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"DEXA" AS A TOOL FOR BODY COMPOSITION PREDICTION, AND EFFECTS OF METHIONINE SOURCES ON BROILERS FROM STARTER, GROWER AND FINISHER PHASES

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Doctor Scientiae

VIÇOSA - MINAS GERAIS
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Adviser: Melissa Izabel Hannas

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“Es inmoral pretender que una cosa deseada se realice mágicamente, simplemente porque la deseamos. Solo es moral el deseo acompañado de la severa voluntad de proveer los medios para su ejecución.”

(José Ortega y Gasset)

ABSTRACT

AGUIAR, Gustavo Augusto Carvalho Costacurta de, D.Sc., Universidade Federal de Viçosa, September, 2024. **"DEXA" AS A TOOL FOR BODY COMPOSITION PREDICTION, AND EFFECTS OF METHIONINE SOURCES ON BROILERS FROM STARTER, GROWER AND FINISHER PHASES**. Adviser: Melissa Izabel Hannas.

Three trials were conducted to evaluate two sources of methionine supplementation in a basal diet in starter, grower, and finisher broilers, respectively. Each trial used 1,820 Cobb500 male, and was performed in a completely randomized design (2 sources × 6 levels), 7 replicates and a common basal diet deficient in methionine. The methionine sources were liquid Met hydroxy-analogue (HMTBA) and DL-methionine (DLM) included in equimolar basis to obtain the levels of 0.04, 0.08, 0.13, 0.2, 0.3, and 0.4% for starter; 0.04, 0.08, 0.12, 0.17, 0.25, and 0.33% for grower and 0.04, 0.08, 0.12, 0.17, 0.24, and 0.32% for finisher. For all trials, growth performance and body chemical composition were evaluated, while carcass and commercial cuts yields were evaluated only in grower and finisher phases. Three chapters were created with the results obtained in all trials. The first, comprehends the development and validation of linear regression equations for body composition prediction using Dual-energy X-ray absorptiometry (DEXA). As results, prediction equations for weight, fat mass, ash mass, lean mass, water mass and protein mass were validated indicating that DEXA is an alternative to measure the body composition of broilers in a non-invasive and non-destructive manner. The second chapter is about the effect of increasing SID Met+Cys:Lys ratios on growth, carcass, and meat yield responses of broilers, and estimate the optimal ratio for starter, grower, and finisher phases. The SID Met+Cys:Lys ratios used were different for starter (53, 57, 61, 67, 75 and 83%), grower (54, 58, 62, 67, 75 and 83%), and finisher (54, 58, 63, 68, 75 and 83%). Results suggest that as birds mature, Met+Cys requirements initially increase, and then slightly decrease. Met supplementation resulted in broiler carcasses with lower fat, but higher protein and mineral. For starter, optimal ratios were 58% for final body weight (FBW) and average daily weight gain (ADWG), 71% for feed conversion ratio (FCR), and 69% for average daily protein deposition. For grower, the ratios were 76% for FBW and ADWG, 81% for FCR, 70% for average daily ash deposition, and 63% for carcass weight (CW). For finisher, requirements were 77% for FCR, 61% for CW, and 58% for carcass yield. Formulating diets to meet growth performance requirements will also attend the carcass composition and meat yields. The third chapter includes the effect of different Met sources on broilers, and the relative bioavailability value (RBV) in equimolar basis of HMTBA to DLM estimated with multi-exponential model. Birds fed DLM presented better results for growth performance in grower and finisher broilers, while the starter birds presented higher protein and ash deposition in their body. The RBV for starter is 99% for growth parameters. In grower is 79% for FBW, 80% for ADWG, 76% for FCR, 66% for CW, 236% for carcass yield, 70% for breast meat yield, and 128% for thigh yield. And for finisher is 79% for FCR, 77% for CW, and 65% for thigh yield. Therefore, DLM provides a better body composition quality for young birds, and the replacement of DLM with HMTBA should consider the animal's growth phase for choosing appropriate RBV.

Keywords: amino acids; requirement; bioefficacy; methionine hydroxy-analogue; body analysis.

RESUMO

AGUIAR, Gustavo Augusto Carvalho Costacurta de, D.Sc., Universidade Federal de Viçosa, setembro de 2024. **"DEXA" COMO FERRAMENTA PARA PREDIÇÃO DA COMPOSIÇÃO CORPORAL E OS EFEITOS DE FONTES DE METIONINA SOBRE FRANGOS DE FASE INICIAL, CRESCIMENTO E TERMINAÇÃO.**. Orientadora: Melissa Izabel Hannas.

Foram conduzidos três experimentos para avaliar duas fontes de suplementação de metionina em uma dieta basal em frangos de fase inicial, crescimento e terminação, respectivamente. 1.820 Cobb500 machos foram utilizados em cada experimento, os quais foram conduzidos em delineamento inteiramente casualizado (2 fontes × 6 níveis), com 7 repetições e uma dieta basal deficiente em metionina. As fontes de metionina foram a metionina hidroxi-análoga liquida (HMTBA) e a DL-metionina (DLM) incluídas em base equimolar para obtenção dos níveis de 0,04; 0,08; 0,13; 0,2; 0,3 e 0,4% para iniciais; 0,04; 0,08; 0,12; 0,17; 0,25; e 0,33% para crescimento e 0,04; 0,08; 0,12; 0,17; 0,24; e 0,32% para terminação. Em todos os experimentos, o desempenho produtivo e a composição química corporal foram avaliadas, enquanto que os rendimentos de carcaça e cortes foram avaliados somente nas fases de crescimento e terminação. Foram criados três capítulos com todos resultados obtidos. O primeiro, abrange o desenvolvimento e a validação de equações de regressão linear para predição da composição corporal, utilizando absorciometria de raios-X de dupla energia (DEXA). Como resultados, equações de predição para peso, massa de gordura, massa mineral, massa magra, massa de água e massa proteica foram validadas, indicando que o DEXA é uma alternativa para mensurar a composição corporal de frangos de maneira não-invasiva e não-destrutiva. O segundo capitulo é sobre o efeito do aumento de relações Met+Cis:Lis dig. sobre o crescimento, rendimentos de carcaça e cortes de frangos, e estimar a relação ótima para as fases iniciais, crescimento e terminação. As relações Met+Cis:Lis dig. usadas foram diferentes para iniciais (53, 57, 61, 67, 75 e 83%), crescimento (54, 58, 62, 67, 75 e 83%), e terminação (54, 58, 63, 68, 75 e 83%). Os resultados sugerem que conforme o amadurecimento da ave a exigência de Met+Cis inicialmente aumenta, e então sofre um leve declínio. A suplementação de Met resultou em carcaças apresentando menor gordura, e maior proteína e mineral. Para iniciais, relações ótimas foram 58% para peso final (PF) e ganho de peso diário (GPD), 71% para conversão alimentar (CA), e 69% para deposição diária de proteína. Para crescimento, as relações foram 76% para PF e GPD, 81% para CA, 70% para deposição diária de mineral e 63% para peso de carcaça (PC). Para terminação, as exigências foram 77% para CA, 61% para PC e 58% para rendimento de carcaça. Formular dietas que atendam a exigência do desempenho produtivo, também vão atender as exigências para respostas de composição de carcaça e rendimentos. O terceiro capitulo inclui o efeito de diferentes fontes de Met sobre os frangos, e o valor de biodisponibilidade relativa (RBV) em base equimolar da HMTBA em relação a DLM, estimado com modelo multi-exponencial. Frangos alimentados com DLM apresentaram melhores resultados de desempenho produtivo nas fases de crescimento e terminação, enquanto que aves iniciais apresentaram maior deposição corporal de proteína e mineral. O RBV para iniciais é de 99% para desempenho produtivo. Para frangos em crescimento é 79% para PF, 80% para GPD, 76% para CA, 66% para PC, 236% para rendimento de carcaça, 70% para rendimento de peito e 128% para rendimento de coxa. E para terminação o RBV é de 79% para CA, 77% para PC e 65% para rendimento de coxa. Portanto, o DLM proporciona uma melhor qualidade de composição corporal para aves jovens, e a substituição do DLM por HMTBA deve considerar a fase de crescimento do animal ao escolher o RBV adequado.

Palavras-chave: amino ácidos; exigência; bioeficácia; metionina hidroxi-análoga; análise corporal

SUMMARY

INTRODUCTION

Dual-Energy X-ray Absorptiometry scanner (**DEXA**) in contrast to chemical analysis, emerges as a non-destructive and non-invasive method that enables longitudinal studies with a restricted number of birds, eliminating the need for animal sacrifice (Gonçalves et al., 2018). It is also essential to underscore that while chemical analysis remains the gold standard, it entails heightened labor, time consumption, extensive reagents and machinery usage, escalated costs, and the production of polluting residuals (Kasper et al., 2021). Consequently, predictive equations are highly recommended to accurately calibrate DEXA estimates for broiler chickens. Additionally, the methodology employed in developing and validating the prediction equations in the current study is adaptable for use in other species and DEXA scanner systems.

Furthermore, broilers digestible methionine + cysteine to lysine ratio (**Met+Cys:Lys**) requirements for each growth phase was checked to evaluate the need of these amino acids for the recent broilers strain. Optimizing the Met+Cys:Lys ratio in broilers is crucial for enhancing broiler performance. Ensuring an optimal ratio preserves these amino acids for protein synthesis rather than energy generation. This not only improves efficiency but also leads to reduced nitrogen emissions, lesser litter accumulation, and decreased water consumption (Lemme et al., 2019). Economically, it translates to lower feed costs, and from a consumer standpoint, results in high-quality meat with reduced fat content (Zhai et al., 2016). To the best of our knowledge, in literature the Met+Cys:Lys requirements range from 66 to 82% (Rostagno et al., 2017; NRC, 1994; Rodrigueiro et al., 2000; Kalinowski et al., 2003; Chamruspollert et al., 2004; Neto et al., 2005; Neto et al., 2007; FEDNA, 2008; Tavernari et al., 2014; Cobb-vantress, 2018; Millecam et al., 2021 and Macelline et al., 2022). Such discrepancy can be attributed to several factors, among them, broiler strain, growth phase, and statistical model used for requirement estimation. In this sense, we considered opportune to investigate broiler growth and carcass responses to increasing SID Met+Cys:Lys ratios in the starter, grower, and finisher phases; and based on such responses, estimate the requirements for both amino acids utilizing different regression models: quadratic, linear broken-line, quadratic broken-line, and exponential asymptotic.

In addition, two sources of methionine, DL-methionine (**DLM**) and methionine hydroxy analogue (**HMTBA**), was used to evaluate the relative bioavailability value (**RBV**) of HMTBA. Methionine (**Met**) is the initiating amino acid in protein synthesis in eukaryotic cells, and it serves as a methyl donor in the synthesis of essential molecules like creatine and choline and in the methylation of DNA, RNA, and histones, influencing gene expression (Brosnan et al., 2007). When not remethylated, Met is converted into cysteine (**Cys**) through trans-sulfuration reactions. Cys is important for antioxidant defense, taurine synthesis, and feather composition, where it is found in much higher concentrations than in other body tissues (Jong et al., 2012). Poultry diets are based on vegetable sources of protein, and these sources are poor in Met. Hence, the supplementation in the diets using Met sources is important to meet the requirements of sulphurous amino acids (Felix et al., 2023). DLM and HMTBA are the most common sources of Met, and they present some differences. DLM have 99% of purity and is produced in crystalized form, while HMTBA have 88% of purity, is produced in liquid form and presents a hydroxyl group instead an amino group in their alfa-carbon of molecular structure, characterizing it as an organic acid precursor of Met (Dibner, 2003). Due these differences between the sources, some biochemical and metabolic distinctions may affect their biological role as Met source (Payne et al., 2006), and the RBV is important to indicate the replacement ratio of these products in the feed, and consequently the cost-effectiveness in the feed manufacturing business (Lemme et al., 2020).

Considering all of the above, the current thesis was divided into three chapters written in a scientific paper format.

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CHAPTER I

Poultry Science

Dual-energy X-ray absorptiometry: an effective approach for predicting broiler chicken body composition

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ABSTRACT Two trials were carried out to develop and validate linear regression equations for body composition prediction using Dual-energy X-ray absorptiometry (**DEXA**). In Trial 1, 300 Cobb500 male chickens raised from 1 to 42 d of age were scanned in DEXA to estimate total weight, fat mass, soft lean tissue (**SLT**) mass, bone mineral content (**BMC**), and fat percentage. DEXA estimates were compared to body ash, crude fat, SLT (sum of protein and water) and scale body weight. The dataset was split, with 70% used for prediction equations development and 30% for testing, and the 5k-fold cross-validation analysis was used to optimize the equations. The R^2, mean absolute error (**MAE**), and root-mean-squared error (**RMSE**) were used as precision and accuracy indicators. A negative correlation (ρ = -0.27) was observed for ash content, while no correlation was observed for protein content ($P > 0.05$). Predictive linear equations were developed to assess broiler weight (R^2 = 0.999, MAE = 25.12, RMSE = 38.99), fat mass (R^2 = 0.981, MAE = 13.87, RMSE = 21.28), ash mass (R^2 = 0.956, MAE = 3.98, RMSE = 5.61), SLT mass (R^2 = 0.997, MAE = 35.73, RMSE = 52.45), water mass (R^2 = 0.997, MAE = 29.56, RMSE = 43.94), protein mass (R^2 = 0.989, MAE = 12.94, RMSE = 19.05), fat content (R^2 = 0.855, MAE = 0.81, RMSE = 1.05), SLT content (R^2 = 0.658, MAE = 1.01, RMSE = 1.28), and water content (R^2 = 0.678, MAE = 0.99, RMSE = 1.27). All equations passed the test. In Trial 2, 395 Cobb500 male chickens were raised from 1 to 42 d of age and used for validation of prediction equations. The equations developed for weight, fat mass, ash mass, SLT mass, water mass, and protein mass were validated. In conclusion, DEXA was found to be an effective approach for measuring the body composition of broilers when using predictive equations validated in this study for estimate calibration.

Key words: DEXA prediction, chicken, carcass body composition, chemical analysis, validation

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INTRODUCTION

Over the years, the poultry industry has undergone changes to meet increasing demand for meat from the global population and minimize economic losses. Furthermore, in commercially-raised birds, such as broilers and laying hens, understanding body composition is valuable for various purposes, including breeding and selection programs, nutritional recommendations, and poultry management. Birds with fast growth have been successfully selected to achieve target body weight earlier and for feed efficiency (Havenstein et al., 2003). However, these selection programs have led to several indirect consequences, such as metabolic disorders, leg problems, and increased fat deposition (Shim et al., 2012; Schallier et al., 2019). In order to detect these inherent problems and help breeders take appropriate actions, methods for measuring chickens' body compositions have been employed. Carcass chemical analysis is the most commonly used method to assess body composition and is considered the gold standard for this process. However, this method is time-consuming, complex, invasive, and requires the animal to be sacrificed to establish the content of protein, fat, water, and ash (Kasper et al., 2021; Martinez et al., 2022b). Although carcass chemical analysis produces reliable measurements, it does not allow for repeated measurements on the same animal throughout its growth, which is feasible with live animal methods. Furthermore, live animal methods meet the ethical demands of certain groups of society for animal care and use, by preventing slaughter of several individuals; moreover, this method can reduce costs and environmental pollution associated with chemical analysis (Gonçalves et al., 2018).

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Dual-energy X-ray absorptiometry (**DEXA**) is a noninvasive method of measuring body composition that was originally designed for humans, but in recent decades, has been used as a tool in livestock research (Scholz et al., 2015), and in the food industry for meat quality inspection (Pipe, 2023). The equipment software uses the reduction of the dual X-ray beam caused by distinct absorbing materials to estimate the values of total tissue, soft lean tissue (**SLT**), fat tissue, bone mineral content (**BMC**), and fat percentage in the projected figures (Schallier et al., 2019). DEXA has been proven useful in animal science; its application varies between prediction of intramuscular fat in beef steak (Nunes et al., 2023), body composition of swine (Soladoye et al., 2016; Kasper et al., 2021), body composition and phosphorus retention in rainbow trout (Ndiaye et al., 2020), mineral density and content in laying hens (Hester et al., 2004; Schreiweis et al., 2004), broiler bone resistance (Shim et al., 2012), and growth performance (Castro et al., 2019; Meyer et al., 2019). Furthermore, DEXA is a promising noninvasive method for longitudinal studies to determine fast heating production, and consequently net energy requirements of chicken growth (Martinez et al., 2022a; Sucsuttajit et al., 2022), and longitudinal studies about potential body growth of broiler and pullet strains (Alves et al., 2019; Gonçalves et al., 2020).

Despite these advantages, DEXA needs to be calibrated to estimate the body composition of birds because the technique is not capable of directly estimating protein and water contents. Some works have shown that DEXA is effective at estimating body composition of chickens by comparing DEXA results with chemical analysis (Mitchell et al., 1997; Swennen et al., 2004; Salas et al., 2012; Gonçalves et al., 2018; Schallier et al., 2019). According to Mitchell et al. (2011), chicken feathers are not detected by DEXA. In this sense, the DEXA results must be related to chemical composition of the feather-free body (**FFB**) of broilers (Alves et al., 2019). To the best of our knowledge, Schallier et al. (2019) is the most recent study to use DEXA to predict body composition of chickens, for which the authors developed reliable prediction regressions; however, in that study, the birds' ages ranged from 21 to 56 d. Nonetheless, there are different brands of DEXA devices and they are all equipped with specific software versions, necessitating the development of individual prediction regression models for all DEXA devices and software combinations (Swennen et al., 2004; Kasper et al., 2021).

For this reason, 2 trials were carried out in the present study. Trial 1 aimed to develop appropriate prediction equations for broiler body composition using a Lunar Prodigy Advance DEXA fan beam scanner (software enCORE v18SP2) and chemical analysis, with broilers of different body compositions ranging from 1 to 42 d of age. Trial 2 aimed to externally validate the models developed to predict the body composition of broilers obtained in Trial 1.

MATERIALS AND METHODS

Ethics

Experiments were conducted to create regression equations (Trial 1) and for equation validation (Trial 2). All procedures adopted for both trials were previously approved by the Ethics Committee in the Use of Farming Animals at the Universidade Federal de Viçosa (**CEUAP-UFV**) under the accession number 029/2020 for Trial 1 and the accession number 057/2022 for Trial 2.

DEXA Scanning

Birds' body composition was measured using the standard mode of the small animal module of the DEXA scanner model Lunar Prodigy Advance (PRODIGY, GE HEALTHCARE, Madison, WI) equipped with enCORE v18SP2 software in Laboratory of Body Composition and Densitometry of the Department of Animal Science of the Universidade Federal de Viçosa (**UFV**). In each day prior to the scanner analyses of the birds, a quality assurance (**QA**) program was performed using a phantom standard to ensure an accurate calibration of the equipment. Next, the birds were placed in the dorsal position on the scanner table with spread wings and stretched legs to avoid overlap of body parts. Birds were scanned from the cranial to caudal direction. After the scanning, the "region of interest" (**ROI**) was selected and personalized by drawing a custom rectangular area around the whole body of the bird image, as shown in Figure 1. The following DEXA traits were recorded: SLT (g), fat tissue (g), BMC (g), and fat percentage (%). The sum of soft lean and fat tissue with the BMC of the DEXA measurements was assumed to be the total body weight (g). SLT (%) and BMC (%) were calculated using the respective gram values as a ratio of total body weight.

Trial 1: Development of Linear Regression Equations

For regression equation development a total of 300 Cobb500 male broilers were raised from 1 to 42 d of age. Diets were formulated for starter (1−14 d of age), grower (14−28 d of age), and finisher (28−42 d of age) feeding phases according to Rostagno et al. (2017) (as shown in Supplemental Table S1), with various levels of methionine inclusion. The application of these ages and diet variations were important for achieving a wide range of body weights and compositions, which is essential for establishing accurate regression equations. During the experimental period, birds were reared on floor pens under standard temperature and light conditions according to genetics guidelines, with diets and water provided ad libitum. Nine 1-day-old birds and 97 birds for each age group of 14, 28, and 42 d of age were selected, respectively, for a fasting period of 12 h, and

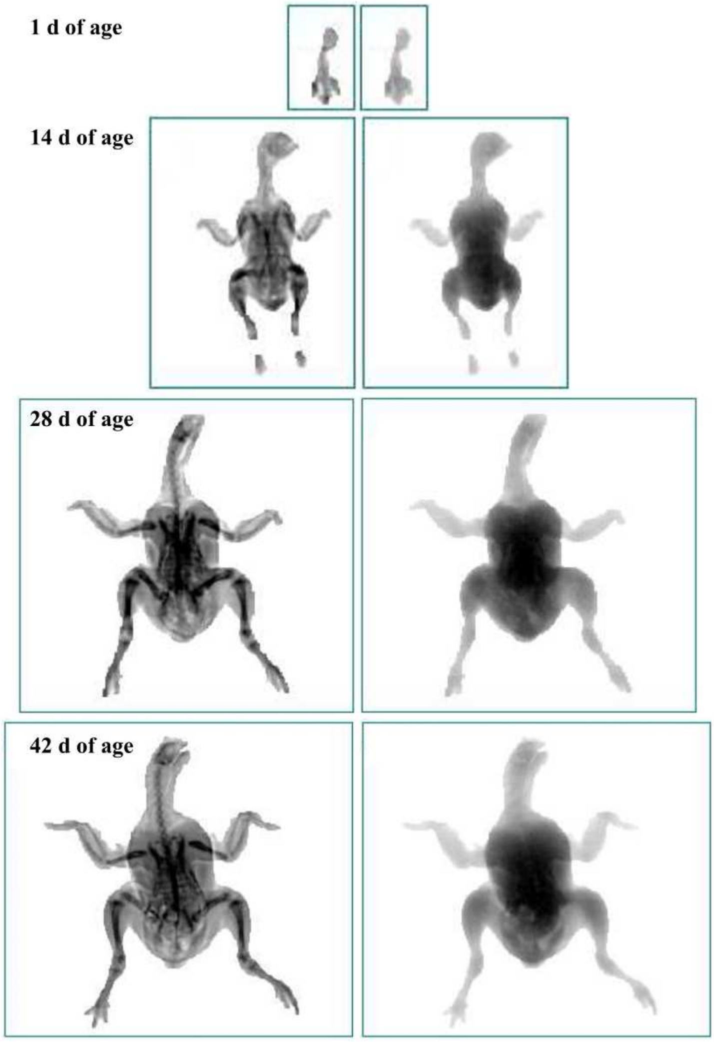

Figure 1. DEXA scan image of a broiler. Square lines define the region of interest (ROI) applied to starter, grower and finisher groups of scanned birds.

then euthanized by cervical dislocation and defeathered. As DEXA does not detect feathers, we chose to pluck and freeze carcasses prior to scanning to facilitate future processing for chemical analysis, and to allow scanning of several number of birds at the same age. The whole carcasses FFB (including organs, heads, and limbs) were weighted, then frozen (−20°C) and later defrosted for DEXA scanning analysis according to the aforementioned method.

After scanning, each of the FFB carcasses were frosted again and were further cut with a band saw (SI-282HD, SKYMSEN, Volta Grande, SC, Brazil), grounded twice with industrial grinder (PBM081, BECCARO, Rio Claro, SP, Brazil), homogenized, and a sample was collected to be lyophilized for chemical analysis conducted at the Laboratory of Animal Nutrition of the Department of Animal Science of the UFV. One-day-old birds were pooled before lyophilization to obtain 3 representative samples. Lyophilized samples were ground in a ball mill and used for analysis of dry matter (INCT-CA, 2021), crude protein Kjeldahl N × 6.25 (INCT-CA, 2021), ether extract (AOCS, Am 5-04, 2009), and ash (INCT-CA, 2021). All chemical values obtained were converted into natural matter to express the quantitative amount of total ash, ether extract, and the protein of total FFB composition (with water) in order to be compared with DEXA scan results. Regarding the 1-day-old birds DEXA scan results, the mean of 3 chicks used for the pool was compared with the wet chemical counterpart (total $n = 294$). Due to the fact that DEXA SLT mass considers the amount of protein and water of tissue, the chemical SLT was considered the sum of crude protein and water.

Trial 2: Linear Regression Equations Validation

A second trial was conducted to evaluate the accuracy of the regression equations developed in Trial 1. A total of 395 Cobb500 male broilers were raised from 1 to 42 d of age on floor pens. During the rearing period, the animals received corn and soybean-meal based diets in order to meet or exceed the requirements established by Rostagno et al. (2017) from 1 to 21 and from 22 to 42 d of age, with variations in mineral sources and quantities (shown in Supplemental Table S2). Birds received diet and water ad libitum and the ambient temperature and the light program were controlled following the genetics guidelines. At the beginning of Trial 2, a group of 10 one-day-old birds were selected, fasted 12 h, and euthanized by cervical dislocation. In order to acquire sufficient data from chickens with different ages for validation of the prediction equations developed in Trial 1, at the end of each 14, 21, 28, 35 and 42 d of rearing, a total of 77 birds were selected, fasted 12 h, euthanized, defeathered, and frozen, respectively. Afterward, the FFB broiler carcasses were defrosted and the FFB carcasses were scanned using the DEXA method mentioned previously, and then all FFB carcasses were frozen for further chemical analysis procedures like those described in Trial 1. For all body composition traits, 77 chickens per age group (14, 21, 28, 35, and 42 d of age) were used for chemical analyses of protein, water, and ash (total $n = 385$). However, 55 chickens for each age groups, were used for fat traits (total $n = 275$). Ten 1-day-old birds were not processed for chemical analysis, a decision that is explained below.

