The higher hemoglobin in T5 suggests improved blood oxygenation, potentially due to better red blood cell production and overall nutritional support from the SVL-containing diet, and the current result agrees with (Jones, 2015). Mean corpuscular hemoglobin concentration (MCHC), which reflects the amount of hemoglobin present within red blood cells, revealed a slight upward trend across treatments, increasing from 28.26 g/dL in T4 to 29.93 g/dL in T5; however, the differences among treatments were not statistically significant. Likewise, no significant variations were detected among treatments for mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). The MCH values ranged from 35.0 pg in T4 to 38.72 pg in T5, while MCV values varied from 124.37 fL in T4 to 129.85 fL in T2, indicating only minor numerical differences among the treatment groups. A likely result was indicated by Straková et al. (2008), who reported that the hematological values, including MCH, MCHC, and MCV, remained within physiological ranges when broilers were fed with lupine meal, whether thermally treated or untreated.

**Serum chemistry parameters**

Table 8 of the present study provided important information regarding the effects of the different dietary treatments on the serum biochemical parameters of the experimental chickens. The mean serum chemistry values across different

treatment groups offer insights into the effects of different dietary or experimental treatments on key serum parameters of chickens. The key serum parameters include creatinine, total protein, total cholesterol, triglycerides, uric acid, and glucose. In the current study, significant differences were observed among experimental treatments for all parameters except triglyceride and glucose ($p < 0.05$). According to Campbell and Ellis (2013), the values of total protein, total cholesterol, triglycerides, glucose, uric acid, and creatinine are generally within normal physiological ranges, although they may vary depending on broiler strain, age, health status, and environmental conditions.

Certain treatments had a more profound impact on physiological processes than others. The T4 indicated the most notable effects, significantly ($p < 0.05$) increasing total protein levels (11.27 g/L), and a tendency to decrease in uric acid concentrations compared to the other treatments in the present study. Total protein levels are a marker of the overall protein metabolism in the body, which can reflect liver function and nutritional status, and this is in line with Richard et al. (2022), who reported that serum protein levels were positively influenced in some studies, suggesting that sweet lupine can enhance protein metabolism in broilers.

**Table 8.** Serum biochemical parameters of chickens during the 90-day feeding trial

| Serum parameters \ Treatments | T1 | T2 | T3 | T4 | T5 | SEM | P -value |
|---|---|---|---|---|---|---|---|
| Total protein (g/L) | 8.00$^{b}$ | 8.80$^{b}$ | 9.13$^{ab}$ | 11.27$^{a}$ | 9.98$^{ab}$ | 0.328 | 0.005 |
| Total cholesterol (mg/dL) | 102.28$^{b}$ | 129.88$^{ab}$ | 110.58$^{b}$ | 111.30$^{b}$ | 162.98$^{a}$ | 5.875 | 0.004 |
| Triglyceride (mg/dL) | 188.68$^{a}$ | 165.65$^{a}$ | 205.28$^{a}$ | 221.58$^{a}$ | 221.45$^{a}$ | 8.563 | 0.181 |
| Uric acid (mg/dL) | 5.96$^{a}$ | 5.59$^{a}$ | 5.33$^{ab}$ | 4.07$^{b}$ | 5.17$^{ab}$ | 0.192 | 0.009 |
| Creatinine (mg/dL) | 1.02$^{a}$ | 0.56$^{ab}$ | 0.36$^{b}$ | 0.50$^{b}$ | 0.52$^{ab}$ | 0.069 | 0.012 |
| Glucose (mg/dL) | 161.85$^{a}$ | 145.90$^{a}$ | 133.08$^{a}$ | 169.43$^{a}$ | 171.65$^{a}$ | 6.508 | 0.286 |

$^{a, b}$ Different superscript letters in a row indicate significantly different ($p < 0.05$). mg: Milligram, dL: Deciliter, SEM: Standard error of the mean, T1: 0% SVL + 100% SBM based, T2: 15% SVL + 85% SBM based, T3: 25% SVL + 75% SBM based, T4: 30% SVL + 70% SBM based, T5: 50% SVL + 50% SBM based.

Creatinine concentration, an indicator of renal function and muscle metabolism Ávila (2025), varied significantly ($p < 0.05$) among treatments. The highest creatinine level was observed in T1 (1.02 mg/dL), which was significantly higher ($p < 0.05$) than those in T3, T4, and T5, while T2 exhibited an intermediate value (0.56 mg/dL). The lower creatinine levels in SVL-containing diets (T3-T5) may indicate reduced renal load or improved filtration efficiency, whereas the elevated level in T1 suggests potential renal or metabolic stress associated with exclusive soybean meal (SBM) inclusion. This contrasts with previous reports indicating that sweet lupine can replace SBM without adverse effects on creatinine levels (Olver, 2009; Lim and Choi, 2023). Ávila (2025) noted that creatinine concentration may also be influenced by dietary protein level, age, and other physiological factors, which may explain the present variation.

