R. Bras. Zootec., 53:e20240056, 2024
https://doi.org/10.37496/rbz5320240056

**Non-ruminants**
Full-length research article

Revista Brasileira de Zootecnia

*Brazilian Journal of Animal Science*
e-ISSN 1806-9290
www.rbz.org.br

# Metabolizable energy levels and L-arginine supplementation in diets for broilers under heat stress from 29 to 42 days of age

**David Henrique de Oliveira**[1], **Fábio Loures Cruz**[1], **Sérgio Turra Sobrane Filho**[1], **Breno Resende Alves**[1], **Márvio Lobão Teixeira de Abreu**[1], **Luciana de Paula Naves**[1], **Paulo Borges Rodrigues**[1]*

[1] Universidade Federal de Lavras, Faculdade de Zootecnia e Medicina Veterinária, Departamento de Zootecnia, Lavras, MG, Brasil.

***Corresponding author:**
pborges@ufla.br

**Received:** May 15, 2024
**Accepted:** August 26, 2024

**How to cite:** Oliveira, D. H.; Cruz, F. L.; Sobrane Filho, S. T.; Alves, B. R.; Abreu, M. L. T.; Naves, L. P. and Rodrigues, P. B. 2024. Metabolizable energy levels and L-arginine supplementation in diets for broilers under heat stress from 29 to 42 days of age. Revista Brasileira de Zootecnia 53:e20240056.
https://doi.org/10.37496/rbz5320240056

**Editors:**
Ines Andretta
Vincenzo Tufarelli



**ABSTRACT** - The objective of this study was to evaluate the L-arginine supplemental effects for broilers in the final rearing phase (29 and 42 days), during which they were exposed to heat stress and fed diets with different metabolizable energy levels. Performance, carcass parameters, and physiological and biochemical parameters related to lipid metabolism were evaluated. A completely randomized design was used, in a 3 × 2 factorial arrangement, corresponding to three metabolizable energy levels (3000, 3150, and 3300 kcal/kg) in diets supplemented or not with L-arginine (0.66%). Eight replicates composed by eight broilers each (experimental units; boxes) per treatment were evaluated, totaling 48 experimental units. Performance was evaluated in the period from 29 to 42 days old, and carcass and blood parameters were measured at 42 days old. L-arginine supplementation improved feed conversion and blood profile, mainly due to the reduction of total and LDL-cholesterol levels. Broilers that received 3300 kcal/kg of metabolizable energy showed the greatest weight gain, regardless of supplementation or not with arginine. The dietary supplementation with 0.66% of L-arginine for heat-stressed broilers can be recommended, regardless of the feed energy level.

**Keywords:** blood parameters, carcass yield, energy conversion, functional amino acid, performance

## 1. Introduction

The modern broiler production systems are highly productive, with high carcass and cuts yield. However, broilers are highly susceptible to climatic variations, especially to high temperatures, affecting performance and lean tissue deposition in the carcass. Moreover, many production units are located in tropical regions, subject to high temperatures, as well as issues related to global warming. This led nutritionists to look for options to minimize the heat stress effects on the production system.

One of the options considered is supplementing the diet with functional amino acids, mainly arginine, above recommended levels. Broilers are unable to synthesize arginine via the *de novo* pathway due to the lack or low enzymes activity such as carbamoyl phosphate synthase I, hepatic arginase, and ornithine carbamoyltransferase in the urea cycle (Fouad et al., 2013). Thus, broilers are highly dependent on dietary arginine supply for protein synthesis and other physiological processes essential to growth and development (Khajali and Wideman, 2010).

Under heat stress, the morphology of the small intestine may change, such as a reduction in villus height (Chamruspollert et al., 2004), which may be responsible for the reduction in the nutrient absorption, especially amino acids (Balnave and Brake, 2002). Thus, heat stress possibly increases the need for arginine, and its supplementation above recommended levels can alter the physiology and metabolism of broilers in the form of increasing protein synthesis in muscle tissue and altering lipid metabolism.

According to Filho et al. (2021), arginine can reduce lipogenesis and increase the lean tissue deposition in the carcass. It is not known whether these effects occur under heat stress conditions, and reports in the literature evaluating this nutritional strategy in these conditions are scarce. In addition, no current studies have reported possible interaction between metabolizable energy levels and supplemental arginine in broiler diets.

Thus, it is necessary investigate the effects of L-arginine supplementation for broilers under heat stress on performance and carcass traits, especially with regard to fat deposition and carcass yield. The present study aimed at evaluating the effects of L-arginine supplementation in broilers under heat stress fed different metabolizable energy levels on the performance, carcass, physiological and biochemical parameters related to lipid metabolism.

## 2. Material and Methods

The present research was carried out according to the institutional committee on animal use (protocol number 059/16) and was conducted in Lavras, South region of the Minas Gerais state, Brazil (920 m altitude, 21°14'45" South latitude, 44°59'59" West longitude of Greenwich).

Three hundred eighty-four male Cobb500 broilers, at 29 days old at the start of the experiment, were used. During the pre-experimental period (1 to 28 days of age), the broilers were reared in a masonry shed, with standard *ad libitum* feeding, following the nutritional requirements described in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011) and in a controlled thermal environment to offer the best welfare conditions for the broilers during this period. On day 29, the broilers were weighed individually, homogenized by weight range, and transferred to an experimental shed where they were distributed in 48 boxes (experimental units) with dimensions of 1.5 × 2.0 m.

