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Evaluation of lysine and metabolizable energy in diets for broilers

Maria Rogervânia Silva de Farias
Doctor Scientiae

**VIÇOSA - MINAS GERAIS
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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Melissa Izabel Hannas

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ABSTRACT

FARIAS, Maria Rogervânia Silva de, D.Sc., Universidade Federal de Viçosa, March, 2025. **Evaluation of lysine and metabolizable energy in diets for broilers.** Adviser: Melissa Izabel Hannas.

Two experiments were conducted to evaluate the optimal levels of metabolizable energy and standardized ileal digestible lysine in broiler diets, focusing on performance and body composition. Two chapters were created based on the results of the trials. In the first chapter, a study was conducted to evaluate the optimal levels of metabolizable energy and standardized ileal digestible lysine on the performance, body composition, blood parameters and liver enzyme activity of broilers from 1 to 14 days of age. A total of 768 male broilers were allocated in a completely randomized design in a 3x4 factorial scheme, with 8 replicates. The levels varied in ME (2950, 3050 and 3150 kcal/kg) and digestible lysine (1.30, 1.39, 1.48 and 1.57%). Broilers aged 1 to 14 days receiving a diet with 3150 Kcal/kg of EM and 1.57% of SID Lys, corresponding to a ratio of 4.98 g/Mcal, presented better CA (1.04 g/g). However, the level of 3150 Kcal/kg of EM should be used with caution, as it increases fat deposition in the carcass, leading to changes in the serum biochemical profile and in the activity of liver enzymes. While the best ratio to reduce body fat deposition was 5.32 g/Mcal corresponding to the level of 2950 Kcal/kg of EM and 1.57% of SID Lys. In the second chapter, 1920 male chickens were distributed in a completely randomized design in a 3 (3100, 3200 and 3300 Kcal/kg of energy - ME) x 4 (0.98; 1.07; 1.16 and 1.25% of lysine) factorial scheme, with 8 replicates. Broilers aged 28 to 40 days receiving feed with 3300 Kcal/kg and 1.25% had better CA. However, it increased fat deposition in the carcass. While the best relationship for protein mass deposition, weight gain and carcass and cutting yield was 3.90 g/Mcal corresponding to the level of 3200 Kcal/kg and 1.25%.

Keywords: energy; lysine; amino acids; broilers

RESUMO

FARIAS, Maria Rogervânia Silva de, D.Sc., Universidade Federal de Viçosa, março de 2025. **Avaliação de lisina e energia metabolizável em dietas para frangos de corte.** Orientadora: Melissa Izabel Hannas.

Dois experimentos foram conduzidos para avaliar os níveis ótimos de energia metabolizável e lisina digestível ileal padronizada em dietas de frangos de corte, com foco em desempenho e na composição corporal. Dois capítulos foram criados baseados nos resultados dos ensaios. No primeiro capítulo, foi conduzido um estudo para avaliar os níveis ótimos de energia metabolizável e lisina digestível ileal padronizada sobre o desempenho, composição corporal, parâmetros sanguíneos e atividade das enzimas hepáticas de frangos de corte de 1 a 14 dias de idade. Foram utilizados 768 frangos de corte machos, alocados em delineamento inteiramente casualizado em esquema fatorial 3x4, com 8 repetições. Os níveis variaram em EM (2950, 3050 e 3150 kcal/kg) e lisina digestível (1,30, 1,39, 1,48 e 1,57%). Frangos de corte de 1 a 14 dias de idade recebendo dieta com 3150 Kcal/kg de EM e 1,57% de SID Lys, correspondendo a uma relação de 4,98 g/Mcal, apresentaram melhor CA (1,04 g/g). Entretanto, o nível de 3150 Kcal/kg de EM deve ser utilizado com cautela, pois aumenta a deposição de gordura na carcaça, levando a alterações no perfil bioquímico sérico e na atividade das enzimas hepáticas. Enquanto a melhor relação para reduzir a deposição de gordura corporal foi de 5,32 g/Mcal correspondendo ao nível de 2950 Kcal/kg de EM e 1,57% de SID Lys. No segundo capítulo, foram utilizados 1920 frangos machos distribuídos em delineamento inteiramente casualizado em esquema fatorial 3 (3100, 3200 e 3300 Kcal/kg de energia - EM) x 4 (0,98; 1,07; 1,16 e 1,25% de lisina), com 8 repetições. Os frangos de corte de 28 a 40 dias recebendo ração com 3300 Kcal/kg e 1,25% apresentam melhor CA. No entanto, aumenta a deposição de gordura na carcaça. Enquanto a melhor relação para deposição de massa proteica, ganho de peso e rendimento de carcaça e corte foi de 3,90 g/Mcal correspondendo ao nível de 3200 Kcal/kg e 1,25%.

Palavras-chave: energia; lisina; aminoácidos; frangos de corte

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INTRODUCTION

Intensive genetic selection of modern broiler lines has altered growth curves, protein and fat deposition rates, as well as the efficiency of energy and amino acid utilization (Tallentire et al., 2016). Comparing the nutritional recommendations proposed by Rostagno et al. (2005) with those from 2017 reveals a 29.88% increase in weight gain rate, reflecting the evolution of broiler performance over time (Rostagno et al., 2005; 2017). In this context, the early (1 to 14 days) and final (28 to 40 days) stages of rearing are considered critical for performance and profitability, with the first being responsible for establishing the birds' muscle growth potential and the latter accounting for a significant portion of total weight gain (Gawel et al., 2022; Duhra et al., 2025; Toghyani et al., 2025).

Lysine is the second limiting amino acid in corn and soybean meal-based diets and is essential for protein synthesis and muscle growth (Hirai et al., 2022). An imbalance in dietary lysine levels can impair protein deposition and bird performance, negatively affecting body composition (Musigwa et al., 2020). However, its action must be considered alongside the level of metabolizable energy (ME) in the diet, which directly influences productive performance and carcass composition in broilers (Ge et al., 2019; Maharjan et al., 2020a).

Different levels of standardized ileal digestible lysine (SID Lys) and ME affect weight gain, protein deposition (PD), and body fat (BF) deposition, impacting cut yield, feed efficiency, and economic parameters of production (Maharjan et al., 2020b; 2021; Mansilla et al., 2022; Barekataan et al., 2021). However, evaluating these nutrients in isolation may not accurately reflect the dynamic effect of the SID Lys:ME ratio on the birds' response. Diets with a low SID Lys:ME ratio promote body fat accumulation due to excess available energy and limit protein deposition due to lysine deficiency (Jariyahatthakij et al., 2018; Musigwa et al., 2020). On the other hand, a high ratio may result in insufficient energy for protein synthesis and increased energy expenditure for excreting metabolites generated from excess lysine (Chrystal et al., 2020; Maharjan et al., 2021a).

Cobb's nutritional recommendation (2022) for the final growth phase is 1.06% SID Lys and 3050 Kcal/kg ME, with a ratio of 3.45 g/Mcal. However, more recent studies have shown that higher ratios may improve performance, body composition, and carcass yield (Maharjan et al., 2020; Mansilla et al., 2022). Thus, adjusting the SID Lys:ME ratio must be a continuous process to meet the genetic potential of modern broiler strains (Toghyani et al., 2025).

In addition to productive and body composition parameters, evaluating serum biomarkers and the activity of liver enzymes involved in protein and lipid metabolism can

provide sensitive indicators of the birds' nutritional and metabolic status (Hu et al., 2021). Changes in these parameters can help interpret the dietary effects on performance and carcass composition.

Thus, we hypothesize that optimizing the SID Lys:ME ratio, rather than focusing solely on individual nutrient levels, will enhance broiler performance, body composition, and feed efficiency while reducing excessive fat deposition. The objective of this study was to evaluate the optimal levels of dietary metabolizable energy and standardized ileal digestible lysine for broilers aged 1 to 14 days and 28 to 40 days, assessing their effects on performance, body composition, biochemical serum profile, liver enzyme activity, and carcass and cut yields.

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ENERGY AND LYSINE FOR YOUNG BROILERS

Lysine and energy levels on growth, body composition and blood parameters of young broilers

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ABSTRACT: A study was conducted to evaluate the optimal levels of dietary metabolizable energy (ME) and standardized ileal digestible lysine (SID Lys) on the performance, body composition, blood parameters, and hepatic enzyme activity of broiler chickens from 1 to 14 days of age. 768 male broiler chickens were allocated in a completely randomized design in a 3×4 factorial arrangement, with 8 replicates. The levels varied in ME (2950, 3050, and 3150 kcal/kg) and digestible lysine (1.30, 1.39, 1.48, and 1.57%). Performance was evaluated. Body composition was analyzed using the Lunar iDXA device. Blood was collected via cardiac puncture, was then analyzed for total protein, albumin, globulin, uric acid, HDL, LDL, VLDL, triglycerides, total cholesterol and lipase. Liver samples were taken to evaluate hepatic lipase, alanine aminotransferase and aspartate aminotransferase activity. The data were subjected to ANOVA, followed by regression analysis to evaluate the linear and quadratic adjustments based on the levels of energy and SID Lys. FI, FCR, BP:BF and hepatic lipase activity showed a decreasing linear effect in response to increasing ME levels, while energy intake, BF, FD, total cholesterol and HDL showed an increasing linear effect with increasing ME levels ($P<0.05$). BF and FD showed a decreasing linear response in response to increasing SID Lys, while lysine intake, BP:BF, uric acid, total protein and albumin concentrations showed an increasing linear response in response to increasing SID Lys ($P<0.05$). Broilers from 1 to 14 days of age receiving a diet with 3150 Kcal/kg of ME and 1.57% SID Lys, corresponding to a ratio of 4.98 g/Mcal, exhibited better FCR (1.04 g/g). However, the level of 3150 Kcal/kg of ME should be used with caution, as it increases FD in the carcass, leading to changes in the serum biochemical profile and liver enzyme activity. The best ratio to reduce FD was 5.32 g/Mcal, corresponding to the level of 2950 Kcal/kg of ME and 1.57% SID Lys.

Keywords: amino acid, energy density, protein deposition, fat deposition.

INTRODUCTION

Lysine is the second limiting amino acid (**AA**) in corn and soybean meal-based diets and is essential for protein synthesis and muscle growth (Hirai et al., 2022). Thus, an imbalance of lysine in the diet can compromise protein synthesis and, consequently, muscle growth, negatively impacting the performance and body composition of chickens. In addition to its relevance, lysine should be considered in conjunction with other nutritional factors, such as the level of metabolizable energy (**ME**) in the diet, which also plays a fundamental role in the nutrition of broilers, directly influencing the productive performance and carcass composition of birds (Ge et al., 2019). Diets unbalanced in terms of metabolizable energy levels promote greater deposition of body fat to the detriment of body protein. In this context, more relevant than analyzing the isolated effects of these nutrients is understanding how the relationship between digestible lysine and metabolizable energy (**SID Lys:ME**) influences the performance and body composition of birds.

It is well documented in the literature that different densities of standardized ileal digestible lysine (**SID Lys**) and metabolizable energy in broiler diets promote improvements in performance and body composition (Maharjan et al., 2021a; Maharjan et al., 2021b). However, most studies focus on the isolated effects of digestible lysine and ME levels, disregarding the dynamic effect of the Lys:ME ratio on the growth response and body composition of the animals, which can lead to divergent interpretations.

Diets with a low SID Lys:ME ratio favor the deposition of adipose tissue due to the excess available ME, which is stored as body fat (**BF**) (Jariyahatthakij et al., 2018). Furthermore, this condition negatively affects the performance and body protein deposition (**PD**) of birds due to the limitation of lysine for protein synthesis (Musigwa et al., 2020), compromising the commercial yield of the carcass, increasing the percentage of losses during the slaughter process and, consequently, negatively impacting the profitability of production (Ge et al., 2019).

On the other hand, when the SID Lys:ME ratio is high, there may be an insufficient supply of energy for protein synthesis, limiting bird growth (Maharjan et al., 2021a). In addition, the excess unused lysine needs to be degraded, requiring energy for the excretion of the generated metabolites (Chrystal et al., 2020). This results in performance losses and a negative economic impact, since if the SID Lys:ME ratio is excessively high without additional benefits to performance, the cost of the diet increases without financial return for the production system. Therefore, the study of the SID Lys:ME ratio, and not just the isolated levels of these

nutrients, is important for the formulation of more effective diets that aim to promote PD and reduce fat accumulation in the commercial carcass.

Considering that the effects of the SID Lys:ME ratio can vary according to the rearing phase, the importance of investigating such adjustments is highlighted, especially in the initial phase, characterized by a high growth rate, mainly pectoral muscle deposition, combined with low feed intake (Gaweł et al., 2022). Thus, the initial phase becomes critical for defining the bird's muscle growth potential, and nutritional interventions, such as increasing the density of essential amino acids, impact productive performance and carcass yield in subsequent phases (Lamot et al., 2019).

In addition to performance and body composition parameters, the analysis of blood biomarkers and the concentration of liver enzymes that regulate protein and lipid metabolism in broilers subjected to different dietary levels of lysine and energy allows monitoring of the nutritional and metabolic status of the birds, providing sensitive indicators of the efficiency of the use of these nutrients (Hu et al., 2021). Changes in these biomarkers may reflect nutritional imbalances, providing support for the interpretation of the results observed in the performance and body composition of the birds.

Thus, we hypothesize that adjustments in the SID Lys:ME ratio will influence the performance, body composition and feed efficiency of broilers from 1 to 14 days of age, optimizing body protein deposition and reducing fat accumulation in the carcass. Given the above, the objective of this study was to evaluate the optimal levels of dietary ME and SID Lys:ME on the performance, body composition, serum biochemical profile and liver enzyme activity of broiler chickens aged 1 to 14 days.

MATERIALS AND METHODS

Animal Care and Use Ethics

All procedures were approved by the Institutional Animal Use Committee of the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (CEUAP-023/2023), and followed the standards outlined by the National Council for the Control of Animal Experimentation (CONCEA).

Experimental design, diets and animals

Seven hundred and sixty-eight (768) male Cobb 500® broiler chickens (42.24 ± 0.17 g) at 1 day of age were allocated in a completely randomized design in a 3 (metabolizable energy

levels) × 4 (digestible lysine levels) factorial arrangement with 8 replications and 8 birds per experimental unit (EU).

The birds were housed in metal cages equipped with trough-type feeders and nipple-type drinkers (600 cm² per bird). A 24-hour light program was adopted. The temperature of the barn was recorded daily at 7:00 a.m., 12:00 p.m., and 5:00 p.m. using thermometers (Incoterm 7427.02.0.00) installed in the climate-controlled battery room. The birds were provided with feed and water *ad libitum* throughout the entire experimental period, from 1 to 14 days of age.

Experimental treatments

Three dietary ME levels (2950, 3050 and 3150 kcal/kg) and four SID Lysine levels (1.30, 1.39, 1.48 and 1.57%) were evaluated. The dilution method was used to obtain the intermediate lysine levels; for this purpose, two basal diets were formulated (1.30% and 1.57%), subsequently mixed to create two intermediate levels of SID Lys (ranging from 1.39% to 1.48%), resulting in a total of four levels of SID Lys at each ME level, totaling 12 experimental diets.

Mineral and vitamin supplementation was performed as recommended by Rostagno et al. (2017). The ingredients were purchased from the same batch to avoid differences in nutrient content. The dry matter, crude protein and amino acid profile contents of the experimental diets are presented in Table 2. The requirement for all essential AA was established following the relationship with lysine, based on the ideal protein concept (Rostagno et al., 2017). The diets were provided in mash form.

Productive Performace

Feed and animals were weighed at the beginning and end of the experimental period to calculate body weight (**BW, g**), feed intake (**FI, g/bird**), body weight gain (**BWG, g/bird**), and feed conversion ratio (**FCR**) (**g/g**).

Slaughter, Blood, and Tisseu Collection

At the beginning, 10 reference birds were weighed and euthanized by cervical dislocation for body composition analysis. At the end of the experiment, one bird from each experimental unit was euthanized by cervical dislocation for body composition analysis, while a second bird from each experimental unit was randomly selected for blood collection by cardiac puncture into Vacutainer® tubes containing a clot activator for subsequent analysis of serum biochemical parameters. The collected liver tissue samples were frozen at -80 °C until subsequent analysis. All birds were slaughtered by cervical dislocation.

Body Composition

The determination of body composition was performed using the comparative slaughter method. For this purpose, at 1 day of age, a reference group of ten birds was selected, and at 14 days of age, one bird per EU was randomly selected, weighed, and euthanized by cervical dislocation. The 14-day-old birds underwent a 12-hour fasting period (water provided *ad libitum*).

The carcass was subjected to body composition analysis using the Lunar iDXA unit (encore 2011, version 13.60.033, GE Lunar, General Electric, Madison, WI, USA), using the full body scan option in standard mode (~20 minutes per full DXA chart scan) to measure total mass (g), lean mass percentage (%), fat mass and bone mass. The equations of Aguiar et al. (2024) were used to estimate body protein (**BP**) and BF, while fat deposition (**FD**) and **PD** were calculated. The relationship between protein and body fat (**BP:BF**) was calculated by dividing BP by BF.