Statistical Analysis

DEXA measurements and wet chemical data were organized into the following variables: weight, fat, ash, SLT, water, and protein of the FFB broiler expressed as weight (g) and content (%) (Table 1). All statistical procedures were performed using R software (R Core Team, 2022), the script of which is available in the supplemental material (Table S3). In all statistical tests described below, results were considered significant when $P < 0.05$.

The first step ($n = 294$) for the development of regression equations for each variable was to verify the confidence interval (**CI**) and significance of the least-squares linear regression parameters: intercept ($\boldsymbol{\beta_0}$) and slope ($\boldsymbol{\beta_1}$). Afterward, a Pearson correlation ($\boldsymbol{\rho}$) analysis between DEXA measurements and wet chemical data was performed.

The second step was to select, based on β_0, β_1, and ρ significances, what body composition variables were able for development of the linear regressions. Therefore, to verify that the model was not overfitted, a random split was performed on the Trial 1 dataset for a 5k-fold cross-validation analysis; 70% of dataset ($n = 206$) was used for training to create the linear regression equations, and the remaining 30% ($n = 88$) was used to test the prediction of linear regressions. Additionally, the mean absolute error (**MAE**) and the root-mean-squared error (**RMSE**) was estimated for each variable, and together with the coefficient of determination ($\mathbf{R^2}$), were used as indicators of precision and accuracy.

The third step was to validate the linear regression equations created and tested with Trial 1 dataset using the Trial 2 dataset. DEXA measurements of Trial 2 were used in linear regression equations to obtain the predicted body composition values. The 1-day-old birds from Trial 2 data were not used for validation because the prediction results were not reliable (e.g., negative values observed for protein mass) (Table 1). The predicted values were then compared to the wet chemical counterparts with a regression analysis (predicted vs. observed) and R^2, MAE, RMSE, and P were assessed as precision and accuracy indices ($n = 385$, except fat = 275).

RESULTS

Variation in Body Composition of Broiler Chickens

Variation in body composition of broiler chickens of different ages for Trial 1 and Trial 2 was achieved; the means with the coefficient of variation are shown in Table 1. Regardless of birds' ages or analysis method (DEXA or chemical), and in both trials, body measurements from highest to lowest variation were: fat, ash, protein, weight, SLT, and water.

Trial 1: Development of Linear Regression Equations

The linear regression parameters of variables measured by DEXA scanner and chemical carcass analysis are presented in Table 2. β_0 indicates the intercept and β_1 the slope of linear regression, and their respective CI are also exhibited. The degree of correlation is indicated by ρ as the correlation coefficient and the R^2 are also shown in this table.

The total weight measured by DEXA scanner was the sum of SLT, fat, and BMC tissues and was compared with the carcasses scale weight. For weight mass linear regression parameters verification, both β_0 and β_1 were significant ($P < 0.001$) and a high positive correlation ($\rho = 1$) and R^2 value (0.999) between DEXA weight and scale weight was observed (Table 2). Based on these results, a linear regression equation was developed to estimate the broiler carcass weight ($R^2 = 0.999$, MAE = 25.12, RMSE = 38.99) and is shown in Figure 2A. The test of regression equations of the weight mass with Trial 1 data split is shown in Table 3, and a high R^2 (0.999) value was observed ($P < 0.001$, MAE = 18.20, RMSE = 25.22).

For both fat content and fat mass, β_0 and β_1 were significant ($P < 0.001$) and a high positive correlation was observed, with the R^2 value of parameter verification for

Table 1. Data divided in age groups of Trial 1 used for development of linear regression equations, and of Trial 2 used for validation. DEXA estimates, chemical analysis and predicted values are presented in means, and in parenthesis the coefficient of variation (%).

	Trial 1[1]			Trial 2[2]		
Item	DEXA	Chemical	Predicted	DEXA	Chemical	Predicted
1 day old						
Weight, g	30.88 (1.25)	44.44 (0.43)		36.51 (1.91)		55.67 (1.35)
Fat, %	5.41 (39.34)	5.58 (13.59)		11.34 (16.09)		9.65 (8.93)
Fat, g	1.67 (40)	2.48 (13.24)		4.14 (16.66)		14.95 (3.49)
Ash, %	0.32 (1.26)	2 (4.28)				
Ash, g	0.1 (0)	0.89 (3.88)		0.37 (13.14)		7.73 (1)
SLT[3], %	94.29 (2.23)	91.44 (1.56)		87.64 (2.18)		86.86 (0.68)
Water, %		75.89 (1.5)				71.16 (0.86)
Protein, %		15.55 (4.8)				
SLT[3], g	29.11 (1.32)	40.64 (1.99)		32 (2.55)		5.22 (18.11)
Water, g		33.73 (1.91)				7.14 (10.75)
Protein, g		6.91 (5.02)				−1.94 (9.14)
14 days old						
Weight, g	434.2 (7.32)	481.2 (6.67)	481.5 (6.99)	500.7 (7.94)	547.5 (7.34)	556.5 (7.71)
Fat, %	7.06 (23.12)	7.81 (14.35)	7.65 (12.17)	10.03 (14.82)	7.69 (16.78)	9.09 (8.02)
Fat, g	30.53 (22.49)	37.59 (16.5)	34.77 (16.84)	50.38 (18.03)	42.24 (20.43)	50.33 (14.1)
Ash, %	1 (11.38)	3.02 (11.08)				
Ash, g	4.33 (12.58)	14.53 (12.21)	14.23 (5.46)	5.66 (11.87)	13.45 (15.24)	16.07 (6.59)
SLT[3], %	91.94 (1.8)	87.86 (1.53)	88.16 (0.69)	88.84 (1.69)	88.65 (1.35)	87.23 (0.53)
Water, %		72.3 (2.12)	72.52 (0.87)		74.48 (1.35)	71.55 (0.68)
Protein, %		15.56 (5.08)				
SLT[3], g	399.3 (7.89)	422.8 (6.81)	426.8 (8.86)	444.7 (7.64)	485.2 (7.23)	482.7 (8.14)
Water, g		347.8 (6.56)	349.8 (8.79)		407.7 (7.22)	395.1 (8.08)
Protein, g		74.96 (9.68)	77.05 (9.2)		77.53 (8.95)	87.49 (8.41)
21 days old						
Weight, g				1069 (6.55)	1141 (5.89)	1170 (6.46)
Fat, %				10.35 (14.77)	7.93 (17.22)	9.21 (7.83)
Fat, g				110.7 (16.12)	90.4 (17.21)	95.89 (14.12)
Ash, %						
Ash, g				13.59 (9.05)	27.95 (12.07)	28.58 (6.79)
SLT[3], %				88.38 (1.72)	88.84 (1.13)	87.09 (0.54)
Water, %					73.99 (1.38)	71.4 (0.68)
Protein, %						
SLT[3], g				944.9 (6.75)	1013 (6.09)	1062 (6.95)
Water, g					844.1 (6.26)	865.3 (6.93)
Protein, g					169.4 (6.31)	195.9 (7.05)
28 days old						
Weight, g	1395 (5.24)	1525 (4.93)	1519 (5.49)	1700 (6.52)	1785 (6.3)	1850 (6.47)
Fat, %	16.1 (14.61)	11.36 (11.7)	11.82 (10.32)	12.04 (12.34)	9.85 (16.27)	10.01 (6.82)
Fat, g	223.9 (13.18)	172.9 (10.7)	178.9 (12.89)	204.74 (14.28)	175.46 (17.26)	167.1 (12.87)
Ash, %	1.6 (9.66)	2.71 (9.58)				
Ash, g	22.3 (8.29)	41.42 (10.91)	42.38 (6.4)	22.31 (9.98)	42.43 (12.11)	42.35 (8.29)
SLT[3], %	82.29 (2.95)	85.28 (1.78)	85.26 (0.96)	86.65 (1.71)	87.95 (1.3)	86.55 (0.53)
Water, %		69.34 (1.92)	69.49 (1.23)		72.85 (1.63)	70.84 (0.67)
Protein, %		15.95 (5.03)				
SLT[3], g	1149 (6.96)	1301 (5.91)	1299 (7.97)	1473 (6.56)	1570 (6.49)	1672 (6.69)
Water, g		1058 (5.76)	1058 (7.94)		1300 (6.56)	1361 (6.67)
Protein, g		243.5 (8.15)	240.3 (8.06)		269.6 (7.76)	310.3 (6.75)
35 days old						
Weight, g				2475 (6.85)	2582 (6.7)	2687 (6.81)
Fat, %				11.39 (16.46)	10.26 (15.57)	9.54 (9.07)
Fat, g				281.3 (18.79)	264.7 (17.67)	219.6 (18.13)
Ash, %						
Ash, g				29.54 (8.46)	59.51 (9.72)	53.75 (7.34)
SLT[3], %				87.47 (2.1)	87.29 (1.33)	86.81 (0.65)
Water, %					72.25 (1.81)	71.1 (0.83)
Protein, %						
SLT[3], g				2164 (6.69)	2253 (6.77)	2472 (6.78)
Water, g					1865 (7.12)	2012 (6.77)
Protein, g					388.1 (6.46)	460.1 (6.82)
42 days old						
Weight, g	2800 (3.28)	3034 (2.84)	3035 (3.84)	3091 (7.71)	3240 (7.62)	3352 (7.68)
Fat, %	18 (14.1)	13.05 (14.65)	12.78 (7.6)	12.94 (17.62)	11.06 (15.26)	10.45 (9.81)
Fat, g	503.6 (13.93)	395.4 (14.17)	391.6 (10.69)	401.1 (20)	361.8 (17.57)	318.9 (17.6)
Ash, %	1.53 (8.34)	2.48 (11.19)				
Ash, g	42.81 (7.94)	75.2 (11.8)	74.98 (7.62)	38.37 (10.33)	75.7 (10.88)	67.69 (9.24)
SLT[3], %	80.47 (3.16)	85 (2.2)	84.65 (0.74)	85.82 (2.63)	86.76 (1.6)	86.3 (0.81)
Water, %		69.2 (2.5)	68.86 (0.95)		71.86 (2.05)	70.57 (1.03)
Protein, %		15.8 (5.23)				
SLT[3], g	2254 (4.95)	2579 (4.02)	2575 (5.32)	2652 (7.72)	2810 (7.56)	3036 (7.8)
Water, g		2099 (4.14)	2095 (5.31)		2328 (7.75)	2470 (7.79)

(continued)

Table 1 (*Continued*)

Item	Trial 1[1]			Trial 2[2]		
	DEXA	Chemical	Predicted	DEXA	Chemical	Predicted
Protein, g		479.4 (6.4)	479.4 (5.35)		482.4 (8.38)	565.8 (7.84)
1–42 days old						
Weight, g	1517 (64.88)	1651 (64.23)	1572 (64)	1709 (57.06)	1798 (56.21)	1860 (56.56)
Fat, %	13.57 (39.24)	10.66 (25.23)	10.64 (23.1)	11.31 (18.11)	9.29 (21.59)	9.64 (9.86)
Fat, g	247.9 (80.77)	198.3 (76.82)	186.9 (76.27)	202.5 (66.7)	181.4 (67.5)	162.1 (62.74)
Ash, %	1.36 (23.35)	2.73 (13.67)		1.22 (9.66)		
Ash, g	22.72 (70.52)	42.98 (60.03)	41.5 (58.05)	21.15 (57.48)	42.39 (55.47)	40.52 (47.35)
SLT[3], %	85.07 (6.57)	86.12 (2.45)	86.1 (1.95)	87.47 (2.35)	87.97 (1.67)	86.81 (0.73)
Water, %		70.4 (3.09)	70.37 (2.49)		73.23 (2.4)	71.1 (0.93)
Protein, %		15.76 (5.19)				
SLT[3], g	1246 (62.15)	1410 (63.67)	1344 (63.19)	1485 (56.23)	1574 (55.49)	1687 (57.29)
Water, g		1149 (63.52)	1095 (63.03)		1305 (55.26)	1373 (57.17)
Protein, g		261.3 (64.63)	248.8 (63.93)		268.2 (56.86)	313 (57.82)

[1]$n = 294$.
[2]$n = 385$; except fat chemical analysis ($n = 275$).
[3]SLT: soft lean tissue.

Table 2. Linear regression parameters (β_0 and β_1) and confidence interval (CI) of broilers body composition DEXA vs. chemical analysis. Birds aging 1 to 42-day old. Correlation coefficient (ρ) and coefficient of determination (R^2) are shown ($n = 294$).

Item	Intercept			Slope				
	β_0	CI	P	β_1	CI	P	R^2	ρ
Weight, g	17.33	9.48–25.18	<0.001	1.08	1.07–1.08	<0.001	0.999	1*
Fat, %	4.40	4.06–4.75	<0.001	0.46	0.44–0.49	<0.001	0.833	0.91*
Fat, g	11.45	7.66–15.25	<0.001	0.75	0.74–0.77	<0.001	0.982	0.99*
Ash, %	3.16	2.98–3.34	<0.001	−0.31	−0.44 to −0.18	<0.001	0.070	−0.27*
Ash, g	7.28	6.15–8.40	<0.001	1.57	1.53–1.61	<0.001	0.953	0.98*
SLT[1], %	60.67	58.41–62.93	<0.001	0.30	0.27–0.33	<0.001	0.628	0.79*
Water, %	44.01	41.71–46.31	<0.001	0.31	0.28–0.34	<0.001	0.635	0.80*
Protein, %	16.66	15.23–18.09	<0.001	−0.01	−0.03 to 0.01	0.22	0.005	−0.07NS
SLT[1], g	−32.14	−42.97 to −21.31	<0.001	1.16	1.15–1.17	<0.001	0.997	1*
Water, g	−23.23	−32.49 to −13.97	<0.001	0.94	0.93–0.95	<0.001	0.997	1*
Protein, g	−8.90	−12.79 to −5.01	<0.001	0.22	0.21–0.22	<0.001	0.989	0.99*

[1]SLT: soft lean tissue.
*Correlation is significant with $P < 0.001$.
NSCorrelation is not significant ($P > 0.05$).

fat content at 0.833 and for Fat mass 0.982 (Table 2). The linear regression equations developed to estimate broiler fat content ($R^2 = 0.855$, MAE = 0.81, RMSE = 1.05) and fat mass ($R^2 = 0.981$, MAE = 13.87, RMSE = 21.28) are shown in Figures 3A and 2B, respectively. The test of the regression equations of fat content ($R^2 = 0.781$, $P < 0.001$, MAE = 0.93, RMSE = 1.21) and fat mass ($R^2 = 0.984$, $P < 0.001$, MAE = 13.07, RMSE = 18.33) with the Trial 1 data split is shown in Table 3. The ash content's β_0 and β_1 were significant ($P < 0.001$); nevertheless, the R^2 value was low (0.070) and a negative correlation ($\rho = -0.27$) was observed (Table 2). Based on these results, a linear regression equation for ash content was not developed. For ash mass, the β_0 and β_1 were also significant ($P < 0.001$), and a high positive correlation ($\rho = 0.98$) and R^2 value (0.953) was observed (Table 2). The linear regression equation developed for ash mass ($R^2 = 0.956$, MAE = 3.98, RMSE = 5.61) is also shown in Figure 2C. The test of linear regression equations using the Trial 1 data split for ash mass ($R^2 = 0.947$, $P < 0.001$, MAE = 3.91, RMSE = 5.60) is shown in Table 3.

The SLT content and SLT mass were compared to the sum of water and protein obtained through humidity (via dry matter) and wet crude protein chemical analysis. β_0 and β_1 were significant ($P < 0.001$) for both SLT content and SLT mass; therefore, a positive correlation ($\rho = 0.79$) was observed for SLT content ($R^2 = 0.628$) and a high positive correlation ($\rho = 1$) was observed for SLT mass ($R^2 = 0.997$) (Table 2). A linear regression equation was developed for SLT content ($R^2 = 0.658$, MAE = 1.01, RMSE = 1.28) and for SLT mass ($R^2 = 0.997$, MAE = 35.73, RMSE = 52.45) and is shown in Figures 3B and 2D, respectively. The test of regression equations of SLT content ($R^2 = 0.566$, $P < 0.001$, MAE = 1.08, RMSE = 1.31) and SLT mass ($R^2 = 0.998$, $P < 0.001$, MAE = 29.66, RMSE = 40.46) with Trial 1 data split is shown in Table 3. As SLT measured by the DEXA scanner represented the muscle tissue composed of protein plus water, the SLT was considered to be the counterpart of chemical-based water and protein data for the linear regression equations development. Considering this, for water content and water mass, both β_0 and β_1 were significant ($P < 0.001$) and a positive correlation ($\rho = 0.80$) for water content ($R^2 = 0.635$) and a high positive correlation ($\rho = 1$) for water mass

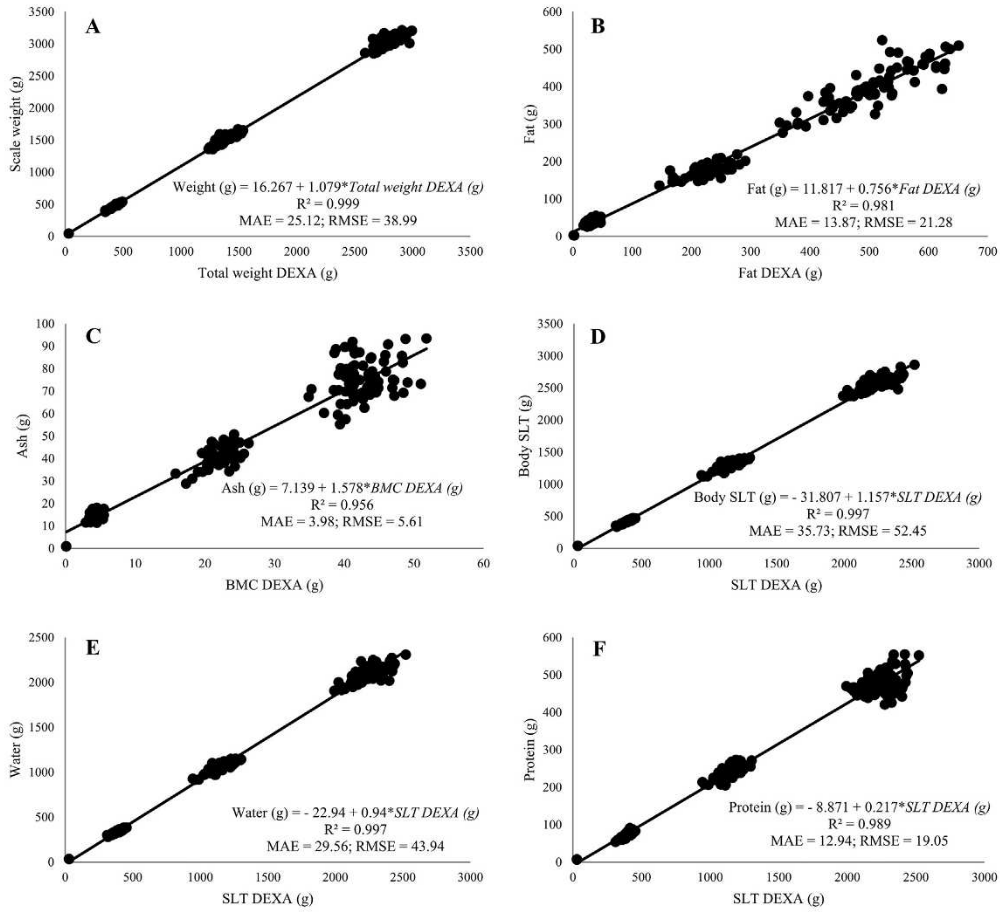

Figure 2. Least-squares regressions developed using 70% of Trial 1 dataset for prediction of weights of broiler body composition: weight (A), fat (B), ash (C), soft lean tissue (SLT) (D), water (E), and protein (F). The DEXA measurements are independent variables on x-axis and corresponding chemical analysis values are dependent variables on y-axis. Regression equations, coefficients of determination (R^2), mean absolute error (MAE) and the root-mean-squared error (RMSE) are shown in every graphic ($n = 206$).

($R^2 = 0.997$) was observed (Table 2). A linear regression equation was also developed for water content ($R^2 = 0.678$, MAE = 0.99, RMSE = 1.27) and for water mass ($R^2 = 0.997$, MAE = 29.56, RMSE = 43.94) and is shown in Figures 3C and 2E, respectively. The test of regression equations of water content ($R^2 = 0.532$, $P < 0.001$, MAE = 1.15, RMSE = 1.42) and water mass ($R^2 = 0.997$, $P < 0.001$, MAE = 26.60, RMSE = 37.55) with Trial 1 data is shown in Table 3. Regarding protein content, β_0 was significant ($P < 0.001$) but β_1 was not significant ($P = 0.22$) and no correlation between DEXA measurements and chemical data was observed (Table 2). Based on these results, a linear regression equation for protein content was not developed. However, in relation to protein mass, both β_0 and β_1 were significant ($P < 0.001$) and a high positive correlation ($\rho = 0.99$) and R^2 value (0.989) was observed (Table 2). The linear regression equation developed to estimate broiler protein mass ($R^2 = 0.989$, MAE = 12.94, RMSE = 19.05) is shown in Figure 2F. The test of linear regression equation using Trial 1 data for protein mass ($R^2 = 0.991$, $P < 0.001$, MAE = 10.95, RMSE = 15.32) is shown in Table 3.

Table 3. Test of regression equations for body composition prediction using 30% of DEXA measurements and chemical analysis of Trial 1 data. Significance level (P) of regression between DEXA estimates and corresponding chemical analysis values. Coefficient of determination (R^2), mean absolute error (MAE) and root-mean-squared error (RMSE) are shown ($n = 88$).

Item	P	R^2	MAE	RMSE
Weight, g	<0.001	0.999	18.20	25.22
Fat, %	<0.001	0.781	0.93	1.21
Fat, g	<0.001	0.984	13.07	18.33
Ash, %	-	-	-	-
Ash, g	<0.001	0.947	3.91	5.60
SLT[1], %	<0.001	0.566	1.08	1.31
Water, %	<0.001	0.533	1.15	1.42
Protein, %	-	-	-	-
SLT[1], g	<0.001	0.998	29.66	40.46
Water, g	<0.001	0.997	26.60	37.55
Protein, g	<0.001	0.991	10.95	15.32

[1]SLT: soft lean tissue.

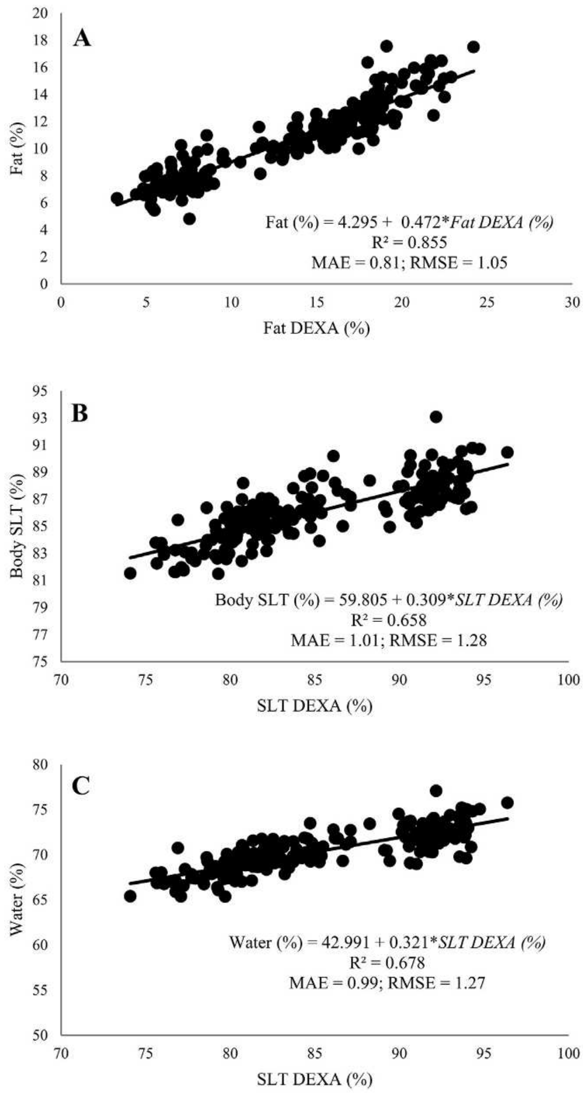

Figure 3. Least-squares regressions developed using 70% of Trial 1 dataset for prediction of contents of broiler body composition: fat (A), soft lean tissue (SLT) (B), and water (C). The DEXA measurements are independent variables on x-axis and corresponding chemical analysis values are dependent variables on y-axis. Regression equations, coefficients of determination (R^2), mean absolute error (MAE) and the root-mean-squared error (RMSE) are shown in every graphic ($n = 206$).