A completely randomized design was used, in a 3 × 2 factorial arrangement, corresponding to three metabolizable energy levels (3000, 3150, and 3300 kcal/kg) in diets supplemented or not with L-arginine (0.66%) (Table 1). This level of supplemental L-arginine was previously determined by Filho et al. (2021). Eight replicates composed of eight broilers each (experimental units; boxes) per treatment were evaluated, totalling 48 experimental units. Except for the supplementation or not with L-arginine and the energy levels of the diets, the experimental diets were isonutrient.

Heat stress was induced every day of the experimental period (29 to 42 days of age), between 10:00 and 18:00 h by keeping the curtains and heaters present in each experimental unit respectively closed and lit. The mean temperature to which the animals were subjected during the period of induction of heat stress was 35±1 °C. Temperature monitoring was performed with the aid of four thermometers located at the four ends of the shed. Temperature was checked and recorded every 30 minutes during the heat stress period. The mean relative humidity in the shed throughout the experimental period was 64.8±5.1%. During the experimental period, feed and water were provided *ad libitum* in tube feeders and pendulum drinkers, respectively, and the feed was weighed before the beginning and at the end of the experiment to calculate the feed intake.

At 42 days of age, the broilers of each experimental unit were weighed to evaluate weight gain, as was the feed surplus in the feeders of each experimental unit, to calculate feed intake and feed conversion ratio. Feed intake, weight gain, and feed conversion ratio were evaluated from 29 to 42 days of age of the broilers according to the methodology of Del Castilho et al. (2013). Total energy intake, daily energy intake, and energy conversion were calculated. The energy intake per broiler was obtained by multiplying the feed intake per bird during the experimental period (29 to 42 days) by the metabolizable energy level of the diet. The daily energy intake per broiler was the relationship between the energy intake per bird throughout the experimental period and the number of days in this period (13 days). Finally, energy conversion was calculated as the daily energy intake per bird multiplied by the daily weight gain per broiler.

**Table 1** - Composition and calculated nutritional values of experimental diets for broilers from 29 to 42 days of age

| Ingredient (%) | Arginine | | | | | |
|---|---|---|---|---|---|---|
| | Without | | | With | | |
| | Metabolizable energy (kcal/kg) | | | | | |
| | 3000 | 3150 | 3300 | 3000 | 3150 | 3300 |
| Corn | 58.75 | 58.75 | 58.75 | 58.75 | 58.75 | 58.75 |
| Soybean meal, 46% | 29.70 | 29.70 | 29.70 | 29.70 | 29.70 | 29.70 |
| Inert | 4.72 | 3.01 | 1.30 | 4.06 | 2.35 | 0.64 |
| Soybean oil | 3.59 | 5.30 | 7.01 | 3.59 | 5.30 | 7.01 |
| Dicalcium phosphate | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| Calcitic limestone | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| L-Arginine | - | - | - | 0.66 | 0.66 | 0.66 |
| Common salt | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 |
| DL-Methionine | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 |
| L-Lysine HCl | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| L-Threonine | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 | 0.07 |
| L-Valine | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Choline chloride | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Salinomycin | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Mineral supplement[1] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin supplement[2] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 |
| Calculated nutritional values | | | | | | |
| Metabolizable energy (kcal/kg) | 2998 | 3149 | 3299 | 2998 | 3149 | 3299 |
| Crude protein (%) | 18.53 | 18.53 | 18.53 | 18.53 | 18.53 | 18.53 |
| Calcium (%) | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | 0.68 |
| Crude fiber (%) | 3.08 | 3.08 | 3.08 | 3.08 | 3.08 | 3.08 |
| Available phosphorus (%) | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| Sodium (%) | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Digestible arginine (%) | 1.15 | 1.15 | 1.15 | 1.81 | 1.81 | 1.81 |
| Digestible phenylalanine + tyrosine (%) | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 |
| Total glycine + serine (%) | 1.55 | 1.55 | 1.55 | 1.55 | 1.55 | 1.55 |
| Digestible histidine (%) | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 | 0.46 |
| Digestible isoleucine (%) | 0.72 | 0.72 | 0.72 | 0.72 | 0.72 | 0.72 |
| Digestible leucine (%) | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 |
| Digestible lysine (%) | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Digestible methionine + cystine (%) | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Digestible threonine (%) | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 | 0.70 |
| Digestible tryptophan (%) | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Digestible valine (%) | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| Arginine:lysine ratio | 1.07 | 1.07 | 1.07 | 1.69 | 1.69 | 1.69 |

[1] Supplemented per kilogram of feed: Zn, 55 mg; Se, 0.18 mg; I, 0.70 mg; Cu, 10 mg; Mn, 78 mg; Fe, 48 mg.
[2] Supplemented per kilogram of feed: folic acid, 0.48 mg; pantothenic acid, 8.70 mg; biotin, 0.018 mg; butylhydroxytoluene (BHT), 1.5 mg; niacin, 11.1 mg; vitamin A, 6000 IU; vitamin B1, 0.8 mg; vitamin E, 12.15 IU; vitamin B12, 8.10 µg; vitamin B2, 3.6 mg; vitamin B6, 1.80 mg; vitamin D3, 1500 IU; vitamin K3, 1.44 mg.