Serum Biochemical Parameters

Blood samples were centrifuged at $3000 \times g$ at 4°C for 15 minutes and stored at -20°C (Ge et al., 2019) to obtain the serum, which was then analyzed for total protein (**TP**, mg/dL), albumin (**ALB**, mg/dL), globulin (**GLO**, mg/dL), uric acid (**UA**, mg/dL), high-density lipoprotein cholesterol (**HDL**, mg/dL), low-density lipoprotein cholesterol (**LDL**, mg/dL), very low-density lipoprotein cholesterol (**VLDL**, mg/dL), total serum triglycerides (**TG**, mg/dL), total cholesterol (**TC**, mg/dL) and lipase (U/L).

Total protein and its fractions were determined by the Biuret and Bromocresol Green method. Analyses of UA, LDL, VLDL, TG and TC were performed by the colorimetric enzymatic method. And HDL was determined by the direct Cholesterol Oxidase method.

Hepatic Enzyme Activity

The activity of enzymes was analyzed: hepatic lipase (**HLIP**, U/L; Bioclin K025-1), alanine aminotransferase (**ALT**, U/L; Bioclin K049-6), and aspartate aminotransferase (**AST**, U/L; Bioclin K048-6). Approximately 0.3 g of liver tissue was homogenized in a chilled NaCl solution using an ultraturrax homogenizer, and the supernatant was obtained by centrifugation at $2500 \times g$ for 10 minutes at 4°C according to the methodology described in Ge et al. (2019).

Statistical Analysis

The mathematical model of the study was: $Y_{ijk} = F_1 + F_2 + (F_1 * F_2) + e_{ijk}$; Where, Y_{ijk} = observation of the response variable; μ = overall mean; F_1 = effect of ME; F_2 = effect of SID Lys levels; $(F_1 * F_2)$ = interaction effect; e_{ijk} = random error. The data were subjected to

analysis of variance (ANOVA) using the F-test at a 5% significance level. Regression analysis was performed to evaluate linear and quadratic fits as a function of energy levels and ileal digestible lysine. The fitted regressions were used to estimate the requirements for ME and SID Lys.

The effect of varying SID Lys levels within each ME level was analyzed to explain the effect of different SID Lys:ME ratios, given that these are quantitative data, and the models adjusted for performance and body composition variables at each ME level were compared by contrasting model parameters.

RESULTS

Analysis of experimental diets

The analyzed nutrient levels in the experimental diets were consistent with the calculated values.

Productive Performance

There was no interaction ($P>0.05$) between Lys SID and ME for the performance variables. There was a decreasing linear effect of ME on CR and FCR, with gradual improvements in FCR as the SID Lys level increased (1.30 to 1.57%). And an increasing linear effect on energy intake. There was an increasing linear effect of SID Lys levels on lysine intake (Table 3, Figures 1A, 1B, 1I and 1J, respectively).

For energy intake, the intercepts and slopes of the fitted equations at the 3050 kcal/kg and 3150 kcal/kg levels were statistically similar ($P>0.05$), indicating no difference in the starting point or rate of change of the response (Table 8).

Body Composition

No interaction was observed between SID Lys and ME for body composition variables ($P>0.05$). Energy and lysine levels had no effect ($P>0.05$) on BP and PD. There was an increasing linear effect of ME ($P<0.05$) on BF and FD, and linear decreasing over the BP:BF ratio (Table 4, Figure 1C, 1E and 1G). Conversely, SID Lys showed a decreasing linear effect on these same variables and linear increasing over the BP:BF ratio (Table 4, Figures 1D, 1F and 1H). At the 3050 kcal/kg and 3150 kcal/kg levels, SID Lys also showed a decreasing linear effect on BF and FD (Table 7).

For BF and FD, the intercepts and slopes of the fitted equations at the 3050 kcal/kg and 3150 kcal/kg levels were statistically similar ($P>0.05$), indicating no difference in the starting point or rate of change of the response (Table 8).

Serum biochemical parameters

No interaction was observed between SID Lys and ME for blood parameters ($P>0.05$). The concentrations of LDL, VLDL, TG were not affected by energy or lysine levels ($P>0.05$). An increasing linear effect of ME was observed on the concentrations of TC and HDL while a quadratic effect was found for GLO, with the lowest globulin concentration (0.97 mg/dL) estimated at a dietary ME content of 3035 kcal ($P<0.05$). There was a increasing linear effect of SID Lys shown on UA, TP, and ALB concentrations, as well as a quadratic effect on lipase, with the lowest lipase concentration (35.52 U/L) estimated at a dietary SID Lys content of 1.44% (Table 5, Figures 2A to 2E).

Hepatic Enzyme Activity

No interaction was observed between SID Lys and ME for hepatic enzyme activity ($P>0.05$). The activity of AST, ALT and HLIP was not influenced by SID Lys levels, and ALT activity was not affected by ME levels ($P>0.05$). There was a linear decreasing effect of ME on HLIP activity and a linear increasing effect on AST activity ($P<0.05$) (Table 6, Figures 3A and 3B).

DISCUSSION

The growth response during the first two weeks of life in modern broilers accounts for over 20% of the production cycle and relies heavily on the concentration of digestible amino acids and ME in the diet to support the accelerated rate of protein synthesis during early developmental stages (Na and Kong, 2024). This is primarily due to the increased nutrient requirements for critical physiological processes at this stage, such as cellular hyperplasia and hypertrophy, development and differentiation of the gastrointestinal tract, and maturation of the thermoregulatory and immune systems (Oke et al., 2020). Therefore, nutritionally imbalanced diets particularly regarding energy and digestible lysine levels during early phases can compromise performance and body composition during the finishing period (Lamot et al., 2019).

It is well known that birds are capable of adjusting their feed intake based on the nutrient density of the diet, especially energy and AA (Massuquetto et al., 2020; Hu et al., 2021; Maharjan et al., 2020). Typically, feed intake increases when dietary nutrient density decreases, meaning that the more diluted the nutrient concentration, the greater the feed intake required to meet nutritional demands (Brickett et al., 2007).

In our results, we observed a linear increase in energy intake with increasing dietary ME levels and a linear decrease in FI, indicating that broilers from 1 to 14 days of age are able to

regulate feed consumption to meet their energy requirements. Similar results were reported by Hu et al. (2021), who found that chicks from 0 to 10 days of age reduced energy intake and improved FCR when fed high energy density diets.

In this context, adjusting the SID Lys:ME ratio in the diet is important to prevent energy-driven regulation of FI from compromising the intake of essential AA required for protein synthesis (Yang et al., 2015), especially during early growth phases, thereby avoiding negative impacts on growth rate and FCR in later phases (Lamot et al., 2019). In our study, the best FCR of 1.04 g/g was observed with a SID Lys:ME ratio of 4.98 g/Mcal, corresponding to 1.57% SID Lys and 3150 kcal/kg ME, indicating that increasing the SID Lys:ME ratio improves feed efficiency in broilers from 1 to 14 days of age.

Similar results were found in the study by Johnson et al. (2020), in which there was an improvement in the feed conversion of birds aged 0 to 12 days fed increasing levels of lysine (1.24 to 1.42%), maintaining fixed energy, that is, with higher SID Lys:ME ratios there was better FCR. Findings consistent with those of Hirai et al. (2022) in which the authors observed gradual reductions in FCR as SID Lys increased from 0.88 to 1.20%. These results reinforce the relevance of defining the ideal SID Lys:ME ratio, as demonstrated in our study, to mainly improve the FCR of birds during the initial growth phase.

The adverse effects of imbalances in the SID Lys:ME ratio on FCR in broilers are also reported by Ko et al. (2023), who observed poorer feed efficiency in broilers aged 1 to 14 days when dietary ME was reduced by 100 kcal/kg below Cobb 500 recommendations. The ME level was reduced from 2,980 kcal/kg to 2,880 kcal/kg while SID Lys was maintained at 1.22%, resulting in SID Lys:ME ratios of 4.09 and 4.23 g/Mcal, respectively, highlighting the importance of an adequate SID Lys:ME ratio for optimal broiler performance.

It is well established that diets with high ME levels and/or low SID Lys:ME ratios promote fat deposition (Jariyahatthakij et al., 2018). In our results, ME had a linear increasing effect on FD, while SID Lys had a linear decreasing effect. Therefore, excess ME combined with low SID Lys levels, that is, the lowest SID Lys:ME ratio tested in this study (4.13 g/Mcal) resulted in greater carcass fat deposition, whereas the lowest ME and highest SID Lys levels corresponding to the highest tested ratio (5.32 g/Mcal) led to the lowest fat deposition.

Increased FD negatively affects carcass yield by raising the percentage of processing losses and, consequently, reducing profitability (Ge et al., 2019). Although these negative effects are more pronounced during the finishing phase, energy density in the diet is a critical factor from the earliest stages, as demonstrated in our findings.

The study by Ko et al. (2023) also found that a 100 kcal/kg reduction in ME, combined with a higher SID Lys:ME ratio from 1 to 35 days of age, reduced body fat at day 35. These findings emphasize that reducing ME levels while increasing the SID Lys:ME ratio helps decrease body fat deposition.

Lipid metabolism in birds can be influenced by the AA density of the diet (Fouad, 2014), and the reduced body fat deposition in response to higher dietary SID Lys levels observed in this study supports this statement. It is assumed that higher lysine levels provided sufficient AA to support protein synthesis, thereby reducing the proportion of energy allocated to fat deposition (Musigwa et al., 2020).

When analyzing the BP:BF ratio, we observed a linear reduction with increasing ME in the diet, indicating that higher levels of ME promote an increase in FD and reduce the BP:BF ratio. In contrast, SID Lys levels showed a linear increase in the BP:BF ratio, as higher lysine intake increased PD and reduced fat accumulation, enhancing the BP:BF ratio. Although PD was not significantly affected by SID Lys levels alone, the BP:BF ratio clearly demonstrates the positive effect of higher SID Lys levels on protein accretion in broilers.

Combined analysis of serum biochemical parameters and key enzyme activity related to protein and lipid metabolism helps explain the physiological mechanisms underlying performance and body composition responses to increasing dietary ME and SID Lys (Hu et al., 2021).

In this context, the linear increase in total cholesterol and HDL concentrations with increasing ME levels suggests that excess dietary energy not used for protein accretion or maintenance was stored as BF. This accumulation is associated with alterations in lipid metabolism, including increased synthesis of lipoproteins such as HDL, which transports cholesterol from peripheral tissues to the liver (Albitar et al., 2024). Our findings are consistent with those of Hu et al. (2021), who reported increased plasma cholesterol, HDL, and LDL concentrations when ME levels were increased from 3200 to 3500 kcal/kg during the 1 to 13-day period.

The liver is a central organ for energy, protein, and lipid metabolism in birds, acting as the main site of lipolysis, lipogenesis, amino acid metabolism, and gluconeogenesis (Liu et al., 2017). Therefore, measuring hepatic enzyme activity is a valuable indicator of both liver health and the adaptive metabolic responses of birds fed high ME and AA levels.

In this regard, HLIP is responsible for hydrolyzing triglycerides from remnant and HDL lipoproteins, releasing fatty acids that can be used as energy sources or for other metabolic

functions (Quiroga and Lehner, 2012). Thus, the reduced HLIP activity in birds fed high-energy diets may indicate adequate energy supply, reducing the need for fatty acid mobilization from lipoproteins. Similar results were reported by Li et al. (2024), where high dietary ME reduced HLIP activity in laying hens.

The effect of dietary ME on AST activity is notable, as it may suggest impaired capacity of the organism to metabolize excess energy, potentially indicating liver damage caused by energy overload (Gao et al., 2019). Total serum protein, composed of ALB and GLO, is often used in nutritional studies, as plasma proteins are sensitive to dietary changes and reflect both the quantity and quality of ingested protein (Jariyahatthakij et al., 2018), and are also indicators of hepatic protein synthesis and muscle growth rate (Hu et al., 2021).

In our results, higher SID Lys levels increased both TP concentration and the ALB fraction in serum. This may be due to sufficient AA supply to support protein synthesis in tissues (Ospina-Rojas et al., 2014).

In birds, UA is the final product of nitrogen metabolism, meaning that excess nitrogen from amino acid catabolism is excreted as uric acid in the excreta (Barzegar et al., 2020; Cappelaere et al., 2021). Therefore, plasma UA is a useful indicator of amino acid utilization efficiency (Donsbough et al., 2010). In this context, the increased plasma UA concentration observed at higher SID Lys levels likely reflects AA excess, since greater lysine intake led to increased catabolism and nitrogen excretion (Cella et al., 2009). It is important to note that nitrogen excretion leads to considerable energy loss, which may impair overall energy efficiency (Selle et al., 2023).

The absence of an effect of SID Lys levels on plasma GLO concentration is intriguing, as an impact was expected considering that TP is composed of ALB and GLO fractions. However, the effect of ME levels on GLO concentration is noteworthy, as the GLO fraction includes several lipid-transporting lipoproteins, such as α 1-lipoprotein, α 2-lipoprotein, and pre- β -lipoprotein, which may be related to the quadratic response observed for globulin in relation to dietary ME levels (Tóthová et al., 2019).

CONCLUSION

Broilers from 1 to 14 days of age receiving a diet with 3150 Kcal/kg of ME and 1.57% SID Lys, corresponding to a ratio of 4.98 g/Mcal, exhibited better FCR (1.04 g/g). However, the level of 3150 Kcal/kg of ME should be used with caution, as it increases FD in the carcass, leading to changes in the serum biochemical profile and liver enzyme activity. The best ratio to

reduce body fat deposition was 5.32 g/Mcal, corresponding to the level of 2950 Kcal/kg of ME and 1.57% SID Lys.

These results have important implications for the industry, as they prove that adjusting the SID Lys:ME ratio improves FCR and reduces fat deposition in the carcass, leading to more efficient production, in addition to potential reduction in production costs.

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FIGURES

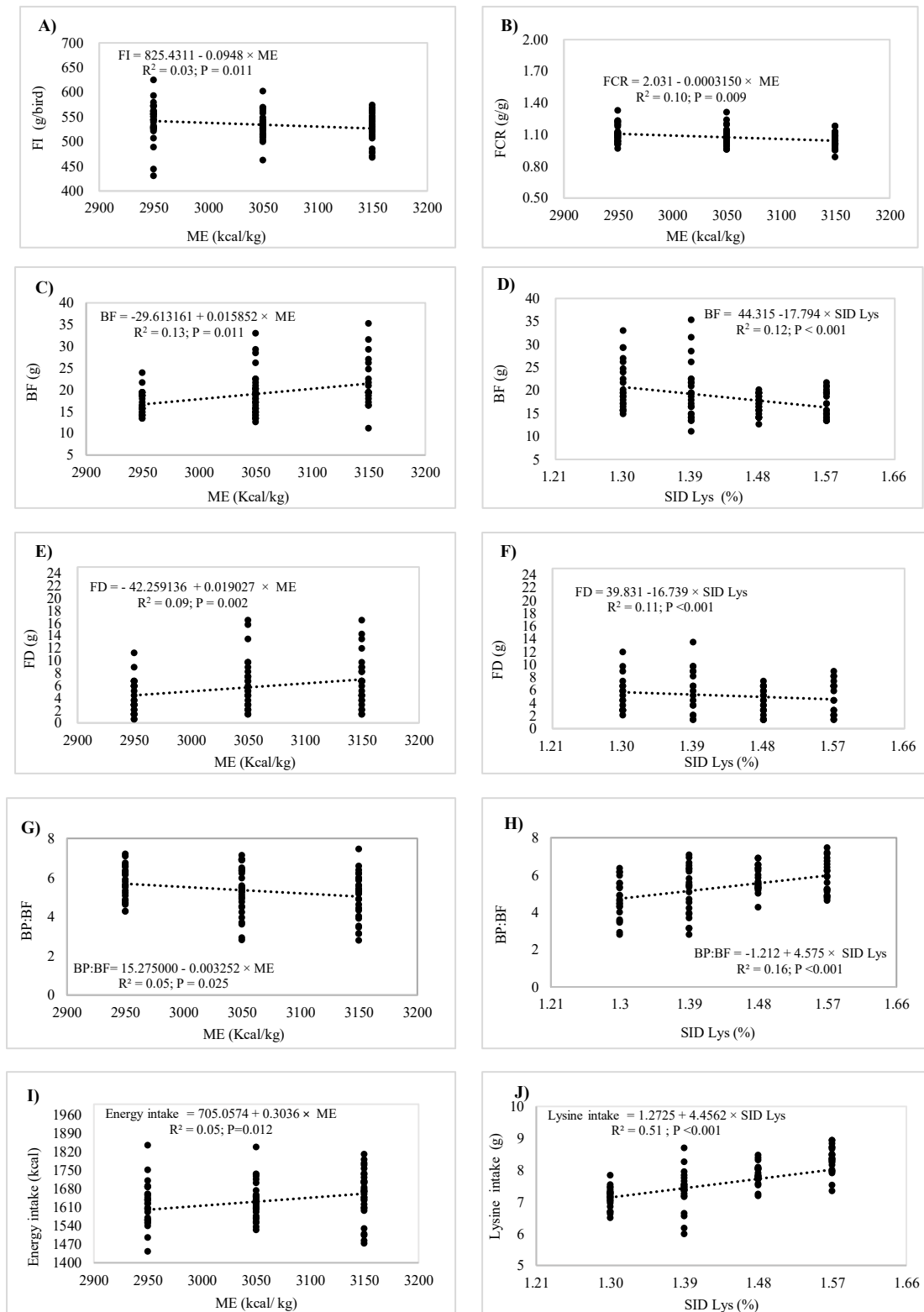


Figure 1: Effect of digestible lysine and metabolizable energy levels on performance and body composition of broilers from 1 to 14 days of age. Feed intake - FI (1A), feed conversion rate - FCR (1B), body fat - BF (1C and 1D), fat deposition - FD (1E and 1F), protein and fat deposited ratio – BP:BF (1G and 1H), energy intake (1I) and lysine intake (1J).