Trial 2: Linear Regression Equations Validation

The linear regression equations validation was performed by comparing the values estimated using the equations developed in Trial 1 (predicted values) against the values observed via chemical analysis through a regression. For all variables expressed in weight, high R^2 values were observed (>90%), whereas, for all variables expressed in content, the R^2 values observed were around 50%. The regressions of predicted vs. observed values for each variable expressed in weights and contents are shown in Figures 4 and 5, respectively.

DISCUSSION

The first Trial aimed to compare the DEXA scanner estimates against chemical carcass analysis of broilers aged 1 to 42 d and to establish reliable predictive linear regression equations. Chemical analysis is still the gold standard for measuring chicken broiler composition, and consequently, DEXA accuracy can be determined by comparison (Schallier et al., 2019). Furthermore, Trial 2 evaluated the validation of prediction equations developed in Trial 1 using external data. Validation is highly recommended for verifying whether a mathematical model is useful and accurate to a real system (Inca et al., 2022).

Independent of the method used to measure the FFB composition, DEXA and chemical analysis estimates showed similar tissue pattern of variation in the following (descending) order: fat, ash, protein, weight, SLT, and water. The diets and age conditions applied to the animals in Trials 1 and 2 successfully provided a diverse dataset for application of tests, development of prediction equations, and subsequent validation.

The results of development of the linear regression equations (Trial 1) demonstrated that DEXA was an effective approach for predicting body carcass composition of broilers. The weight determined by DEXA as the sum of SLT mass, fat mass, and BMC showed an almost perfect correlation with scale weight in this study. The equation slope of 1.079 demonstrated that DEXA slightly underestimated the FFB weight of chickens, which may be due to underestimation of SLT mass and ash and the overestimation of fat, which is consistent with the observations of Schallier et al. (2019). Nonetheless, other studies with chickens demonstrated an overestimation of DEXA weight even in the presence of a high positive correlation with scale weight (i.e., Mitchell et al., 1997; Swennen et al., 2004; Salas et al., 2012; Gonçalves et al., 2018; Martinez et al., 2022b). This inconsistency among previous studies, although small, reinforces the importance of calibration of specific instruments, software versions, and ROI methodologies. The criteria for stating equation robustness were to observe high R^2 and low MAE or RMSE error metrics (Martinez et al., 2022b). Therefore, the linear equation for weight demonstrated a high accuracy when we observed the reduction of 6.92 g MAE (or, RMSE = 13.77) value of testing using Trial 1 split data.

The high correlation of fat mass observed in this study is also in agreement with what was observed ($\rho = 0.98$) by Schallier et al. (2019). Furthermore, the R^2 (98%) of this study was higher and was corroborated by other values in the literature ranging from 89 to 96% (Korine et al., 2004; Swennen et al., 2004; Salas et al., 2012; Gonçalves et al., 2018; Schallier et al., 2019). An exception to this were the results of Mitchell et al. (1997), who observed a value of 62% for R^2 that the authors

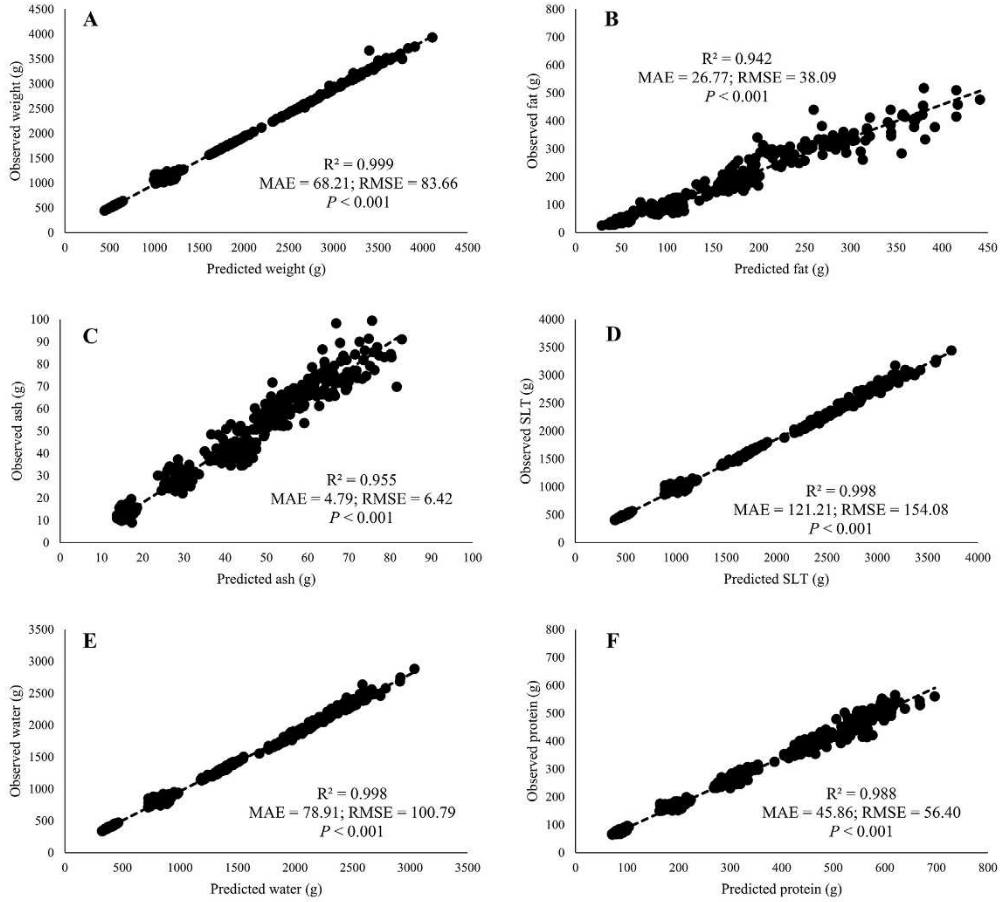

Figure 4. Validation with external data of least-squares regressions using DEXA for prediction of weights of broiler body composition: weight (A), fat (B), ash (C), soft lean tissue (SLT) (D), water (E), and protein (F). The predicted measurements are independent variables on x-axis and corresponding observed chemical values are dependent variables on y-axis. Coefficients of determination (R^2), mean absolute error (MAE), the root-mean-squared error (RMSE) and P are shown in every graphic ($n = 385$, except fat $n = 275$).

attributed to the DEXA scanning mode, program version, and to the weight of the birds, as birds weighing less than 2,000 g had more discrepancies in fat mass. However, Gonçalves et al. (2018), even with a high R^2 (91%), also observed similar discrepancies in birds with low body weight (7 d of age), which had high fat mass; the contrary occurred in heavyweight birds (28 and 77 d of age). In the present study, DEXA overestimated fat mass ($\beta_1 - 0.756$), which is in agreement with other studies (Korine et al., 2004; Swennen et al., 2004; Salas et al., 2012; Schallier et al., 2019). This was previously attributed to variation in soft tissue hydration, as discussed by Pietrobelli et al. (1998), although the authors concluded in the same work that the magnitude of DEXA fat estimation error related to soft tissue hydration is small under normal circumstances, and should not pose any substantial limitations to the accuracy of DEXA technique. Nonetheless, Korine et al. (2004) pointed out the following reasons for DEXA fat overestimation in their study: first, DEXA calibration was based on rat bone density (mouse phantom of Lunar PIXImus 2 model); second, the feathers were mistaken as fat by the DEXA scan; lastly, the ether petroleum used as a solvent for lipid extraction extracted mainly triglycerides (84% of total lipids), whereas the DEXA scanner estimates total lipids. In the present study, the first point can be disregarded due to this DEXA scanner model's and software differences. Although defeathered chickens were used in this study, it is important to note that, while Mitchell et al. (2011) stated that chicken feathers are not detected by DEXA, the study by Korine et al. (2004) focused on migratory birds that may have very different feather compositions than domestic broiler chickens. In this context, the last point about lipid extraction method may explain the fat mass overestimation observed in our study. Another topic discussed by the authors is that DEXA's X-ray attenuation coefficient distinguishes a pixel based on the coefficients of high (e.g., bone tissue) and low (e.g., soft tissue) attenuation energy levels; in areas close to the bones that may not contain adequate amounts of soft tissue, such as the wings, neck, head, and feet, the interpolation process is necessary to estimate the soft tissue composition. In such cases, the process is based on reconstruction of the

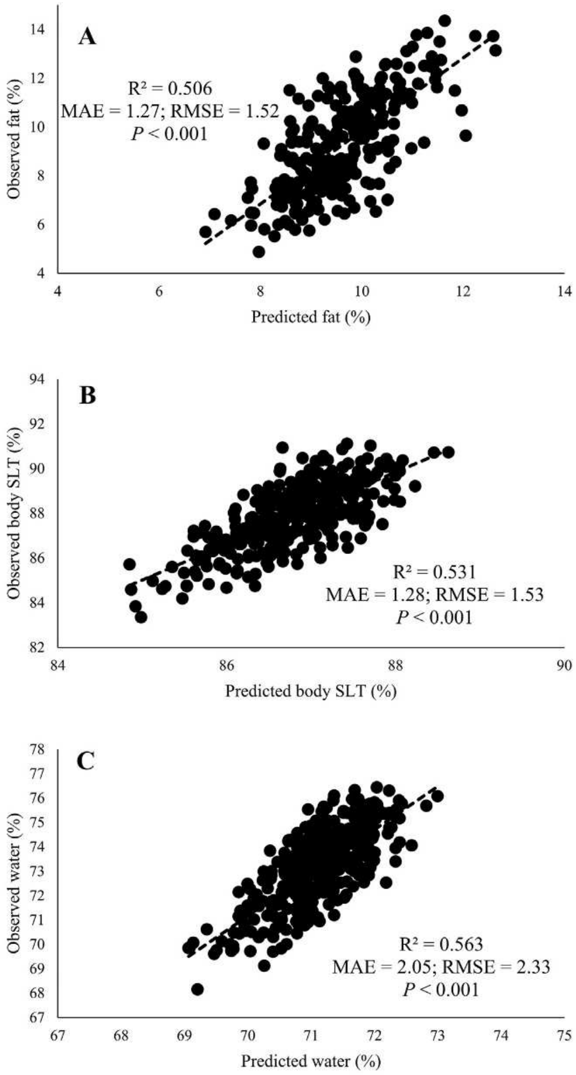

Figure 5. Validation with external data of least-squares regressions using DEXA for prediction of contents of broiler body composition: fat (A), soft lean tissue (SLT) (B), and water (C). The predicted measurements are independent variables on x-axis and corresponding observed chemical values are dependent variables on y-axis. Coefficients of determination (R^2), mean absolute error (MAE), the root-mean-squared error (RMSE) and P are shown in every graphic ($n = 385$, except fat $n = 275$).

soft tissue composition by applying the average body fat percentage of the entire animal; thus, if this percentage is greater than the fat percentage of these areas, the fat will be overestimated (Mitchell et al., 1997; Nagy and Clair, 2000; Korine et al., 2004). Regarding fat content, there was also a positive correlation ($\rho = 0.91$) between DEXA and chemical analysis results, while slightly less than that observed for fat mass. Although this decrease is in agreement with other works, the correlation found in our study was higher compared to Swennen et al. (2004) and Schallier et al. (2019), who found correlation values 0.59 and 0.77, respectively. These differences may be related to evolution of DEXA software, which has been calibrated over the years; additionally, the scanner used in this study was more recently developed. The linear equation for fat mass proved to be robust in the split data Trial 1 test, as no changes in R^2 and a slight reduction of MAE and RMSE values were observed. However, the fat content prediction equation test showed a reduction of 7.43% in the R^2 value and an increase of 0.12% in MAE (or, RMSE = 0.16) value, indicating a possible inaccuracy of this prediction model.

Ash content was compared using the DEXA BMC percentage as an independent variable, and as result, a negative correlation and a small R^2 value were observed in this study, thereby discarding the possibility of prediction model development and corroborating results observed in other studies (Swennen et al., 2004; Schallier et al., 2019). A possible explanation for these results is that ash content comprises a small range of values among all age groups of chicken, reaching 0.3 to 1.6% for BMC (fold difference of 5.3) and 2 to 3% for chemical ash (fold difference of 1.5). On this basis, a linear regression model, contrasting ash chemical and BMC content, with a low coefficient of determination, was expected. However, a high correlation was observed for ash mass, as well as reported by Swennen et al. (2004) and Schallier et al. (2019). A high R^2 was likewise observed by these authors, as well as by Salas et al. (2012) and Gonçalves et al. (2018). Only 1 study observed a low coefficient of determination of 46% between ash mass and DEXA BMC, and the authors were unable to clarify the absence of correlation (Mitchell et al., 1997). Observing the equation slope ($\beta_1 = 1.578$), DEXA greatly underestimated the ash quantity of broiler bodies compared to chemical ash weight. This finding was also reported in other studies (Swennen et al., 2004; Salas et al., 2012; Gonçalves et al., 2018; Schallier et al., 2019). The observed discrepancy between BMC and ash chemical weight may be attributed to the fact that DEXA exclusively measures the mineral content of bones; conversely, mineral chemical analysis, carried out by burning the sample in a muffle furnace, takes into account the entire broiler body, including bones and nonbone tissues, which also contain substantial mineral content (such as organs, muscle, and liver) (Johnson et al., 2016; Gonçalves et al., 2018). Additionally, based on the results of the linear regression test using split data of Trial 1, DEXA BMC allowed a good estimation of total body ash mass of broilers when corrected with the corresponding linear regression. A slight reduction of 0.92% in R^2 was observed, whereas the MAE value was reduced to 0.07 g (or, RMSE = 0.01), indicating the robustness of the prediction model.

Water comprises more than 70% of the whole bodyweight of broilers. Because bone and (hydrophobic) fat have less water content due to their chemical nature, it is assumed that SLT is composed of protein and a larger proportion of water (Brommage, 2003). Hence, the comparison between DEXA SLT and water plus protein obtained chemically was accepted as "chemical soft lean tissue mass." In this study, a high positive correlation between DEXA SLT mass and chemical SLT, water, or protein mass was consistent with previous works on chickens (Mitchell et al., 1997; Swennen et al., 2004; Salas et al., 2012; Gonçalves et al., 2018; Schallier et al., 2019). In spite of this, an overestimation was observed by Swennen et al. (2004) in SLT determined by DEXA, and the same was found by Gonçalves et al. (2018) in chickens with heavy weights. On the other hand, similar to the present study ($\beta_1 = 1.157$), Schallier et al. (2019) observed DEXA underestimated the SLT mass ($\beta_1 = 1.047$), which the authors attribute to characteristics of every unique scanner and software, emphasizing the need for proper calibration. Furthermore, the linear regression developed with $R^2 = 0.997$ and MAE = 35.73 g (RMSE = 52.45) allowed a proper SLT prediction based on DEXA estimations; as when tested with Trial 1 split data, a reduction of 6.07 g in MAE (or, RMSE = 11.99) was observed. The same behavior was observed when linear equations for water and protein mass were tested using Trial 1 split data, as reductions in MAE of 2.96 g (or, RMSE = 6.39) and 1.99 g (or, RMSE = 3.73) were observed, respectively.

Similarly to fat content, SLT ($\rho = 0.79$) and water ($\rho = 0.80$) content showed a positive correlation to weight but to a lesser extent ($\rho = 1$, for both). In relation to protein content, no correlation was observed, making it impossible to develop a prediction equation based on DEXA SLT estimates. Although diets guaranteed a body protein content variation (**CV** ~5%) within the broiler ages, the protein content percentages among the different age groups were 15.55% (1 d), 15.56% (14 d), 15.95% (28 d), and 15.80 % (42 d), with practically no variations between the group ages. When compared to protein mass, we observed among the same different age groups, results of 6.91 g (1 d), 74.96 g (14 d), 243.5 g (28 d), and 479.4 g (42 d); this explains why no significance was observed for β_1 of protein content percentages but was observed for protein mass. These results were similarly observed in other studies involving chickens, where the correlation between DEXA values and the water content was better than that for the protein (Mitchell et al., 1997; Schallier et al., 2019). These observations can be attributed to the high content of water relative to protein within the tissue when calculating their relationship with SLT. The equations developed for SLT and water content showed R^2 values of 66 and 68%, respectively, which is not high, and can cause inaccurate prediction. Therefore, the equations tests using Trial 1 split data demonstrate a reduction in R^2 of 9.22% for SLT content and 14.52% for water content, as well as an increase in MAE value of 0.07 and 0.16%, respectively. Consequently, accurately distinguishing minor variations in fat, SLT, and water content within a narrow range of tissue weights is more difficult (Schallier et al., 2019). Thus, a possible explanation for the results observed for the variables, expressed as percentage of fat, water, SLT, and especially for ash and protein, can be attributed to the smaller range of values (0−100%, as a ratio of total body weight) compared to the wide range of absolute data points (Swennen et al., 2004). Nevertheless, validation with external data is still necessary to ensure model efficiency and no overfitting (Martinez et al., 2022c).

The method of validation using unavailable data when the model was developed is considered the most rigorous approach (Harrell, 2015), and it has been demonstrated that this method can identify high prediction errors in models previously considered accurate at the development phase (Inca et al., 2022; Martinez et al., 2022c). In this sense, Trial 2 was conducted to obtain external data and use it for validation of the prediction equations developed in Trial 1. The criteria for validation were the same as those described by Martinez et al. (2022c), which considered a conservative change for external validation if the $\Delta R^2 < 25\%$ ($\Delta R^2 = R^2$ of prediction model - R^2 of validation), when the R^2 is high enough. In this way, the equations for prediction of broiler FFB weight, fat mass, ash mass, SLT mass, water mass, and protein mass based on DEXA estimates were validated (all regressions showed $\Delta R^2 = 0$, except for fat mass, for which $\Delta R^2 = 4\%$) and can be securely used as a tool to measure chicken body composition. An increase in the MAE and RMSE values were observed for all of these variables in validation, although the opposite was observed in the results of the Trial 1 split-data test. The reason for this is that splitting data may cause an overestimate of the efficiency of models, underlining the need for validation using external data (Harrell, 2015; Martinez et al., 2022c). In relation to fat content, the linear regression for prediction was not validated ($\Delta R^2 = 35\%$), and the R^2 between the predicted and real observations was considered low for this purpose ($R^2 = 51\%$). Additionally, SLT and water content showed ΔR^2 values of 13 and 12%, respectively. However, the coefficient of determination for both was low, with values of 53 and 56%, respectively. Based on these results, the prediction equations for any variable expressed as a percentage in this study were not validated, indicating that DEXA is only recommended to estimate the absolute weight of body tissues in broilers. Hence, different model structures to predict these tissue percentages may be explored. Nonetheless, other models validated herein can be used to obtain the percentage of the target tissue as ratio of total weight (e.g., ash content = ash mass/weight × 100).

It should be noted that, when DEXA estimates of 1-day-old birds from Trial 2 were applied in the prediction equations, the body prediction results were not reliable, especially for the protein mass-negative values that were observed. Because of this, we decided not to use these records for validation. A possible explanation for these results could be one disadvantage of GE Prodigy DEXA, which is the lower weight limit of approximately 250 g (Johnson et al., 2016). Despite this lower limit of DEXA, one aim of this study was to test the accuracy of measurements at this early stage, as well as to obtain a wide range of dependent variable for prediction equations, similar to Salas et al. (2012). Another disadvantage mentioned in studies that use DEXA to evaluate the body growth rate of chickens, is that a parallel study using the slaughter technique must be carried out to evaluate the feather growth rate (Gonçalves et al., 2018; Alves et al., 2019). Due to the inability of DEXA to detect chicken feathers, it is impossible to measure the composition of feathers growth of individuals over time (Gonçalves et al., 2020). However, for the meat industry it would not be a problem, as one of the market's interests is the body composition of the FFB carcass, mainly protein and fat.

In the current study, only male broilers were used for Trials 1 and 2. However, the prediction models obtained here can be reliably used for female birds as well, as no trait associated with sex influence on the DEXA measurements has been previously reported in the literature (Salas et al., 2012; Soladoye et al., 2016; Gonçalves et al., 2018). The strain of bird also may not have an influence on DEXA results, as Swennen et al. (2004) developed the prediction equations with the Cobb strain and validated it with the Ross strain. Therefore, further studies evaluating the effect of different chicken genetic strains on DEXA-traits are suggested, as was observed for swine (Soladoye et al., 2016).

In the literature, there are several studies which could benefit from the equations developed here. Castro et al. (2019), who studied the effect of L-arginine supplementation, used the DEXA scanner (Prodigy, GE Healthcare) method to evaluate the body composition of broiler chickens. When applying the equations of our study, in addition to properly calibrating the data, the authors would discover that increasing L-arginine could result in an increasing protein weight of broilers, which would require statistical procedures to verify significance. Wang et al. (2019), investigating the efficacy of different levels of phytase and multicarbohydrase on growth performance and bone mineralization in male broilers, could take advantage of our prediction equations to evaluate the effect of body composition on the body weight gain, since the authors used DEXA scanner but did not used the DEXA results of SLT. In a study by Chen et al. (2020), although the authors did not specify that they used GE Healthcare DEXA scanner and used the small animal module, the use of DEXA results in the prediction equation could contribute to enriching the understanding of the effect of 25-hydroxyvitamin D_3 supplementation for laying hens on the animals' total body compositions, most notably on total body ash, in addition to bone structural development. For broiler breeders, caution should be exercised when using the prediction equations developed in our study. Studies employing DEXA to estimate body composition (Aranibar et al., 2020; Avila et al., 2023), could benefit from these prediction equations for accurate calibration of body composition. Nevertheless, researchers must carefully consider poultry age and body traits before applying prediction equations to avoid extrapolating results.

In summary, we demonstrate here that DEXA is a good method of measuring body carcass composition of broiler chickens. Furthermore, DEXA, in contrast to chemical analysis, is a nondestructive and noninvasive method that allows longitudinal studies to be carried out using a limited number of birds, as there is no need to kill the animals. It is also important to emphasize that chemical analysis, although it remains the gold standard, is associated with increased labor, time consumption, multiple reagents and machinery usage, higher costs, and the generation of pollution residuals. In the poultry industry, dual X-ray technology can be used for meat inspection, quality control, and to typify carcasses. Thus, predictive equations, such as those validated in the present study, are strongly recommended for proper calibration of DEXA estimates.

CONCLUSIONS

The linear models for chicken body composition prediction validated in this study are possibly specific to particular equipment, software version, and procedures (such as bird positioning and ROI specifications). Nonetheless, the same equation structures can be adapted to new data using different DEXA scanner systems by following the same development and validation processes of this study to acquire new customized prediction models. In Trial 1, predictive linear models were developed for broiler weight, fat mass, ash mass, SLT mass, water mass, protein mass, fat content, SLT content, and water content. Therefore, Trial 2 was conducted for validation, and remarkably, all absolute tissues were validated, indicating a high accuracy prediction of these equations. Thus, the prediction equations validated, can be used to obtain the tissue content as ratio of total weight. The DEXA method is an effective approach to measuring the body composition of broilers by using regression equations to calibrate results. The equations developed in this study could greatly contribute to DEXA applicability in poultry research, genetic improvement, meat production, and the poultry industry.

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DISCLOSURES

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

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103363.

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SUPLEMENTARY MATERIALS

Table S1. Experimental diets of Trial 1. In a basal diet, using DL-Met or DL-2-hydroxy-4-methylthio butanoic acid (HMTBA) as source, increasing levels (in parenthesis) of Met was supplemented in substitution of starch. A plus diet with high DL-Met supplementation and more Biolys was also formulated. Totalizing 14 experimental diets.