At 42 days of age, three broilers from each experimental unit were selected (average weight ± 5%), subjected to a preslaughter fast for 12 h, and then slaughtered, according to the methodology of Mendonça et al. (2020) and Filho et al. (2021). Blood was collected (5 mL), and samples were placed in tubes without anticoagulant. The blood samples were centrifuged at 2,000 × $g$ for 6 min at room temperature, and serum was stored at −20 °C for biochemical analyses related to lipid metabolism. Thus, serum levels of total cholesterol, total triglycerides, high-density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (LDL-cholesterol) were determined by colorimetric principles (NUNC F spectrophotometer, Thermo Fischer Scientific Inc., Kamstrup, Denmark) using commercial kits (Labtest®, Lagoa Santa, Brazil). Serum level of very low-density lipoprotein cholesterol (VLDL-cholesterol) was calculated as the difference between total cholesterol and HDL-cholesterol plus LDL-cholesterol. Serum levels of glucose and urea were determined with an Automatic Biochemical Analyser CR-200 using specific commercial kits (Labtest®, Lagoa Santa, Brazil).

Blood collection during the bleeding stage was followed by scalding, plucking, evisceration, and cleaning of the carcass. The carcasses were individually packed in properly identified plastic bags, according to the experimental units and refrigerated at 5 °C in a cold chamber for 24 h for carcass evaluations. During evisceration, edible viscera (heart, liver and gizzard) and abdominal fat (fat from the retroperitoneal region) were collected.

After the cooling period, the carcasses were weighed. The carcass was defined as the body with the neck but without head, feet, abdominal fat, or edible offal. Next, the cuts were divided, separating the breast and thigh with drumstick from the remainder of the carcass. Carcass yield, the yield of the breast and thigh cuts with drumstick, yield of edible offal (liver and heart), and yield of abdominal fat were evaluated. To obtain the carcass yield, slaughter weight and carcass weight were calculated as the average body and carcass weights of the three broilers/box, respectively. Thus, carcass yield was calculated as the ratio between carcass weight and weight at slaughter, and this ratio was multiplied by 100 to obtain the percentage. The yield of the cuts (breast and thigh with drumstick) was the ratio between the weight of a particular cut, corresponding to the average weight of the cut from the three broilers of each experimental unit, and the carcass weight. The yield of edible viscera (liver and heart) and yield of abdominal fat were calculated as the ratio between the weight of the given component, corresponding to the average weight of the component from the three broilers of each plot, and the weight at slaughter. As for the carcass yield, these relationships were multiplied by 100 to obtain the percentage yield data.

The statistical model used was:

$$Y_{ikl} = \mu + A_i + E_k + AE_{ik} + e_{ikl},$$

in which $Y_{ikl}$ = represents the observation on experimental unit *i*, supplemented or not with L-arginine ($A_i$) and metabolizable energy level *k* ($E_k$), on repetition *l*; $\mu$ = the overall mean; $A_i$ = L-arginine supplementation effect (*i* = with or without); $E_k$ = metabolizable energy effect (*k* = 1, 2, 3); $AE_{ik}$ = interaction of the L-arginine supplementation *i* and the metabolizable energy level *k*; and $e_{ikl}$ = the residues corresponding to each observation, which, by hypothesis, has a normal distribution with zero mean and sigma squared variance.

The data were analyzed using the Bartlett and Shapiro–Wilk tests at a 5% significance level to evaluate the assumptions of the analysis of variance (ANOVA) (homogeneity and normality, respectively). If one of the assumptions was not met, the data were transformed using logarithmically for statistical analysis. If both assumptions were met, the data were analyzed by ANOVA using R software, version 3.2.5 (R Core Team, 2017). The variables with responses with significant effects in the analysis of variance for the treatments and/or interactions were subjected to the Scott–Knott test of means at the 5% probability level.

## 3. Results

There was interaction between diet and metabolizable energy level ($P<0.05$) for feed conversion and energy conversion (Table 2). In broilers fed the control diet, the metabolizable energy level of 3300 kcal/kg provided better feed conversion. Similar results were observed for broilers that received the diet with L-arginine, and the levels of 3150 and 3300 kcal/kg provided better feed conversion indices (Table 3). Considering the evaluated metabolizable energy levels, there was effect of the arginine supplementation only when the broilers received the feed formulated with 3150 kcal/kg, so that the arginine supplementation resulted in best feed conversion (Table 3). In terms of energy

**Table 2** - Performance of broilers from 29 to 42 days of age subjected to heat stress, as a function of diet and level of metabolizable energy

| Variable | Diet | | Metabolizable energy (kcal/kg; E) | | | P-value[1] | | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Arginine | 3000 | 3150 | 3300 | D | E | D × E | |
| WG | 1.26 | 1.28 | 1.23b | 1.26b | 1.31a | 0.133 | 0.003 | 0.322 | 0.010 |
| DWG | 104.6 | 106.8 | 102.5b | 105.4b | 109.2a | 0.141 | 0.003 | 0.351 | 0.847 |
| FI | 2.11 | 2.08 | 2.11 | 2.11 | 2.05 | 0.384 | 0.129 | 0.659 | 0.014 |
| FC | 1.68A | 1.63B | 1.72a | 1.67a | 1.57b | 0.011 | 0.001 | 0.034 | 0.014 |
| EI | 6625.2 | 6550.0 | 6334.4b | 6655.3a | 6773.1a | 0.379 | 0.001 | 0.676 | 48.894 |
| DEI | 552.1 | 545.8 | 527.9b | 554.6a | 564.4a | 0.379 | 0.001 | 0.676 | 4.075 |
| CON | 5.28A | 5.12B | 5.15 | 5.27 | 5.17 | 0.001 | 0.223 | 0.031 | 0.034 |