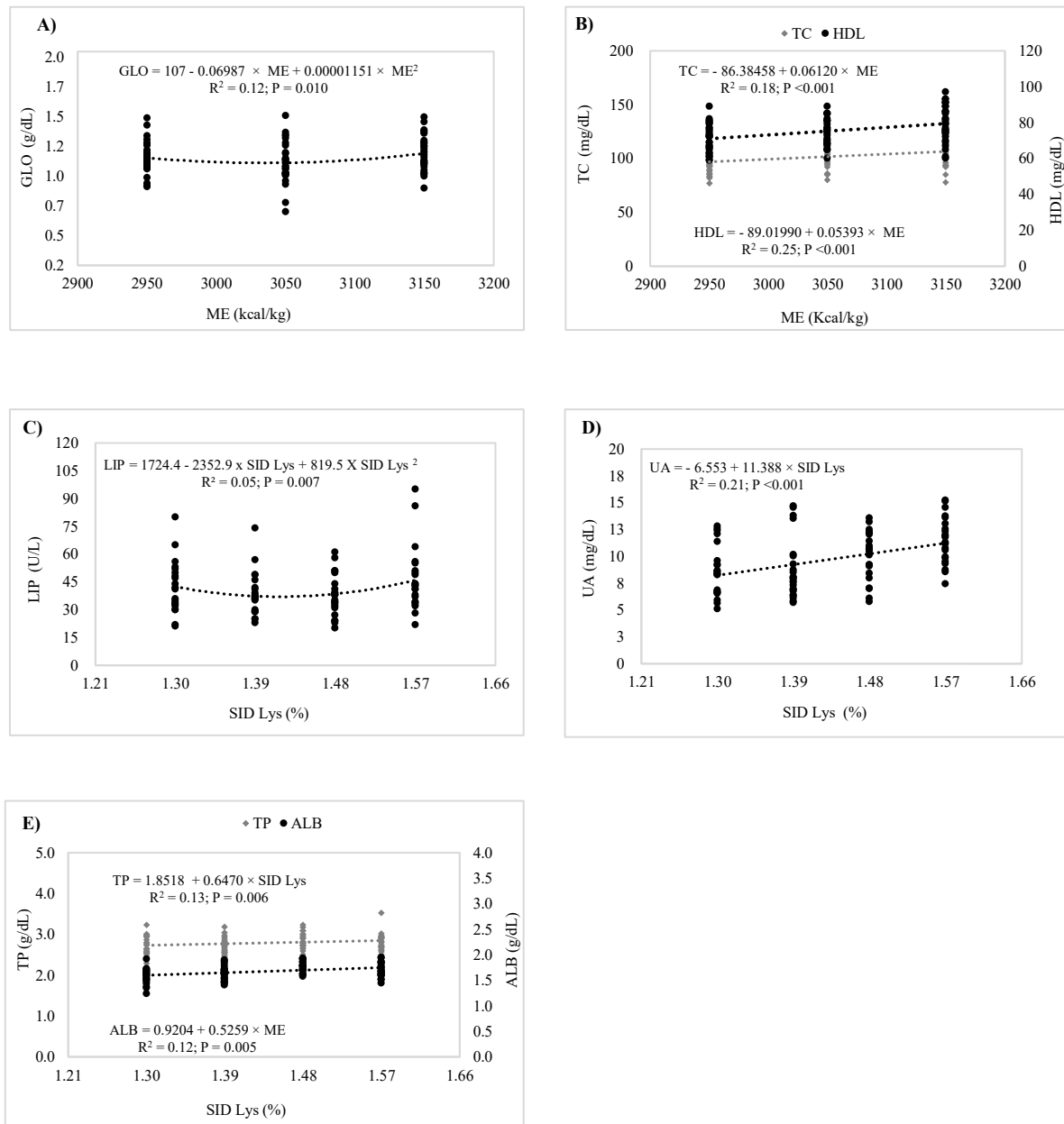


Figure 2: Effect of digestible lysine and metabolizable energy levels on blood parameters of broilers from 1 to 14 days of age. 2A and 2B show the effect of ME on the concentration of globulin (GLO), total cholesterol (TC) and HDL, respectively. 2C, 2D and 2E show the effect of digestible lysine on the concentration of lipase (LIP), uric acid (UA), total protein (TP) and albumin (ALB), respectively.

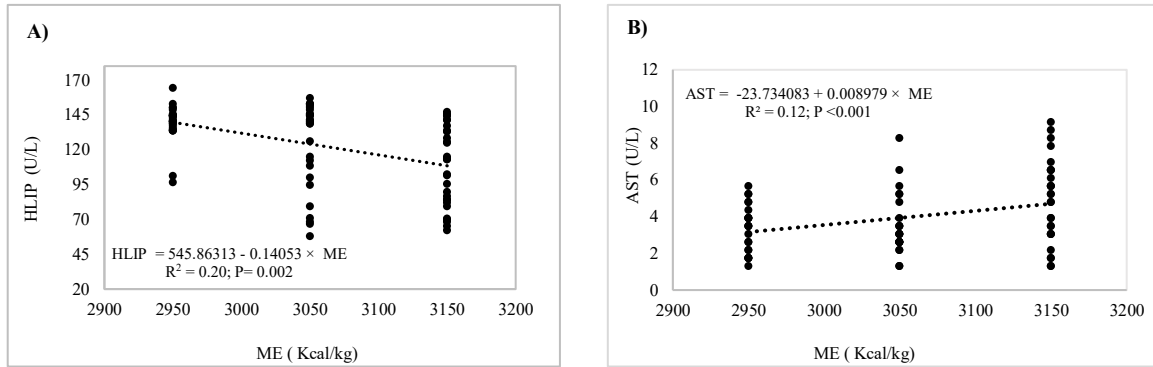


Figure 3: Effect of digestible lysine and metabolizable energy levels on hepatic enzyme activity of broiler chickens from 1 to 14 days of age. 3A and 3B show the effect of ME on hepatic lipase (HLIP) and aspartate aminotransferase (AST) activity, respectively.

Tables

Table 1. Centesimal and calculated composition of experimental diets for broilers aged 1 to 14 days

Ingredients	2950 ME ¹ (Kcal/kg)				3050 ME (Kcal/kg)				3150 ME (Kcal/kg)			
	SID Lysine Levels (%)				SID Lysine Levels (%)				SID Lysine Levels (%)			
	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57
Corn 7.88%	441.08	419.65	398.22	376.80	419.10	397.94	376.76	355.60	397.05	376.17	355.28	334.40
Corn Gluten 60%	20.00	33.33	46.67	60.00	20.00	33.33	46.67	60.00	20.00	33.33	46.67	60.00
Soybean Meal 46%	432.36	444.52	456.68	468.83	435.58	447.43	459.28	471.12	439.05	450.50	461.96	473.42
Soy oil	37.90	36.35	34.80	33.25	56.80	55.22	53.63	52.05	75.70	74.10	72.50	70.90
Dicalcium phosphate	25.10	25.00	24.90	24.80	25.20	25.10	25.00	24.90	25.20	25.10	25.00	24.90
Calcitic limestone	12.30	12.27	12.23	12.20	12.30	12.23	12.17	12.10	12.20	12.17	12.13	12.10
Coccidiostat ²	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin Premix ³	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Micromineral Premix ⁴	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.45
Antioxidant ⁵	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60
L-Glycine	0.00	0.47	0.93	1.40	0.00	0.48	0.97	1.45	0.00	0.48	0.97	1.45
L-Lysine HCl	0.94	1.59	2.24	2.88	0.89	1.54	2.20	2.85	0.83	1.50	2.16	2.83
DL-Methionine	3.18	3.49	3.80	4.11	3.21	3.52	3.84	4.15	3.23	3.55	3.87	4.18
L-Threonine	0.41	0.64	0.88	1.12	0.42	0.66	0.91	1.15	0.43	0.68	0.93	1.17
L-Valine	0.00	0.11	0.23	0.34	0.00	0.12	0.24	0.36	0.00	0.13	0.26	0.39
Choline Chloride 60	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Inert ⁶	16.47	12.31	8.16	4.00	16.24	12.16	8.08	4.00	16.05	12.03	8.02	4.00
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Nutritional levels												
Met. Energy (Kcal/kg)	2950	2950	2950	2950	3050	3050	3050	3050	3150	3150	3150	3150
Fat (%)	6.73	6.55	6.38	6.20	8.53	8.36	8.18	8.00	10.34	10.16	9.98	9.80
Crude Protein (%)	25.10	26.47	27.84	29.21	25.08	26.44	27.80	29.16	25.06	26.41	27.76	29.11
Dig. lysine (%)	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57
Dig. methionine (%)	0.65	0.71	0.76	0.81	0.66	0.71	0.76	0.81	0.66	0.71	0.76	0.81
Met + Cys. dig. (%)	0.99	1.06	1.13	1.20	0.99	1.06	1.13	1.20	0.99	1.06	1.13	1.20
Dig. threonine (%)	0.88	0.95	1.01	1.07	0.88	0.95	1.01	1.07	0.88	0.95	1.01	1.07
Dig. tryptophan (%)	0.29	0.30	0.31	0.32	0.29	0.30	0.31	0.32	0.29	0.30	0.31	0.32
Dig. Arginine (%)	1.56	1.62	1.67	1.73	1.56	1.62	1.68	1.73	1.57	1.62	1.68	1.73
Dig. Glyc. + Ser. (%)	1.96	2.10	2.23	2.37	1.96	2.10	2.23	2.37	1.96	2.10	2.23	2.37
Dig. Valine (%)	1.05	1.12	1.18	1.25	1.05	1.12	1.18	1.25	1.05	1.12	1.18	1.25
Dig. Isoleucine (%)	0.99	1.04	1.09	1.13	0.99	1.04	1.09	1.13	0.99	1.04	1.09	1.13
Dig. Leucine (%)	1.99	2.15	2.31	2.46	1.98	2.14	2.30	2.45	1.97	2.13	2.28	2.44
Dig. Histidine (%)	0.59	0.61	0.64	0.66	0.59	0.61	0.64	0.66	0.59	0.61	0.63	0.66
Dig. Phenylalanine (%)	1.16	1.23	1.30	1.37	1.16	1.23	1.29	1.36	1.16	1.22	1.29	1.36

Dig. Phen. + Tyr. (%)	2.01	2.13	2.25	2.37	2.01	2.13	2.25	2.37	2.00	2.12	2.24	2.36
Gross Fiber (%)	2.64	2.67	2.71	2.74	2.62	2.65	2.68	2.71	2.59	2.62	2.65	2.68
Total calcium (%)	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24
Total Phosphorus (%)	0.84	0.84	0.85	0.86	0.83	0.84	0.85	0.85	0.83	0.84	0.84	0.85
Avail. Phosphorus (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Potassium (%)	0.94	0.96	0.98	1.00	0.94	0.96	0.98	0.99	0.94	0.95	0.97	0.99
Sodium (%)	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Chlorine (%)	0.40	0.40	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Choline (mg/kg)	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00

¹Metabolizable energy

²Salinomycin sodium (12%)

³ Vit. A-9750 U.I.; Vit. D3-2470 U.I.; Vit. E-36.6 U.I.; Vit. B1 – 2.60 mg; Vit. B2 = 6.50 mg; Vit. B6 – 3.64 mg; Vit. B12 – 0.015 mg; Pantothenic acid – 13 g; Biotin – 0.091 mg; Vit. K3 = 1.95 mg; Folic acid – 0.91 mg; Nicotinic acid - 39 mg

⁴ manganese, 58.36 mg; zinc, 54.21 mg; iron, 41.680 mg; copper, 8.310 mg; selenium, 250 mg e iodine 843 mg.

⁵ Hydroxybutyl toluene

⁶ Kaolin

Table 2. Crude protein, dry matter and amino acid profile analyzed in experimental diets of broiler chickens 1 to 14 days of age

Item	2950 ME ¹ (Kcal/kg)				3050 ME (Kcal/kg)				3150 ME (Kcal/kg)			
	SID Lysine Levels ² (%)				SID Lysine Levels (%)				SID Lysine Levels (%)			
	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57	1.30	1.39	1.48	1.57
Dry Matter (%)	90.59	90.54	89.71	89.87	90.59	90.85	90.01	90.10	91.25	91.02	90.44	90.22
Crude Protein (%)	25.72	27.19	28.47	29.19	25.23	26.82	28.14	29.65	25.20	26.86	27.51	28.59
Methionine (%)	0.66	0.73	0.75	0.82	0.70	0.76	0.80	0.85	0.67	0.71	0.83	0.87
Cystine (%)	0.39	0.40	0.43	0.44	0.37	0.39	0.42	0.44	0.39	0.40	0.42	0.42
Met + Cys (%)	1.05	1.13	1.18	1.25	1.07	1.16	1.22	1.29	1.06	1.11	1.24	1.29
Lysine (%)	1.47	1.57	1.68	1.77	1.42	1.58	1.65	1.75	1.49	1.58	1.64	1.73
Threonine (%)	1.03	1.08	1.16	1.20	1.00	1.08	1.15	1.22	1.02	1.08	1.12	1.19
Arginine (%)	1.78	1.80	1.89	1.91	1.69	1.78	1.86	1.91	1.78	1.82	1.82	1.86
Isoleucine (%)	1.17	1.19	1.25	1.28	1.11	1.19	1.23	1.29	1.15	1.20	1.21	1.23
Leucine (%)	2.22	2.28	2.52	2.61	2.13	2.28	2.48	2.63	2.19	2.33	2.42	2.51
Valine (%)	1.26	1.30	1.37	1.40	1.21	1.30	1.35	1.42	1.25	1.31	1.33	1.37
Histidine (%)	0.68	0.69	0.73	0.74	0.65	0.69	0.72	0.74	0.67	0.69	0.70	0.72
Phenylalanine (%)	1.34	1.36	1.47	1.50	1.27	1.36	1.45	1.52	1.33	1.38	1.41	1.45
Glycine (%)	1.07	1.14	1.24	1.31	1.03	1.14	1.24	1.33	1.07	1.15	1.21	1.29
Serine (%)	1.28	1.31	1.42	1.45	1.23	1.30	1.40	1.46	1.29	1.34	1.35	1.41
Proline (%)	1.55	1.58	1.70	1.77	1.47	1.56	1.67	1.78	1.43	1.58	1.65	1.68
Alanine (%)	1.29	1.33	1.46	1.51	1.25	1.33	1.43	1.52	1.26	1.34	1.39	1.45
Aspartate (%)	2.74	2.79	2.96	2.98	2.61	2.77	2.91	3.02	2.71	2.80	2.79	2.92
Glutamate (%)	4.64	4.74	5.09	5.19	4.41	4.70	5.00	5.25	4.59	4.79	4.86	5.03

¹ Metabolizable energy

² Digestible lysine

Table 3. Performance of broilers fed different levels of digestible lysine (%) and metabolizable energy (Kcal/kg) from 1 to 14 days of age