Ingredients (%)	1 - 14 days	14 - 28 days	28 - 42 days
	Initial	Grower	Finisher
Corn	53.32	61.20	59.61
Soybean meal, 46%	32.05	25.55	27.56
Soy Concentrate, 62%	6.37	4.00	0
Starch	(2, 1.96, 1.92, 1.87, 1.8, 1.7, 1.6, 1.34)	(2, 1.96, 1.92, 1.88, 1.83, 1.75, 1.67, 1.47)	(2, 1.96, 1.92, 1.88, 1.83, 1.76, 1.68, 1.48)
Soy oil	2.36	3.14	6.87
Salt	0.28	0.17	0.17
Sodium bicarbonate	0.15	0.49	0.56
Dicalcium phosphate	1.84	1.67	1.40
Limestone	0.71	0.64	0.64
DL – Methionine, 99.7% or HMTBA, 88%[1]	(0, 0.04, 0.08, 0.13, 0.2, 0.3, 0.4)	(0, 0.04, 0.08, 0.12, 0.17, 0.25, 0.33)	(0, 0.04, 0.08, 0.12, 0.17, 0.24, 0.32)
Biolys, 54.6%[2]	(0.12, 0.38)	(0.22, 0.42)	(0.26, 0.46)
L-Threonine, 98%	0.13	0.15	0.16
L-Valine	0.10	0.12	0.14
L-Isoleucine	0.04	0.10	0.10
L-Arginine	0	0.06	0.08
L-Glycine	0.11	0.10	0.11
Choline chloride, 70%	0.08	0.08	0.08
Mineral premix[3]	0.13	0.12	0.09
Vitamin premix[4]	0.13	0.12	0.09
Coccidiostátic[5]	0.06	0.06	0.06
Antioxidant[6]	0.02	0.02	0.02
Calculated composition[7]			
Metabolizable Energy, kcal/kg	2974	3107	3344
Crude protein, %	23.13	19.53	17.98
SID Met + Cys, %	(0.6, 0.64, 0.68, 0.73, 0.8, 0.9, 1)	(0.52, 0.56, 0.6, 0.64, 0.69, 0.77, 0.85)	(0.48, 0.52, 0.56, 0.6, 0.65, 0.72, 0.8)
SID Methionine, %	(0.31, 0.35, 0.39, 0.44, 0.51, 0.61, 0.71)	(0.27, 0.30, 0.34, 0.38, 0.43, 0.51, 0.59)	(0.25, 0.28, 0.32, 0.36, 0.41, 0.48, 0.55)
SID Cysteine, %	0.29	0.26	0.24
SID Lysine, %	(1.2, 1.34)	(1.03, 1.14)	(0.96, 1.07)
SID Threonine, %	0.86	0.75	0.71
SID Tryptophan, %	0.25	0.20	0.19
SID Arginine, %	1.41	1.20	1.14
SID Valine, %	1.06	0.92	0.86
SID Isoleucine, %	0.91	0.81	0.76

SID Leucine, %	1.71	1.48	1.35
SID Histidine, %	0.57	0.46	0.42
SID Phenylalanine, %	1.01	0.84	0.77
Sodium, %	0.16	0.21	0.23
Non-phytic phosphorus, %	0.49	0.42	0.36

[1] Thirteen diets: basal diet without any Met supplementation (zero) and six levels of methionine supplementation as percentage (% of Met) using each DL-methionine or HMTBA as Met source;

[2] One diet with high supplementation of DL-Met also received more Biolys;

[3] Composition per kg of product: manganese, 58.36 mg; zinc, 54.21 mg; iron, 41.68 mg; copper, 8.31 mg; selenium, 250 mg and iodine 843 mg.

[4] Composition per kg of product: vit. A, 9,637,000 UI; vit. D3, 2,409,000 UI; vit. E, 36,100 UI; vit. B1, 2.59 g; vit B2, 6.45 g; vit. B6, 3.61 g; pantothenic acid, 12.95 g; vit K3, 1.93 g; Folic acid, 903 mg; niacin, 39.20 g; vit B12, 15.9 mg; biotin, 89.8 mg;

[5] Salinomycin (12%);

[6] Hydroxybutyltoluene – BHT;

[7] Calculated values were based on the ingredient's nutritional composition (Rostagno et al., 2017).

Table S2. Experimental diets of Trial 2. In a basal diet, using each inorganic or organic mineral sources, increasing levels (in parenthesis) of Mn, Zn, Cu, Fe and Se were supplemented. A diet without mineral supplementation was also formulated. Totalizing 9 experimental diets.

Ingredients (%)	1 - 21 days	21 - 42 days
	Initial/Grower	Finisher
Corn	46	54
Soybean meal, 45%	45	37
Soy Oil	4.53	5.28
Salt	0.53	0.49
Dicalcium phosphate	1.27	0.81
Limestone	0.97	0.84
DL-Methioine, 98%	0.333	0.288
L-Lysine HCl, 80%	0.122	0.156
L-Threonine, 98%	0.056	0.058
Choline chlorine, 60%	0.1	0.1
Mn, ppm[1]	(100, 32.83, 73.98, 53.40, 0)	(100, 25.90, 58.36, 42.13, 0)
Zn, ppm[1]	(80, 30.54, 68.72, 49.63, 0)	(80, 24.09, 54.21, 39.15, 0)
Cu, ppm[1]	(12, 4.58, 10.54, 7.56, 0)	(12, 3.61, 8.31, 5.96, 0)
Fe, ppm[1]	(60, 22.91, 52.84, 37.87, 0)	(60, 18.07, 41.68, 29.88, 0)
Se, ppm[1]	(0.3, 0.138, 0.317, 0.227, 0)	(0.3, 0.109, 0.25, 0.179, 0)
Potassium Iodate (I), ppm	(1.1, 0)	(1.1, 0)
Vitamin Supplement[2]	0.14	0.10
Coccidiostatic[3]	0.06	0.06
Antioxidant[4]	0.02	0.02
Growth promoter	0.01	0.01
Enzyme[5]	0.02	0.02
Inert[6]	0.84	0.77
Calculated composition[7]		
Metabolizable Energy, kcal/kg	3.066	3222
Crude protein, %	24.60	21.60
SID Lysine, %	1.326	1.160
SID Methionine, %	0.653	0.575
SID Met + Cys, %	0.974	0.862
SID Threonine, %	0.868	0.769
SID Tryptophan, %	0.280	0.238
SID Valine, %	1.029	0.900
Calcium, %	0.942	0.755
Total Phosphorus, %	0.699	0.591
Available Phosphorus, %	0.448	0.353
Sodium, %	0.224	0.208
Chlorine, %	0.213	0.202
Potassium, %	0.941	0.814

[1] Inorganic and organic sources of: Mn - Manganese sulfate, 31.78% and Bioplex Mn, 16.13%; Zn - Zinc sulfate, 35.23% and Bioplex Zn, 16.96%; Cu - Copper sulfate, 25.1% and Bioplex Cu, 10.26%; Fe - Iron sulfate, 31.17% and Bioplex Fe, 16.03%; Se - Sodium selenite, 45.42% and Sel-Plex, 0.23%;

[2] Composition per kg of product: vit. A, 9,637,000 UI; vit. D3, 2,409,000 UI; vit. E, 36,100 UI; vit. B1, 2.59 g; vit B2, 6.45 g; vit. B6, 3.61 g; pantothenic acid, 12.95 g; vit

K3, 1.93 g; Folic acid, 903 mg; niacin, 39.20 g; vit B12, 15.9 mg; biotin, 89.8 mg;

[3] Salinomycin (12%);

[4] Hydroxybutyltoluene – BHT;

[5] Alltech SSF. 200 g / ton;

[6] Tixosil;

[7] Calculated values were based on the ingredient's nutritional composition (Rostagno et al., 2017).

Table S3. R Script used for statistical analysis.

```
Trial1=read.table(file.choose(),h=T)  ### Entering the dataset of Trial 1, search .txt file in your computer

#Data must be as follows: E.G.
    #Trial1 <- data.frame(bird=c(1, 2, 3, 4, 5, n),
         #      x=c(100, 200, 300, 400, 500, n),
          #     y=c(110, 190, 320, 405, 499, n))
## Y = chemical analysis values; X = DEXA estimates ##

#Checking the data input
head(Trial1)
Trial1=edit(Trial1) #Editing the dataset if necessary
attach(Trial1)

#Opening packages
library(caret)
library(Metrics)
library(Hmisc)
library(corrplot)

###### First Step - Parameters verification (B0, B1) and correlation#####
model=lm(
  y~x,
  data = Trial1) #Creating the linear model
summary(model) # verifing the parameters values and p-value
confint(model) # confident interval

res= rcorr(
  as.matrix(Trial1),
  type = c("pearson")) ## correlation
res

corrplot.mixed(
  res$r,
  lower = "number",
  upper = "square") ## correlation plot

test=cor.mtest(
  res$r) #obtaining p-value ($p)
test

###### Step 2 - Prediction equations ##########
#### Spliting data for prediction equations development - creating the data and models for cross validation ####

Amostra =createDataPartition(
  Trial1$bird,
  p = .70,
  list = FALSE)  ### spliting the dataset

trein= Trial1[ Amostra,] ## training data [ used for development of prediction equations] -> 70%
teste= Trial1[-Amostra,] ### test data -> 30%

met=trainControl(method = "cv",
                number = 5) #creating the anlysis method of Cross validation

set.seed(123)

#Prediction equation
```

```
mod= train(y ~ x , data=trein,
          method='lm',
          trControl = met,
          )

print(mod) ### Obtaining the error metrics of equations (RMSE, R^2 e MAE)
mod$finalModel ### assessing the parameters (B0,B1)
dat=cbind(
  trein$x,
  trein$y) ## Creating columns to use in Excel plot
dat

##### Test of prediction equations##########
### Using the 30 % of data set ####
predictions=predict(
  mod$finalModel,
  teste) ### calculating the predict based on test data (30%) and using the prediction model developed

model=lm(
  teste$y ~ predictions,
  data=teste) ## observed vs predict of test

summary(model) ### assessing the R^2

data=cbind(
  predictions,
  teste$SWg) ## Creating columns to use in Excel plot
data

rmse(teste$y, predictions); mae(teste$y, predictions) ## error metrics

#### Step 3 - Validation using external data ####
###### Trial2 input data (Predicted values were calculated in Excel using the equations developed in
Trial1) ########
Trial2=read.table(file.choose(),h=T)
head(Trial2)
Trial2=edit(Trial2)
attach(Trial2)

#Data must be as follows: E.G.
#Trial2 <- data.frame(bird=c(1, 2, 3, 4, 5, n),
#      y=c(100, 200, 300, 400, 500, n),
#     z=c(110, 190, 320, 405, 499, n))
#y = chemical analysis; z = predicted values ##

##Predict vs observed
modrvsp=lm(
  y ~ z,
  data=Trial2) ## Observed vs predicted for validation
summary(modrvsp) ### assessing R^2
mae(y, z) # assessing Mean Absolute Error
```

CHAPTER II

Optimal methionine plus cystine to lysine ratio for starter, grower and finisher broilers: growth performance, carcass composition and meat yields⁂

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ABSTRACT

This study investigated the effect of increasing SID Met+Cys:Lys ratios on growth, carcass, and meat yield responses of broilers, and estimated the optimal ratio for starter, grower, and finisher phases. Three trials were conducted with each containing a completely randomized desing, six SID Met+Cys:Lys ratios and seven replicates. The SID Met+Cys:Lys ratios were different for starter (53, 57, 61, 67, 75 and 83%), grower (54, 58, 62, 67, 75 and 83%), and finisher (54, 58, 63, 68, 75 and 83%) trials. Results indicate that as birds mature, Met+Cys:Lys requirements increase initially, then slightly decline. Met supplementation leads to broiler carcasses with lower lipid but higher protein and mineral contents. For starter broilers, optimal ratios were 58% for final body weight (FBW) and average daily weight gain (ADWG), 71% for feed conversion ratio (FCR), and 69% for average daily protein deposition. Grower broilers required 76% for FBW and ADWG, 81% for FCR, 70% for average daily ash deposition, and 63% for carcass weight (CW). Finisher phase requirements were 77% for FCR, 61% for CW, and 58% for carcass yield (CY). In conclusion, formulating diets to meet these requirements for growth performance will also fulfill carcass composition and yield needs.

INTRODUCTION

Methionine (Met) is a sulphur amino acid of crucial importance for poultry survival, growth, and reproduction — one whose postabsorptive metabolism, unlike that of other indispensable amino acids, goes beyond simply serving as a "building block" for protein synthesis. Even when not abundant in all of the proteins, Met is the initiating amino acid in the translation of mRNA into proteins in all known eukaryotic cells [1]. Apart from its structural function, Met plays two other crucial roles in poultry, which to different extents will also reflect the efficiency with which body protein deposition and egg protein synthesis occurs. As a methyl donor, Met is involved in the synthesis of important molecules such as creatine, choline, polyamines, carnitine, melatonin, and epinephrine [1-3] and in the methylation of DNA, RNA, and histones, regulating, for example, gene expression. When not remethylated, homocysteine from Met is consumed in irreversible trans-sulfuration reactions with the participation of serine (Ser) to form cysteine (Cys) [4]. The latter, in addition to being utilized for maintenance functions and body protein accretion, is also essential for antioxidant defense, taurine synthesis, and feather composition [5, 6, 7]. For feathers, Cys is an amino acid of tremendous importance because its concentration in such a tissue might range between 50 and 70 g/kg protein, whereas in the feather-free body, this concentration corresponds to approximately 6 to 11 g/kg protein [8, 9].

Because Met is irreversibly converted into Cys, the latter is considered a nonessential amino acid whose requirements are partially fulfilled by Met. Therefore, broiler requirements for both sulphur amino acids, are expressed as Met+Cys, and usually considered together when designing feeding programs [10]. Regarding to commercial feeds, as far as broiler amino acid nutrition is concerned, Met has been universally acknowledged as the first limiting essential amino acid in feeds. Because feed ingredients usually do not provide enough Met to fulfill the sulphur amino acid needs of poultry [11], synthetic Met sources must be provided in feeds to optimize broiler feeding programs. Conventionally, decisions regarding the concentrations of Met + Cys to be provided in feeds have generally been made on economic grounds, considering the price of Met+Cys doses in feeds, the performance responses to such Met + Cys doses, and the price of broiler final live weight. Without accurate knowledge regarding broiler responses to different levels of Met + Cys, and the doses of both amino acids that optimize the responses of economic interest (e.g., body weight, feed conversion, average daily gain), the profit margin and revenue of the intensive broiler rearing industry could be seriously compromised [12, 13]. To decrease the impacts of poultry meat protein production on the environment, the crude protein content of feeds has been systematically reduced [14], which might consequently restrict the supply of protein-bound Cys, changing the relevance of Met to meet broiler requirements for sulfur amino acids.

The literature describes broiler requirements for SID Met+Cys:Lys ranging from 66% to 82% [11, 15-25]. This discrepancy can be attributed to several factors, including the broiler strain, growth phase, and statistical model used for requirement estimation. Poultry breeding has succeeded in improving broiler growth rates, and it might also affect the dynamics with which the body and feathers grow, the allometric relationships between tissues, and the efficiency with which amino acids such as Met and Cys are partitioned for different functions in modern broiler strains. Given this brief background, we considered opportune to investigate broiler growth and carcass responses to increasing SID Met+Cys:Lys ratios in the starter, grower, and finisher phases, furthermore, based on such responses, to estimate the requirements for both amino acids utilizing different regression models.

RESULTS

The SID Met+Cys:Lys ratio impacts the growth performance of broilers

The results for the broiler starter, grower and finisher phases are shown in Table 1. The final body weight (FBW) and average daily weight gain (ADWG) of broilers fed increasing SID Met+Cys:Lys ratios from Met-supplemented diets in the starter and grower phases showed a quadratic response ($P<0.01$), with 67% and 75% resulting in greater mean values, respectively. However, in broilers during the finisher phase, an increasing linear response ($P<0.01$) was observed for FBW and ADWG, and a decreasing linear response ($P=0.01$) was observed for average daily feed intake (ADFI). Regarding feed conversion ratio (FCR), in the starter, grower and finisher phases, a quadratic response ($P<0.01$) was observed, and better mean values were obtained in broilers fed 67%, 83%, and 83% of the SID Met+Cys:Lys ratios, respectively.

SID Met+Cys:Lys ratios alter carcass composition and broiler meat yields

The carcass composition and broiler meat yields results are shown in Table 2. Regarding carcass composition, in the starter phase, a quadratic response ($P<0.01$) was observed for average daily protein deposition (ADPD), with a greater response in broilers fed 61% SID Met+Cys:Lys. For the grower phase, an ascending linear response was observed for the ADPD ($P<0.01$) of birds fed Met-supplemented diets, and a quadratic response ($P<0.01$)

of average daily ash deposition (ADAD) was observed with greater deposition on birds fed 67% SID Met+Cys:Lys. The average daily fat deposition (ADFD) of birds in the finisher phase decreased linearly (*P*<0.01).

Broiler yields were evaluated in the grower and finisher phases through measurements of carcass weight (CW), carcass yield (CY), viscerosomatic index (VI), and commercial cut yields of the breast, drumstick and thigh. During the grower and finisher phases, the CWs of the broilers fed SID Met+Cys:Lys ratios from the Met-supplemented diets showed a quadratic response (*P*<0.01), with 75% and 68% resulting in greater CWs, respectively. Broilers from the grower phase fed increasing SID Met+Cys:Lys ratios showed an increasing linear response for CY and breast yield (*P*<0.01), whereas for VI, drumstick and thigh yields, a decreasing linear response was observed (*P*<0.01). Regarding the finisher phase, a quadratic response (*P*<0.01) was observed for CY and thigh yield, with 58% and 68% SID Met+Cys:Lys resulting in greater and lesser means, respectively.

Different models estimate the SID Met+Cys:Lys requirement of broilers

The statistical models used to estimate the SID Met+Cys:Lys broiler requirements were quadratic polynomial (QP), linear broken-line (LBL), quadratic broken-line (QBL), and exponential asymptotic (EA) (Table 3). The model that presented the following ranking criterion was chosen: lowest value of the Akaike information criterion (AIC), highest R^2 or the best estimated value of the SID Met+Cys:Lys optimal ratio. The growth performance parameters requirements (Figure 1) of starter broilers were estimated using the LBL model for FBW and ADWG, and for both growth parameters, 58% SID Met+Cys:Lys was estimated as the optimal ratio. However, the FBW and ADWG requirements of grower broilers were estimated using the QP model, and for both growth parameters, 76% SID Met+Cys:Lys was estimated as the optimal ratio. The optimal ratio for FCR of broilers from the starter phase was estimated by the QP model, and the optimal ratio was 71% SID Met+Cys:Lys. Therefore, the EA model better fit the FCR means from the grower and finisher phases, and the estimated requirements were 81% and 77% SID Met+Cys:Lys, respectively.

The optimal SID Met+Cys:Lys ratios for carcass composition and meat yields are shown in Figure 2. For the starter phase, the 69% SID Met+Cys:Lys requirement for the ADPD of broilers was estimated by the QP model. For the grower phase, the QP model better fit the ADAD means, and the requirement was 70% SID Met+Cys:Lys, while for the CW the EA was used to estimate the 63% SID Met+Cys:Lys optimal ratio. Regarding the finisher phase, the CW requirement was 61% SID Met+Cys:Lys, as estimated by the EA model, and the CY requirement was 58% SID Met+Cys:Lys, as estimated by the LBL model. Furthermore, thigh yield data fit better in the QP model with 73% SID Met+Cys:Lys as the estimated level.

DISCUSSION

Responsible consumption and production are the 12th goal of the 2030 Agenda for Sustainable Development [26], and poultry meat is the animal-protein source most widely produced and eaten at the global level [27]. Animal feeding is one of the points at which nutritional strategies can be created for sustainable production through better nutrient retention in meat and less environmental pollution. Among these nutritional strategies, feed formulations using digestible amino acids in reduced crude protein diets to support the desired broiler performance, maximize the use of these amino acids for protein synthesis and not as an energy source, decrease environmental pollution by reducing N emission and litter volume, reduce the water consumption of birds, reduce feed costs, and decrease the requirements for limiting amino acids [14, 28]. This strategy is based on the concept of ideal protein, in which the requirements of all essential SID amino acids are given as percentages of the SID Lys requirement [11]. In broilers, Met is the first limiting amino acid, and together with Cys, it is considered the group of sulfur-containing amino acids in the diet formulation [29]. Therefore, it is crucial to meet the SID Met+Cys:Lys requirements of broilers throughout their productive lifespan.

This study aimed to evaluate the effect of graded levels of SID Met+Cys:Lys on growth performance, carcass composition and meat yields at starter (1 to 14 days of age), grower (14 to 28 days of age) and finisher (28 to 42 days of age) phases and to estimate the optimal SID Met+Cys:Lys ratio for the different variables. In all broiler phases, the supplementation of Met in the diet resulted in better growth parameter responses; however, the patterns differed. For starter and grower phases, a quadratic response was observed for FBW, ADWG, and FCR. For the finisher phase, a linear response was observed for FBW, ADWG, and ADFI. It has been well demonstrated in the literature that Met supplementation improves growth performance parameters [18, 24, 30-32], but in some studies, the authors also verified linear or quadratic effects. In general, the results from this study corroborate findings for starter [10, 19, 25, 33], grower [10, 16, 22, 25, 33, 34], and finisher [10, 20, 22, 33] phases. Nonetheless, differing from this study, for weight gain (WG) of grower phase broilers, an ascending linear response was observed in the research of Neto et al. [19]. In addition, in finisher broilers, several authors reported a quadratic response for WG [10, 16], and Kalinowski et al. [17] reported a decreasing linear response for FCR. These differences among studies may be related to the definition of broiler phases, as the number of days and number of phases varied among the studies, the Met supplementation source used in the diets, the tested levels, and the birds' strains.

The estimated requirements for FBW or ADWG were 58% and 76% SID Met+Cys:Lys for the starter and grower phases, respectively. Concerning the FCR, the estimated requirements were 71%, 81% and 77% SID Met+Cys:Lys for the starter, grower and finisher phases, respectively. Usually, the SID Met+Cys:Lys requirement for FCR is greater than that for body weight gain variables [18, 19, 22]. For broilers during the starter phase, there are few references in the literature about Met+Cys requirements (< 14 days of age). Therefore, the recommendations of Rostagno et al. [11], FEDNA [21] and Cobb-Vantress [23] are 74%, 73% and 74%, respectively, for this growth phase. Moreover, NRC [15] recommendation is 82% SID Met+Cys:Lys, this value is higher than that observed in this study because comprehends birds until 21 days of age, and the genetics of the NRC publication year is very different from that of recent broiler strains. Goulart et al. [10] reported a 71% SID Met+Cys:Lys requirement for WG of broilers until 7 days of age. The findings of our study suggest that modern broiler strains are more efficient at utilizing Met+Cys for improving the growth performance parameters of broilers at the starter phase. Therefore, further investigations about the effect of different SID Met+Cys:Lys ratios on the metabolism of early-stage broilers need to be performed. For grower broilers, the optimal SID Met+Cys:Lys ratio estimated for ADWG in this study is similar to that reported by other authors [10, 25] but is higher than that reported by others: 66% [18], 72% [19], and 75% [22]. Nonetheless, for the FCR requirement, the estimated SID Met+Cys:Lys ratio in this study is far greater than the values reported in the literature: 69% [18], 74% [19], and 76% [22, 25]. This highlights the importance of constantly investigating broiler requirements for proper recommendations. The SID Met+Cys:Lys recommendations for the growth performance of grower broilers from Rostagno et al. [11] and FEDNA [21] is 74%, and that from Cobb-vantress [23] and NRC [15] is 77%, which is close to the optimal SID Met+Cys:Lys ratio estimated for ADWG in this study. For the finisher phase, only the FCR observed results for the optimal SID Met+Cys:Lys was in accordance with the recommendations of Neto et al. [20] and Tavernari et al. [22]. The estimated values in this study are close to those of the FEDNA [21] (76%) and Cobb-vantress [23] (78%) but higher than those of the Rostagno et al. [11] (74%) and NRC [15] (72%) recommendations. In regard to ADWG, the stable results observed in finisher birds fed diets with 68 to 83% SID Met+Cys:Lys ratios indicate that additional Met supplementation could not increase ADWG further if the birds had already approached nutritional adequacy, and 54% of the SID Met+Cys:Lys starting range was not sufficient to promote different stages of growth response for a descriptive response curve for assessment of the requirement [29, 35].

Methionine supplementation not only modulates the growth performance of broiler chickens but also alters their body composition. To the best of our knowledge, there is no published research about optimal SID Met+Cys:Lys for assessing the carcass composition traits of broilers (whole body and plucked) in all growing phases. A graded ratio of SID Met+Cys:Lys increases the ADPD in broiler carcasses from the starter and grower phases. Similar results were observed in eviscerated carcasses, however, for broilers from the starter [19] and finisher [16] phases. Moreover, Neto et al. [19] observed a quadratic response for protein deposition in starter broilers. In the animal body, Met is an essential sulfur amino acid for protein synthesis and influences lipid metabolism via carnitine synthesis and hormone-sensitive lipase (HSL) activity [36]. Most studies have evaluated the influence of Met on the abdominal fat of poultry [10, 13, 17, 20, 33, 37]; therefore, this technique results in a greater coefficient of variation due to human bias and does not represent the whole body of the animal. Hence, chemical analysis methods for investigating lipid deposition in broiler bodies can generate reliable results. In the present study, a reduction in lipid deposition was observed with increasing ratios of SID Met+Cys:Lys in broilers from the grower (trend, $P = 0.09$) and finisher phases. This finding is relevant for the poultry industry and for consumers seeking meat-broilers products with better nutritional quality. Further investigating these results, the protein deposition to lipid deposition (PD:LD) ratio of grower broilers fed 54% SID Met+Cys:Lys (105%) compared to broilers fed 83% SID Met+Cys:Lys (149%) increased 1.41-fold, while for finisher broilers, a 1.47-fold increase was observed. Rodrigueiro et al. [16] also observed a linear decrease in the fat content of eviscerated carcasses of finisher broilers fed increasing SID Met+Cys:Lys. In 35-day-old female birds, a quadratic concave upward response was observed for fat content in eviscerated carcasses [22]. In grower broilers, an increase in ADAD was observed with graded ratios of SID Met+Cys:Lys, which may be related to increased growth performance, with the mineral content being deposited in bone and muscle tissue to a greater extent. The SID Met+Cys:Lys requirement for ADPD in starter broilers was lower (69%) than the 74% found in the literature [19]; therefore, the age of the birds used in the referred study ranged from 1 to 21 days, which may explain this discrepancy. This is the first study to identify a SID Met+Cys:Lys requirement for ADAD in broilers. More studies about the effects of SID Met+Cys:Lys ratios on the carcass composition of broilers throughout their lifespan are needed since most published works have focused on growth performance and meat yield.