WG - weight gain (kg/bird); DWG - daily weight gain (g/bird/day); FI - feed intake (kg/bird); FC - feed conversion (kg/kg); EI - energy intake; DEI - daily energy intake; CON - energy conversion; SEM - standard error of the mean.
[1] Scott–Knott test at 5% probability.
Means followed by different letters indicate difference between diets (A,B - Fischer's test) and between levels of metabolizable energy (a,b - Skott-Knott's test).

conversion, the metabolizable energy level of 3150 kcal/kg resulted in the highest value considering the control diet, while the 3000 and 3300 kcal/kg energy levels were similar to each other. Reinforcing this result, for the broilers fed 3150 kcal/kg of metabolizable energy, the control diet without supplemental arginine provided the highest energy conversion value (Table 3).

There was effect of the metabolizable energy level (P<0.05) on weight gain during the experimental period from 29 to 42 days and on daily weight gain, and broilers fed diet containing 3300 kcal/kg of metabolizable energy had the highest means (Table 2). For the energy intake and daily energy intake, the broilers that received 3150 and 3300 kcal/kg of metabolizable energy in the diet had the highest means (P<0.05) (Table 2).

Regarding the physiological and biochemical parameters related to lipid metabolism, there was interaction between diet and metabolizable energy level for triglycerides (P<0.05) and VLDL-cholesterol (P<0.05) (Table 4). Serum triglyceride levels were affected by diet only in broilers fed 3300 kcal/kg of metabolizable energy, and those receiving L-arginine supplementation had the lowest value (Table 3). Regarding serum VLDL-cholesterol levels, among broilers that received the control diet, the level of 3300 kcal/kg of metabolizable energy provided a higher value than the lowest level (3000 kcal/kg), while birds that received the intermediate level (3150 kcal/kg) were similar to the other groups. Among the broilers that received the diet with L-arginine supplementation, the metabolizable energy level of 3000 kcal/kg provided a higher serum VLDL-cholesterol level than the highest level (3300 kcal/kg), though it was similar to the value in the birds that received the intermediate level (3150 kcal/kg) (Table 3). There was a difference between diets only among broilers that received 3300 kcal/kg of metabolizable energy, and those that received L-arginine supplementation had the lowest concentration of serum VLDL-cholesterol (Table 3).

There was an effect of diet (P<0.05) on the serum concentrations of total cholesterol, HDL-cholesterol, and LDL-cholesterol, and the broilers that received the diet with the inclusion of L-arginine had the lowest values (Table 4). Serum glucose was lowest in broilers that received the control diet (P<0.05). Regarding the effect of metabolizable energy level, the broilers that received 3150 and 3300 kcal/kg had the highest glucose levels (P<0.05) (Table 4).

Regarding the carcass evaluations, metabolizable energy level (P<0.05) only affected abdominal fat yield; broilers fed 3300 kcal/kg had a higher mean than those that received the lowest level (3000 kcal/kg) but a similar mean as broilers fed the intermediate level (3150 kcal/kg) (Table 5).

**Table 3 -** Breakdown of means of variables with significant interaction between diet and level of metabolizable energy

| Variable | Diet (D) | Metabolizable energy (kcal/kg; E) | | | P-value[1] |
|---|---|---|---|---|---|
| | | 3000 | 3150 | 3300 | E |
| FC | Control | 1.72a | 1.74Aa | 1.58b | 0.001 |
| | Arginine | 1.72a | 1.61Bb | 1.55b | 0.001 |
| P-value[1] | D | 1.000 | 0.001 | 0.359 | - |
| CON | Control | 5.15b | 5.47Aa | 5.23b | 0.010 |
| | Arginine | 5.16 | 5.08b | 5.12 | 0.768 |
| P-value[1] | D | 0.972 | 0.001 | 0.300 | - |
| TG | Control | 39.69 | 39.52 | 44.40A | 0.130 |
| | Arginine | 43.04 | 39.57 | 36.67B | 0.070 |
| P-value[1] | D | 0.220 | 0.986 | 0.006 | - |
| VLDL | Control | 7.65b | 7.91ab | 8.88Aa | 0.044 |
| | Arginine | 8.61a | 7.91ab | 7.33Bb | 0.049 |
| P-value[1] | D | 0.063 | 0.986 | 0.004 | - |

FC - feed conversion; CON - energy conversion; TG - triglycerides; VLDL - very low-density lipoprotein.
[1] Scott–Knott test at 5% probability.
Means followed by different letters indicate difference between diets (A,B - Fischer's test) and between levels of metabolizable energy (a,b - Skott-Knott's test).