Treatments	SID Lys:ME (g/Mcal)	BW ¹ (g)	BWG ² (g/bird)	FI ³ (g/bird)	FCR ⁴ (g/g)	Lysine intake (g)	Energy intake (kcal)
2950 ME /1.30 SID Lys ⁶	4.40	529.7	487.5	560.5	1.15	7.28	1653
2950 ME /1.39 SID Lys	4.71	539.3	497.0	534.2	1.10	7.42	1575
2950 ME /1.48 SID Lys	5.02	544.8	502.6	531.6	1.08	7.86	1568
2950 ME /1.57 SID Lys	5.32	551.3	509.1	540.0	1.08	8.48	1593
3050 ME /1.30 SID Lys	4.26	542.1	499.8	541.6	1.08	7.04	1652
3050 ME /1.39 SID Lys	4.56	547.3	505.1	531.5	1.06	7.38	1621
3050 ME /1.48 SID Lys	4.85	539.7	497.4	532.3	1.10	7.87	1623
3050 ME /1.57 SID Lys	5.15	554.4	512.1	528.6	1.04	8.44	1641
3150 ME /1.30 SID Lys	4.13	564.0	521.8	544.5	1.06	7.08	1715
3150 ME /1.39 SID Lys	4.41	541.1	498.8	524.4	1.05	7.29	1652
3150 ME /1.48 SID Lys	4.70	552.2	509.9	527.4	1.03	7.80	1661
3150 ME /1.57 SID Lys	4.98	551.6	509.3	513.1	1.00	8.05	1616
SEM ⁷		3.267	3.266	3.343	0.008	0.065	10.094
Effect							
Energy level (ME)		0.389	0.389	0.040	0.003	0.347	0.043
Lysine level (SID Lys)		0.738	0.739	0.106	0.081	0.001	0.111
ME x SID Lys		0.824	0.823	0.779	0.656	0.824	0.859
ME (kcal/kg)							
2950		541.3	499.1	541.9	1.10	7.73	1598
3050		545.6	503.3	533.6	1.07	7.66	1635
3150		552.2	510.0	526.8	1.04	7.57	1659
Regression							
Linear		ns	ns	*	*	ns	*
Quadratic		ns	ns	ns	ns	ns	ns
SID Lys (%)							
1.30		545.2	503.1	549.0	1.10	7.13	1671
1.39		542.6	500.3	530.0	1.07	7.36	1616
1.48		545.6	503.3	530.3	1.07	7.84	1619
1.57		552.3	510.1	526.7	1.04	8.31	1616
Regression							
Linear		ns	ns	ns	ns	*	ns
Quadratic		ns	ns	ns	ns	ns	ns

¹ Body weight

² Body weight gain

³ Feed intake

⁴ Feed conversion rate

⁵ Metabolizable energy level

⁶ Digestible lysine level

⁷ Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

Table 4. Body composition of broilers fed different levels of digestible lysine (%) and metabolizable energy (Kcal/kg) from 1 to 14 days of age

Treatments	SID Lys:ME (g/Mcal)	BP ¹ (g)	BF ² (g)	PD ³ (g)	FD ⁴ (g)	BP:BF ⁵
2950 ME ⁶ /1.30 SID Lys ⁷	4.40	92.6	16.7	85.74	14.25	5.39
2950 ME /1.39 SID Lys	4.71	94.3	15.7	89.00	13.30	5.88
2950 ME /1.48 SID Lys	5.02	93.8	16.7	86.93	14.19	5.72
2950 ME /1.57 SID Lys	5.32	96.0	15.4	89.12	13.00	5.78
3050 ME /1.30 SID Lys	4.26	90.5	21.7	83.65	19.26	4.28
3050 ME /1.39 SID Lys	4.56	97.6	19.7	90.72	17.27	5.20
3050 ME /1.48 SID Lys	4.85	94.4	16.3	87.53	13.87	5.86
3050 ME /1.57 SID Lys	5.15	97.5	16.8	90.58	14.34	5.96
3150 ME /1.30 SID Lys	4.13	97.6	22.5	90.70	20.11	4.43
3150 ME /1.39 SID Lys	4.41	94.7	22.3	87.80	19.82	4.27
3150 ME /1.48 SID Lys	4.70	97.2	17.2	90.37	14.73	5.62
3150 ME /1.57 SID Lys	4.98	96.4	17.0	89.50	14.53	5.81
SEM ⁸		0.672	0.507	0.666	0.507	0.117
Effect						
Energy level (ME)		0.649	0.018	0.472	0.004	0.044
Lysine level (SID Lys)		0.710	<0.001	0.685	0.004	<0.001
ME x SID Lys		0.492	0.215	0.859	0.276	0.190
2950		94.2	16.1	87.66	13.69	5.69
3050		95.1	18.6	88.19	16.18	5.34
3150		96.5	19.8	89.59	17.38	5.04
Regression						
Linear		ns	*	ns	*	*
Quadratic		ns	ns	ns	ns	ns
1.30		93.7	20.3	86.83	17.87	4.69
1.39		95.5	19.2	89.18	16.80	5.12
1.48		95.1	16.7	88.28	14.25	5.74
1.57		96.6	16.4	89.70	14.00	5.85
Regression						
Linear		ns	*	ns	*	*
Quadratic		ns	ns	ns	ns	ns

¹ Body protein

² Body fat

³ Protein deposition

⁴ Fat deposition

⁵ BP:BF ratio

⁶ Metabolizable energy level

⁷ Digestible lysine level

⁸ Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

- ¹ Lipase
 - ² Total cholesterol
 - ³ HDL cholesterol
 - ⁴ LDL cholesterol
 - ⁵ VLDL cholesterol
 - ⁶ Triglycerides
 - ⁷ Uric acid
 - ⁸ Total protein
 - ⁹ Albumin
 - ¹⁰ Globulin
 - ¹¹ Metabolizable energy level
 - ¹² SID lysine level
 - ¹³ Standard Error of the Mean
- not significant (ns), significant at 5% probability (*)

Table 6. Enzymatic activity of chickens fed different digestible lysine:metabolizable energy ratios from 1 to 14 days of age

Treatments	SID Lys:ME (g/Mcal)	Hepatic lipase (U/L)	AST ¹ (U/L)	ALT ² (U/L)
2950 ME ³ /1.30 SID Lys ⁴	4.40	147.1	3.18	150.2
2950 ME /1.39 SID Lys	4.71	133.4	2.99	145.4
2950 ME /1.48 SID Lys	5.02	142.5	2.49	152.3
2950 ME /1.57 SID Lys	5.32	116.2	3.27	133.8
3050 ME /1.30 SID Lys	4.26	95.8	3.87	144.0
3050 ME /1.39 SID Lys	4.56	136.0	2.94	137.5
3050 ME /1.48 SID Lys	4.85	131.9	3.40	173.8
3050 ME /1.57 SID Lys	5.15	103.8	2.43	149.9
3150 ME /1.30 SID Lys	4.13	113.3	6.47	147.3
3150 ME /1.39 SID Lys	4.41	97.4	5.55	167.0
3150 ME /1.48 SID Lys	4.70	115.5	3.61	153.5
3150 ME /1.57 SID Lys	4.98	98.6	4.14	163.7
SEM ⁵		3.527	0.225	2.746
Effect				
Energy level (ME)		<0.001	<0.001	0.210
Lysine level (SID Lys)		0.162	0.137	0.319
ME x SID Lys		0.054	0.250	0.400
ME (kcal/kg)				
2950		134.2	2.97	145.6
3050		116.3	3.16	151.6
3150		106.0	4.86	157.9
Regression				
Linear		*	*	ns
Quadratic		ns	ns	ns
SID Lys (%)				
1.30		117.7	4.38	147.1
1.39		121.1	3.78	150.2
1.48		129.4	3.14	159.9
1.57		106.2	3.32	149.8
Regression				
Linear		ns	ns	ns
Quadratic		ns	ns	ns

¹ Aspartate aminotransferase

² Alanine aminotransferase

³ Metabolizable energy level

⁴ Digestible lysine level

⁵ Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

Table 7. Adjustment of equations for linear and quadratic models of performance, body composition and enzymatic activity of broilers from 1 to 14 days of age, considering the interaction of digestible lysine at the level of metabolizable energy

Item	Model	Equations	R ²	P value	Req. ¹	Optimal Response
2950 kcal/kg						
Lysine intake (g)	L	Lysine intake = 1.4062 + 4.4307 x SID Lys	0.39	0.001	-	-
3050 Kcal/kg						
Lysine intake (g)	L	Lysine intake = 0.208 + 5.214 × SID Lys	0.72	<0.001		
BF ¹ (g)	L	BF = 47.605 - 20.164 × SID Lys	0.14	0.018	-	-
FD ² (g)	L	FD = 45.122 -20.163 × SID Lys	0.14	0.018	-	-
BP:BF	L	BP:BF = -3.739 + 6.329 ×SID Lys	0.24	0.002		
3150 Kcal/kg						
Lysine intake (g)	L	Lysine intake = 2.0328 + 3.8497 × SID Lys	0.48	< 0.001	-	-
BF ³ (g)	L	BF = 54.316 - 24.030 × SID Lys	0.17	0.010	-	-
FD ⁴ (g)	L	FD = 51.835 - 24.030 × SID Lys	0.18	0.010	-	-
BP:BF	L	BP:BF = -3.535 + 5.976 × SID Lys	0.28	0.001		

¹ Estimated requirement

² Body fat

³ Fat deposition

³ BP:BF ratio

Linear model (L), quadratic model (Q)

Table 8. Comparison of regression parameters

Variable	ME level compared	Z statistic	P value
BF ²			
Intercept	3050 x 3150	-0.392	0.694
Slope	3050 x 3150	0.325	0.745
FD ³			
Intercept	3050 x 3150	-0.598	0.550
Slope	3050 x 3150	0.520	0.602
BP:BF ratio ⁴			
Intercept	3050 x 3150	-0.055	0.956
Slope	3050 x 3150	0.137	0.891
Lysine intake			
Intercept	2950 x 3050	0.724	0.469
	2950 x 3150	-0.358	0.720
	3050 x 3150	0.364	0.172
Slope	2950 x 3050	-0.679	0.497
	2950 x 3150	0.477	0.633
	3050 x 3150	0.468	0.142

¹ Feed conversion rate² Body fat³ Fat deposition⁴ BP:BF ratio

ENERGY AND LYSINE ON THE PERFORMANCE OF BROILERS

Evaluation of metabolizable energy and digestible lysine levels in the diet on the performance and body composition of broilers from 28 to 40 days of age

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ABSTRACT: 1920 male chickens were distributed in a completely randomized design in a 3 (3100, 3200 and 3300 Kcal/kg of energy - ME) x 4 (0.98, 1.07, 1.16 and 1.25% of lysine) factorial arrangement, with 8 replicates. Was measure body weight (BW), feed intake (FI), body weight gain (BWG) and feed conversion rate (FCR), body protein (BP) and body fat (BF), fat deposition (FD), protein deposition (PD), carcass yield (CY) and prime cuts cutting yield. The data were subjected to ANOVA at 5% significance, regression analysis was performed and the interactions were compared using the SNK test, and then response surface and multiple linear regression were analyzed. There was no interaction between the levels of ME and lysine, except for FCR (1.25% and 3300 Kcal/kg), and BP and PD, with better results at the highest levels of lysine and lowest ME. Effect for SID Lys on FI, breast weight (BRW), thigh weight (TW) and breast yield (BRY). ME had a quadratic effect for BW (3178.27 Kcal/kg). There was a linear and quadratic effect of lysine for BW and BWG (1.20% and 1.19%, respectively) and a decreasing linear effect for FI. There was an increasing linear effect of ME on BF and FD a decreasing linear effect of lysine for the same variables, as well as a quadratic effect, with a requirement of 1.05% and 1.06%. Regarding the lysine levels, there was an increasing linear effect for SW, CW, BRW, BRY and TW, as well as a quadratic effect (1.17%, 1.19%, 1.15% and 1.13%, respectively). It is concluded broilers aged 28 to 40 days receiving feed with 3300 Kcal/kg and 1.25% present better FCR. However, it increases fat deposition in the carcass. While the best relationship for protein mass deposition, weight gain and carcass and cut yield was 3.90 g/Mcal corresponding to the level of 3200 Kcal/kg and 1.25%.

Keywords: amino acid, energy density, protein deposition, fat deposition

INTRODUCTION

The constant genetic selection of modern broiler lines alters the birds growth curves, protein and lipid gain rates, as well as the efficiency of energy and amino acid utilization (Tallentire et al., 2016). When we compare the weight gain rates of the nutritional recommendations of Rostagno et al. (2017) with those recommended 12 years ago (Rostagno et al., 2005), we observe an increase of 29.88%, reflecting the evolution in broiler performance over time. The final phase of broiler production is a critical period that accounts for a significant portion of total weight gain. Research consistently shows that optimizing nutritional strategies during this phase can impact growth rate, feed efficiency, and economic outcomes (Duhra et al., 2025; Toghyani et al., 2025; Johnson et al., 2020).

Dietary digestible lysine (**SID Lys**) and metabolizable energy levels (**ME**) directly influence gain rates, protein deposition (**PD**), and lipid deposition (Maharjan et al., 2020a), and consequently, bird performance, cut yield, and body composition (Maharjan et al., 2020b; Maharjan et al., 2021; Mansilla et al., 2022; Barekatain et al., 2021). Conducting research to update energy and SID Lys requirements should ensure adequate fractionation of body protein (**BP**) and body fat (**BF**) (Chrystal et al., 2020) and better nutrient utilization, thus optimizing the potential for genetic improvement of broiler chickens.

Furthermore, analyzing the effects of SID Lys and ME levels separately may not reflect the dynamism of the SID Lys:ME ratio on the animals' body gain and composition response. Therefore, it is preferable to study the SID Lys:ME ratio rather than just the isolated levels of these nutrients, since an imbalance in the SID Lys:ME ratio, whether due to deficiency or excess, impairs the performance and carcass composition of birds, leading to economic losses for the production system (Toghyani et al., 2025). In diets with a low SID Lys:ME ratio, the excess available ME is deposited as body fat (Jariyahatthakij et al., 2018). There may also be a reduction in weight gain due to lysine limitation for protein synthesis (Musigwa et al., 2020). On the other hand, a high SID Lys:ME ratio can also compromise weight gain in chickens, as the energy available for protein synthesis may be insufficient, in addition to the additional energy expenditure required for the excretion of metabolites generated by excess lysine (Chrystal et al., 2020; Maharjan et al., 2021a).

Cobb's nutritional recommendation (2022) for broilers in the finishing phase (29 - 39 days) is 1.06% SID Lys and 3050 Kcal/kg ME, with a ratio of 3.45 g/Mcal. However, subsequent research has shown that higher SID Lys:ME ratios provide better performance, body composition and carcass yield results. As research by Maharjan et al. (2020), identified better results in amino acid retention, productive performance, and carcass yield when chickens

received diets with 1.10% digestible lysine and 3150 Kcal/kg of ME in the feed, which corresponds to a ratio of 3.48 g of SID Lys/Mcal of ME. Also the results of the research by Mansilla et al. (2022) evaluating the reduction of ME and the increase in the SID Lys: ME ratio in broilers in the finishing phase, obtained higher BW, better FCR, as well as higher protein and lower fat in the carcass with the highest SID Lys ratio tested (3.50 g/Mcal). Therefore, to meet the genetic potential and maximize the profitability of new broiler lines, the ideal SID Lys: ME ratio must be constantly reviewed and adjusted (Toghyani et al., 2025).

Thus, we hypothesize that SID Lys:ME ratios more closely adjusted to the actual requirements of current broiler chicken lines influence the performance, body composition, and feed efficiency of broilers aged 28 to 40 days, and may optimize body protein deposition and reduce carcass fat accumulation. Given the above, the objective of this study was to determine the optimal levels of SID Lys and ME in the diet for performance, body composition, as well as cut yield of broilers aged 28 to 40 days.

MATERIAL AND METHODS

Animal Care and Use Ethics

All procedures were approved by the Institutional Animal Use Committee of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (CEUAP-023/2023) and followed the standards outlined by the National Council for the Control of Animal Experimentation (CONCEA).

Experimental design, diets and animals

The 1-day-old birds were placed in a conventional masonry shed and raised according to lineage guidelines until they reached the experimental age of 28 days. At 28 days, 1,920 male Cobb 500® broilers (1572.08 ± 0.005 kg) were allocated in a completely randomized design in a 3 (metabolizable energy levels) \times 4 (digestible lysine levels) factorial arrangement with 8 replications and 20 birds per experimental unit (EU).

The birds were housed in a masonry shed with side curtains, in a concrete pen measuring $0.6\text{m} \times 1.5\text{m} \times 2.0\text{m}$ (height \times width \times length), with the floor covered in wood shavings. A lighting program of 18 hours of light and 6 hours of darkness was used. The shed temperature was recorded daily at 7:00 a.m., 12:00 p.m., and 5:00 p.m. using thermometers (Incoterm 7427.02.0.00) installed in the experimental shed at the height of the animal mass. Temperature was regulated by adjusting curtains and fans. The birds were provided with feed and water *ad libitum* throughout the experimental period from 28 to 40 days of age.

Experimental treatments

Three dietary ME levels (3100, 3200 and 3300 kcal/kg) and four SID Lysine levels (0.98%, 1.07%, 1.16%, and 1.25%) were evaluated. The dilution method was used to obtain the intermediate lysine levels; for this purpose, two basal diets were formulated (1.30% and 1.57%), subsequently mixed to create two intermediate levels of SID Lys (ranging from 0.98 % to 1.16%), resulting in a total of four levels of SID Lys at each ME level, totaling 12 experimental diets.

Mineral and vitamin supplementation was performed as recommended by Rostagno et al. (2017). The ingredients were purchased from the same batch to avoid differences in nutrient content. The dry matter, crude protein and amino acid profile contents of the experimental diets are presented in Table 2. The requirement for all essential AA was established following the relationship with lysine, based on the ideal protein concept (Rostagno et al., 2017). The diets were provided in mash form.