Broilers fed Met-supplemented diets showed greater CW and CY in the grower and finisher phases. It is extensive in the literature that methionine supplementation results in greater carcass weight and yield [13, 16, 24, 30, 32, 33]. In fact, the observed improvements in growth performance were expected to be reflected in the broiler carcasses. The yields of commercial cuts from the breast, drumstick and thighs were also influenced by the graded levels of SID Met+Cys:Lys in grower broilers, while the breast yield increased the drumstick and thigh proportionally decreased, as observed by some researchers [13, 24, 33]. In finisher broilers, only thigh yield was influenced by Met levels in the diet. Although the mean breast yield did not significantly differ, mathematically, greater values of breast yield were observed for the 68% and 75% SID Met+Cys:Lys levels, the same treatments with lower thigh yields. Moreover, these levels are also associated with higher CW, and similar findings were observed in geese [38]. The SID Met+Cys:Lys requirement for CW was 63% and 61% for grower and finisher broilers, respectively, while for the CY of finisher broilers, the SID Met+Cys:Lys requirement was 58%, lower than the 73% found in Rodrigueiro et al. [16]. The variations in SID Met+Cys:Lys requirements observed among the works for all variables discussed in this study can be attributed to the statistical model used for estimation.

Most studies use the QP model to estimate the optimal nutrient level in the diet for optimized broiler performance [10, 16, 19, 20, 22, 25]. However, one model does not necessarily best represent all nutritional responses of broilers [3939] due to variations in tissue deposition and body composition during growth. In this sense, the results of experiments should dictate the model choice [39, 40]. In the present study, we observed that for the same evaluated parameter, the model used to estimate the SID Met+Cys:Lys requirement changes according to the broiler growth phase (e.g., growth parameter of starter and grower phases). In most cases, the QP model superestimates the SID Met+Cys:Lys requirement. Therefore, comparisons with previous research results should consider differences in studied variables such as strain, sex, age, diet formulation, statistical model, and method (factorial vs. empirical) [39].

In summary, the growth performance requirements found in this study indicate that as birds grow and develop, the SID Met+Cys:Lys requirement increases at certain point, and then slightly decreases. Higher requirements are found in the grower phase, which may be related to the feather growth of broilers since the feather growth rate reaches a maximum between 14 and 28 days posthatch [29]. As protein accretion rates increase in feathers, increased amino acid requirements, particularly Cys, are expected during this period [29]. In the animal body, Met is transformed into Cys by a nonreversible process in the homocysteine cycle. Moreover, as assessed in this study, the body composition of broilers changes with the age and development growth state of the animals, and Met supplementation produces carcasses with low lipid but higher protein and mineral contents, which is interesting for the poultry industry. The SID Met+Cys:Lys requirements for starter broilers were 58% for FBW and ADWG, 71% for FCR, and 69% for ADPD; for grower broilers, they were 76% for FBW and ADWG, 81% for FCR, 70% for ADAD, and 63% for CW; and for the finisher phase, they were 77% for FCR, 61% for CW, and 58% for CY. In conclusion, formulating diets to meet the SID Met+Cys:Lys requirement for growth performance parameters will also meet the requirements for carcass composition and yield.

METHODS

Three trials were conducted at the Poultry Sector of the Animal Science Department of Universidade Federal de Viçosa (UFV) for each broiler starter, grower and finisher phase. The trials were previously approved by the Ethics Committee of Animal Production of Universidade Federal de Viçosa (CEUAP – UFV n°029/2020), and the animal handling procedures were performed in strict accordance with the committee guidelines. All trials were carried out in compliance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines 2.0.

Experimental diets

Ingredients were acquired from the same batch to avoid differences in nutrient content. The crude protein and total amino acid contents of corn, soybean meal, and soy concentrate were analyzed by near infrared spectroscopy [41] (NIRS) before feed manufacturing. The experimental diets for the starter, grower and finisher phases were formulated following the nutritional recommendations of Rostagno et al. (2017) [11], except for the methionine levels. The SID lysine was added to reach 94% of its recommendation for the different broiler phases to avoid excess. The other amino acids were supplemented to 10% above the nutritional recommendation to avoid bird performance limitations. For each broiler phase, the SID Met+Cys:Lys ratios were obtained through the dilution method. The experimental diets needed for each trial, were produced using a basal diet without DL-methionine (DLM) supplementation (low SID Met+Cys) and a diet supplemented with DLM (MetAMINO, Evonik Operations GmbH, Essen, Germany, min. 99% DL-methionine) in replacement of starch (high SID Met+Cys) (Table 4). Thereafter, intermediate experimental ratios were obtained through the proportional mixing of low- and high-SID Met+Cys diets. The total amino acid contents of the experimental diets were determined by AminoLab (Evonik Operations GmbH), and the SID amino acid content was estimated using the respective SID amino acid coefficient of Rostagno et al. (2017) [11].

Bird husbandry and experimental design

Three trials were conducted with an experimental period of two weeks each, with bird group phases: starter (1 to 14 days), grower (14 to 28 days), and finisher (28 to 42 days), respectively. The trials were performed in a completely randomized design with 6 SID Met+Cys:Lys ratios and 7 replicates (Table 5).

For the starter phase trial, a total of 840 Cobb500 male chickens (average BW: 43.84 ± 0.03 g) were randomly distributed to 42 pens in a masonry building with curtains on the sides. Twenty birds were placed in concrete pens 0.6 m × 1.5 m × 2.0 m (height × width × length) with one feeder, two drinking nipples with cups, and covered with wood shavings. Each pen was considered the experimental unit. The building lighting program included 12 h of natural light and 12 h of artificial light. The ambient temperature was controlled and varied from 33 to 30°C throughout the entire experimental period. Feed and water were provided *ad libitum*.

For the grower phase trial, Cobb500 male chickens were allotted to conventional mansory buildings from 1 to 14 days of age and fed a commercial starter diet. After 14 days of age, 840 chickens were selected, weighed (average BW: 429.50 ± 0.001 g) and randomly distributed to 42 pens to receive the experimental diets. The bird's husbandry was similar to those of the starter trial, except for the ambient temperature, which varied from 27 to 23°C throughout the entire experimental period.

For the finisher phase trial, Cobb500 male chickens were allotted to conventional mansory buildings from 1 to 28 days of age and fed commercial starter and grower diets. After they reached 28 days of age, 840 chickens were selected, weighed (average BW: 1.54 ± 0.003 kg) and randomly distributed to 42 pens to receive the experimental diets. The birds husbandry was similar to those of the starter and grower trials, except for the ambient temperature, which varied from 23 to 20°C throughout the entire experimental period.

Growth performance

For each trial, which included the starter, grower and finisher phases, the birds and feed were weighed on the first day and at the end of the experimental period, respectively, to obtain the FBW, ADWG, ADFI and FCR. The ADWG of a bird was calculated as the difference between the FBW and initial body weight divided by the number of days in the experimental period. The ADFI of a bird was calculated as the difference between the total amount of feed provided and the amount of feed leftovers divided by the number of days in the experimental period. The FCR was calculated as the ratio of the total feed intake to the total weight gain during the entire experimental period.

Carcass composition and yield traits

At the beginning of the experimental period, 9 birds were randomly selected at days 1, 14, and 28 of age and were euthanized by cervical dislocation to determine the initial carcass composition of the starter, grower and finisher phases, respectively. At the end of each experimental period, one bird (n= 42) that was close to the average BW of the pen was selected, weighed, and euthanized by cervical dislocation after 12 h of fasting with *ad libitum* drinking water, and was used to determine the broiler carcass composition. The process was similar for starter, grower and finisher trials. After slaughter, the bird was plucked, and the whole-body carcass (containing viscera, blood, feet and head) was stored in a freezer at -20°C. The frosted carcasses were cut using a band saw (SI-282HD, Skymsen, Santa Catarina, Brazil), ground twice with an industrial grinder (PBM081, Beccaro, São Paulo, Brazil), and homogenized, and a sample was collected for lyophilization for chemical analysis at the Laboratory of Animal Nutrition of the Department of Animal Science of the UFV. One-day-old birds were pooled before lyophilization to obtain three representative samples. The lyophilized samples were ground in a ball mill and used for analysis of dry matter [42], crude protein Kjeldahl N × 6.25 [42], ether extract [43], and ash [42]. The average daily protein, fat, and ash deposition were calculated as the difference between the carcass composition at the end and at the beginning of each experimental period and then divided by 14 (number of experimental period days) for each starter, grower and finisher trial.

Only for the grower and finisher phases, in addition to the bird selected for carcass composition, two more birds (n=84, each trial) that were close to the average BW of the pen were selected, weighed, and euthanized by cervical dislocation after 12 h of fasting with *ad libitum* drinking water. These birds were used to determine the yields of the carcass, such as CW, CY, VI, breast yield, drumstick yield, and thigh yield. For CW, the bird was plucked and bled, and then the hot carcass containing the head, feet and viscera was weighed. After weighing, the hot carcass was eviscerated, and the viscera weight was obtained as the difference between the carcass weight and the eviscerated carcass. Then, the VI was calculated as the weight of the viscera relative to the fasting weight. The CY was calculated as the eviscerated carcass weight as a percentage of the fasting weight. The breast, drumstick and thighs were considered the commercial cuts of the bird. The eviscerated carcass was cut to obtain the commercial cuts, which were separately weighed. The breast, drumstick and thigh yields were calculated as percentages of the eviscerated carcass.

Statistical analysis

All the statistical analysis procedures were performed using SAS statistical software [44]. The raw data were evaluated for outliers per trial period, and significant outliers (those outside the range of the mean ± 2 times the standard deviation) were excluded from the statistical analysis. The data from the three trials were subjected separately to analysis of variance (ANOVA) at the 5% probability level. Therefore, the six SID Met+Cys:Lys ratios of the Met-supplemented diets were subjected to polynomial contrast analysis to evaluate whether there was a linear or quadratic effect of the tested SID Met+Cys:Lys ratios on the mean parameters ($P<0.05$). When a quadratic effect of polynomial contrast was observed, the nutritional requirement of SID Met+Cys:Lys for broilers was estimated using the QP, LBL, QBL and EA models, where the growth performance, carcass composition, and meat yield trait parameters were regressed against the SID Met+Cys:Lys ratios [39]. The 95% asymptotic response was used to estimate the requirement for the EA model. The criterion for choosing the model that best fit the data were that one presented, in this order: the lowest value of the AIC, the highest R^2 or the best estimated value of the SID Met+Cys:Lys optimal ratio.

Data Availability

The raw data will be available from the corresponding author upon a reasonable request.

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Author contributions statement

G. A. C. C. A. conducted the trials, laboratory and statystical analysis, data visualization, prepared the tables, figures, original manuscript draft, and revised the manuscript; M. A. N. conducted the trials and laboratory analysis; N. R. F. conducted the trials and laboratory analysis; W. J. A. conducted the trials and statystical analysis; G. S. V. revised and edited the manuscript; V. D. N. conceived and supervised the project, designed the study, revised the manuscript; M. I. H. conceived, supervised and administrated the project and measurements, designed the study, data visualization, and revised the manuscript.

Additional information

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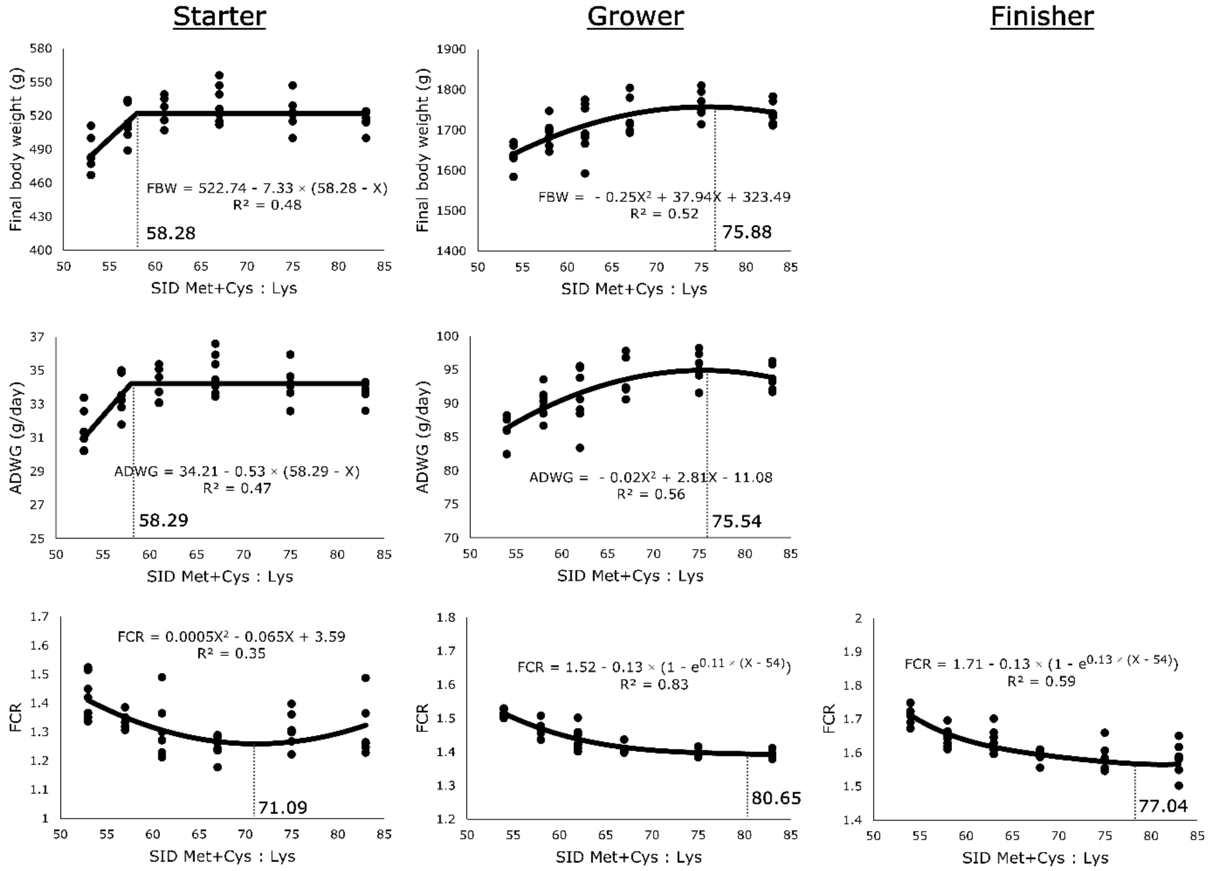

Figure 1. SID Met+Cys:Lys requirement for growth performance of starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers. FBW = final body weight; ADWG = average daily weight gain; FCR = feed conversion ratio. Optimal SID Met+Cys:Lys values (requirement) are shown in every graphic by a vertical pointed line.

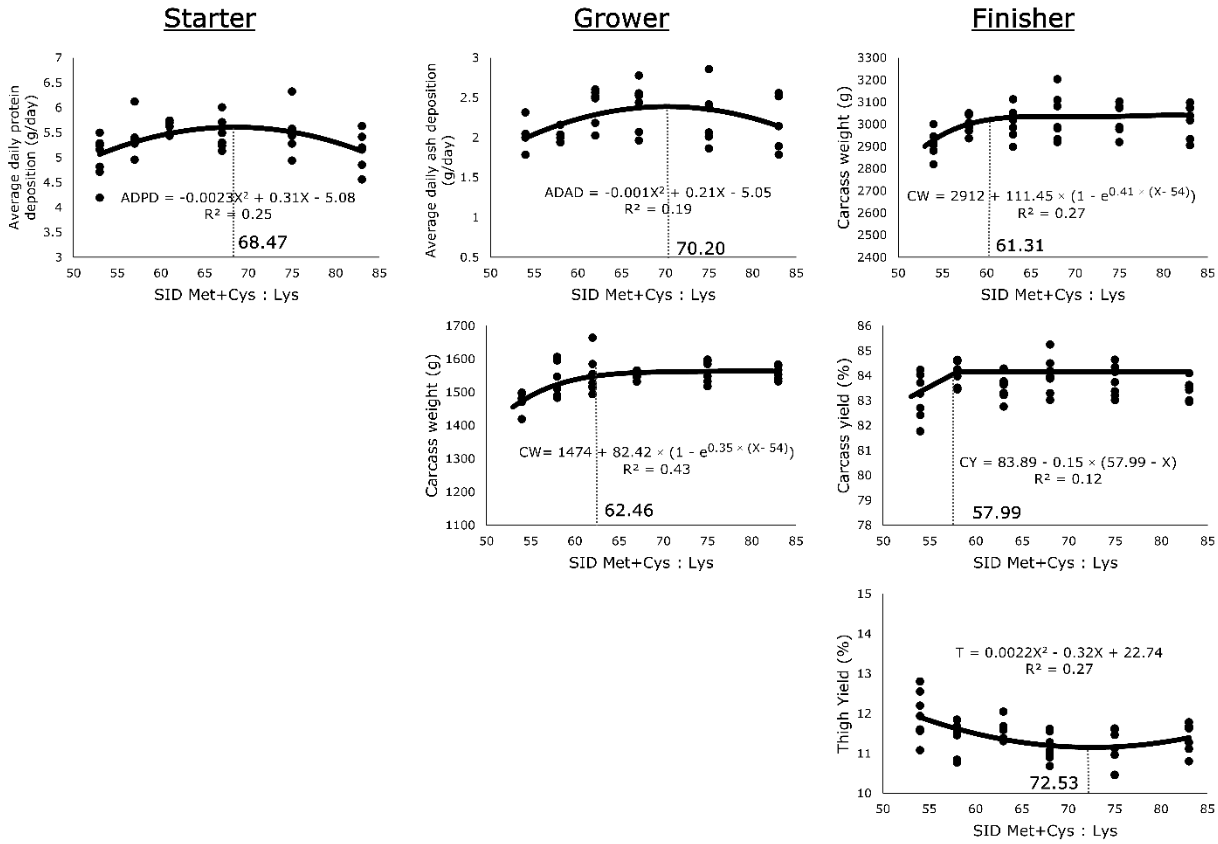

Figure 2. SID Met+Cys:Lys requirement for carcass composition and yield of starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers. ADPD = average daily protein deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield; T = thigh yield. Optimal SID Met+Cys:Lys values (requirement) are shown in every graphic by a vertical pointed line.

SID Met+Cys:Lys (%)	FBW (g)	ADWG (g/day)	ADFI (g/day)	FCR (g feed/g weight gain)
Starter (Day 1 – 14)				
53	484 ± 16.4	31.4 ± 1.17	44.7 ± 1.35	1.42 ± 0.08
57	514 ± 17.3	33.5 ± 1.23	44.9 ± 1.40	1.34 ± 0.03
61	521 ± 13.0	34.1 ± 0.93	44.3 ± 3.25	1.30 ± 0.10
67	531 ± 16.8	34.8 ± 1.20	43.6 ± 1.80	1.25 ± 0.04
75	522 ± 14.2	34.2 ± 1.01	44.3 ± 2.69	1.30 ± 0.07
83	515 ± 8.69	33.7 ± 0.61	44.1 ± 3.48	1.31 ± 0.10
linear	<0.01	<0.01	0.55	<0.01
quadratic	<0.01	<0.01	0.73	<0.01
Grower (Day 14 – 28)				
54	1631 ± 30.1	85.8 ± 2.01	130 ± 2.36	1.52 ± 0.01
58	1690 ± 32.9	90.0 ± 2.19	132 ± 3.43	1.47 ± 0.02
62	1699 ± 65.4	90.7 ± 4.38	130 ± 4.68	1.44 ± 0.03
67	1741 ± 46.2	93.7 ± 3.15	132 ± 5.31	1.41 ± 0.02
75	1765 ± 32.5	95.4 ± 2.19	133 ± 2.47	1.40 ± 0.01
83	1740 ± 26.7	93.6 ± 1.75	130 ± 2.65	1.39 ± 0.01
linear	<0.01	<0.01	0.53	<0.01
quadratic	<0.01	<0.01	0.20	<0.01
Finisher (Day 28 – 42)				
54	3267 ± 76.4	124 ± 5.22	212 ± 5.19	1.72 ± 0.03
58	3324 ± 44.5	128 ± 3.43	210 ± 2.86	1.65 ± 0.03
63	3320 ± 66.7	127 ± 4.63	208 ± 5.51	1.64 ± 0.04
68	3363 ± 49.7	130 ± 3.46	208 ± 4.26	1.60 ± 0.02
75	3352 ± 52.2	130 ± 3.90	206 ± 3.91	1.59 ± 0.04
83	3362 ± 95.3	130 ± 6.78	206 ± 8.55	1.58 ± 0.05
linear	<0.01	<0.01	0.01	<0.01
quadratic	0.25	0.25	0.54	<0.01

Table 1. Growth performance of broilers fed graded SID Met+Cys:Lys ratios in starter, grower and finisher phases. FBW = final body weight; ADWG = average daily weight gain; ADFI = average daily feed intake; FCR = feed conversion ratio. Values are presented as means ± standard deviation.

SID Met+Cys:Lys (%)	Carcass composition			Yield traits					
	ADPD (g/day)	ADFD (g/day)	ADAD (g/day)	CW (g)	CY (%)	VI (%)	Breast (%)	Drumstick (%)	Thigh (%)
Starter (Day 1 – 14)									
53	4.99 ± 0.44	2.70 ± 0.31	1.04 ± 0.10						
57	5.40 ± 0.39	2.41 ± 0.15	1.11 ± 0.17						
61	5.61 ± 0.12	3.00 ± 0.42	1.16 ± 0.06						
67	5.48 ± 0.33	2.41 ± 0.18	1.02 ± 0.05						
75	5.51 ± 0.46	2.77 ± 0.67	1.02 ± 0.06						
83	5.14 ± 0.35	2.65 ± 0.45	1.03 ± 0.13						
linear	0.38	0.85	0.27						
quadratic	<0.01	0.95	0.24						
Grower (Day 14 – 28)									
54	12.0 ± 1.11	11.4 ± 1.01	2.02 ± 0.22	1472 ± 26.7	80.5 ± 0.78	11.6 ± 0.54	28.3 ± 1.42	14.3 ± 0.90	12.6 ± 0.68
58	13.1 ± 0.21	10.6 ± 1.60	2.01 ± 0.09	1537 ± 53.0	81.8 ± 1.64	12.4 ± 1.21	29.8 ± 1.54	13.0 ± 0.35	11.7 ± 0.54
62	13.8 ± 0.72	11.1 ± 0.49	2.39 ± 0.24	1549 ± 58.0	81.6 ± 1.10	11.1 ± 0.90	30.1 ± 1.73	13.3 ± 0.83	11.9 ± 0.85
67	13.8 ± 1.37	9.76 ± 1.79	2.41 ± 0.29	1552 ± 14.5	81.4 ± 0.98	11.1 ± 0.38	31.4 ± 0.32	13.2 ± 0.77	11.6 ± 0.48
75	14.3 ± 0.34	10.3 ± 1.97	2.28 ± 0.33	1565 ± 34.1	81.9 ± 0.92	10.3 ± 0.51	33.4 ± 1.36	13.1 ± 0.33	11.5 ± 0.22
83	14.8 ± 1.18	9.94 ± 0.68	2.18 ± 0.35	1558 ± 20.7	82.1 ± 0.53	10.5 ± 0.49	33.2 ± 0.92	12.8 ± 0.73	11.3 ± 0.55
linear	<0.01	0.09	0.12	<0.01	0.03	<0.01	<0.01	<0.01	<0.01
quadratic	0.28	0.66	0.02	0.02	0.47	0.92	0.75	0.17	0.25
Finisher (Day 28 – 42)									
54	16.2 ± 0.95	22.4 ± 3.14	2.91 ± 0.61	2911 ± 55.6	83.2 ± 0.91	9.24 ± 0.45	33.9 ± 1.15	13.2 ± 0.61	12.0 ± 0.60
58	18.4 ± 0.80	18.8 ± 0.89	2.43 ± 0.13	3005 ± 43.7	84.2 ± 0.51	8.67 ± 0.46	34.9 ± 1.04	13.3 ± 0.37	11.4 ± 0.45
63	17.2 ± 1.32	17.3 ± 3.34	2.82 ± 0.21	3007 ± 69.1	83.5 ± 0.49	9.11 ± 0.39	34.4 ± 0.94	13.0 ± 0.55	11.6 ± 0.26
68	17.0 ± 1.03	18.4 ± 4.11	2.77 ± 0.97	3030 ± 104	84.0 ± 0.74	8.71 ± 0.65	35.0 ± 1.24	13.5 ± 0.45	11.1 ± 0.35
75	17.3 ± 1.53	17.0 ± 1.15	3.18 ± 0.73	3030 ± 68.7	83.8 ± 0.62	8.60 ± 0.37	35.2 ± 1.27	13.3 ± 0.58	11.1 ± 0.50
83	18.5 ± 3.00	17.4 ± 1.91	2.66 ± 0.58	3020 ± 74.2	83.5 ± 0.39	9.01 ± 0.33	34.7 ± 1.03	13.5 ± 0.34	11.4 ± 0.36
linear	0.15	0.02	0.64	<0.01	0.68	0.25	0.22	0.22	<0.01
quadratic	0.91	0.11	0.95	0.02	0.04	0.12	0.37	0.48	0.03

Table 2. Carcass composition and yields of broilers fed graded SID Met+Cys:Lys ratios in starter, grower and finisher phases. ADPD = average daily protein deposition; ADFD = average daily fat deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield; VI = viscerossomatic index. Values are presented as means ± standard deviation.