Significant differences were also observed in total cholesterol concentrations, which ranged from 102.28 to 162.98 mg/dL ($p < 0.05$). The T5 exhibited the highest cholesterol level significantly, whereas T1 recorded the lowest value ($p < 0.05$). No significant differences were detected among T2, T3, and T4 ($p > 0.05$). Triglyceride concentrations did not differ significantly among treatments, ranging from 165.65 to 221.58 mg/dL ($p > 0.05$). Although elevated cholesterol may indicate altered lipid metabolism, the increase observed in T5 remained within normal physiological limits, suggesting enhanced lipid intake or synthesis associated with higher SVL inclusion, and this was consistent with earlier studies reporting that diets containing lupine can positively influence lipid profiles by elevating high-density lipoprotein (HDL) levels and lowering low-density lipoprotein (LDL) cholesterol, providing potential metabolic benefits (Kim et al., 2023). Similarly, Lim and Choi (2023) reported increased polyunsaturated fatty acid (PUFA) concentrations in blood and muscle tissues of poultry fed lupine-containing diets.

The increased polyunsaturated fatty acid (PUFA) results suggest that increasing SVL inclusion may promote lipid accumulation and energy reserves, which could be advantageous depending on production objectives. However, careful formulation is required to avoid potential imbalances in mineral metabolism at higher inclusion levels (Hassan et al., 2018). In contrast, Dousa et al. (2011) reported no significant effects of legume-based diets on cholesterol levels, indicating variability among studies.

Uric acid levels differed significantly ($p < 0.05$) among treatments. Higher concentrations were observed in T1 (5.96 mg/dL) and T2 (5.59 mg/dL) than in T3, T4, and T5, whereas the lowest level occurred in T4 (4.07 mg/dL), with T5 indicating an intermediate value (5.17 mg/dL). Elevated uric acid levels in T1 may reflect increased protein catabolism or renal stress, while reduced levels in T4 suggest improved nitrogen utilization and metabolic efficiency, potentially associated with the combined inclusion of SVL and SBM. The elevated uric acid levels observed in T1 are consistent with those reported by Samofalova et al. (2023), who improved blood protein profiles and metabolic health following

SBM replacement with lupine. Although the uric acid concentration was comparatively higher in T1 and lower in T4, the values recorded in the present study remained within the normal physiological reference range and agree with Lumeij (2008), who reported that the normal serum uric acid concentration in chickens generally ranges from 2.0 to 7.0 mg/dL. Furthermore, Islam et al. (2020) reported that the physiological reference interval in avian species, including chickens, may extend up to 10 mg/dL depending on factors such as age, breed, diet, and management conditions.

Blood glucose concentrations did not differ significantly ($p > 0.05$) among treatments, although a numerical increase was observed from T3 (133.08 mg/dL) to T5 (171.65 mg/dL). The absence of significant differences indicated that varying proportions of SBM and SVL did not markedly affect glucose metabolism, suggesting stable glucose homeostasis across treatments ($p > 0.05$). However, the absence of significant differences in glucose metabolism in the current study contrasts with Zapletal et al. (2020), who reported reduced glucose and lipid metabolites following partial or complete replacement of SBM with whole lupine seeds.

## CONCLUSION

The present study demonstrated that SVL can effectively replace up to 50% of SBM in the diets of Sasso $T_{44}$ dual-purpose chickens without compromising growth performance, carcass characteristics, or hematological and biochemical health indicators. Dietary inclusion of SVL improved feed intake, body weight, carcass weight, and dressing percentage with most hematological and biochemical indices. However, significant reductions in hematocrit and increases in total cholesterol were observed at the highest SVL inclusion level, indicating that not all measured parameters responded uniformly. Therefore, while SVL indicated strong potential as an alternative protein source for dual-purpose chicken diets, careful consideration of inclusion levels is necessary. A limitation of the present study is that the effects of sweet lupine (SVL) inclusion were evaluated over the overall experimental period without separately assessing its impact during the starter, grower, and finisher phases, despite the varying nutrient requirements across growth stages. Consequently, the optimal SVL inclusion level for each production phase could not be established. Furthermore, although hematological and biochemical parameters were examined, other important indicators, such as gut health and microbiota composition, were not investigated, limiting a more comprehensive understanding of the physiological responses to SVL-based diets. Future studies should evaluate the effects of SVL inclusion during the starter and grower phases separately to determine phase-specific optimal inclusion levels that maximize growth performance and health outcomes. In addition, comprehensive investigations into gut health, intestinal morphology, and microbiota composition are warranted to better elucidate the physiological mechanisms underlying the responses of chickens to SVL-based diets.

## DECLARATIONS

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**Authors' contributions**

Belayneh Bazie designed and performed the experiment, analyzed and interpreted the data, wrote the original draft, reviewed, edited, and validated the paper; Yeshambel Mekuriaw designed and performed the experiment, analyzed and interpreted the data, reviewed, edited, and validated the paper; Bimrew Asmare designed and performed the experiment, analyzed and interpreted the data, reviewed, edited, and validated the paper; Semahegn Yilkal designed the experiment, reviewed, and resourced. All authors read and approved the last edition of the manuscript before publication.

**Availability of data and materials**

All the raw data involved for analysis during the current study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors have declared that there are no competing interests.

**Ethical considerations**

The author has checked the ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy. The authors confirmed that no artificial intelligence (AI) tools were used to generate or assist in the writing of this manuscript. The original article, data interpretation, and analysis were conducted solely by the authors.

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