Productive performance

Feed and animals were weighed at the beginning and end of the experimental period to calculate body weight (**BW**, g), feed intake (**FI**, g/bird), body weight gain (**BWG**, g/bird) and feed conversion rate (**FCR**, g/g). The consumption of ME and SID Lys was calculated by multiplying the CR by the level of ME and SID Lys in the diet.

Body composition

The determination of the protein and body fat deposition was performed by the comparative slaughter method. For this purpose, at 28 days of age, a reference group of ten birds, and at 40 days of age, one bird per EU were randomly selected, weighed and euthanized after a 12 hour fasting period (water provided *ad libitum*).

The carcass was subjected to body composition analysis using the Lunar iDXA unit (encore 2011, version 13.60.033, GE Lunar, General Electric, Madison, WI, USA), using the full body scan option in standard mode (~20 minutes per full DXA chart scan) to measure total mass (g), lean mass percentage (%), fat mass and bone mass. The equations of Aguiar et al. (2024) The equations of Aguiar et al. (2024) were used to estimate body protein (**BP**) and body fat (**BF**), while fat deposition (**FD**), protein deposition (**PD**) were calculated. The relationship between protein deposition and body fat (**BP:BF**) was assessed by calculating the ratio of body protein content to body fat content.

Carcass and cut yield

The carcass and cut yield were evaluated at the end of the experimental period. One bird from each experimental unit was randomly selected and fasted for 12 hours (with water

provided *ad libitum*). After fasting, the birds were weighed and euthanized following approved procedures. After fasting, the birds were weighed (slaughter weight – **SW**) and euthanized following approved procedures. For carcass yield (**CY**), the weight of the clean, eviscerated carcass without feet, head and neck (**CW**) was considered. The prime cuts were weighed (breast - **BRW**, thigh – **TW** and drumstick - **DW**) and the cutting yield (breast yield - **BRY**, thigh yield - **TY** and drumstick yield - **DY**) calculated as a percentage of the eviscerated carcass.

Statistical analysis

The mathematical model of the study was: $Y_{ijk} = F_1 + F_2 + (F_1 * F_2) + e_{ijk}$; Where, Y_{ijk} = observation of the response variable; μ = general means; F_1 = effect of EM levels; F_2 = effect of lysine levels; $(F_1 * F_2)$ = interaction effect; e_{ijk} = random error. The data were subjected to analysis of variance (ANOVA) using the F test. Regression analysis, including both linear and quadratic adjustments, was performed to compare treatments. Adjusted regressions were used to estimate the requirements for ME and SID Lys. The linear plateau, quadratic plateau, and exponential models were tested; however, none provided an adequate fit to the data.

In the case of interactions, means were compared using the Tukey test. An effect was considered significant when $P < 0.05$. The effect of varying SID Lys levels within each ME level was analyzed to explain the effect of different SID Lys:ME ratios, given that these are quantitative data. And the models adjusted for performance and body composition variables at each ME level were compared by contrasting model parameters.

For variables showing significant interaction, multiple linear regression analysis was conducted to determine the models that best explained the results. Additionally, to better understand the behavior of the variables, response surface methodology was applied. This created an interactive graph that illustrated the effects of the variables relative to dietary ME and SID Lys levels, allowing for visual evaluation of the observed interactions between the factors.

RESULTS

Analysis of experimental diets

The analyzed nutrient levels in the experimental diets were consistent with the calculated values (Table 2).

Productive Performance

An interaction between ME and Lys was observed for FCR ($P < 0.05$), with better FCR at higher levels of ME and SID Lys (Table 4, Figure 4). In the surface graph we can see the variation in the color range, that is, the contours show the effect of the interaction between SID Lys and ME, such as better FCR values with an increase in SID Lys and ME. A quadratic effect

of ME levels on BW was found, with a requirement of 3178.27 kcal/kg of ME for a maximum weight of 3214.91 g, and a quadratic effect on BWG ($P<0.05$), with a requirement of 3179.45 kcal/kg for a maximum gain of 1638.76 g. There was an increasing linear effect of ME levels on energy intake (Table 3, Figure 1).

An increasing linear effect of SID Lys levels on BW was observed, and a quadratic effect on BWG ($P<0.05$), with a level of 1.19% SID Lys for a gain of 1755.5 g. there was an increasing linear effect of SID Lys levels on lysine intake and a decreasing linear effect on FI and energy intake ($P<0.05$) (Table 3, Figure 1).

Considering the effect of SID Lys levels at each ME level, an increasing linear effect ($P<0.05$) of SID Lys was observed on BW and BWG at 3100 kcal/kg and 3300 kcal/kg ME. A quadratic effect ($P<0.05$) was found at 3200 kcal/kg ME on both BW and BWG, with a requirement of 1.16% SID Lys for the maximum response of 3310.06 g for BW and 1737.90 g for BWG, respectively. Additionally, a decreasing linear effect ($P<0.05$) was observed in FCR at the three ME levels. Furthermore, a decreasing linear effect on FI was observed at the 3200 kcal/kg and 3300 kcal/kg levels ($P<0.05$). There was an increasing linear effect of SID lysine levels on lysine intake at the 3100 Kcal/kg and 3300 Kcal/kg levels ($P<0.05$) and a quadratic effect at the 3200 kcal/kg level, with 1.25 lysine for 30.73 g intake. There was a decreasing linear effect of Lys SID levels on energy intake at the 3300 kcal/kg level. And a quadratic effect at the 3200 kcal/kg level, with 1.07 lysine for an intake of 8,451.4 kcal (Tables 9, 10 and 11).

For FCR, the intercepts and slopes of the equations for the levels of 3100 Kcal/kg and 3300 Kcal/kg are statistically equal, that is, there is no difference between the initial point and the speed of response variation, while at the level of 3200 Kcal/kg, the FCR at the intersection point presents a higher initial value, that is, it presents a worse FCR ($P<0.05$).

Through multiple linear equation analysis, the FCR of broilers can be explained by the following equation: $FCR = 6.1545908366 - 0.00035807 * ME - 5.41169922 * SID \text{ Lys} + 2.039825922 * SID \text{ Lys}^2$, $R^2 \text{ adjusted} = 0.713$ (ME ranging from 3100 a 3300 and SID Lys ranging from 0.98 a 1.25, with an optimized value of 1.40 g/g for the levels of 3300 Kcal/kg of ME and 1.25% of SID Lys).

Body composition

An interaction between ME and SID Lys was observed for BP and BD ($P<0.05$), with the lowest ME levels and highest SID Lys levels yielding the best results (Tables 6 and 7; Figures 5 and 6). In the surface graph we can observe the greatest variation in the color range, that is, the contours parallel to the x-axis (SID Lys) showing that the level of SID Lys has a greater effect on BP and PD, as evidenced in the multiple regression. An increasing linear effect

of ME levels was observed on BF and FD. And quadratic effect ($P<0.05$) of SID Lys levels on BF and FD, as levels of 1.05% and 1.06% of SID Lys for 323.16 g and 220.13 g, respectively. And increasing linear effect of SID Lys levels on BP:BF ($P<0.05$) (Table 5; Figure 2).

Considering the effect of Lys SID levels at each ME level, an increasing linear effect of Lys SID on BP and PD was observed at the levels of 3100 kcal/kg and 3300 kcal/kg. A quadratic effect was observed at the level of 3200 kcal/kg, with 1.21% Lys SID level for a response of 584.70 g and 325.40 g, respectively. Furthermore, a decreasing linear effect was observed at the levels of 3100 kcal/kg and 3,200 kcal/kg ($P<0.05$) on BF and DF. Regarding BP:BF, there was a quadratic effect at the level of 3200 Kcal/kg, with a level of 1% SID Lys for a ratio of 1.615. In addition to a decreasing linear effect at the level of 3300 Kcal/kg (Tables 9, 10 and 11).

For BP and PD the intercepts and slopes of the equations for the 3100 Kcal/kg and 3300 Kcal/kg ME levels are statistically equal to each other and different from the coefficients for the 3200 Kcal/kg ME level. The intercept at the 3200 Kcal/kg level is lower than at the other levels, which means a lower starting point for body protein. However, with a greater slope, which indicates a greater speed of variation in body protein as digestible lysine increased ($P<0.05$).

In the multiple regression analysis, only SID Lys was significant in explaining the responses of BP content and PD, according to the following equations: $BP = 252.72716 + 0.021902004 * ME + 217.336702 * SID \text{ Lys}$ ($R^2 = 0.39$) and $PD = -13.7905763 + 0.028061905 * ME + 205.0272962 * SID \text{ Lys}$ ($R^2 = 0.36$), respectively, with ME ranging from 3100 Kcal/kg to 3300 Kcal/kg and SID Lys ranging from 0.98% to 1.25%, with optimized values of 594.23g and 332.29g for BP and PD, respectively, for the levels of 3200 Kcal/kg of ME and 1.25% of SID Lys.

In the response surface graphs for BP and PD, it can be observed that at all ME levels, the highest values for these variables were achieved at the highest dietary SID Lys levels.

Carcass and cut yield

There no interaction was observed between the ME and SID Lys for carcass yield and cuts ($P<0.05$). A quadratic effect ($P<0.05$) of ME levels on slaughter weight (SW) and carcass weight (CW) was found, with levels of 3,179.41 Kcal/kg and 3,178.86 Kcal/kg achieving 2,652.55 g and 2,527.2 g of weight, respectively. Regarding Lys SID levels, there was a linear effect for drumstick weight ($P<0.05$) and quadratic effect ($P<0.05$) on SW, CW, BRW and BRY, as levels of 1.17%, 1.19%, 1.15% and 1.13% of Lys SID for a maximum response of 3,296.24 g, 2,626.39 g, 992.86 g and 37.90%, respectively (Table 8; Figure 3).

Furthermore, an increasing linear effect ($P<0.05$) of Lys SID levels on DW and a quadratic effect on SW, CW, BRW and BRY were observed. The estimated requirements for maximum responses were 1.17%, 1.19%, 1.15% and 1.13% Lys SID for 3296.24 g, 2626.39 g, 992.86 g and 37.90%, respectively (Table 8; Figure 3).

Considering the effect of SID Lys levels at each ME level, there was an increasing linear effect ($P<0.05$) of SID Lys on SW at the 3100 kcal/kg and 3300 kcal/kg levels, and a quadratic effect at the 3200 kcal/kg level, with 1.16% of SID Lys for 3260.98 g of weight. At the 3100 kcal/kg level, there was a quadratic effect ($P<0.05$) of SID Lys levels on BRW and BRY, with a level of 1.13% for 1012.82 g and 38.93 g, respectively. At the 3200 kcal/kg level, there was an increasing linear effect ($P<0.05$) of SID Lys levels on CW, TW and DW and a quadratic effect on BRW, with a level of 1.15% for 1007 g of weight (Tables 9, 10 and 11). The slopes and intercepts of the equations were statistically similar for all yield variables.

DISCUSSION

To ensure optimal performance and carcass yield in broiler chickens, thereby maximizing production profitability, it is crucial to formulate balanced diets, particularly concerning the requirements for SID Lys and ME (Johnson et al., 2020). Achieving the balance between these nutrients primarily involves using the ideal protein concept and considering the SID Lys:ME ratio. This approach aims to prevent both deficiency and excess of digestible amino acids and metabolizable energy, enhancing weight gain in the form of muscle tissue while reducing fat deposition in the carcass (Maharjan et al., 2021a).

Feed intake regulation in animals is influenced by a complex interplay of physiological mechanisms (Classen, 2017). The impact of dietary energy levels on FI regulation is well established, particularly through the glucostatic theory (Ferket and Gernat, 2006). Analyzing the effect of SID Lys levels at different ME levels, it is evident that dietary ME continues to regulate FI in broilers, with a linear reduction in FI as ME levels increase, with the highest energy intake, even in mash diets. Another noteworthy finding was the reduction in FI with increasing dietary AA density. This observation aligns with the aminostatic theory, which suggests that FI is regulated by dietary amino acid availability (Lamot et al., 2017). Similar findings were reported by Mushtaq et al. (2015), where FI decreased as dietary digestible lysine levels increased.

The FCR is a reliable metric for assessing the productive efficiency of a batch. Interestingly, the calculation of FCR disregards the animal's maintenance energy, as it only considers BWG and FI, focusing solely on the energy used for growth (Prakash et al., 2020).

The positive effects of increased BWG in response to higher levels of SID Lys and ME become evident when we observe the significant improvement in FCR due to the interaction between these factors.

This highlights the idea that higher levels of digestible lysine, when paired with a higher dietary energy density, enhance the efficiency of both energy and amino acid utilization (Ge et al., 2019), likely through the optimal adjustment of the SID Lys:ME ratio. The response surface plot and multiple linear regression analysis for FCR further support that increased lysine levels have a more substantial impact on improving FCR, with an optimized value of 1.40 g/g identified through response surface methodology (Kidd and Tillman, 2016).

Our results revealed that in male broilers of high genetic potential, dietary lysine levels above 1.16% promoted a better FCR response at low ME level (3100 kcal), while levels of 1.25% SID Lys promoted the best FCR responses in medium and high ME (3200 and 3300 kcal/kg), with the best FCR being verified with the highest level of ME and SID Lys (FCR= 1.40 g/g).

These results should be related to the responses of PD and FD, where the increase in ME from 3100 kcal/kg to 3300 kcal/kg did not impact protein deposition while increasing the body fat deposition of broilers by 18.4%. While SID Lys levels of 0.98% and 1.25% promoted increases of 21% in protein deposition and reduction of 24.2% in body fat deposition, respectively, a result that is reinforced by the increasing linear effect of SID Lys levels on the relationship between protein and body fat.

In our results indicated that reducing SID Lys levels and increasing dietary ME negatively impacted the carcass composition of modern broilers. This combination drastically reduced body protein deposition while increasing the percentage of fat in the carcass. Excess ME in the diet is known to be directly related to increased fat deposition, as the bird, once reaching the maximum level of protein synthesis, directs the surplus energy towards storage in the form of adipose tissue (Maharjan et al., 2021a). This study clearly demonstrates the limitations of insufficient dietary lysine for protein development in broilers, as well as the high lysine requirements of birds with high genetic potential.

An increase in body fat deposition has been one of the major challenges faced by the poultry industry, as it negatively affects commercial carcass yield, leading to increased processing losses and, ultimately, reducing production profitability (Ge et al., 2019). Similar findings were reported by Maharjan et al. (2021a), where higher dietary energy levels led to increased fat mass.

Reducing dietary energy density, as observed with lower levels of ME, results in a lower accumulation of body fat. This effect is thought to be related to the inhibitory impact of lower energy density on enzymes associated with hepatic lipogenesis, such as nicotinamide adenine dinucleotide phosphate-malate dehydrogenase (NAD-MDH), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase, and fatty acid synthase (FAS), as noted by Fouad et al. (2014). Therefore, as demonstrated in the body composition results, the ME level of the diet should be carefully balanced. While increasing energy content may improve feed conversion, it also leads to greater body fat deposition.

Dietary amino acid density, particularly lysine, plays a crucial role in the development of skeletal muscle in birds (Maharjan et al., 2020a). The findings in this study, where higher BW, BWG, BP, CY and BRY were observed alongside lower fat deposition in birds receiving diets with 1.25% SID Lys, indicate a more efficient use of energy for protein metabolism (Musigwa et al., 2020).

This suggests an adequate supply of AA for muscle protein synthesis, thus reducing the proportion of energy directed toward body fat deposition. The observed interaction between SID Lys and ME levels for BP and PD further supports this concept. It is also important to note that modern broiler chicken lines have been genetically selected for higher yields of prime cuts, such as the breast, thigh, and drumstick (Vieira and Angel, 2012). Given that lysine is the primary AA involved in muscle protein synthesis, these genetic improvements necessitate an increased SID Lys requirement to support the greater weight of these cuts, ultimately contributing to enhanced BW and BWG (Aftab, 2019).

There is ongoing discussion among nutritionists that the current amino acid requirements may not be ideal for optimizing growth and feed efficiency in modern broilers. This is based on the idea that contemporary breeds are more efficient in their use of amino acids and are increasingly responsive to higher amino acid density in the diet (Aftab, 2019).

Our findings support this hypothesis and align with research such as that of Sharma et al. (2018), who observed a linear increase in body weight and breast yield, alongside a reduction in feed conversion rate, in broilers aged 14 to 34 days. In their study, the authors evaluated digestible lysine levels ranging from 0.95% to 1.15%, approximately 10% above the recommended levels for this phase, further reinforcing this concept.

The results of the multiple linear regression analysis and the optimization of interactions using response surface graphs for the variables exhibiting interaction between SID Lys and ME (FCR, BP, and PD) reinforce that high nutrient and energy densities favor FCR. However, the main impact is related to the availability of lysine and other amino acids for protein synthesis

in improved broilers, evidenced by a progressive increase in body protein deposition at higher SID Lys levels across all ME levels.