Parameter	QP			LBL			QBL			EA		
	Optimal ratio	**AIC**	**R^2**	**Optimal ratio**	**AIC**	**R^2**	**Optimal ratio**	**AIC**	**R^2**	**Optimal ratio**	**AIC**	**R^2**
Starter (Day 1 – 14)												
FBW	72	336.4	0.49	**58**	**327.1**	**0.48**	61	327.1	0.48	61	327.2	0.47
ADWG	71	124.2	0.49	**58**	**121.3**	**0.47**	61	121.3	0.47	61	121.3	0.47
FCR	**71**	**-91.4**	**0.35**	60	-88	0.33	64	-88.1	0.33	60	-87.7	0.32
ADPD	**69**	**35.7**	**0.25**	57	39.7	0.17	58	39.7	0.17	56	39.7	0.17
Grower (Day 14 – 28)												
FBW	**76**	**413.9**	**0.52**	69	415.4	0.50	75	414.6	0.51	77	414.9	0.50
ADWG	**76**	**198.6**	**0.56**	67	200.4	0.53	75	199.1	0.54	77	199.5	0.54
FCR	76	-190.5	0.81	66	-190	0.81	75	-194.5	0.83	**81**	**-195**	**0.83**
ADAD	**70**	**139.4**	**0.19**	-	-	-	-	-	-	-	-	-
CW	74	391.4	0.36	59	387.5	0.42	61	387.5	0.42	**63**	**387.3**	**0.43**
Finisher (Day 28 – 42)												
FCR	77	-141.6	0.62	61	-136.8	0.51	75	-143.5	0.59	**77**	**-144.2**	**0.59**
CW	73	482.2	0.23	59	480.2	0.27	61	480.2	0.27	**61**	**480.1**	**0.27**
CY	-	-	-	**58**	**89.0**	**0.12**	58	89.0	0.12	55	89.1	0.12
Thigh	**73**	**54.3**	**0.27**	59	56.4	0.24	61	56.4	0.24	67	55.8	0.25

Table 3. Models used for SID Met+Cys:Lys requirement estimation of growth performance, carcace composition and yields parameters for starter, grower and finisher broilers. FBW = final body weight; ADWG = average daily weight gain; FCR = feed conversion ratio; ADPD = average daily protein deposition; ADAD = average daily ash deposition; CW = carcass weight; CY = carcass yield. AIC = Akaike information criterion. Bold values represent the model chosen, based on following order criterion: lowest value of the AIC, highest R^2 or best value of SID Met+Cys:Lys optimal ratio estimated. If model fit were not accepted (P>0.05), no values are given in the table and “-” is displayed.

Ingredients (%)	Starter		Grower		Finisher	
	Low SID Met+Cys	High SID Met+Cys	Low SID Met+Cys	High SID Met+Cys	Low SID Met+Cys	High SID Met+Cys
Corn	53.32	53.32	61.20	61.20	59.61	59.61
Soybean meal (46%)	32.05	32.05	25.55	25.55	27.56	27.56
Soy Concentrate (62%)	6.37	6.37	4.00	4.00	0	0
Starch	2.00	1.60	2.00	1.67	2.00	1.68
Soy oil	2.36	2.36	3.14	3.14	6.87	6.87
Salt	0.28	0.28	0.17	0.17	0.56	0.56
Sodium bicarbonate	0.15	0.15	0.49	0.49	0.17	0.17
Dicalcium phosphate	1.84	1.84	1.67	1.67	1.40	1.40
Limestone	0.71	0.71	0.64	0.64	0.64	0.64
DLMet (99.7%)	0	0.40	0	0.33	0	0.32
Biolys (54.6%)	0.12	0.12	0.22	0.22	0.26	0.26
L-Threonine	0.13	0.13	0.15	0.15	0.16	0.16
L-Valine	0.10	0.10	0.12	0.12	0.14	0.14
L-Isoleucine	0.04	0.04	0.10	0.10	0.10	0.10
L-Arginine	0	0	0.06	0.06	0.08	0.08
L-Glycine	0.11	0.11	0.10	0.10	0.11	0.11
Choline chloride 70%	0.08	0.08	0.08	0.08	0.08	0.08
Mineral premix[1]	0.13	0.13	0.12	0.12	0.09	0.09
Vitamin premix[2]	0.13	0.13	0.12	0.12	0.09	0.09
Coccidiostatic[3]	0.06	0.06	0.06	0.06	0.06	0.06
Antioxidant[4]	0.02	0.02	0.02	0.02	0.02	0.02
Composition[5]						
ME, kcal/kg	2974	2974	3107	3107	3344	3344
Crude protein, %	24.20 (23.10)	24.68 (23.10)	19.70 (19.53)	19.35 (19.53)	18.35 (17.98)	18.79 (17.98)
SID Met + Cys, %	0.70 (0.60)	1.03 (1.00)	0.56 (0.52)	0.92 (0.85)	0.55 (0.48)	0.86 (0.80)
SID Met, %	0.34 (0.31)	0.67 (0.71)	0.27 (0.27)	0.63 (0.59)	0.26 (0.25)	0.56 (0.55)
SID Cys, %	0.37 (0.29)	0.36 (0.29)	0.29 (0.26)	0.29 (0.26)	0.28 (0.24)	0.29 (0.24)
SID Lys, %	1.38 (1.20)	1.34 (1.20)	1.15 (1.03)	1.14 (1.03)	1.07 (0.96)	1.10 (0.96)
SID Thr, %	1.00 (0.86)	1.00 (0.86)	0.83 (0.75)	0.83 (0.75)	0.77 (0.71)	0.80 (0.71)
SID Arg, %	1.63 (1.41)	1.58 (1.41)	1.30 (1.20)	1.29 (1.20)	1.22 (1.14)	1.27 (1.14)
SID Val, %	1.19 (1.06)	1.18 (1.06)	0.98 (0.92)	0.98 (0.92)	0.96 (0.86)	0.98 (0.86)
SID Ile, %	1.06 (0.91)	1.04 (0.91)	0.89 (0.81)	0.86 (0.81)	0.84 (0.76)	0.85 (0.76)
SID Leu, %	1.95 (1.71)	1.92 (1.71)	1.52 (1.48)	1.51 (1.48)	1.50 (1.35)	1.52 (1.35)
SID His, %	0.62 (0.57)	0.60 (0.57)	0.49 (0.46)	0.48 (0.46)	0.46 (0.42)	0.47 (0.42)
SID Phe, %	1.16 (1.01)	1.15 (1.01)	0.90 (0.84)	0.90 (0.84)	0.86 (0.77)	0.88 (0.77)
Na, %	0.16	0.16	0.21	0.21	0.23	0.23
Non-phytate P, %	0.49	0.49	0.42	0.42	0.36	0.36

Table 4. Feed formulation of basal diet (Low SID Met+Cys) and High SID Met+Cys diet used for intermediate experimental diets obtention from starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers. [1]Composition per kg of product: manganese, 58.36 mg; zinc, 54.21 mg; iron, 41.68 mg; copper, 8.31 mg; selenium, 250 mg and iodine 843 mg. [2]Composition per kg of product: vit. A, 9,637,000 UI; vit. D3, 2,409,000 UI; vit. E, 36,100 UI; vit. B1, 2.59 g; vit B2, 6.45 g; vit. B6, 3.61 g; pantothenic acid, 12.95 g; vit K3, 1.93 g; Folic acid, 903 mg; niacin, 39.20 g; vit B12, 15.9 mg; biotin, 89.8 mg. [3]Salinomycin (12%). [4]Hydroxybutyltoluene – BHT. [5]Analyzed values are presented, and calculated values based on the ingredient's nutritional composition are shown in parenthesis.

Diet	Starter		Grower		Finisher		Replicates	n
	SID Met	SID Met+Cys:Lys	SID Met	SID Met+Cys:Lys	SID Met	SID Met+Cys:Lys		
1	0.37 (0.35)	53 (53)	0.31 (0.30)	53 (54)	0.31 (0.28)	56 (54)	7	140
2	0.42 (0.39)	56 (57)	0.35 (0.34)	56 (58)	0.34 (0.32)	58 (58)	7	140
3	0.47 (0.44)	61 (61)	0.40 (0.38)	62 (62)	0.40 (0.36)	63 (63)	7	140
4	0.51 (0.51)	66 (67)	0.45 (0.43)	64 (67)	0.44 (0.41)	69 (68)	7	140
5	0.60 (0.61)	74 (75)	0.49 (0.51)	68 (75)	0.49 (0.48)	72 (75)	7	140
6	0.67 (0.71)	77 (83)	0.63 (0.59)	81 (83)	0.56 (0.55)	79 (83)	7	140

Table 5. Analyzed SID Met levels and SID Met+Cys:Lys ratios of experimental diets from starter (1 to 14 days of age), grower (14 to 28 days of age), and finisher (28 to 42 days of age) broilers used in the studies. Calculated level or ratio are presented inside the parenthesis.

CHAPTER III

Session of periodic: Metabolism and Nutrition

METHIONINE SOURCES IN BROILER GROWTH PHASES

Effect of two methionine sources on body composition of broiler chickens and relative bioavailability value⁂

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ABSTRACT

Three trials were conducted to compare liquid Met hydroxy-analogue (**HMTBA**) and DL-methionine (**DLM**) supplementation in broilers from starter, grower, and finisher phases. Each trial used 1,820 Cobb500 male broilers, and was performed in a completely randomized design (2 sources × 6 levels), 7 replicates and a common basal diet deficient in methionine. The Met-sources inclusion was made in equimolar basis, and six incrementation levels of 0.04, 0.08, 0.13, 0.2, 0.3, and 0.4% for starter; 0.04, 0.08, 0.12, 0.17, 0.25, and 0.33% for grower and 0.04, 0.08, 0.12, 0.17, 0.24, and 0.32% for finisher broilers was obtained. Growth performance, body composition, carcass and commercial cuts yields were analyzed to evaluate the effect of levels and Met-source. A multi-exponential model was used to estimate the relative bioavailability value (**RBV**) considering DLM as reference. Met supplementation improved all the parameter evaluated, therefore some differences between the sources were observed ($P<0.05$). In grower broilers, DLM presented better results than HMTBA for final body weight (**FBW**), average daily weight gain (**ADWG**), and feed conversion ratio (**FCR**), in finisher broilers similar result was observed for FCR, and starter broilers fed 0.2% DLM presented better FCR. Starter broilers fed DLM presented higher protein and ash deposition conferring a better body quality than broilers fed HMTBA. Grower broilers achieved higher breast meat yield with 0.25% DLM inclusion, at the same level significant difference with HMTBA was observed. The RBV in equimolar basis for starter broiler is 99% for growth parameters. In grower broilers is 79% for FBW, 80% for ADWG, 76% for FCR, 66% for carcass weight, 236% for carcass yield, 70% for breast meat yield, and 128% for thigh yield. And for finisher is 79% for FCR, 77% for carcass weight, and 65% for thigh yield. Therefore, DLM provides a better body composition quality for young birds, and the replacement of DLM with HMTBA should consider the animal's growth phase for choosing appropriate RBV.

Key words: bioefficacy; hydroxy-analogue; amino acid; growth performance; broiler carcass.

INTRODUCTION

Methionine (Met) is the first limiting amino acid in commercial poultry diets, and is an essential amino acid with functions in poultry metabolism beyond serving merely as substrate for protein synthesis throughout the broiler chicken's life. Met is irreversibly converted into Cys, which is considered a nonessential amino acid, as its requirements are partially met through Met intake. Consequently, the nutritional needs of broilers for both sulfur amino acids are typically expressed together as Met+Cys, and they are often addressed jointly when developing feeding strategies (Pacheco et al., 2018). In commercial poultry feeds, Met is recognized as the primary limiting essential amino acid. Since natural feed ingredients generally do not provide adequate Met to fulfill the sulfur amino acid requirements of broilers (Rostagno et al., 2017), the inclusion of synthetic Met sources in the diet is necessary to ensure optimal nutrition.

Conventionally, these sources include crystalline DL-Methionine (**DLM**; 99% purity) or liquid methionine hydroxy analog (**HMTBA**; 88% active substance) (Babazadeh & Simab, 2022). HMTBA and DLM presents biochemical and metabolic distinctions that affect their biological role as Met source (Payne et al. 2006). Because HMTBA presents a hydroxyl group instead an amino group in their alfa-carbon of molecular structure, it is characterized as an organic acid precursor of Met, that must be firstly converted to L-Met in the liver and then utilized (Dibner 2003, Vázquez-Añón et al. 2017). Besides both sources are suitable to support birds' growth, misconceptions related to the matrix and equivalence of these compounds in feed formulations can lead to suboptimal Met+Cys utilization, resulting in economic losses and environmental impacts, particularly in terms of fat deposition in poultry carcasses. Regarding effect of Met sources in the body composition of birds, most studies focused only on breast meat composition (Liu et al 2006, Ullrich et al. 2019, Yenice et al 2023) and abdominal fat (Liu et al., 2007, Aggrey et al. 2017, Kim et al. 2019, Yenice et al. 2023).

The relevant question whether the intrinsic differences regarding the chemical structure and metabolism of both Met-sources could affect their utilization by birds have been addressed in dose response assays, and led to controversial and different conclusions. This debate extends to questions about the appropriate statistical methods for evaluating the bioefficacy of essential nutrient sources (Vázquez-Añón et al. 2006b, Sauer et al. 2008, Becquet et al. 2023, Lemme et al., 2024). As described by Littell et al. (1997), relative bioavailability value (**RBV**) or bioefficacy, can be obtained by slope-ratio (linear response) or by nonlinear models (multi-exponential function) depending on the data structure of the respective dose-response trial. As described in studies of Huyghebaert (1993) and Lemme et al. (2020), the dose-response of HMTBA and DLM present a non-linear response, therefore the data fit better in a nonlinear model such an exponential or multiexponential function (Liu et al., 2004). The multi-exponential function model contain three main elements: a common starting point describing the birds' performance with basal diet; a maximum level of performance (asymptote), which is common for all methionine sources in comparison; and a steepness coefficient for each methionine source, describing the curve shape, when the higher is the coefficient value the less of methionine source is needed to maximize performance, and the relationship of the steepness values of DLM and HMTBA reveal the RBV (Niu et al., 2018). The HMTBA RBV ranges from 70% to 80% in equimolar basis (Hyughebaert, 1993; Jansman et al., 2003; Hoehler et al., 2005; Sauer et al., 2008), and literature suggests an RBV of 65% in product basis (Lemme et al., 2002; Hoehler et al., 2005; Lemme et al., 2022; Yenice et al., 2023; Lemme et al., 2024).

Throughout the birds' life, feathers and muscle growth occurs at different rates affecting nutritional requirements for maintenance and growth (Sakomura et al., 2015). The less marked feather growth in starter phase compared to grower and finisher phases, may reduce the need for Cys (abundant amino acid in feathers protein) and enables Met to support other metabolic functions (Reis et al., 2018; Leme et al., 2022). Most of the studies focused on RBV for grower and finisher phases, and little is discussed if RBV is different for starter phase.

With the current study we aimed to evaluate the effect of Met supplementation with two different Met sources (DLM and HMTBA) on the growth performance, body composition, carcass and commercial cuts yields (breast, drumstick and thigh), thus, determine the RBV of HMTBA relative to DLM using a multi-exponential model in broilers from starter, grower, and finisher phases.

MATERIALS AND METHODS

Ethics

All procedures involving animal care and use described along this section were previously approved by the institutional Ethics Committee in the Use of Farming Animals of the Universidade Federal de Viçosa (029/2020).

Birds and housing

A total of 5,460 male Cobb500 chicks were used in three experiments conducted in different growth phases: starter (from 1 to 14 days, average initial BW: 43.84 ± 0.03g), grower (from 14 to 28 days, average initial BW: 429.50 ± 0.001g), and finisher (from 28 to 42 days old, average initial BW: 1.54 ± 0.003kg). For each experiment, 1,820 birds were housed in 0.6m × 1.5m × 2.0m (height x width x length) pens covered with wood shavings. Feed and water were be provided *ad libitum* during the entire experimental period. Birds utilized in the experiments in the grower, and finisher phases were housed maintained in the same environmental conditions described above and fed commercial diets before the beginning of the trial, at d 14, and d 28 post-hatch, respectively.

Experimental design and diets

In each trial, 7 replicate pens of 20 birds were randomly assigned to one 13 treatments that consisted of 6 levels of Met supplementation provided by 2 different Met sources and a common Met deficient basal diet (Table 1). The Met sources under study, DLM (Evonik, Germany) and HMTBA (Adisseo, France), were included in the experimental diets according to the levels previously established for each treatment (Table 2), at the expense of starch. The equimolar concentration of DLM and HMTBA was considered as 99.7% and 88%, respectively. Liquid HMTBA was included with a carrier (finely ground corn) in order to derive a dry product. The supplementation of both sources provided the concentration of 0.60, 0.64, 0.68, 0.73, 0.80, 0.90 and 1.00% of digestible methionine + cysteine for birds in the starter phase, levels of 0.52, 0.56, 0.60, 0.64, 0.69, 0.77 and 0.85% in the grower phase, and levels of 0.48, 0.52, 0.56, 0.60, 0.65, 0.72 and 0.80% in the finisher phase. The ENAA:TN ratio followed the recommendations of Rostagno et al. (2017).

Growth performance

In the end of each phase, birds and feed leftovers were weighted to calculate the average daily feed intake (**ADFI**), average daily weight gain (**ADWG**), and feed conversion ratio (**FCR**).

Body chemical composition

In the beginning of experimental period, 9 birds were randomly selected with 1 d, 14 d and 28 d of age to be euthanized to determine the initial birds' body chemical composition in the starter, grower, and finisher phases, respectively. At the end of experimental period, for each phase 7 birds per treatment closest to the average experimental unit body weight were selected (n = 91 per phase) to body chemical composition analysis. After weighed, birds were humanely sacrificed by cervical dislocation, plucked and weighed to obtain the total carcass weight. The whole-body carcasses, i.e., with viscera, blood, feet and head, were immediately stored under -20°C. The frozen whole-body carcasses were cut in band saw (SI-282HD, Skymsen, Santa Catarina, Brazil), twice ground in an industrial meat grinder (PBM081, Beccaro, São Paulo, Brazil) and finally homogenized. The samples were lyophilized, and analyzed for dry matter (INCT-CA, G-003/1, 2021), nitrogen (INCT-CA, N-001/2, 2021), ether extract (AOCS, Am 5-04, 2009) and ash (INCT-CA, M-001/2, 2021) analysis at the Animal Nutrition Laboratory of the Animal Science Department of Universidade Federal de Viçosa. The fat, protein and ash deposition in the carcass were calculated as the difference between the carcass chemical composition at the end and at the beginning of each experimental period for the starter, grower, and finisher experiments. Crude protein was calculated by multiplying N content in samples by 6.25.

Carcass and commercial cuts yield

In the end of the grower and finisher phases, two birds with similar BW to the average BW of its respective pen were selected (n = 182 per phase), weighed, and sacrificed in a poultry slaughterhouse from Universidade Federal de Viçosa. The carcass, i.e., with head, feet, viscera, bled, scald, and plucked was weighed. The carcass was further eviscerated, and the viscerosomatic index (**VI**) calculated as the ratio between the weight of the viscera and the fasting (12h of fasting) live BW. The carcass yield (**CY**) was calculated as the eviscerated carcass weight (with head and feet) as a percentage of the fasting weight. Then, the eviscerated carcass was cut to obtain the commercial cuts breast, drumstick and thigh, which were separately weighed to obtain their respective yields calculated as percentages of the eviscerated carcass.

Statistical Analysis

Raw data were evaluated for outliers per measurement period and significant outliers (outside the range of mean ± 2 times SD) were excluded from statistical analysis. If at least one of the response parameters evaluated was identified as an outlier, the entire record for that experimental unit was excluded.

Collected data from the three experiments were subjected separately. Means were compared to the basal diet treatment by Dunnett's test with the significance of 5% ($P \leq 0.05$). A two-way ANOVA was performed to investigate the effects of 2 sources of Met (DLM and HMTBA), 6 levels of supplementation, and their interactions. After, means were compared by the Tukey test at the 5% probability level ($P \leq 0.05$). Polynomial contrasts were used to assess the linear or quadratic effect ($P \leq 0.05$).

In all the statistical analysis, the initial weight at 1, 14 and 28 days of age in the starter, grower and finisher experiments, respectively, were considered as a covariate.

For the RBV determination of HMTBA compared to DLM, data of growth performance, carcass and commercial cuts yield were analyzed by simultaneous multi-exponential function (Littell et al., 1997):

$$Y = B1 + B2 \times (1 - e^{(B31 \times X1 + B32 \times X2)})$$

Where X1 is the level of DLM, X2 the level of HMTBA, B1 is the intercept, B1 + B2 is the asymptote, B31 is the steepness coefficient for DLM and B32 is the steepness coefficient for HMTBA. The RBV value of HMTBA relative to DLM was indicated by the ratio of B32/B31.

All statistical analysis procedures were performed using the SAS statistical software (SAS Institute, 1990). The parameters B1, B2, B31, and B32 were estimated simultaneously by using a modified Gauss-Newton iterative method of SAS.

RESULTS AND DISCUSSION

Effect of methionine sources on growth performance, chemical body composition, carcass and commercial cuts yields

Means of growth performance variables are shown in Table 3a (starter), Table 4 (grower), and Table 5 (finisher). In the starter phase, irrespective of the Met source under study, all birds fed diets supplemented either with DLM or HMTBA exhibited improvements in final body weight (**FBW**) and ADWG compared with birds fed basal diet. Conversely, FCR of birds fed the lowest DLM supplemental level and three treatments of HMTBA group (53, 57 and 67 % of SID Met+Cys:Lys) did not differ from that of birds fed basal diet. The highest FBW and ADWG were observed in broilers fed diets in which SID Met+Cys:Lys concentration was equivalent or higher than 57% regardless the Met source. Interactive effects between Met sources and levels were noticed on FCR (Table 3b). Birds fed 67% of SID Met+Cys:Lys from DLM had better FCR (1.25), compared with birds fed the same SID Met+Cys:Lys level provided by HMTBA (1.41). In the grower phase, FBW, ADWG and FCR from birds Met supplemented diets were different from those fed with the basal diet. In this phase, analyzing the source effect we noticed that birds fed DLM showed better performance traits (+20.84g of FBW, +1.49g/day of ADWG and -0.02 of FCR) than broilers fed HMTBA diets. Analyzing the SID Met+Cys:Lys level effect, better results were observed from levels of 67% or above. Similarly, the performance traits of finisher broilers were affected by the Met sources under study compared to those birds fed the basal diet. Although no differences were observed between DLM and HMTBA for FBW and ADWG, FCR of birds fed HMTBA was poorer compared with that in birds fed DLM supplemented diets (1.65 vs. 1.63, respectively). Analyzing the SID Met+Cys:Lys level effect, FBW and ADWG achieved the greatest values in broilers fed diets containing levels of 67 and 83%, whereas best FCR was optimized in broilers fed the highest level under study (83%).