**Table 4** - Physiological and biochemical parameters of broilers at 42 days of age subjected to heat stress, as a function of diet and level of metabolizable energy

| Variable | Diet (D) | | Metabolizable energy (kcal/kg; E) | | | P-value[1] | | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Arginine | 3000 | 3150 | 3300 | D | E | D × E | |
| TG | 41.21 | 39.76 | 41.37 | 39.54 | 40.54 | 0.358 | 0.634 | 0.017 | 0.823 |
| TC | 145.5A | 121.0B | 139.4 | 129.26 | 131.13 | 0.001 | 0.194 | 0.901 | 2.946 |
| HDL | 60.15A | 51.87B | 58.29 | 56.28 | 53.46 | 0.001 | 0.070 | 0.316 | 1.049 |
| LDL | 74.72A | 61.17B | 72.80 | 61.85 | 69.19 | 0.001 | 0.066 | 0.218 | 2.205 |
| VLDL | 8.14 | 7.95 | 8.13 | 7.91 | 8.11 | 0.512 | 0.798 | 0.004 | 0.158 |
| Glucose | 182.7B | 195.7A | 183.5b | 191.14a | 192.90a | 0.001 | 0.009 | 0.079 | 1.676 |
| Urea | 6.68 | 7.99 | 6.83 | 7.44 | 7.74 | 0.056 | 0.527 | 0.080 | 0.352 |

TG - triglycerides; TC - total cholesterol; HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein; SEM - standard error of the mean.
[1] Scott–Knott's test at 5% probability.
Means followed by different letters indicate difference between diets (A,B - Fischer's test) and between levels of metabolizable energy (a,b - Skott-Knott's test).

**Table 5** - Carcass evaluations of broilers slaughtered at 42 days of age subjected to heat stress, as a function of diet and level of metabolizable energy

| Variable | Diet (D) | | Metabolizable energy (kcal/kg; E) | | | P-value[1] | | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Arginine | 3000 | 3150 | 3300 | D | E | D × E | |
| CY | 75.51 | 75.49 | 75.33 | 75.85 | 75.31 | 0.962 | 0.423 | 0.226 | 0.188 |
| BY | 37.48 | 37.84 | 37.69 | 37.70 | 37.59 | 0.426 | 0.973 | 0.473 | 0.215 |
| TDY | 26.85 | 26.89 | 26.85 | 26.69 | 27.05 | 0.849 | 0.393 | 0.519 | 0.102 |
| AFY | 1.37 | 1.28 | 1.21b | 1.36ab | 1.41a | 0.168 | 0.034 | 0.426 | 0.035 |
| LY | 1.43 | 1.39 | 1.42 | 1.42 | 1.40 | 0.101 | 0.665 | 0.659 | 0.013 |
| HY | 0.46 | 0.45 | 0.45 | 0.47 | 0.45 | 0.413 | 0.080 | 0.255 | 0.006 |

CY - carcass yield (%); BY - breast yield (%); TDY - thigh and drumstick yield (%); AFY - abdominal fat yield (%); LY - liver yield (%); HY - heart yield (%); SEM - standard error of the mean.
[1] Scott–Knott's test at 5% probability.
Means followed by different letters (a,b) indicate difference between levels of metabolizable energy.

## 4. Discussion

Improvements in broilers' performance parameters by L-arginine supplementation have been reported in several studies, with an increase in body weight (Emadi et al., 2010; Murakami et al., 2012; Ebrahimi et al., 2014; Xu et al., 2018; Brugaletta et al., 2023) and weight gain (Ebrahimi et al., 2014; Xu et al., 2018; Sirathonpong et al., 2019), and improvement in feed conversion (Murakami et al., 2012; Xu et al., 2018; Zampiga et al., 2018; Filho et al., 2021; Brugaletta et al., 2023). There are some reasons why arginine, when supplemented in broiler diets, can positively affect animal performance. The first is that this amino acid is a primary component of proteins, increasing protein synthesis and inhibiting proteolysis in the skeletal muscle, thus promoting body growth as a whole (Yao et al., 2008; Jahanian, 2009). A second reason is that arginine acts as a secretagogue of some hormones, stimulating the release of insulin, growth hormone, and insulin-like growth factor-1 into the bloodstream, and thus may indirectly stimulate feed intake and increase protein synthesis in muscle tissue, since these hormones have an anabolic effect (Jahanian, 2009; Xu et al., 2018). Finally, polyamines (putrescine, spermidine, and spermine), which are products resulting from the metabolism of arginine, have anabolic actions, such as improving the cellular absorption of amino acids, thus stimulating protein synthesis (Khajali and Wideman, 2010). In the present study, there was only one change in feed conversion ratio, being an improvement in this index observed when the diet of 3150 kcal/kg was supplemented with 0.66% L-arginine, which corroborates the fact that this amino acid, when supplemented at ideal levels, can improve the performance of broilers, even under heat stress conditions.

In this study, broilers that received L-arginine supplementation showed lower energy conversion. This parameter measures how much energy consumed is converted into weight gain. The fact that the supplemented broilers had lower energy conversion values indicates more efficiency at converting feed energy into weight gain, requiring lower energy intake for weight gain than broilers receiving the control diet without arginine.

Regarding the metabolizable energy level effects, improved weight gain and feed conversion ratio were observed in broilers fed the highest metabolizable energy level evaluated. These results are in agreement with Massuquetto et al. (2020), which reported an increase in gain and improvement in the feed conversion as the energy level increased from 12.73 to 13.73 MJ/kg. Thus, diets with higher energy levels can improve broiler performance, even in heat stress situations, by providing more energy for events related to protein synthesis and animal growth in general.

L-arginine supplementation may affect the levels of blood biochemical parameters related to lipid metabolism. In the present study, with L-arginine supplementation, total cholesterol content and the HDL- and LDL-cholesterol fractions were reduced, as were the triglyceride and VLDL-cholesterol contents in broilers fed diet with 3300 kcal/kg metabolizable energy. Similar results were obtained by Pirsaraei et al. (2018), as a reduction in the blood levels of cholesterol, triglycerides, and LDL-cholesterol was found with L-arginine supplementation in broiler diets. Other studies (Emadi et al., 2011; Ebrahimi et al., 2014; Filho et al., 2021) reported a reduction in serum cholesterol and triglyceride as L-arginine levels in the diet of broilers increased.