Optimized values of BP and PD were observed at 3200 kcal/kg and 1.25% SID Lys, along with a reduction in fat deposition, which modulates the relationship between protein mass and body adipose tissue. These results express the improvement in growth efficiency and body composition of modern broilers and validate the need for constant adjustment in dietary AA density to optimize the performance and deposition of protein and body fat in these birds (Kidd and Tillman, 2016).

The beneficial effects of increased dietary lysine on performance parameters and carcass characteristics have also been documented in several studies, such as that carried out by Maharjan et al. (2020b) where the increase in dietary lysine from 0.80% to 1.10% resulted in greater breast yield and reduced body fat in birds, which was consistent with our findings, where a reduction in BF and FD was observed with the increase in digestible lysine from 0.98% to 1.25%.

When analyzing the lysine requirement for gain, we observed that, at the level of 1.16% SID Lys the requirement was 16.62 g lysine/kg of BWG, and at the level of 1.25% SID Lys it was 17.49 g lysine/kg of BWG. In comparison, using equation to estimate the lysine requirement proposed by Rostagno et al. (2017) the value was of 19.06 g lysine/kg of BWG. In our results we have a difference of 2.44 and 1.57 points for the levels of 1.16% and 1.25% of SID Lys, respectively, these results highlight that modern broilers are more efficient in using lysine for gain (Aftab, 2019), that is, they make greater use of lysine in the diet to support gain, whether in the form of performance, with greater body weight and weight gain and lower FCR, in the greater carcass yield and prime cuts or in the greater deposition of body protein.

Considering the independent effects of ME and SID Lys levels, as well as the interactions observed in several key parameters with distinct response intensities, it was found that at the lowest ME level studied (3100 kcal/kg), the best performance and body composition responses—manifested in greater protein gain and lower fat gain, were achieved at a SID Lys:ME ratio of 4.03 g/Mcal. For carcass and cut yield, however, the optimal ratio was 3.64 g/Mcal. At the intermediate ME level of 3200 kcal/kg, the best responses were observed at the following ratios: 3.62 g/Mcal for body weight gain (BWG), 3.78 g/Mcal for body composition and protein gain, and 3.90 g/Mcal for fat deposition and body fat reduction. At the highest ME level (3300 kcal/kg), the optimal performance and body composition responses were achieved with a ratio of 3.78 g/Mcal. These findings highlight the effects of varying levels of ME and

SID Lys in the diet and their distinct impacts on critical zootechnical parameters in improved broiler chickens.

CONCLUSION

Broilers aged 28 – 40 days fed a diet containing 3,300 kcal/kg ME and 1.25% Lys SID (equivalent to a ratio of 3.78 g/Mcal) showed the best feed conversion ratio (FCR) at 1.40 g/g. However, caution is advised when using the 3,300 kcal/kg ME level, as this leads to increased carcass fat deposition. In contrast, the optimal balance for protein mass deposition, weight gain, and carcass and cut yield, found by multiple regression, was achieved with a Lys:ME ratio of 3.90 g/Mcal, corresponding to a diet of 3,200 kcal/kg ME and 1.25% Lys SID.

For future research, an economic analysis combined with the measurement of biochemical and molecular markers is necessary to confirm the reduction in production costs and to provide a better understanding of the animal's physiological mechanisms in response to the optimal SID Lys:ME ratio.

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Figures

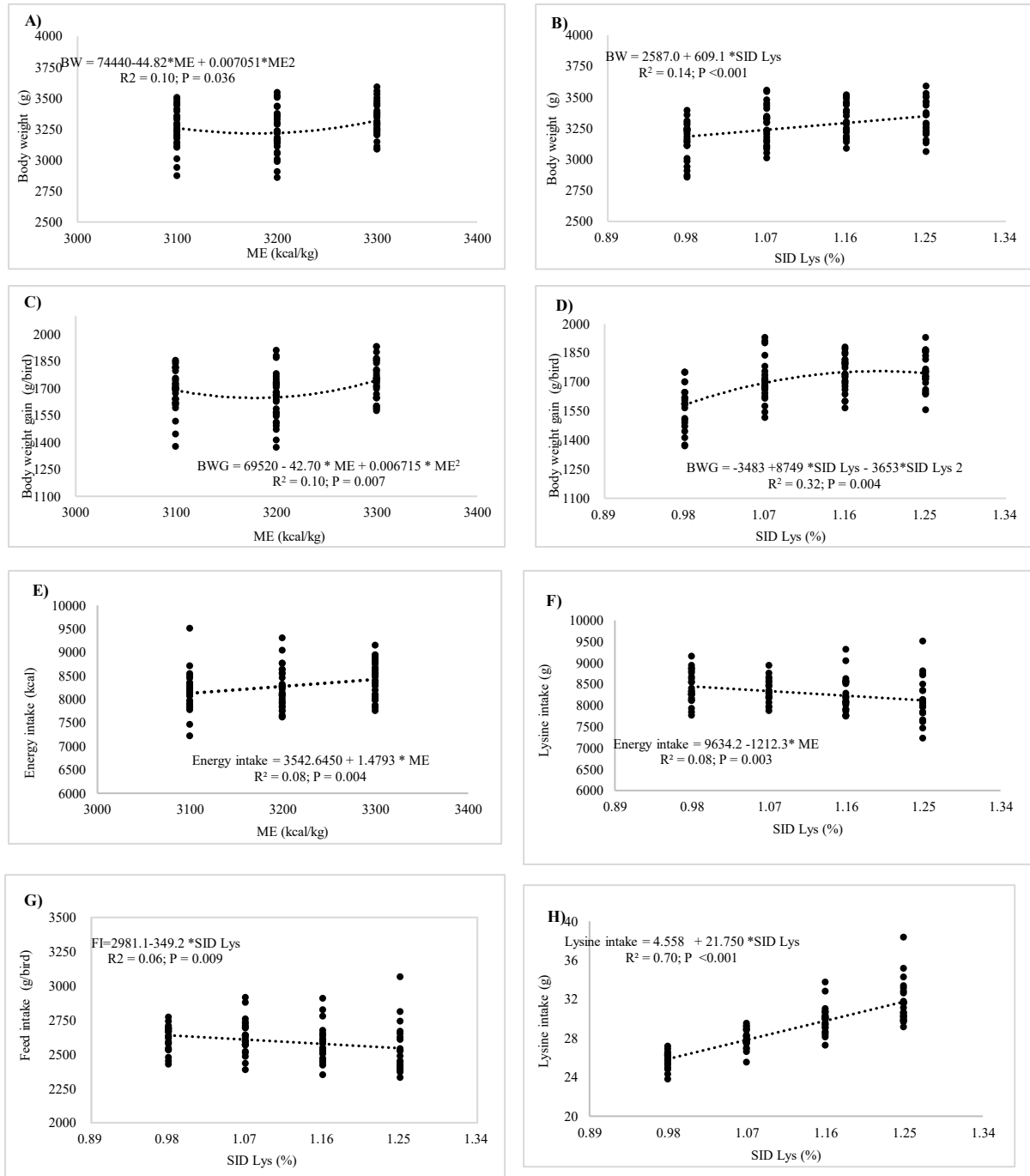


Figure 1: Effect of digestible lysine and metabolizable energy levels on the performance of 28- to 40-day-old broilers. Figures 1A and 1B show the effect of energy and lysine on body weight (BW), respectively. Figures 1C and 1D show the effect of energy and lysine on body weight gain (BWG), respectively. Figures 1E and 1F show the effect of energy and lysine on energy intake, respectively. Figures 1G and 1H show the effect of lysine on feed intake (FI) and lysine intake, respectively.

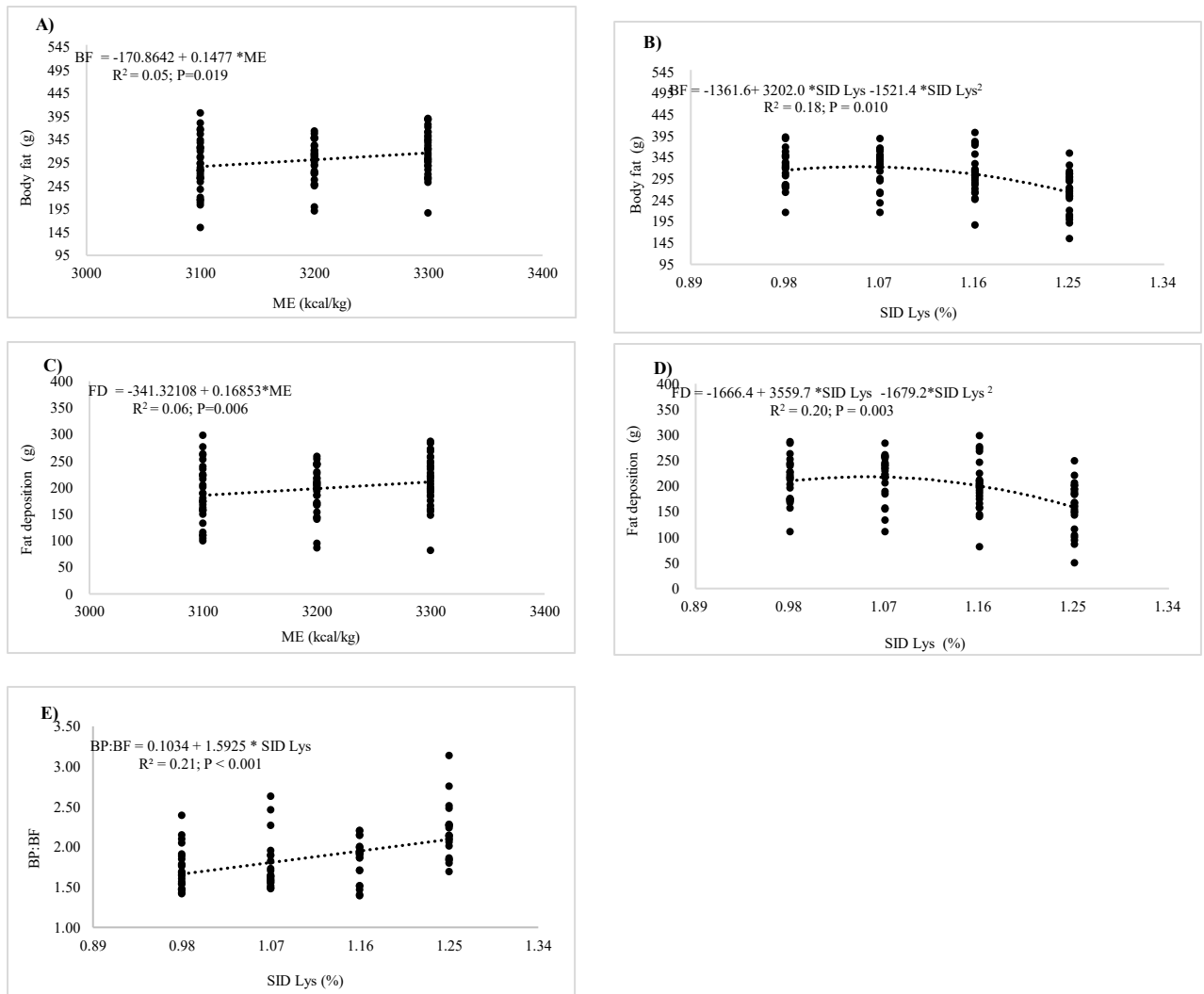


Figure 2: Effect of digestible lysine and metabolizable energy levels on body composition of 28- to 40-day-old broilers. Figures 2A and 2B show the effect of energy and lysine on body fat (BF), respectively. Figures 2C and 2D show the effect of energy and lysine on body fat deposition (BF), respectively. Figure 2E shows the effect of lysine on the ratio of protein to body fat deposition (BP:BF).

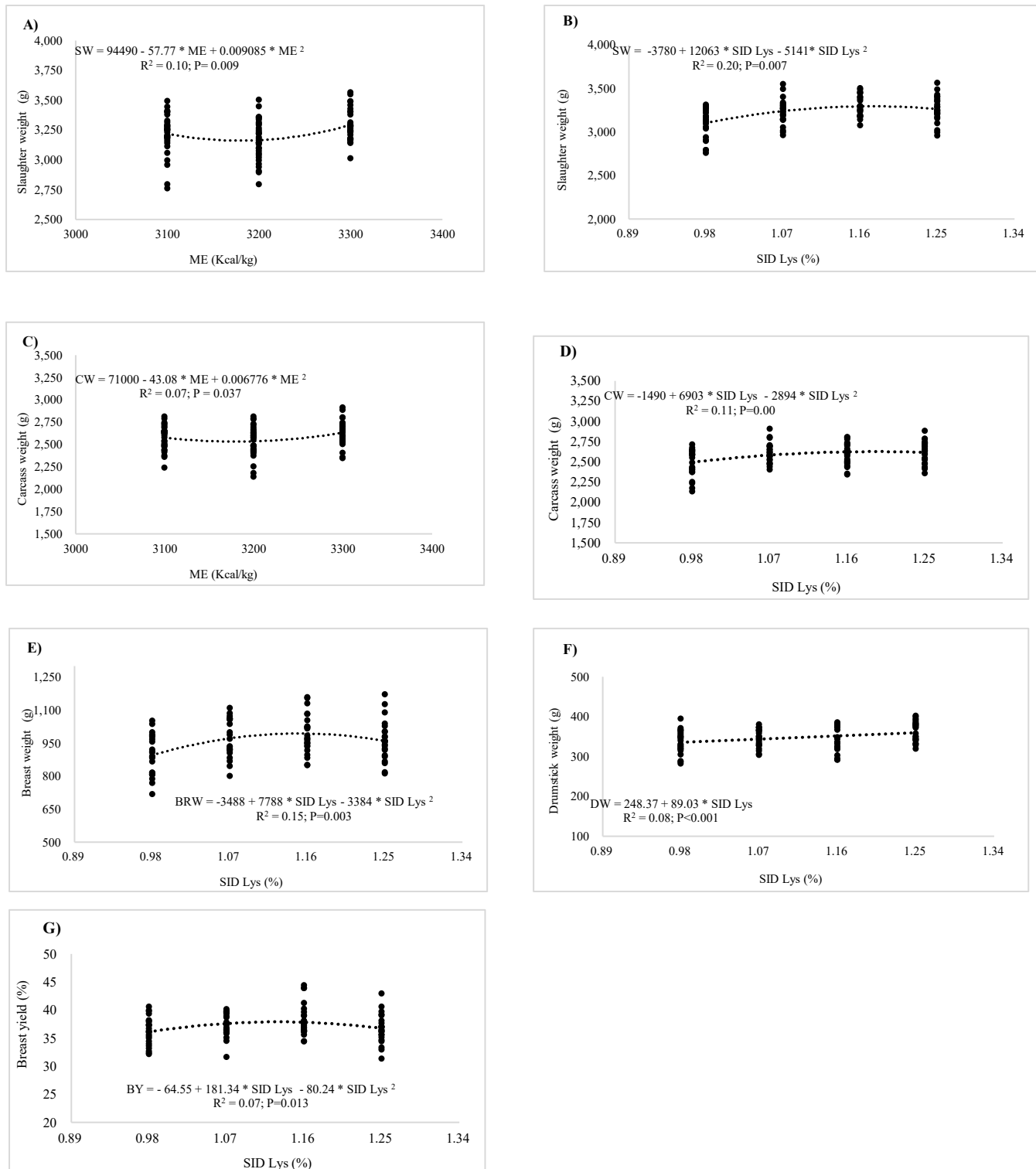


Figure 3: Effect of digestible lysine and metabolizable energy levels on carcass and cut yield of broilers from 28 to 40 days of age. Figures 3A and 3B show the effect of energy and lysine on slaughter weight (SW), respectively. Figures 3C and 3D show the effect of energy and lysine on carcass weight (CW), respectively. The figures show the effect of lysine on breast weight (BRW), drumstick weight (DW), and breast yield (BRY), respectively.