The increment noticed in performance response as Met concentration increased demonstrate that the basal diet was indeed deficient in methionine, and that both Met sources can support broiler growth. Overall, in the current study, broilers fed DLM showed better growth performance results compared to broiler fed diets containing HMTBA. Our outcomes support previous research findings (Huyghebaert 1993, Kim et al. 2019, Felix et al. 2023), but differ from those reported by Liu et al. (2006), Liu et al. (2007), and Ullrich et al. (2019) that found no differences in the performance traits between the Met sources. In both Liu studies (Liu et al., 2006 and Liu et al., 2007) the authors tested three equimolar levels of Met supplementation (starter: 0.08, 0.16, 0.24% of Met; grower/finisher: 0.07, 0.14, 0.21% of Met) using DLM or HMTBA as source, whereas the present study tested six equimolar levels of Met supplementation in a greater range, and this is what might have made it possible to observe the differences between the sources in our study. While Ullrich et al. (2019) methodology compared four feeding groups offered in a protein-reduced complete diets. The groups were composed by HMTBA, DLM, L-methionine as Met sources and a group they named as reduced HMTBA, with the minimum Met required level provided using HMTBA as Met source. Alternatively, Vázquez-Añón et al. (2006a) describes in their study that by a quadratic effect, HMTBA may outperforms DLM at levels of supplementation near the optimal, whereas DLM may outperform HMTBA at suboptimal levels. Other authors, consider that comparing HMTBA and DLM near the commercial level might lead to conclusions that are not entirely accurate due to these feeding conditions are often slightly above birds' requirements, therefore close to the asymptote of performance response (Lemme et al. 2024). Lemme et al. (2020), demonstrated that pairwise comparisons of DLM and HMTBA treatments is more pronounced at lower supplementation levels (below recommendation) and then it narrows as supplementation levels increases (close or above the recommendation). The authors attribute this phenomenon to the law of diminishing returns, where the marginal benefits of supplementation decrease as the requirements of broilers are progressively met. In our study, besides both sources were considered equivalent by supplementation in equimolar levels, the differences observed in performance traits between DLM and HMTBA groups might be attributed to the nutritional value of these sources. HMTBA might have a lower bioequivalence compared to DLM, which might be the cause for poorer performance observed in birds fed HMTBA diets.

The chemical body composition of starter, grower and finisher broilers are shown in Table 6. Birds from starter phase fed diets containing Met source showed greater protein deposition compared to birds fed with basal diet. Therefore, those birds which were fed DLM exhibited greater bodily protein deposition compared with birds fed HMTBA. Analyzing the SID Met+Cys:Lys level effect, better response was observed in 67%. Similarly, birds fed DLM as Met source showed greater mineral deposition in their body compared to birds fed with HMTBA. In broilers from grower phase, Met supplementation elicited an increase in bodily protein deposition achieving a peak at 75% SID Met+Cys:Lys. Bodily mineral deposition was the greatest at 63% SID Met+Cys:Lys. For finisher broilers, regardless of the Met source investigated, the greatest bodily protein deposition was achieved at 83% SID Met+Cys:Lys, while birds fed 58% SID Met+Cys:Lys or levels above showed reduced bodily fat deposition.

Methionine is related to protein synthesis and lipid metabolism. It was expected that methionine-deficient diets would result in lower protein deposition and higher accumulation of adipose tissue, thus methionine addition present a positive effect on protein synthesis due several pathways, including regulating genes related to growth (Del Vesco et al. 2013, Yenice et al. 2024). The current study provided an interesting result regarding the use of methionine sources on protein deposition in chickens during the initial phase; the animals that received DLM showed greater deposition compared to those that received HMTBA. It is not possible to state whether this difference would be maintained throughout the entire production cycle of the bird, or if the animals fed with HMTBA would show any compensatory gain later, as the experiments were divided into phases and conducted independently. This difference in protein deposition in the initial phase can be explained by the conversion of these compounds into the L-methionine isomer, which is the form used by the body for various functions.

DLM contains 50% of its composition as D-form and the remaining as L-form, readily available for use after absorption. On the other hand, HMTBA contains both D-HMTBA and L-HMTBA isomers, and both need to be converted into L-met for utilization after absorption with action of deaminase and transaminase enzymes (Zhang et al. 2018). These HMTBA reactions produce more reactive oxygen species (H_2O_2) compared to DLM reactions for conversion to L-met, and may results in an overloading of the cellular antioxidant system (Stange et al. 2023). In the study of Klünemann et al. (2024), the authors observed that results of pathway activation indicated greater inhibition of the oxidative stress pathway due to DLM, and HMTBA promotes greater expression of GSH gene. As described in Felix et al. (2023) study, the GSH activity is related to antioxidant activity, which is increased in stress condition to mitigate the deleterious effects of free radicals (Aggrey et al. 2017, Zeitz et al. 2018). Furthermore, it was found that chickens in the initial phase fed with DLM showed higher GABA neurotransmitter activity than those fed with HMTBA (Klünemann et al. 2024). Animals in the initial phase are more sensitive to stress, and GABA is related to the adaptation of chickens on environmental stress situations such as heat or high stocking density (Park et al., 2023). GABA activity can influence muscle hypertrophy by affecting muscle contraction and the growth hormone signaling (Mills 2021). Additionally, due to the HMTBA chemical nature not characterized as aminoacid, a Nitrogen is required to complete the L-methionine conversion. In reduced crude protein diets, such as those in the present study (starter: 23%, grower: 20%, finisher: 18%), the Nitrogen available to complete the HMTBA transformation might be insufficient in higher levels of supplementation, and therefore, the bird will not be able to present their better performance. This could explain the differences between DLM and HMTBA observed in performance and chemical body composition traits. Further investigation is needed about this topic with new studies.

Through its role in balancing protein synthesis or as a methyl donor and participation in choline, betaine, folic acid, and B vitamin metabolism, Met can influence the lipid metabolism (Young et al. 1955). Therefore, Met-deficient diets tend to increase adipose tissue as observed in finisher broilers. In the current study, diets were isoenergetic among the treatments. Therefore, in diets with lower Met density, the energy provided may surpass the requirements for growth, as limited amino acid availability constrains protein synthesis and, consequently, growth. Excess energy is commonly directed to lipid deposition, although this process is subject to genetic limitations. Similar result was observed for relative weight of abdominal fat (Liu et al. 2006, Liu et al. 2007, Zeitz et al. 2018).

The carcass and commercial cuts yield results are shown in Table 7a (grower) and Table 8a (finisher). In the grower phase, Met supplemented in the diet resulted in great CW of broilers, and the 75% SID Met+Cys:Lys level showed the best result. However, the CY of birds fed diets containing low level of SID Met+Cys:Lys (54%) regardless the Met source, was not different from birds fed basal diet. Therefore, the supplementation level of 75% showed the best result. An interaction effect was observed for VI (Table 7b), the diet with 58% SID Met+Cys:Lys from DLM showed higher VI (12.38) compared to others DLM diets, and compared to 58% SID Met+Cys:Lys from HMTBA (10.65). For breast meat yield, interaction between source and level was observed (Table 7c), the greater breast meat yield was observed in birds fed DLM diet containing 75% SID Met+Cys:Lys, and birds fed HMTBA with 83%. Also, a significant difference between 75% SID Met+Cys:Lys from DLM (33.40%) and the same level from HMTBA (30.13%) was observed. Greater drumstick and thigh yields values were observed in birds fed 54% SID Met+Cys:Lys, regardless the Met source. In the finisher phase, Met added in the diets from level 58% SID Met+Cys:Lys or above resulted in greater CW either using DLM or HMTBA. Interaction effect was observed for VI (Table 8b), birds fed diets with levels of 54, 63, and 83% SID Met+Cys:Lys from DLM showed higher values compared to birds fed diets containing the same levels from HMTBA. Regardless the Met soured, breast meat yield of finisher broilers were greater when birds were fed diet containing 75% SID Met+Cys:Lys level, and birds fed 54% SID Met+Cys:Lys showed greater thigh yield.

The carcass and commercial cuts yields are in line with growth performance data, where Met-supplementation improved carcass and cuts yields. Similar results of Met supplementation over a Met-deficient diet are reported (Lemme et al. 2002, Payne et al. 2006, Lemme et al. 2020, Yenice et al. 2023). Decreased drumstick and thigh yield with Met supplementation may be mainly related to increased breast meat yield, as reported in other studies (Pontin et al., 2018, Yenice et al. 2023). The greater breast meat yield observed in birds fed with DLM at a lower supplementation level compared to those fed with HMTBA supports the findings of Lemme et al. (2020). This study suggests that DLM-fed birds reach the asymptote for growth earlier than those fed with HMTBA, as DLM is a more efficient methionine source for Met+Cys deposition in the body.

Relative bioavailability value

The results presented previously successfully achieved the main pre-requisites for an appropriate RBV determination as described by Lemme et al. (2020): (1) a common starting point (basal diet) clearly Met-deficient, (2) three or more supplementation levels of each Met source products included in the study, and (3) graded supplementation of the Met source product resulted in graded dose-responses with the first inclusion level not nearby asymptotic performance avoiding possible misinterpretations of RBV. The Met+Cys:Lys reccommendation of Rostagno et al. (2017) used as reference for diets formulation of present study is 74. Our design (Table 2) consisted of a common basal diet deficient in methionine (50% Met+Cys:Lys), and for each one of the Met sources, four supplementation levels below the recommendation, one level close (75% Met+Cys:Lys) and the last above (83% Met+Cys:Lys) in order to obtain a dose-response curve containing the common asymptote. The multi-exponential regression analysis is used for RBV determination when responses are non-linear (Huyghebaert 1993).

The RBV was estimated using multi-exponential regression analysis based on equimolar weights of HMTBA to DLM for growth performance, carcass and commercial cuts yields. The RBV for growth performance parameters in birds for starter phase were 99% for FBW and ADWG (Figure 1). Interestingly, the RBV of growth parameters found for grower (Figure 2) and finisher (Figure 3) phases were similar. For grower broilers a value of 79 and 80% was found for FBW and ADWG, respectively. While, for FCR an RBV of 76% was found for grower broilers, and a value of 79% was found for finisher.

The bioavailability of HMTBA for growth parameters in starter birds was different from that determined in the grower and finisher phases. These results indicates that the efficiency with which younger broilers utilize methionine from HMTBA and DLM is rather similar. Since HMTBA is classified as an organic acid, its absorption differs from that of DLM. The diffusion process is a major contributor to organic acids absorption, which is enhanced in their dissociated lipophilic state at low pH levels, facilitating their uptake (Dibner and Buttin, 2002). In their study, Richards et al. (2005) found that approximately 85% of HMTBA is absorbed before it reaches the gizzard, within the acidic portion of the gastrointestinal tract. Younger broilers have a more acidic crop pH compared to grower and finisher broilers (Dono et al., 2014).

Furthermore, the growth of broiler chickens involves dynamic and phase-specific amino acid demands due to differences in the composition of muscle, viscera, and feathers, each with distinct AA profiles. Feather proteins are particularly rich in sulfur amino acids, especially cysteine (**Cys**), whereas muscle growth relies more broadly on essential amino acids like Met (Leme et al., 2022). The growth of feathers and muscles occurs at different rates throughout a broiler's life, affecting nutritional requirements in distinct phases. During the starter phase, feather growth is less pronounced, reducing the demand for Cys and allowing Met to fulfill other metabolic roles (Reis et al., 2018). This metabolic prioritization might explain why the bioavailability differences between DLM and HMTBA are less evident during this phase. However, in grower and finisher phases, when feather growth intensifies, the increased demand for Cys may lead to a metabolic prioritization of dietary and endogenously derived

Cys for feather synthesis, potentially limiting its availability for muscle growth if dietary supplementation is inadequate (Pacheco et al., 2018; Leme et al., 2022). Factorial models' studies explain that amino acid efficiency is influenced by tissue-specific deposition rates and the broiler's age, emphasizing the importance of phase-specific dietary formulations to optimize growth (Sakomura et al., 2015). The findings in our study suggest that the efficiency of HMTBA as a Cys precursor may be lower compared to DLM during phases of high feather growth, further emphasizing the necessity of targeted nutritional strategies. This could explain the differences in bioavailability values observed for growth performance, and why the RBV in starter phase is higher than those for grower and finisher phases.

The average RBV of grower phase is 78% on equimolar basis, and considering the 88% purity of HMTBA is 69% on product basis, while for finisher phase is 79% on equimolar basis and 70% on product basis. The RBV of growth parameters found for grower and finisher phases are in accordance with literature. Hyughebaert (1993), found a RBV of 73% for weight gain and 68% for FCR on equimolar basis. Sauer et al. (2008), in their meta-analysis study found a RBV of 81 and 79% for ADWG and growth:feed ratio on equimolar basis. Jansman et al. (2003) found 77 and 76% for weight gain and FCR on equimolar basis. Hoehler et al. (2005), evaluated the RBV in five trials conducted in different locations and found and RBV of 82% for FCR in equimolar basis, however, for weight gain the author found an RBV of 52%; and an overall mean of 65% in a product basis. Lemme et al. (2002) found in product basis an RBV of 68% for weight gain, and 67% for FCR. In their study, Lemme et al. (2022) found and RBV of 58% for weight gain, and 66% for FCR in product basis. The scientific literature suggests an RBV of 65% on product basis, based on several approaches such as 65% DLM diluted diet (Lemme et al. 2002, Hoehler et al. 2005, Lemme et al., 2022,), HMTBA replacing DLM in a weight-to-weight ratio of 100:65 (1.54 times higher amount of HMTBA inclusion) (Yenice et al., 2023), or meta-analysis (Lemme et al., 2024). In general, considering the average of performance traits (FBW, ADWG and FCR), the RBV in equimolar basis of HMTBA is 99% for starter, 78% for grower and 79% for finisher broilers, with an overall RBV of 85%, or 75% in a product basis.

CONCLUSIONS

The supplementation of Met improved growth performance, body composition, carcass and commercial cuts yields of broilers in all growth phases. Whether assumed to have the same equivalence, DLM presented greater growth performance compared to HMTBA in starter

(FCR), grower (FBW, ADWG, and FCR) and in finisher (FCR) broilers. The source of methionine can shape the body composition of starter broilers, birds fed DLM diet presented higher protein and ash deposition, compared to those fed HMTBA diet. The RBV vary according to growth phase and the parameter in evaluation. Considering the average of performance traits, the RBV in equimolar basis is 99% for starter, 78% for grower and 79% for finisher broilers. Overall, the results from the current study demonstrate that the replacement of DLM with HMTBA needs to consider the growth phase of the bird for choosing an appropriate RBV, and attention is needed to the quality of the body composition of young birds when using the hydroxy analog, as the source of methionine influenced the body protein deposition.

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Table 1. Basal diet formula and composition.

Ingredients (%)	Starter (1 to 14 d)	Grower (14 to 28 d)	Finisher (28 to 42 d)
Corn	53.32	61.20	59.61
Soybean meal (46%)	32.05	25.55	27.56
Soy Concentrate (62%)	6.37	4.00	0
Starch	2.00	2.00	2.00
Soy oil	2.36	3.14	6.87
Salt	0.28	0.17	0.17
Sodium bicarbonate	0.15	0.49	0.56
Dicalcium phosphate	1.84	1.67	1.40
Limestone	0.71	0.64	0.64
Biolys (54.6%)	0.12	0.22	0.26
L-Threonine (98%)	0.13	0.15	0.16
L-Valine	0.10	0.12	0.14
L-Isoleucine	0.04	0.10	0.11
L-Arginine	0.00	0.06	0.10
L-Glycine	0.11	0.10	0.08
Choline chloride 70%	0.08	0.08	0.08
Mineral premix[1]	0.13	0.12	0.09
Vitamin premix[2]	0.13	0.12	0.09
Coccidiostátic[3]	0.06	0.06	0.06
Antioxidant[4]	0.02	0.02	0.02
Calculated composition[5]			
Met. Energy (kcal/kg)	2974	3107	3344
Crude protein (%)	23.13	19.53	17.98
Dig. Methionine+Cysteine (%)	0.60	0.52	0.48
Dig. Methionine (%)	0.31	0.27	0.25
Dig. Cysteine	0.29	0.26	0.24
Dig Lysine (%)	1.20	1.03	0.96
Dig Threonine (%)	0.86	0.75	0.71
Dig Tryptophan (%)	0.25	0.20	0.19
Dig Arginine (%)	1.41	1.20	1.14
Dig Valine (%)	1.06	0.92	0.86
Dig Isoleucine (%)	0.91	0.81	0.76
Dig Leucine (%)	1.71	1.48	1.35
Dig Histidine (%)	0.57	0.46	0.42
Dig Phenylalanine (%)	1.01	0.84	0.77
Sodium (%)	0.16	0.21	0.23
Non-phytic phosphorus (%)	0.49	0.42	0.36

[1] Composition per kg of product: manganese, 58.360 mg; zinc, 54.210 mg; iron, 41.680 mg; copper, 8.310 mg; selenium, 250 mg e iodine 843 mg.

[2] Composition per kg of product: vit. A, 9.637.000 UI; vit. D3, 2.409.000 UI; vit. E, 36.000 UI; vit. B1, 2.590 mg; vit B2, 6.450 mg; vit. B6, 3.610 mg; pantothenic acid, 12.950 mg; vit K3, 1.930 mg; Folic acid, 903 mg; niacin, 39.200 mg; vit B12, 15,9 mg; biotin, 89,8 mg

[3] Salinomycin (12%);

[4] Hydroxybutyltoluene – BHT;

[5] Calculated values were based on the ingredient's nutritional composition (Rostagno et al., 2017).

Table 2. Experimental design and supplementation levels of DL-methionine (DLM, 99% of Met) and DL-2-hydroxy-4-methylthio butanoic acid (HMTBA, 88% of Met).

Treatment	Met Source	Addition of Methionine Sources (%)[1]			Replicates	Birds
		0-14 day[*]	14-28 day[*]	28-42 day[*]		
1	Basal diet	0 (50)	0 (50)	0 (50)	7	140
2	DLM	0.040 (53)	0.040 (54)	0.040 (54)	7	140
3	DLM	0.080 (57)	0.080 (58)	0.080 (58)	7	140
4	DLM	0.130 (61)	0.120 (62)	0.120 (63)	7	140
5	DLM	0.200 (67)	0.170 (67)	0.170 (68)	7	140
6	DLM	0.300 (75)	0.250 (75)	0.240 (75)	7	140
7	DLM	0.400 (83)	0.330 (83)	0.324 (83)	7	140
8	HMTBA	0.045 (53)	0.045 (54)	0.045 (54)	7	140
9	HMTBA	0.091 (57)	0.091 (58)	0.091 (58)	7	140
10	HMTBA	0.148 (61)	0.136 (62)	0.136 (63)	7	140
11	HMTBA	0.227 (67)	0.193 (67)	0.193 (68)	7	140
12	HMTBA	0.341 (75)	0.284 (75)	0.273 (75)	7	140
13	HMTBA	0.454 (83)	0.375 (83)	0.364 (83)	7	140

[1]Addition on equimolar basis.

[*]Met+Cys:Lys ratio in percentage of diets are inside parenthesis.

Table 3a. Effect of increasing levels of Met sources on final body weight (FBW), average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of starter broilers from 1 to 14 days of age.

Met Source	SID Met+Cys:Lys (%)	FBW (g/bird)[1]	ADWG (g/bird.day^{-1})[1]	ADFI (g/bird.day^{-1})	FCR (g/g)[1]
Basal diet	50	436.91	28.08	41.34	1.47
DLM	53	483.86 *	31.43 *	44.65	1.42
DLM	57	513.46 *	33.54 *	44.94	1.34 *
DLM	61	521.31 *	34.10 *	44.25	1.30 *
DLM	67	531.13 *	34.80 *	43.56	1.25 *
DLM	75	522.29 *	34.18 *	44.30	1.30 *
DLM	83	515.34 *	33.69 *	44.10	1.31 *
HMTBA	53	492.17 *	32.02 *	43.71	1.37
HMTBA	57	505.15 *	32.95 *	45.53	1.38
HMTBA	61	516.15 *	33.74 *	45.04	1.34 *
HMTBA	67	516.64 *	33.78 *	47.70	1.41
HMTBA	75	511.84 *	33.43 *	44.68	1.34 *
HMTBA	83	516.49 *	33.76 *	44.70	1.33 *
	SEM[2]	8.12	0.578	1.81	0.043
	P-value	<0.01	<0.01	0.060	<0.01
Source effect					
DLM		514.30	33.62	44.30	1.32
HMTBA		508.49	33.28	45.23	1.36
	SEM[3]	2.31	0.232	0.435	0.012
Level effect					
	53	486.00 b	31.72 b	44.17	1.40
	57	508.83 a	33.25 a	45.24	1.36
	61	516.50 a	33.91 a	44.64	1.32
	67	523.86 a	34.29 a	45.63	1.33
	75	517.14 a	33.81 a	44.49	1.32
	83	516.05 a	33.73 a	44.40	1.32
	SEM[3]	4.00	0.406	1.03	0.022
Source of variation		*Probability*			
	source*level	0.405	0.405	0.261	0.021
	source	0.143	0.144	0.134	0.023
	level	<0.01	<0.01	0.730	0.053
Polynomial contrast					
DLM and HMTBA	linear	<0.01	<0.01	0.988	<0.01
	quadratic	<0.01	<0.01	0.252	0.137
DLM	linear	<0.01	<0.01	0.554	<0.01
	quadratic	<0.01	<0.01	0.730	<0.01
HMTBA	linear	<0.01	<0.01	0.569	0.299
	quadratic	0.044	0.044	0.054	0.365

[*] Values with an asterisk are different from the control group by Dunnett's test (P≤0,05).
[1]Values without a common letter within a column differ significantly by Tukey's test (P≤0,05).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 3b. Unfolding of the interaction (Source x Level) for feed conversion ratio (FCR) of starter broilers from 1 to 14 days of age.

Level[2]	Source[1]		*P-value*
	DLM	HMTBA	
53	1.42 a	1.37	0.191
57	1.34 ab	1.38	0.386
61	1.30 ab	1.34	0.355
67	1.25 bB	1.41 A	<0.01
75	1.30 ab	1.34	0.339
83	1.31 ab	1.33	0.698
P-value	<0.01	0.260	
SEM	0.040		

[1]Means in a row followed by uppercase letters differ significantly (P≤0.05) by F test;
[2]Means in a column followed by lowercase letters differ significantly (P≤0.05) by F test.

Table 4. Effect of increasing levels of Met sources on final body weight (FBW), average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of grower broilers from 14 to 28 days of age.

Met source	Met level (%)	FBW (g/bird)[1]		ADWG (g/bird.day^{-1})[1]		ADFI (g/bird.day^{-1})		FCR (g/g)[1]	
Basal diet	50	1503.63		76.72		126.27		1.65	
DLM	54	1630.72	*	85.80	*	130.06		1.52	*
DLM	58	1689.91	*	90.03	*	132.30	*	1.47	*
DLM	62	1698.70	*	90.66	*	130.39		1.44	*
DLM	67	1740.61	*	93.65	*	132.28	*	1.41	*
DLM	75	1765.33	*	95.41	*	133.31	*	1.40	*
DLM	83	1740.34	*	93.63	*	130.28		1.39	*
HMTBA	54	1625.56	*	85.43	*	130.60		1.53	*
HMTBA	58	1668.56	*	88.50	*	131.58	*	1.49	*
HMTBA	62	1685.11	*	89.68	*	131.75	*	1.47	*
HMTBA	67	1738.67	*	93.51	*	132.70	*	1.42	*
HMTBA	75	1717.87	*	92.02	*	130.17		1.41	*
HMTBA	83	1703.19	*	90.98	*	129.44		1.42	*
	SEM[2]	18.17		1.30		1.76		0.012	
	P-value	<0.01		<0.01		0.031		<0.01	
Source effect									
DLM		1710.50	a	91.51	a	131.41		1.44	b
HMTBA		1689.66	b	90.02	b	131.04		1.46	a
	SEM[3]	7.71		0.551		0.732		0.005	
Level effect									
	54	1627.26	c	85.56	c	130.25		1.52	d
	58	1678.47	b	89.22	b	131.86		1.48	c
	62	1691.49	b	90.15	b	131.04		1.46	b
	67	1740.29	a	93.63	a	132.59		1.42	a
	75	1741.61	a	93.73	a	131.77		1.40	a
	83	1721.37	ab	92.28	ab	129.83		1.41	a
	SEM[3]	12.99		0.953		1.26		0.008	
Source of variation		*Probability*							
	source*level	0.471		0.472		0.553		0.479	
	source	<0.01		<0.01		0.619		<0.01	
	level	<0.01		<0.01		0.254		<0.01	
Polynomial contrast									
DLM and HMTBA	linear	<0.01		<0.01		0.916		<0.01	
	quadratic	<0.01		<0.01		0.036		<0.01	
DLM	linear	<0.01		<0.01		0.528		<0.01	
	quadratic	<0.01		<0.01		0.200		<0.01	
HMTBA	linear	<0.01		<0.01		0.428		<0.01	
	quadratic	<0.01		<0.01		0.084		<0.01	

[*] Values with an asterisk are different from the control group by Dunnett's test (P≤0,05).
[1]Values without a common letter within a column differ significantly by Tukey's test (P≤0,05).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 5. Effect of increasing levels of Met sources on final body weight (FBW), average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of finisher broilers from 28 to 42 days of age.