These results may be related to the fact that the amino acid arginine can alter lipid metabolism reducing the lipogenesis in the liver. Thus, Ebrahimi et al. (2014) and Pirsaraei et al. (2018) observed reduction of the hepatic lipogenic genes expression in broilers fed diets supplemented with L-arginine, regardless of the supplementation level. With the slowing of hepatic lipogenesis, there is a lower lipidic flow in the bloodstream, which may be responsible for the lower production and release of lipoproteins by the liver, especially VLDL-cholesterol and LDL-cholesterol, which lead to lower total cholesterol levels (Pirsaraei et al., 2018). In addition, arginine may alter lipid metabolism by increasing blood thyroid hormone concentrations, especially triiodothyronine (T3) (Ebrahimi et al., 2014), thus reducing blood lipid concentrations (Jobgen et al., 2006).

In the present study, there was an increase in blood glucose with supplementation of 0.66% L-arginine. Two other studies (Emadi et al., 2010; Pirsaraei et al., 2018) reported an increase in serum glucose as the level of L-arginine supplementation in the diet of broilers increased. The arginine effect on blood glucose can be attributed to its increase of gluconeogenesis, which causes greater glucose production through other substrates, such as amino acids and glycerol, and consequently greater release into the bloodstream (Foye et al., 2006).

Serum glucose concentration was also affected by metabolizable energy level. With increasing energy level, higher blood glucose values were observed. This may be related to increased gluconeogenesis, and as dietary energy increases, it may generate greater accumulation of lipids in tissues and, in turn, may enable a higher lipolysis rate, with consequent release of glycerol from triglycerides. Thus, this possible increase in the blood availability of glycerol may have resulted in increased hepatic gluconeogenesis pathway because glycerol is a gluconeogenic precursor.

The results of the present study show that arginine is capable of altering the lipid metabolism of broilers, even under heat stress, to reduce blood lipid concentrations, which may contribute to a reduction in the carcass fat deposition. However, there was no change in abdominal fat by arginine supplementation, differing from an earlier study (Wu et al., 2011), which supplemented ducks with 10 g/kg of L-arginine in a basal diet of corn and soybean and found reduced abdominal fat. Likewise, Ebrahimi et al. (2014) evaluated different supplementation levels (100, 153, 168, and 183%) of digestible L-arginine, based on the Ross lineage recommendation in broiler diets, and reported reduced abdominal fat accumulation as a function of the increased arginine supplementation. Pirsaraei et al. (2018) evaluated supplementation levels (100, 124, 139, and 154%) of L-arginine, based on the recommendation of Arian broilers – Corporation Support of Animal Affairs (2008) and observed a reduction in abdominal fat content when this amino acid was included in the diet of broilers at any level above of the recommended.

When we fed broilers the highest metabolizable energy level, they had higher abdominal fat content than broilers fed the lowest energy level. These results are in agreement with Leeson et al. (1996), in which the abdominal fat increased progressively from 2.7 to 3.2% when the broilers were fed diets containing 8.16 to 13.40 MJ/kg of metabolizable energy. As metabolizable energy increases, there is a greater intake of energy by the broilers, as observed in this study, which can increase carcass lipid deposition and cause fat accumulation, so that the excess energy consumed induces lipogenesis in the liver and adipose tissue.

Regarding the carcass traits, there are some reasons why L-arginine supplementation in the diet of broilers can positively alter these traits, thus increasing the lean mass content in the carcass as well as the yield of carcass and of the main cuts. The first is that there is evidence that the regulation of intracellular protein turnover by arginine favours skeletal muscle gain (Castro et al., 2019). The second is that this amino acid acts as a secretagogue of some hormones, such as growth hormone and insulin-like growth factor-1, which in turn induce various anabolic effects on skeletal muscle metabolism, such as myofibrillar protein aggregation, which is one of the events related to protein synthesis in muscle tissue (Jobgen et al., 2009). A third reason may be related to the fact that arginine plays a role in cell signalling in muscle tissue (Wu et al., 2011). In pigs, arginine activates the mTOR protein cellular signalling pathway in skeletal muscle, which in turn activates translation proteins, thus increasing protein synthesis in muscle tissue and, in general, the body growth of animals (Yao et al., 2008). Another reason is that nitric oxide, derived from arginine metabolism, acts in the formation of new blood vessels through the process of angiogenesis (Wu et al., 2009) and stimulates vasodilation (Mateo et al., 2008; Wu et al., 2009), which can generate increased blood flow under L-arginine supplementation in the diet of non-ruminant animals. Thus, arginine may be indirectly responsible for the increase in blood flow in the animal body, which may increase the uptake of nutrients by muscle tissue, such as amino acids, stimulating protein synthesis in this tissue. Therefore, the increase in protein synthesis in muscle tissue due to the various physiological events presented may be directly related to the increase in lean mass in the carcass and thus to the increase in carcass weight and yield of the main cuts, such as the breast, thigh, and drumstick.