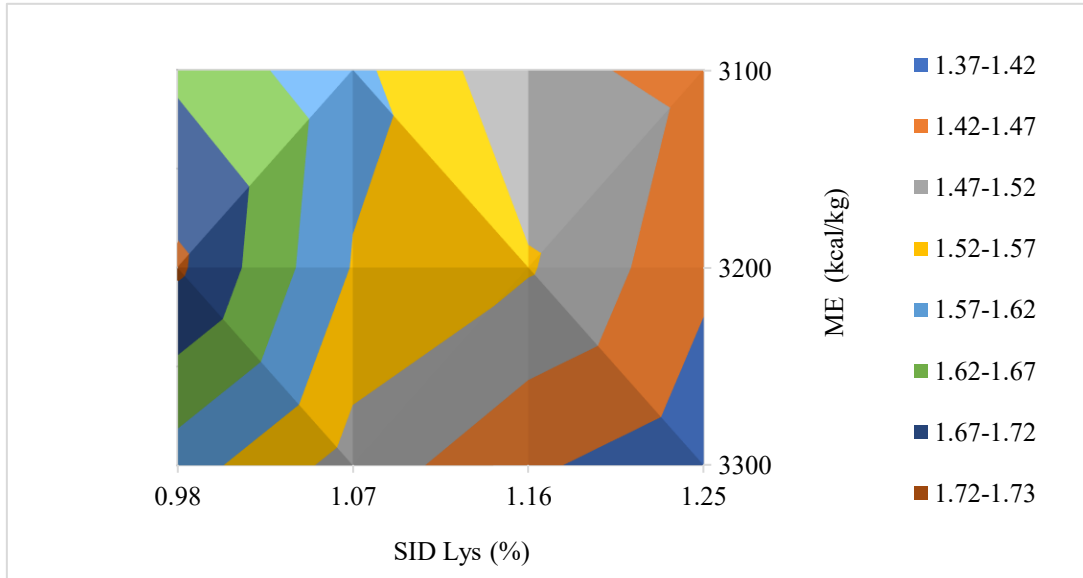


Figure 4: Response surface describing the relationship between ME, SID Lys and FCR levels in 28- to 40-day-old broilers

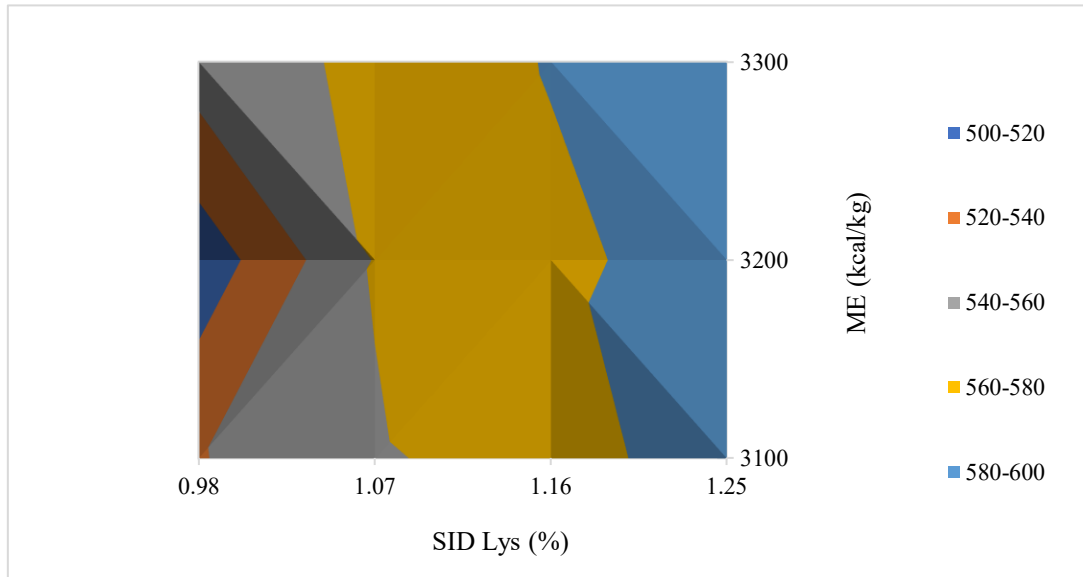


Figure 5: Response surface describing the relationship between ME, SID Lys and body protein in broilers aged 28 to 40 days.

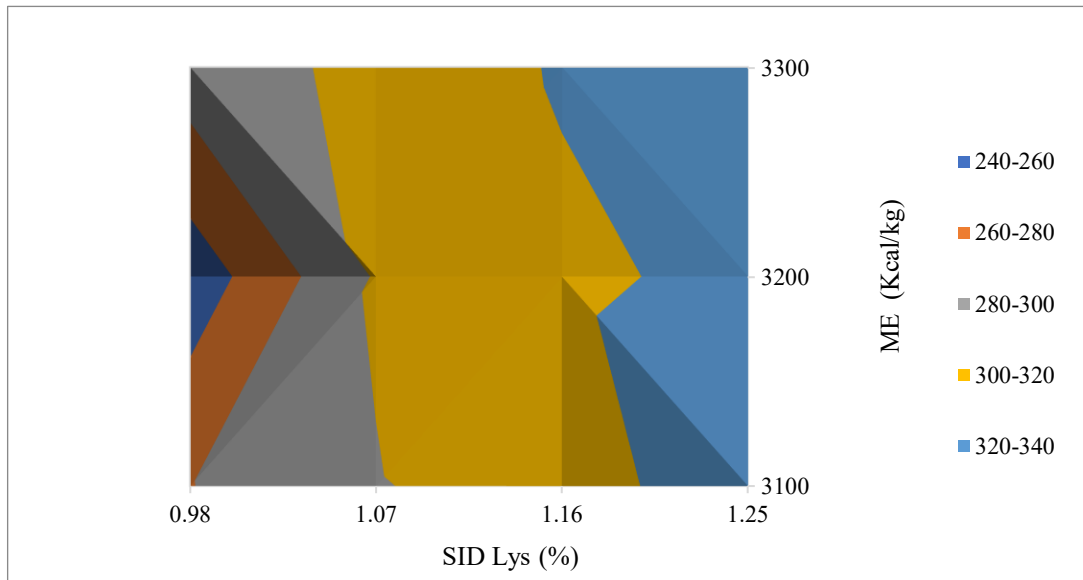


Figure 6: Response surface describing the relationship between ME, SID Lys and body protein deposition in 28- to 40-day-old broilers.

Tables

Table 1: Centesimal and calculated composition of experimental diets for broiler chickens aged 28 to 40 days

Ingredients	3100 ME ¹ (kcal/kg)				3200 ME (kcal/kg)				3300 ME (kcal/kg)			
	SID Lys ² (%)				SID Lys (%)				SID Lys (%)			
	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25
Corn 7.88%	674.35	629.98	585.6	541.24	656.64	612.26	567.87	523.49	583.07	556.91	530.75	504.59
Soybean Meal 46%	266.07	303.91	341.76	379.61	268.20	306.07	343.95	381.83	278.36	313.63	348.90	384.17
Soy oil	19.05	25.33	31.62	37.90	36.60	42.90	49.20	55.50	73.40	73.40	73.40	73.40
Dicalcium phosphate	13.90	13.60	13.30	13.00	13.90	13.60	13.30	13.00	14.00	13.70	13.40	13.10
Calcitic limestone	8.10	7.97	7.83	7.70	8.10	7.93	7.77	7.60	7.90	7.80	7.70	7.60
Cocciostat ³	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin Premix ⁴	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Micromineral Premix ⁵	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Antioxidant ⁶	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	5.20	5.18	5.17	5.15	5.20	5.18	5.17	5.15	5.20	5.18	5.17	5.15
L-Arginine	0.04	0.03	0.02	0.00	0.03	0.03	0.01	0.00	0.00	0.00	0.00	0.00
L-Glycine	0.00	0.08	0.16	0.24	0.00	0.08	0.17	0.25	0.00	0.09	0.18	0.28
L-Lysine HCl	2.04	2.04	2.04	2.04	2.01	2.01	2.01	2.01	1.86	1.90	1.94	1.99
DL-Methionine	2.44	2.82	3.19	3.57	2.47	2.84	3.22	3.60	2.56	2.91	3.27	3.63
L-Threonine	0.54	0.69	0.84	0.99	0.56	0.71	0.86	1.00	0.615	0.75	0.89	1.02
L-Valine	0.39	0.48	0.58	0.68	0.40	0.50	0.59	0.69	0.43	0.52	0.61	0.70
Choline Chloride 60	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Inert ⁷	4.00	4.00	4.00	4.00	2.00	2.00	2.00	2.00	28.73	19.32	9.91	0.50
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Nutritional levels												
Met. Energy (kcal/kg)	3100	3100	3100	3100	3200	3200	3200	3200	3300	3300	3300	3300
Fat (%)	5.22	5.79	6.35	6.92	6.91	7.48	8.04	8.61	10.32	10.33	10.33	10.33
Crude Protein (%)	18.07	19.53	20.98	22.44	18.03	19.49	20.95	22.41	17.91	19.40	20.88	22.37
Dig. lysine (%)	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25
Dig. methionine (%)	0.49	0.55	0.60	0.65	0.50	0.55	0.60	0.65	0.50	0.55	0.60	0.65
Met + Cys. dig. (%)	0.75	0.82	0.88	0.95	0.75	0.82	0.88	0.95	0.75	0.82	0.88	0.95
Dig. threonine (%)	0.67	0.73	0.79	0.85	0.67	0.73	0.79	0.85	0.67	0.73	0.79	0.85
Dig. tryptophan (%)	0.20	0.22	0.24	0.26	0.20	0.22	0.24	0.26	0.20	0.22	0.24	0.26
Dig. Arginine (%)	1.08	1.18	1.29	1.39	1.08	1.18	1.29	1.39	1.08	1.19	1.29	1.39
Dig. Glyc. + Ser. (%)	1.38	1.51	1.63	1.76	1.38	1.51	1.63	1.76	1.38	1.51	1.63	1.76
Dig. Valine (%)	0.78	0.85	0.92	0.99	0.78	0.85	0.92	0.99	0.78	0.85	0.92	0.99
Dig. Isoleucine (%)	0.68	0.74	0.80	0.86	0.68	0.74	0.80	0.86	0.68	0.74	0.80	0.86
Dig. Leucine (%)	1.46	1.55	1.63	1.71	1.46	1.54	1.62	1.70	1.42	1.51	1.60	1.69
Dig. Histidine (%)	0.44	0.47	0.50	0.53	0.44	0.47	0.50	0.53	0.43	0.46	0.50	0.53
Dig. Phenylalanine (%)	0.80	0.86	0.93	1.00	0.80	0.86	0.93	1.00	0.79	0.86	0.93	1.00

Dig. Phen. + Tyr. (%)	1.39	1.51	1.62	1.74	1.39	1.51	1.62	1.73	1.39	1.50	1.62	1.73
Gross Fiber (%)	2.31	2.39	2.48	2.56	2.29	2.37	2.46	2.54	2.20	2.31	2.41	2.52
Total calcium (%)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Total Phosphorus (%)	0.58	0.58	0.59	0.59	0.57	0.58	0.59	0.59	0.56	0.57	0.58	0.59
Avail. Phosphorus (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Potassium (%)	0.70	0.76	0.81	0.87	0.70	0.76	0.81	0.87	0.70	0.75	0.81	0.86
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Chlorine (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.37	0.38	0.38	0.37	0.37
Choline (mg/kg)	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00	477.00

¹Metabolizable energy

² Digestible lysine

³ Salinomycin sodium (12%)

⁴ Vit. A-9750 U.I.; Vit. D3-2470 U.I.; Vit. E-36.6 U.I.; Vit. B1 - 2,60 mg; Vit. B2 = 6,50 mg; Vit. B6 - 3,64 mg; Vit. B12 - 0,015 mg; Pantothenic acid - 13,0 g; Biotin - 0,091 mg; Vit. K3 = 1,95 mg; Folic acid - 0,91 mg; Nicotinic acid - 39,0 mg

⁵ manganese, 58,360 mg; zinc, 54,210 mg; iron, 41,680 mg; copper, 8,310 mg; selenium, 250 mg e iodine 843 mg.

⁶ Hydroxybutyl toluene

⁷ Kaolin

Table 2. Crude protein, dry matter and amino acid profile analyzed in experimental diets of broiler chickens 28 to 40 days of age

Item	3100 ME ¹ (kcal/kg)				3200 ME (kcal/kg)				3300 ME (kcal/kg)			
	SID Lysine Levels ² (%)				SID Lysine Levels (%)				SID Lysine Levels (%)			
	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25	0.98	1.07	1.16	1.25
Dry Matter (%)	89.99	89.75	90.11	90.37	89.98	90	90.27	90.20	90.65	90.67	90.51	90.32
Crude Protein (%)	17.59	19.13	20.99	21.79	16.81	18.9	20.61	21.98	17.6	18.81	20.69	22.35
Methionine (%)	0.48	0.55	0.56	0.62	0.47	0.52	0.58	0.65	0.48	0.53	0.58	0.61
Cystine (%)	0.28	0.29	0.33	0.33	0.27	0.29	0.32	0.32	0.27	0.29	0.31	0.33
Met + Cys (%)	0.76	0.84	0.89	0.95	0.74	0.81	0.90	0.98	0.76	0.82	0.90	0.95
Lysine (%)	1.03	1.16	1.23	1.31	1.00	1.11	1.23	1.35	1.02	1.12	1.25	1.32
Threonine (%)	0.70	0.79	0.88	0.91	0.68	0.76	0.85	0.90	0.72	0.78	0.84	0.92
Arginine (%)	1.10	1.23	1.37	1.43	1.08	1.21	1.37	1.41	1.12	1.22	1.34	1.45
Isoleucine (%)	0.72	0.80	0.90	0.94	0.69	0.79	0.89	0.91	0.72	0.79	0.87	0.93
Leucine (%)	1.54	1.65	1.81	1.85	1.41	1.55	1.75	1.78	1.51	1.59	1.72	1.83
Valine (%)	0.87	0.96	1.05	1.10	0.82	0.94	1.04	1.09	0.85	0.93	1.02	1.09
Histidine (%)	0.46	0.50	0.55	0.57	0.44	0.49	0.54	0.56	0.46	0.49	0.53	0.57
Phenylalanine (%)	0.85	0.94	1.04	1.08	0.81	0.91	1.04	1.05	0.86	0.92	1.00	1.09
Glycine (%)	0.70	0.79	0.88	0.91	0.69	0.77	0.87	0.89	0.71	0.78	0.85	0.92
Serine (%)	0.83	0.92	1.04	1.06	0.80	0.89	1.01	1.03	0.86	0.91	0.99	1.08
Proline (%)	1.07	1.14	1.23	1.26	1.03	1.10	1.21	1.22	1.07	1.12	1.19	1.26
Alanine (%)	0.92	0.98	1.07	1.08	0.86	0.93	1.03	1.04	0.89	0.94	1.01	1.07
Aspartate (%)	1.70	1.91	2.18	2.23	1.63	1.86	2.11	2.19	1.74	1.89	2.08	2.27
Glutamate (%)	3.03	3.32	3.70	3.82	2.85	3.19	3.61	3.70	3.03	3.24	3.53	3.80

¹ Metabolizable energy, ² Digestible lysine

Table 3: Performance of broiler chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

Treatments	SID Lys:ME (g/Mcal)	BW ¹ (g)	BWG ² (g/bird)	FI ³ (g/bird)	FCR ⁴ (g/g)	Lysine intake (g)	Energy intake (kcal)
3100 ME ⁵ /0.98 SID Lys ⁶	3.16	3154	1581	2617	1.66	25.89	8189
3100 ME /1.07 SID Lys	3.45	3230	1660	2628	1.58	28.41	8233
3100 ME /1.16 SID Lys	3.74	3333	1764	2613	1.48	30.04	8028
3100 ME /1.25 SID Lys	4.03	3316	1746	2639	1.47	32.99	8183
3200 ME /0.98 SID Lys	3.06	3055	1488	2588	1.74	25.36	8282
3200 ME /1.07 SID Lys	3.34	3269	1699	2659	1.56	28.06	8392
3200 ME /1.16 SID Lys	3.63	3286	1715	2615	1.52	30.33	8368
3200 ME /1.25 SID Lys	3.91	3265	1694	2452	1.43	30.65	7848
3300 ME /0.98 SID Lys	2.97	3246	1670	2661	1.59	26.08	8783
3300 ME /1.07 SID Lys	3.24	3327	1757	2594	1.49	27.32	8426
3300 ME /1.16 SID Lys	3.52	3322	1743	2527	1.42	29.32	8340
3300 ME /1.25 SID Lys	3.79	3385	1806	2503	1.38	31.29	8260
SEM ⁷		16.089	12.223	13.715	0.011	0.277	43.203
Effect							
ME		< 001	< 001	0.168	< 001	0.021	0.003
SID Lys		< 001	< 001	0.027	< 001	<.001	0.016
ME x SID Lys		0.091	0.083	0.075	0.025	0.067	0.064
ME (kcal/kg)							
3100		3258	1688	2624	1.55	29.46	8159
3200		3219	1649	2578	1.57	28.62	8217
3300		3320	1744	2571	1.47	28.54	8453
Regression							
Linear		ns	ns	ns	ns	ns	*
Quadratic		*	*	ns	ns	ns	ns
SID Lys (%)							
0.98		3152	1579	2622	1.66	25.77	8428
1.07		3275	1705	2627	1.55	27.93	8350
1.16		3314	1741	2585	1.48	29.89	8255
1.25		3322	1748	2531	1.42	31.64	8097
Regression							
Linear		*	ns	*	ns	*	*
Quadratic		ns	*	ns	ns	ns	ns

¹ Body weight

² Body weight gain

³ Feed intake

⁴ Feed conversion rate

⁵ Metabolizable energy level

⁶ Digestible lysine level

⁷ Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

Table 4. Result of the breakdown of factors (ME x SID Lys) for FCR of chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

SID Lys level ¹	ME Level ²			SEM ³	<i>P</i> value
	3100	3200	3300		
0.98	1.66 bA	1.74 aA	1.59 cA	0.001	0.006
1.07	1.58 aB	1.56 aB	1.49 bB		0.005
1.16	1.48 aC	1.52 aB	1.42 bB		<0.001
1.25	1.47 aC	1.43 abC	1.38 bC		0.003
<i>P</i> value	< 0.001	0.003	0.002		

Different letters, uppercase in the column and lowercase in the row, differ from each other by the 5% SNK test.