Met source	Met level (%)	FBW (g/bird)[1]		ADWG (g/bird.day^{-1})[1]		ADFI (g/bird.day^{-1})[1]		FCR (g/g)[1]	
Basal diet	50	3123.34		113.26		209.68		1.85	
DLM	54	3267.28	*	123.54	*	212.33		1.72	*
DLM	58	3324.21	*	127.61	*	209.95		1.65	*
DLM	63	3319.52	*	127.27	*	207.88		1.64	*
DLM	68	3362.96	*	130.38	*	208.01		1.60	*
DLM	75	3352.21	*	129.61	*	205.74		1.59	*
DLM	83	3361.47	*	130.27	*	205.90		1.58	*
HMTBA	54	3280.99	*	124.52	*	216.61	*	1.74	*
HMTBA	58	3329.66	*	128.00	*	212.08		1.66	*
HMTBA	63	3327.93	*	127.87	*	209.13		1.64	*
HMTBA	68	3340.38	*	128.76	*	206.98		1.61	*
HMTBA	75	3328.04	*	127.88	*	208.86		1.63	*
HMTBA	83	3346.04	*	129.17	*	206.63		1.60	*
	SEM[2]	34.44		2.46		2.93		0.020	
	P-value	<0.01		<0.01		0.023		<0.01	
Source effect									
DLM		3330.69		128.05		208.25		1.63	b
HMTBA		3326.11		127.73		210.11		1.65	a
	SEM[3]	14.41		1.03		1.19		0.008	
Level effect									
	54	3273.53	b	123.97	b	214.42	a	1.7289	a
	58	3326.89	ab	127.78	ab	211.01	ab	1.651	b
	63	3324.71	ab	127.63	ab	208.60	ab	1.6362	bc
	68	3351.89	a	129.57	a	207.52	b	1.6041	cd
	75	3339.73	ab	128.70	ab	207.27	b	1.6121	cd
	83	3353.65	a	129.69	a	206.26	b	1.5926	d
	SEM[3]	24.43		1.75		1.98		0.013	
Source of variation		*Probability*							
	source*level	0.943		0.943		0.842		0.624	
	source	0.752		0.752		0.125		0.030	
	level	0.022		0.022		<0.01		<0.01	
Polynomial contrast									
DLM and HMTBA	linear	<0.01		<0.01		<0.01		<0.01	
	quadratic	0.142		0.142		0.123		<0.01	
DLM	linear	<0.01		<0.01		0.012		<0.01	
	quadratic	0.246		0.246		0.544		<0.01	
HMTBA	linear	0.105		0.105		<0.01		<0.01	
	quadratic	0.352		0.352		0.109		<0.01	

[*] Values with an asterisk are different from the control group by Dunnett's test ($P \leq 0.05$).
[1]Values without a common letter within a column differ significantly by Tukey's test ($P \leq 0.05$).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 6. Levels of Met sources on protein deposition (DP), fat deposition (DF), and ash deposition (DA) of starter, grower, and finisher broilers.

Met source	SID Met+Cys:Lys (%) starter/grower/finisher	Starter DP (g)[1]	Starter DF (g)[1]	Starter DA (g)[1]	Grower DP (g)[1]	Grower DF (g)[1]	Grower DA (g)[1]	Finisher DP (g)[1]	Finisher DF (g)[1]	Finisher DA (g)[1]
Basal diet	50/ 50/ 50	57.63	30.69	13.07	149.15	163.13	25.47	225.92	286.45	37.94
DLM	53/ 54/ 54	69.80 *	37.73	14.56	168.08 *	159.91	28.34	226.14	313.53	40.71
DLM	57/ 58/ 58	75.61 *	33.66	15.51 *	183.02 *	148.24	28.18	257.38 *	263.33	34.04
DLM	61/ 62/ 63	78.49 *	42.01	16.23 *	193.26 *	154.80	33.45 *	240.50	241.52	39.48
DLM	67/ 67/ 68	76.74 *	33.77	14.35	193.25 *	136.69	33.78 *	238.05	257.55	38.70
DLM	75/ 75/ 75	77.11 *	38.83	14.33	199.87 *	143.66	31.93 *	242.02	238.51	44.56
DLM	83/ 83/ 83	71.94 *	37.04	14.45	206.88 *	139.21	30.50 *	258.29 *	243.62	37.20
HMTBA	53/ 54/ 54	67.83 *	39.82	14.43	166.43 *	155.42	27.28	239.07	303.46	38.77
HMTBA	57/ 58/ 58	69.54 *	39.47	13.94	179.77 *	147.83	31.35 *	247.94	257.28	45.13
HMTBA	61/ 62/ 63	71.76 *	35.76	14.58	186.68 *	143.13	35.16 *	240.08	213.70 *	46.14
HMTBA	67/ 67/ 68	75.55 *	40.42	15.39 *	195.28 *	146.60	31.62 *	262.24 *	252.87	44.02
HMTBA	75/ 75/ 75	70.74 *	39.22	14.04	209.27 *	153.18	30.86 *	222.25	210.09 *	36.38
HMTBA	83/ 83/ 83	71.62 *	38.32	12.45	199.39 *	145.80	31.67 *	254.75 *	261.82	40.74
	SEM[2]	1.81	2.38	0.68	8.48	13.62	2.40	13.07	29.50	4.91
	P-value	<0.01	0.08	0.02	<0.01	0.6	<0.01	0.030	<0.01	0.3
Source effect										
DLM		74.93 a	37.16	14.9 a	190.70	146.92	31.03	243.73	260.12	39.08
HMTBA		71.18 b	38.84	14.14 b	189.51	148.74	31.33	244.28	250	41.89
	SEM[3]	1.08	1.46	0.38	2.92	4.58	0.86	5.37	11.53	2
Level effect										
	53/ 54/ 54	68.80 c	38.76	14.50	167.13 e	157.20	27.8 d	232.61 cd	308.98 a	39.7
	57/ 58/ 58	72.58 ab	36.57	14.72	181.36 d	147.84	29.77 cd	252.60 ab	260.56 b	39.59
	61/ 62/ 63	75.08 ab	38.86	15.41	189.97 cd	148.91	34.31 a	240.14 abcd	227.63 b	42.86
	67/ 67/ 68	76.15 a	37.10	14.87	194.39 bc	142.00	32.72 ab	250.05 abc	255.38 b	41.38
	75/ 75/ 75	73.91 ab	39.01	14.18	204.61 a	148.49	31.4 bc	232.13 d	224.73 b	40.43
	83/ 83/ 83	71.81 bc	37.71	14.44	203.15 ab	142.56	31.1 bc	256.48 a	253.07 b	38.96
	SEM[3]	1.89	2.49	0.66	4.99	8.24	1.47	8.98	19.26	3.33
Source of variation		*Probability*								
	source*level	0.34	0.16	0.17	0.56	0.72	0.44	0.21	0.84	0.08
	source	<0.01	0.26	0.05	0.69	0.69	0.73	0.92	0.38	0.16
	level	<0.01	0.89	0.08	<0.01	0.52	<0.01	0.03	<0.01	0.88
Polynomial contrast										
DLM and HMTBA	linear	0.07	0.98	0.06	<0.01	0.12	0.04	0.21	<0.01	0.89
	quadratic	<0.01	0.86	0.02	0.04	0.46	<0.01	0.99	<0.01	0.29
DLM	linear	0.38	0.85	0.27	<0.01	0.09	0.12	0.15	0.02	0.64
	quadratic	<0.01	0.95	0.24	0.28	0.66	0.02	0.91	0.11	0.95
HMTBA	linear	0.09	0.87	0.11	<0.01	0.67	0.15	0.75	0.05	0.50
	quadratic	0.06	0.76	0.03	0.04	0.53	<0.01	0.91	<0.01	0.15

* Values with an asterisk are different from the control group by Dunnett's test ($P \leq 0.05$).

[1]Values without a common letter within a column differ significantly by Tukey's test ($P \leq 0.05$).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 7a. Effect of increasing levels of Met sources on carcass weight (CW), carcass yield (CY), viscerossomatic index (VI), breast meat yield (B), drumstick tield (D) and thighs yield (T) of broilers from 14 to 28 days of age.

Met source	Met level (%)	CW (g)[1]	CY (%)[1]	VI (%)[1]	B (%)[1]	D (%)[1]	T (%)[1]
Basal diet	50	1341.63	80.24	11.74	27.71	13.27	12.54
DLM	54	1472.12 *	80.53	11.63	28.25	14.33 *	12.63
DLM	58	1537.31 *	81.83 *	12.38	29.81 *	13.01	11.74 *
DLM	62	1548.76 *	81.60 *	11.08	30.05 *	13.30	11.90 *
DLM	67	1551.67 *	81.44 *	11.06	31.37 *	13.24	11.57 *
DLM	75	1564.64 *	81.87 *	10.30 *	33.40 *	13.11	11.54 *
DLM	83	1558.14 *	82.09 *	10.50 *	33.16 *	12.83	11.29 *
HMTBA	54	1444.57 *	80.74	12.44	27.61	13.85	12.04
HMTBA	58	1495.55 *	82.55 *	10.65	29.23 *	13.72	12.11
HMTBA	62	1537.67 *	81.75 *	11.77	30.68 *	13.25	11.61 *
HMTBA	67	1548.55 *	81.98 *	10.73	29.91 *	13.36	11.49 *
HMTBA	75	1584.62 *	83.79 *	11.16	30.13 *	13.37	11.34 *
HMTBA	83	1529.04 *	81.32 *	11.48	33.20 *	12.86	11.29 *
	SEM[2]	20.76	0.55	0.61	0.76	0.38	0.29
	P-value	<0.01	<0.01	0.02	<0.01	0.02	<0.01
Source effect							
DLM		1538.58	81.56	11.16	31.01	13.31	11.77
HMTBA		1523.19	82.03	11.37	30.12	13.40	11.65
	SEM[3]	9.11	0.240	0.270	0.33	0.16	0.96
Level effect							
	54	1458.10 d	80.64 d	12.04	27.95	14.09 a	12.33 a
	58	1516.17 c	82.19 abc	11.52	29.54	13.36 b	11.92 ab
	62	1543.05 bc	81.68 c	11.42	30.36	13.28 b	11.75 b
	67	1550.10 ab	81.71 c	10.88	30.61	13.31 b	11.54 b
	75	1574.53 a	82.83 ab	10.73	31.74	13.24 b	11.44 c
	83	1543.39 abc	81.71 c	10.99	33.18	12.85 b	11.29 d
	SEM[3]	15.42	0.43	0.47	0.54	0.28	0.23
Source of variation				*Probability*			
	source*level	0.45	0.07	0.03	0.03	0.43	0.43
	source	0.10	0.06	0.44	<0.01	0.54	0.34
	level	<0.01	<0.01	0.070	<0.01	<0.01	<0.01
Polynomial contrast							
DLM and HMTBA	linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	quadratic	<0.01	0.010	0.220	0.890	0.34	0.3
DLM	linear	<0.01	0.030	<0.01	<0.01	<0.01	<0.01
	quadratic	0.020	0.470	0.920	0.750	0.17	0.25
HMTBA	linear	<0.01	0.050	0.270	<0.01	<0.01	<0.01
	quadratic	<0.01	<0.01	0.070	0.610	0.99	0.73

[*] Values with an asterisk are different from the control group by Dunnett's test (P≤0,05).
[1]Values without a common letter within a column differ significantly by Tukey's test (P≤0,05).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 7b. Unfolding of the interaction (Source x Level) for viscerossomatic index (VI) of broilers from 14 to 28 days of age.

Level[2]	Source[1]		*P-value*
	DLM	HMTBA	
54	11.63 ab	12.44	0.220
58	12.38 aA	10.65 B	<0.01
62	11.08 b	11.77	0.300
67	11.06 bc	10.73	0.620
75	10.30 c	11.16	0.210
83	10.50 bc	11.48	0.140
P-value	0.04	0.070	
SEM	0.660		

[1]Means in a row followed by uppercase letters differ significantly (P≤0.05) by F test;
[2]Means in a column followed by lowercase letters differ significantly (P≤0.05) by F test.

Table 7c. Unfolding of the interaction (Source x Level) for breast meat yield (B) of broilers from 14 to 28 days of age.

Level[2]	Source[1]		*P-value*
	DLM	HMTBA	
54	28.25 c	27.61 c	0.420
58	29.81 c	29.23 b	0.480
62	30.05 bc	30.68 b	0.470
67	31.37 bc	29.91 b	0.060
75	33.40 aA	30.13 bB	<0.01
83	33.16 a	33.20 a	0.980
P-value	<0.01	<0.01	
SEM	0.800		

[1]Means in a row followed by uppercase letters differ significantly (P≤0.05) by F test;
[2]Means in a column followed by lowercase letters differ significantly (P≤0.05) by F test.

Table 8a. Effect of increasing levels of Met sources on carcass weight (CW), carcass yield (CY), viscerossomatic index (VI), breast meat yield (B), drumstick yield (D) and thighs yield (T) of broilers from 28 to 42 days of age.

Met source	Met level (%)	CW (g)[1]	CY (%)[1]	VI (%)[1]	B (%)[1]	D (%)[1]	T (%)[1]
Basal diet	50	2814.07	82.58	9.88	32.48	13.26	11.90
DLM	54	2910.86	83.17	9.24	33.89	13.20	11.95
DLM	58	3005.31 *	84.15 *	8.67 *	34.85 *	13.28	11.42
DLM	63	3006.65 *	83.52	9.11 *	34.37	12.93	11.58
DLM	68	3030.39 *	84.02 *	8.71 *	34.97 *	13.46	11.15 *
DLM	75	3030.09 *	83.78 *	8.60 *	35.22 *	13.31	11.09 *
DLM	83	3019.83 *	83.46	9.01 *	34.69	13.47	11.42
HMTBA	54	2900.24	83.92 *	8.56 *	32.82	13.71	11.90
HMTBA	58	2985.15 *	83.60	8.85 *	34.66	13.28	11.50
HMTBA	63	3004.4 *	84.13 *	8.42 *	34.23	12.84	11.71
HMTBA	68	2987.98 *	83.55	8.81 *	35.13 *	13.09	11.56
HMTBA	75	3026.12 *	83.54	8.78 *	36.36 *	12.83	11.28
HMTBA	83	2993.19 *	83.19	8.38 *	36.67 *	13.07	11.21
	SEM[2]	34.92	0.39	0.27	0.80	0.269	0.25
	P-value	<0.01	<0.01	<0.01	<0.01	0.1	<0.01
Source effect							
DLM		3000.36	83.68	8.89	34.67	13.28	11.43
HMTBA		2982.85	83.66	8.63	34.99	13.14	11.53
	SEM[3]	14.42	0.27	0.110	0.33	0.11	0.1
Level effect							
	54	2905.38 b	83.54	8.90	33.36 d	13.46	11.92 a
	58	2995.08 a	83.87	8.76	34.76 c	13.28	11.46 bc
	63	3005.62 a	83.84	8.76	34.31 bc	12.89	11.64 ab
	68	3009.20 a	83.79	8.76	35.06 abc	13.28	11.35 abc
	75	3027.94 a	83.66	8.69	35.79 ac	13.07	11.19 c
	83	3006.40 a	83.33	8.69	35.69 ac	13.27	11.31 abc
	SEM[3]	24.70	0.27	0.19	0.56	0.19	0.18
Source of variation		*Probability*					
	source*level	0.96	0.06	0.02	0.11	0.12	0.6
	source	0.23	0.93	0.03	0.33	0.22	0.37
	level	<0.01	0.30	0.90	<0.01	0.07	<0.01
Polynomial contrast							
DLM and HMTBA	linear	<0.01	0.270	0.270	<0.01	0.3	<0.01
	quadratic	<0.01	0.040	0.710	0.440	0.04	0.18
DLM	linear	<0.01	0.680	0.250	0.220	0.22	<0.01
	quadratic	0.020	0.040	0.120	0.370	0.48	0.03
HMTBA	linear	<0.01	0.060	0.660	<0.01	0.01	<0.01
	quadratic	0.030	0.330	0.310	0.840	0.02	0.85

[*] Values with an asterisk are different from the control group by Dunnett's test ($P \leq 0.05$).
[1]Values without a common letter within a column differ significantly by Tukey's test ($P \leq 0.05$).
[2]Standard error mean (n=91, df=90).
[3]Standard error mean (n=84. df=83).

Table 8b. Unfolding of the interaction (Source x Level) for viscerossomatic index (VI) of broilers from 28 to 42 days of age.

Level[2]	Source[1]		*P-value*
	DLM	HMTBA	
54	9.24 A	8.56 B	0.020
58	8.67	8.85	0.510
63	9.11 A	8.42 B	0.010
68	8.71	8.81	0.710
75	8.60	8.78	0.490
83	9.01 A	8.38 B	0.020
P-value	0.09	0.330	
SEM	0.270		

[1]Means in a row followed by uppercase letters differ significantly (P≤0.05) by F test;
[2]Means in a column followed by lowercase letters differ significantly (P≤0.05) by F test.

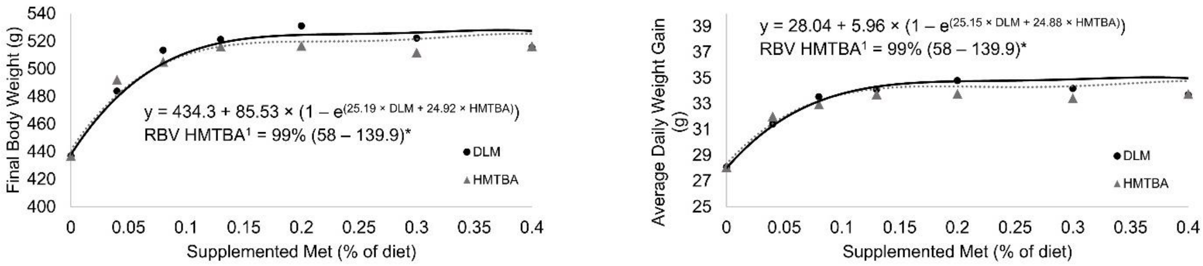

Figure 3. Final Body Weight and Average Daily Weight Gain of broilers in starter phase fed increasing levels each of DL-Met (99% DLM) and Methionine Hidroxy-Analogue (88%, liquid HMTBA). [1] RBV = Relative bioavailability value, determined as ratio of steepness value for HMTBA to DLM. [*] Confidence interval at $P<0.05$ inside the parentheses.

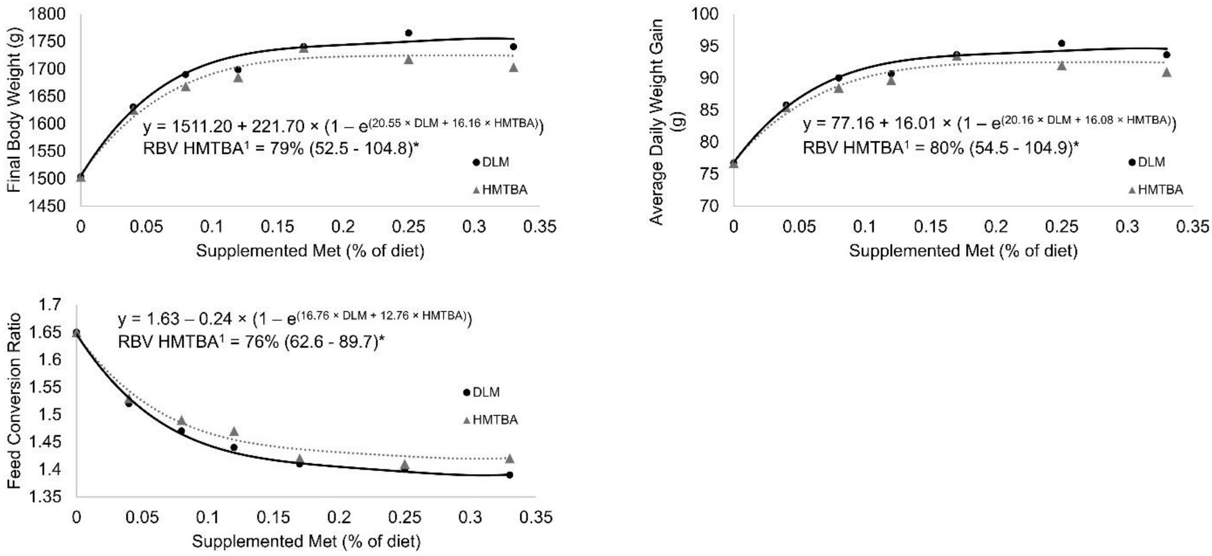

Figure 2. Final Body Weight, Average Daily Weight Gain, Feed Conversion Ratio, Carcass Weight, Carcass Yield, Breast Meat Yield and Thighs Yield of broilers in grower phase fed increasing levels each of DL-Met (99% DLM) and Methionine Hidroxy-Analogue (88%, liquid HMTBA). [1] RBV = Relative bioavailability value, determined as ratio of steepness value for HMTBA to DLM. [*] Confidence interval at P<0.05 inside the parentheses.

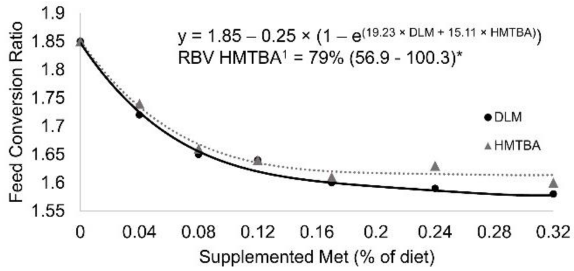

Figure 3. Feed Conversion Ratio of broilers in finisher phase fed increasing levels each of DL-Met (99% DLM) and Methionine Hidroxy-Analogue (88%, liquid HMTBA). [1] RBV = Relative bioavailability value, determined as ratio of steepness value for HMTBA to DLM. [*] Confidence interval at P<0.05 inside the parentheses.

PRACTICAL IMPLICATIONS

Dual energy X-Ray absorptiometry (DEXA)

DEXA scanning can bring the birds lean mass (protein and water), bone content (mineral), and fat content in minutes. However, as DEXA was firstly developed for human body, the calibration of these results for birds is essential. It is possible to obtain these calibrated body composition measurements by using proper equations, as those presented in the Chapter I. Besides the less time consumed to analyze the body composition, the usage of DEXA to estimate the body composition of broilers present several advantages compared to chemical analysis, notably, the possibility of analysis in live animals. DEXA permits repeated measurements on the same bird throughout its growth, enabling a more accurate monitoring of the intrinsic characteristics of that individual helping breeders to take appropriate actions.

Optimal level of Met+Cys:Lys

Methionine (Met) is a crucial sulfur amino acid for poultry survival, growth, and reproduction, with its metabolism extending beyond protein synthesis. Broiler requirements for both Met and Cys are typically considered together when designing feeding programs due to the irreversibly conversion of Met into Cys. Since natural feed ingredients often lack sufficient Met to meet sulfur amino acid needs, synthetic Met sources are required in feeds. Decisions on Met+Cys concentrations are typically based on economic factors, but without accurate knowledge of how broilers respond to different levels of these amino acids, optimal growth and feed efficiency may be compromised, impacting profitability in intensive broiler production.

In results of Chapter II, it is possible to observe that as birds grow and develop, the SID Met+Cys:Lys requirement (optimal level) increases at certain point, and then slightly decreases. The optimal levels for starter broilers were 58% for Final Body Weight and Daily Weight Gain, and 71% for Feed Conversion Ratio. For grower broilers, they were 76% for Final Body Weight and Daily Weight Gain, and 81% for Feed Conversion Ratio. For finisher phase, they were 77% for Feed Conversion Ratio. In summary, considering the average of performance traits we have a Met+Cys:Lys requirement of 65% for starter, 79% for grower, and 77% for finisher phases.

Relative bioavailability value of methionine hydroxy analogue

Methionine hydroxy analogue (MHA, 88% purity) is one of the sources of Met included in poultry diets to fulfill the sulfur amino acid requirements of broilers. Another source conventionally used is DL-methionine (DLM, 99% purity). As both methionine sources are suitable for supporting broiler growth, the nutritionist's decision should be based on the economic value of using one source over the other. Furthermore, due to their inherent differences, the formulator must pay attention to the equivalence and matrix of the sources and avoid misconceptions regarding the use of MHA. The relative bioavailability value is an important indicator of equivalence, and is presented in equimolar or product basis.

In Chapter III, it is possible to observe that RBV value is different in starter phase, compared to those found in grower and finisher phases. In equimolar basis, the MHA RBV for starter phase is 99%, for grower phase is 78% and for finisher phase is 79%. To convert these values into product basis, we simply multiply the RBV by 88%, which is the purity of MHA. Therefore, in product basis the RBV for starter is 87%, for grower is 69% and for finisher is 70%. Taking the average of RBV from all growing phases we found an overall RBV of 85% in equimolar basis, or 75% in product basis. In practical terms, an equivalent replacement of DLM by MHA in starter phase is 100:87 (1.15 times higher amount of MHA inclusion), in grower phase is 100:69 (1.45 times higher amount of MHA inclusion) and in finisher phase is 100:70 (1.43 times higher amount of MHA inclusion).