Some studies in the literature have reported an increase in broiler carcass yield (Al-Daraji and Salih, 2012; Ebrahimi et al., 2014; Pirsaraei et al., 2018) and in cuts such as breast (Fernandes et al., 2009;

Al-Daraji and Salih, 2012; Pirsaraei et al., 2018) and thigh with drumstick (Pirsaraei et al., 2018) with L-arginine supplementation, regardless of the supplementation level. However, in the present study, L-arginine supplementation did not cause changes in the carcass traits of the chickens, such as carcass yield and main cuts (breast and thigh with drumstick). The fact that there was no effect on carcass characteristics may have been due to the effect of heat stress to which the animals were subjected, since, according to Chamruspollert et al. (2004), high temperatures reduce the absorption of arginine in the small intestine due to changes in the morphology of this portion of the digestive system. Thus, too little arginine may have been absorbed to cause physiological changes that could affect the carcass characteristics of the broilers that received supplementation with this amino acid. Another issue may be due to the level of supplementation, as the level of 0.66% in the diet of broilers was lower than that used by some other authors.

## 5. Conclusions

Diet supplemented with 0.66% L-arginine supplied for broilers exposed to heat stress, in the period from 29 to 42 days of age, improves the performance and the blood profile related to lipid metabolism. Regarding the levels of metabolizable energy, broilers that receive 3300 kcal/kg in the diet show better performance than the birds fed the lower energy level evaluated.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

**Conceptualization:** Abreu, M. L. T. and Rodrigues, P. B. **Data curation:** Oliveira, D. H. **Formal analysis:** Oliveira, D. H.; Sobrane Filho, S. T. and Alves, B. R. **Funding acquisition:** Rodrigues, P. B. **Investigation:** Oliveira, D. H.; Cruz, F. L.; Sobrane Filho, S. T.; Alves, B. R.; Abreu, M. L. T. and Naves, L. P. **Supervision:** Rodrigues, P. B. **Writing – original draft:** Oliveira, D. H. **Writing – review & editing:** Cruz, F. L. and Rodrigues, P. B.

## Acknowledgments

The authors acknowledge the financial support of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA).

## References

Al-Daraji, H. J. and Salih, A. M. 2012. Effect of dietary L-arginine on carcass traits of broilers. Research Opinions in Animal and Veterinary Sciences 2:40-43.

Balnave, D. and Brake, J. 2002. Re-evaluation of the classical dietary arginine: lysine interaction for modern poultry diets: a review. World's Poultry Science Journal 58:275-289. https://doi.org/10.1079/WPS20020021

Brugaletta, G.; Zampiga, M.; Laghi, L.; Indio, V.; Oliveri, C.; De Cesare, A. and Sirri, F. 2023. Feeding broiler chickens with arginine above recommended levels: effects on growth performance, metabolism, and intestinal microbiota. Journal of Animal Science and Biotechnology 14:33. https://doi.org/10.1186/s40104-023-00839-y

Castro, F. L. S.; Su, S.; Choi, H.; Koo, E. and Kim, W. K. 2019. L-Arginine supplementation enhances growth performance, lean muscle, and bone density but not fat in broiler chickens. Poultry Science 98:1716-1722. https://doi.org/10.3382/ps/pey504

Chamruspollert, M.; Pesti, G. M. and Bakalli, R. I. 2004. Chick responses to dietary arginine and methionine levels at diferent environmental temperatures. British Poultry Science 45:93-100. https://doi.org/10.1080/00071660410001668914

Del Castilho, C. C.; Santos, T. T.; Rodrigues, C. A. F. and Torres Filho, R. A. 2013. Effects of sex and genotype on performance and yield characteristics of free range broiler chickens. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65:1483-1490. https://doi.org/10.1590/S0102-09352013000500029

Ebrahimi, M.; Shahneh, A. Z.; Shivazad, M.; Pirsaraei, Z. A.; Tebianian, M.; Ruiz-Feria, C. A.; Adibmoradi, M.; Nourijelyani, K. and Mohamadnejad, F. 2014. The effect of feeding excess arginine on lipogenic gene expression and growth performance in broilers. British Poultry Science 55:81-88. https://doi.org/10.1080/00071668.2013.864381

Emadi, M.; Kaveh, K.; Bejo, M. H.; Ideris, A.; Jahanshiri, F.; Ivan, M. and Alimon, R. A. 2010. Growth performance and blood parameters as influenced by different levels of dietary arginine in broiler chickens. Journal of Animal and Veterinary Advances 9:70-74.

Emadi, M.; Jahanshiri, F.; Kaveh, K.; Hair-Bejo, M.; Ideris, A. and Alimon, A. R. 2011. Nutrition and immunity: the effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. Avian Pathology 40:63-72. https://doi.org/10.1080/03079457.2010.539590

Fernandes, J. I. M.; Murakami, A. E.; Martins, E. N.; Sakamoto, M. I. and Garcia, E. R. M. 2009. Effect of arginine on the development of the pectoralis muscle and the diameter and the protein:deoxyribonucleic acid rate of its skeletal myofibers in broilers. Poultry Science 88:1399-1406. https://doi.org/10.3382/ps.2008-00214

Filho, S. T. S.; Lima, E. M. C.; Oliveira, D. H.; Abreu, M. L. T.; Rosa, P. V.; Laurentiz, A. C.; Naves, L. P. and Rodrigues, P. B. 2021. Supplemental L-arginine improves feed conversion and modulates lipid metabolism in male and female broilers from 29 to 42 days of age. Animal 15:100120. https://doi.org/10.1016/j.animal.2020.100120