¹ Digestible lysine

² Metabolizable energy

³ Standard Error of the Mean

Table 5: Body composition of broiler chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

Treatments	SID Lys:ME (g/Mcal)	BP ¹ (g)	BF ² (g)	PD ³ (g)	FD ⁴ (g)	BP:BF ⁵
3100 ME ⁶ /0.98 SID Lys ⁷	3.16	538.7	304.4	279.4	199.3	1.812
3100 ME /1.07 SID Lys	3.45	558.6	296.0	299.2	190.8	1.955
3100 ME /1.16 SID Lys	3.74	565.7	313.8	306.4	208.7	1.863
3100 ME /1.25 SID Lys	4.03	600.6	238.6	335.3	133.5	2.208
3200 ME /0.98 SID Lys	3.06	507.1	314.7	247.7	209.6	1.625
3200 ME /1.07 SID Lys	3.34	561.0	338.7	301.6	233.6	1.662
3200 ME /1.16 SID Lys	3.63	575.1	290.9	315.7	185.7	1.921
3200 ME /1.25 SID Lys	3.91	585.1	253.4	325.7	148.3	2.363
3300 ME /0.98 SID Lys	2.97	550.8	325.5	291.5	220.4	1.745
3300 ME /1.07 SID Lys	3.24	563.7	336.1	304.3	231.0	1.630
3300 ME /1.16 SID Lys	3.52	581.3	309.2	321.9	221.6	1.755
3300 ME /1.25 SID Lys	3.79	586.0	300.0	326.7	194.9	1.992
SEM ⁸		3.591	5.214	3.554	5.122	0.035
Effect						
ME		0.032	0.022	0.033	0.003	0.104
SID Lys		< 001	< 001	< 001	< 001	<.001
ME x SID Lys		0.018	0.206	0.023	0.202	0.139
ME (kcal/kg)						
3100		564.7	288.2	305.0	183.1	1.934
3200		557.0	298.9	297.7	193.8	1.901
3300		570.2	317.7	310.9	216.8	1.786
Regression						
Linear		ns	*	ns	*	ns
Quadratic		ns	ns	ns	ns	ns
SID Lys (%)						
0.98		531.4	314.9	272.0	209.8	1.732
1.07		561.1	323.6	301.7	218.5	1.754
1.16		574.1	304.6	314.7	204.6	1.847
1.25		590.3	264.0	329.3	158.9	2.185
Regression						
Linear		ns	ns	ns	ns	*
Quadratic		ns	*	ns	*	ns

¹ Body protein

² Body fat

³ Protein deposition

⁴ Fat deposition

⁵ Protein and body fat ratio

⁶ Metabolizable energy level

⁷ Digestible lysine level

⁸ Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

Table 6. Result of the breakdown of factors (SID Lys x ME) for BP of chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

SID Lys level ¹	ME Level ²			SEM ³	P value
	3100	3200	3300		
0.98	538.7 aB	507.1 bB	557.3 a	3.681	0.001
1.07	558.6 B	561.0 A	563.7		0.849
1.16	565.7 B	575.0 A	569.9		0.717
1.25	600.6 A	585.1 A	592.9		0.601
P value	0.024	<0.001	0.109		

Different letters, uppercase in the column and lowercase in the row, differ from each other by the 5% SNK test

¹ Digestible lysine

² Metabolizable energy

³ Standard Error of the Mean

Table 7. Result of the breakdown of factors (SID Lys x ME) for PD of chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

SID Lys level ¹	ME Level ²			SEM ³	<i>P</i> value
	3100	3200	3300		
0.98	279.4 aB	247.7 bB	298.0 a	3.644	0.002
1.07	299.2 B	301.6 A	304.3		0.850
1.16	306.4 AB	315.7 A	310.5		0.719
1.25	335.3 A	325.7 A	333.5		0.900
<i>P</i> value	0.001	<0.001	0.112		

Different letters, uppercase in the column and lowercase in the row, differ from each other by the 5% SNK test.

¹ Digestible lysine

² Metabolizable energy

³ Standard Error of the Mean

Table 8: Carcass and cut yield of chickens fed different levels of SID Lys and ME from 28 to 40 days of age.

Treatments	SID Lys:ME (g/Mcal)	SW ¹ (g)	CW ² (g)	BRW ³ (g)	TW ⁴ (g)	DW ⁵ (g)	CY ⁶ (%)	BRY ⁷ (%)	TY ⁸ (%)	DY ⁹ (%)
3100 ME ¹⁰ /0.98 SID Lys ¹¹	3.16	3088	2476	870.7	321.7	338.8	77.88	35.03	12.99	13.67
3100 ME /1.07 SID Lys	3.45	3247	2644	988.2	335.1	345.8	78.75	37.92	12.61	13.29
3100 ME /1.16 SID Lys	3.74	3285	2576	1010	313.2	341.3	77.51	39.16	12.17	13.54
3100 ME /1.25 SID Lys	4.03	3250	2612	942.5	333.5	357.1	79.34	36.37	12.75	13.66
3200 ME /0.98 SID Lys	3.06	3002	2378	845.7	298.6	321.0	78.05	36.58	12.56	13.50
3200 ME /1.07 SID Lys	3.34	3188	2550	960.7	302.3	338.7	78.03	37.26	12.09	13.70
3200 ME /1.16 SID Lys	3.63	3266	2614	1014	333.2	355.7	78.90	38.86	12.75	13.61
3200 ME /1.25 SID Lys	3.91	3201	2603	948.0	330.1	354.8	79.02	36.41	12.69	13.63
3300 ME /0.98 SID Lys	2.97	3224	2621	959.2	321.5	351.4	78.74	36.98	12.39	13.84
3300 ME /1.07 SID Lys	3.24	3267	2642	973.3	319.3	354.4	79.28	36.75	12.14	13.77
3300 ME /1.16 SID Lys	3.52	3353	2599	945.3	326.7	334.7	78.31	36.36	12.34	13.05
3300 ME /1.25 SID Lys	3.79	3335	2665	990.8	323.0	381.4	77.84	37.09	12.43	14.39
SEM ¹²		16.819	15.540	9.867	2.712	2.990	0.176	0.263	0.085	0.084
Effect										
ME		< 001	0.006	0.543	0.309	0.102	0.867	0.720	0.348	0.529
SID Lys		< 001	< 001	< 001	0.174	0.004	0.622	0.045	0.366	0.269
ME x SID Lys		0.430	0.124	0.060	0.074	0.127	0.180	0.152	0.598	0.307
3100		3217	2575	953.2	325.6	345.8	78.36	37.15	12.64	13.54
3200		3164	2536	944.8	316.1	342.5	78.52	37.28	12.53	13.61
3300		3293	2632	967.2	322.6	355.5	78.57	36.80	12.33	13.77
Regression										
Linear		ns	ns	ns	ns	ns	ns	ns	ns	ns
Quadratic		*	*	ns	ns	ns	ns	ns	ns	ns
0.98		3105	2486	893.7	313.9	336.4	78.25	36.20	12.65	13.68
1.07		3234	2613	974.7	318.2	346.0	78.72	37.31	12.28	13.59
1.16		3299	2596	990.1	324.4	344.3	78.24	38.13	12.43	13.41
1.25		3262	2627	961.2	328.8	363.7	78.74	36.64	12.64	13.88
Regression										
Linear		ns	ns	ns	ns	*	ns	ns	ns	ns
Quadratic		*	*	*	ns	ns	ns	*	ns	ns

¹ Slaughter weight² Carcass weight

³ Breast weight

⁴ Thigh weight

⁵ Drumstick weight

⁶ Carcass yield

⁷ Breast yield

⁸ Thigh yield

⁹ Drumstick yield

¹⁰ Metabolizable energy level

¹¹ Digestible lysine level

¹² Standard Error of the Mean

not significant (ns), significant at 5% probability (*)

Table 9. Adjustment of equations for linear and quadratic models of the analysis of performance and body composition of broilers fed different levels of SID Lys and ME from 28 to 40 days of age, considering the interaction of SID Lys at the level of 3100 Kcal of ME.

Item	Model	Equations		R ²	P value	Req. ¹	Optimal Response
		Performance					
BW ² (g)	L	BW = 2530.1 + 653.5 * SID Lys		0.15	0.014	-	-
BWG ³ (g/bird)	L	BWG = 946.4 + 665.3 * SID Lys		0.36	< 0.001	-	-
FCR ⁴ (g/g)	L	FCR = 2.3767 - 0.7420* SID Lys		0.55	< 0.001	-	-
Lysine intake (g)	L	Lysine intake = 0.8103 + 25.5919 * SID Lys		0.73	< 0.001		
Body composition							
BP ⁵ (g)	L	BP = 328.55 + 213.03* SID Lys		0.40	< 0.001	-	-
PD ⁶ (g)	L	PD = 87.47 + 195.41* SID Lys		0.35	< 0.001	-	-
BF ⁷ (g)	L	BF = 510.60 -199.42 * SID Lys		0.09	0.048	-	-
FD ⁸ (g)	L	FD = 405.48 -199.42* SID Lys		0.09	0.048	-	-
Carcass and cut yield							
SW ⁹ (g)	L	SW = 2566.8 + 584 * SID Lys		0.09	0.044	-	-
BRW ¹⁰ (g)	Q	BRW = -6386 + 13003 * SID Lys - 5713 * SID Lys ²		0.24	0.007	1.13	1012.82
BRY ¹¹ (%)	Q	BRY = -184.64 + 395.03 * SID Lys - 174.49 * SID Lys ²		0.29	0.001	1.13	38.93

¹ Estimated requirement

² Body weight

³ Body weight gain

⁴ Feed conversion rate

⁵ Body protein

⁶ Protein deposition

⁷ Body fat

⁸ Fat deposition

⁹ Slaughter weight

¹⁰ Breast weight

¹¹ Breast yield

linear model (L), quadratic model (Q)

Table 10. Adjustment of equations for linear and quadratic models of the analysis of performance and body composition of broilers fed different levels of SID Lys and ME from 28 to 40 days of age, considering the interaction of SID Lys at the level of 3200 Kcal of ME.

Item	Model	Equations		R ²	P value	Req. ¹	Optimal Response
		Performance					
BW ² (g)	Q	BW = -6517 + 16878* SID Lys -7247 * SID Lys ²		0.26	0.029	1.16	3310.06
FI ³ (g/bird)	L	FI = 3138.2 - 501.7 * SID Lys		0.10	0.042	-	-
BWG ⁴ (g/bird)	Q	BWG = -7955 + 16651* SID Lys -7151* SID Lys ²		0.48	0.001	1.16	1737.90
FCR ⁵ (g/g)	L	FCR = 2.77184 -1.08188 * SID Lys		0.81	<0.001	-	-
Lysine intake (g)	Q	Lysine intake = - 85.26 + 185.47 * SID Lys - 74.14 * SID Lys ²		0.73	<0.001	1.25	30.73
Energy intake (kcal)	Q	Energy intake = -14357 + 42344* SID Lys -19653 * SID Lys ²		0.23	0.031	1.07	8451.40
Body composition							
BP ⁶ (g)	Q	BP = -1420.3 + 3295.8 * SID Lys -1354.4 * SID Lys ²		0.50	0.041	1.21	584.70
PD ⁷ (g)	Q	PD = -1679.6 + 3295.8 * SID Lys -1354.4 * SID Lys ²		0.50	0.041	1.21	325.40
BF ⁸ (g)	L	BF = 597.17 - 266.39 * SID Lys		0.36	< 0.001	-	-
FD ⁹ (g)	L	FD = 492.05 -266.39* SID Lys		0.36	< 0.001	-	-
BP:BF	Q	BP:BF = 14.283 -25.200 * SID Lys + 12.532 * SID Lys ²		0.58	<0.001	1.00	1.615
Carcass and cut yield							
SW ¹⁰ (g)	Q	LW = - 7257 + 18085 * SID Lys - 7774 * SID Lys ²		0.30	0.017	1.16	3260.98
CW ¹¹ (g)	L	CW = 1611.7 + 828.1 * SID Lys		0.23	0.003	-	-
BRW ¹² (g)	Q	BRW = -6505 + 13060 * SID Lys - 5676 * SID Lys ²		0.40	0.001	1.15	1007.49
TW ¹³ (g)	L	TW = 160.77 + 139.31 * SID Lys		0.20	0.005	-	-
DW ¹⁴ (g)	L	DW = 195.63 + 131.81 * SID Lys		0.17	0.010	-	-

¹ Estimated requirement

² Body weight

³ Feed intake

⁴ Body weight gain

⁵ Feed conversion rate

⁶ Body protein

⁷ Protein deposition

⁸ Body fat

⁹ Fat deposition

¹⁰ Slaughter weight

¹¹ Carcass weight

¹² Breast weight

¹³ Thing weight

¹⁴ Drumstick weight

Linear model (L), quadratic model (Q),

Tabela 11. Adjustment of equations for linear and quadratic models of the analysis of performance and body composition of broilers fed different levels of SID Lys and ME from 28 to 40 days of age, considering the interaction of SID Lys at the level of 3300 Kcal of ME.

Item	Model	Equations		R ²	P value	Req. ¹	Optimal Response
		Performance					
BW ² (g)	L	BW = 2811.4 + 456.5 *SID Lys		0.09	0.049		
FI ³ (g/ ave)	L	FI = 3243.5 - 602.6 * SID Lys		0.22	0.003		
BWG ⁴ (g/ ave)	L	BWG = 1255.5 + 438.5* SID Lys		0.16	0.012		
FCR ⁵ (g/g)	L	FCR = 2.34436 - 0.77679 * SID Lys		0.78	<0.001		
Lysine intake (g)	L	Lysine intake = 6.745 + 19.524 * SID Lys		0.77	<0.001		
Energy intake (kcal)	L	Energy intake = 10524.9 -1855.3 * SID Lys		0.24	0.002		
Body composition							
BP ⁶ (g)	L	BP = 417.75 + 136.97 * SID Lys		0.27	0.001	-	-
PD ⁷ (g)	L	PD = 158.39 + 136.97 * SID Lys		0.27	0.001	-	-
BP:BF	L	BP:BF = 0.7171 + 0.9592 * SID Lys		0.11	0.038		
Carcass and cut yield							
SW ⁸ (g)	L	LW = 2779.5 + 461.5 * SID Lys		0.10	0.042	-	-

¹ Estimated requirement

² Body weight

³ Feed intake

⁴ Body weight gain

⁵ Feed conversion rate

⁶ Body protein

⁷ Protein deposition

⁸ Slaughter weight

Linear model (L)

Table 12. Comparison of regression parameters

Variable	ME level compared	Z statistic	P value	
FI ¹				
Intercept	3200 x 3300	-0.310	0.757	
Slope	3200 x 3300	0.332	0.740	
BW ²				
Intercept	3100 x 3200	0.267	0.790	
	3100 x 3300	-0.745	0.456	
	3200 x 3300	-0.996	0.319	
	Slope	3100 x 3200	-0.171	0.864
		3100 x 3300	0.584	0.559
		3200 x 3300	0.741	0.458
BWG ³				
Intercept	3100 x 3200	0.304	0.761	
	3100 x 3300	-1.225	0.221	
	3200 x 3300	-1.391	0.164	
	Slope	3100 x 3200	-0.163	0.871
		3100 x 3300	1.007	0.314
		3200 x 3300	1.058	0.290
FCR ⁴				
Intercept	3100 x 3200	-2.378	0.017	
	3100 x 3300	0.262	0.793	
	3200 x 3300	3.358	0.008	
	Slope	3100 x 3200	2.267	0.023
		3100 x 3300	0.288	0.773
		3200 x 3300	-2.631	0.008
BP ⁵				
Intercept	3100 x 3200	0.985	0.985	
	3100 x 3300	-1.298	0.194	
	3200 x 3300	-2.238	0.025	
	Slope	3100 x 3200	-0.874	0.382
		3100 x 3300	1.234	0.217
		3200 x 3300	2.066	0.039
PD ⁶				
Intercept	3100 x 3200	1.216	0.224	
	3100 x 3300	-1.034	0.301	
	3200 x 3300	-2.237	0.025	
	Slope	3100 x 3200	-1.124	0.261
		3100 x 3300	0.953	0.340
		3200 x 3300	2.066	0.038
BF ⁷				
Intercept	3100 x 3200	-0.670	0.503	
Slope	3100 x 3200	0.581	0.561	
FD ⁸				
Intercept	3100 x 3200	-0.670	0.503	
Slope	3100 x 3200	0.581	0.561	
Lysine intake				
Intercept	3100 x 3200	0.501	0.133	
Slope	3100 x 3200	0.724	0.084	

- ¹ Feed intake
- ² Body weight
- ³ Body weight gain
- ⁴ Feed conversion rate
- ⁵ Body protein
- ⁶ Protein deposition
- ⁷ Body fat
- ⁸ Fat deposition