Fouad, A. M.; El-Senousey, H. K.; Yang, X. J. and Yao, J. H. 2013. Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. Animal 7:1239-1245. https://doi.org/10.1017/S1751731113000347

Foye, O. T.; Uni, Z.; McMurty, J. P. and Freket, P. R. 2006. The effects of amniotic nutrient administration, "*In ovo* feeding" of arginine and/or β-hydroxy-β-methyl butyrate (HMB) on insulin-like growth factors, energy metabolism and growth in turkey poults. International Journal of Poultry Science 5:309-317. https://doi.org/10.3923/ijps.2006.309.317

Jahanian, R. 2009. Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks. Poultry Science 88:1818-1824. https://doi.org/10.3382/ps.2008-00386

Jobgen, W. S.; Fried, S. K.; Fu, W. J.; Meininger, C. J. and Wu, G. 2006. Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates. The Journal of Nutritional Biochemistry 17:571-588. https://doi.org/10.1016/j.jnutbio.2005.12.001

Jobgen, W.; Meininger, C. J.; Jobgen, S. C.; Li, P.; Lee, M. J.; Smith, S. B.; Spencer, T. E.; Fried, S. K. and Wu, G. 2009. Dietary L-arginine supplementation reduces white fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. The Journal of Nutrition 139:230-237. https://doi.org/10.3945/jn.108.096362

Khajali, F. and Wideman, R. F. 2010. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. World's Poultry Science Journal 66:751-766. https://doi.org/10.1017/S0043933910000711

Leeson, S.; Caston, L. and Summers, J. D. 1996. Broiler response to energy or energy and protein dilution in the finisher diet. Poultry Science 85:522-528. https://doi.org/10.3382/ps.0750522

Massuquetto, A.; Panisson, J. C.; Schramm, V. G.; Surek, D.; Krabbe, E. L. and Maiorka, A. 2020. Effects of feed form and energy levels on growth performance, carcass yield and nutrient digestibility in broilers. Animal 14:1139-1146. https://doi.org/10.1017/S1751731119003331

Mateo, R. D.; Wu, G.; Moon, H. K.; Carroll, J. A. and Kim, S. W. 2008. Effects of dietary arginine supplementation during gestation and lactation on the performance of lactating primiparous sows and nursing piglets. Journal of Animal Science 86:827-835. https://doi.org/10.2527/jas.2007-0371

Mendonça, N. B. S. N.; Sobrane Filho, S. T.; Oliveira, D. H.; Lima, E. M. C.; Rosa, P. V.; Faria, P. B.; Naves, L. P. and Rodrigues, P. B. 2020. Dietary chia (*Salvia hispanica* L.) improves the nutritional quality of broiler meat. Asian-Australasian Journal of Animal Sciences 33:1310-1322.

Murakami, A. E.; Fernandes, J. I. M.; Hernandes, L. and Santos, T. C. 2012. Effects of starter diet supplementation with arginine on broiler production performance and on small intestine morphometry. Pesquisa Veterinária Brasileira 32:259-266. https://doi.org/10.1590/S0100-736X2012000300014

Pirsaraei, Z. A.; Rahimi, A.; Deldar, H.; Sayyadi, A. J.; Ebrahimi, M.; Shahneh, A. Z.; Shivazad, M. and Tebianian, M. 2018. Effect of feeding arginine on the growth performance, carcass traits, relative expression of lipogenic genes, and blood parameters of Arian broilers. Brazilian Journal of Poultry Science 20:363-370. https://doi.org/10.1590/1806-9061-2017-0620

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3.ed. UFV, Viçosa, MG.

Sirathonpong, O.; Ruangpanit, Y.; Songserm, O.; Koo, E. J. and Attamangkune, S. 2019. Determination of the optimum arginine:lysine ratio in broiler diets. Animal Production Science 59:1705-1710. https://doi.org/10.1071/AN18049

Wu, G.; Bazer, F. W.; Davis, T. A.; Kim, S. W.; Li, P.; Marc Rhoads, J.; Carey Satterfield, M.; Smith, S. B.; Spencer, T. E. and Yin, Y. 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153-168. https://doi.org/10.1007/s00726-008-0210-y

Wu, L. Y.; Fang, Y. J. and Guo, X. Y. 2011. Dietary L-arginine supplementation beneficially regulates body fat deposition of meat-type ducks. British Poultry Science 52:221-226. https://doi.org/10.1080/00071668.2011.559452

Xu, Y. Q.; Guo, Y. W.; Shi, B. L.; Yan, S. M. and Guo, X. Y. 2018. Dietary arginine supplementation enhances the growth performance and immune status of broiler chickens. Livestock Science 209:8-13. https://doi.org/10.1016/j.livsci.2018.01.001

Yao, K.; Yin, Y. L.; Chu, W. Y.; Liu, Z. Q.; Deng, D.; Li, T. J.; Huang, R. L.; Zhang, J. S.; Tan, B.; Wang, W. C. and Wu, G. Y. 2008. Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. The Journal of Nutrition 138:867-872. https://doi.org/10.1093/jn/138.5.867

Zampiga, M.; Laghi, L.; Petracci, M.; Zhu, C.; Meluzzi, A.; Dridi, S. and Sirri, F. 2018. Effect of dietary arginine to lysine ratios on productive performance, meat quality, plasma and muscle metabolomics profile in fast-growing broiler chickens. Journal of Animal Science and Biotechnology 9:79. https://doi.org/10.1186/s40104-018-0294-5