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Supplemental L-arginine improves feed conversion and modulates lipid metabolism in male and female broilers from 29 to 42 days of age

S.T.S. Filho [a], E.M. da C. Lima [a], D.H. de Oliveira [a], M.L.T. de Abreu [a], P.V. Rosa [a], A.C. de Laurentiz [b], L. de P. Naves [a], P.B. Rodrigues [a,*]

[a] Department of Animal Science, Federal University of Lavras, 37200-900 Lavras, Minas Gerais, Brazil
[b] Department of Biology and Animal Science, São Paulo State University, 15385-000, Ilha Solteira, São Paulo, Brazil

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ABSTRACT

The excessive accumulation of abdominal fat in broilers is an economic waste. Supplemental dietary L-arginine has been shown to reduce lipogenesis in broilers, but studies on this subject are still scarce. Two experiments were conducted in a 5 × 2 factorial design, with five L-arginine concentrations in diets (0, 3, 6, 9 and 12 g/kg) provided to male and female broilers, from 29 to 42 days of age, reared in boxes or cages, with six replicates of 23 broilers per box and six replicates of three broilers per cage, totalling 1560 broilers. Data on performance, carcass and cuts yield, abdominal fat deposition, chemical composition of the breast, lipid profile and liver enzyme activity were evaluated in experiment 1. In experiment 2, the balance and retention of nitrogen, metabolizability coefficients and metabolizable energy of feed were determined and the treatments were evaluated in six replicates of three broilers per cage, totalling 180 broilers. In both experiments, there was no interaction ($P > 0.05$) between L-arginine concentration in the diet and sex of the broilers for all parameters evaluated. Male broilers showed higher ($P < 0.05$) weight gain, noble cuts yield, levels of CP and mineral matter in the breast, better feed conversion ($P < 0.05$) and lower ($P < 0.05$) malic enzyme activity in the liver and abdominal fat deposition. On the other hand, regardless of sex, the increase in L-arginine concentration in the diet improved ($P < 0.05$) the feed conversion in addition to reducing ($P < 0.05$) serum levels of total cholesterol and low-density lipoprotein cholesterol, the malic enzyme activity in the liver and abdominal fat deposition. In conclusion, male broilers had better productive results than females. However, supplementing the diet of male or female broilers from 29 to 42 days of age with L-arginine at a concentration of 6.87 g/kg represents a nutritional strategy to improve feed conversion and reduce circulating triacylglycerol and cholesterol levels, NADPH synthesis by liver malic enzyme and abdominal fat deposition, without negatively affecting the carcass and noble cuts yield, the amount of nitrogen excreted by the broilers and the energy value of the feed.

Implications

Broilers from fast-growing lines divert more nutrients from the diet to the accumulation of abdominal fat, which is an economic waste. The effect of supplemental dietary L-arginine on decreasing lipogenesis has been confirmed when L-arginine is used for 21 days of broiler rearing. Therefore, the main contribution of this study is to demonstrate that the use of supplemental dietary L-arginine for 14 days is sufficient to reduce abdominal fat deposition without increasing the amount of nitrogen excreted by broilers in the environment.

Introduction

Broilers of modern commercial lines exhibit rapid muscle growth. However, they also have a genetic predisposition to divert more nutrients from the diet to triacylglycerol biosynthesis and subsequently accumulate more fat in the abdominal region (Cui et al., 2012). According to Choct et al. (2000), in modern broiler lines, approximately 85% of all body fat is not physiologically necessary for the organism to function. As the excessive deposition of abdominal fat can reduce carcass yield and quality (Fouad and El-Senousey, 2014), nutritional strategies that modulate lipid metabolism in broilers need to be established. In this context, the effects of dietary L-arginine supplementation on the regulation of lipogenesis have been investigated.

In an experiment with broilers fed diets supplemented with L-arginine over the period of 21 to 42 days of age, Fouad et al. (2013)

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[*] Corresponding author.
E-mail address: pborges@ufla.br (P.B. Rodrigues).

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reported reduced gene expression of the liver enzymes fatty acid synthase (**FAS**) and 3-hydroxy-3-methylglutaryl CoA (**HMG-CoA** reductase), which participate in the biosynthesis of triacylglycerol and cholesterol, respectively. Similarly, Ebrahimi et al. (2014) also observed a reduction in the expression of the gene encoding FAS when the diet of broilers was supplemented with L-arginine in the period of 25 to 46 days of age.

However, according to Guo et al. (2011), up until the fourth week of age of the broilers, the accumulation of fat is mainly due to the increase in the number of adipocytes (hyperplasia events). After that, adipocyte hypertrophy events predominate. Considering that shorter periods of use of industrial amino acids result in lower feed costs, the question arose whether dietary L-arginine could maintain its action in reducing the accumulation of abdominal fat when supplemented in the diet only after 29 days of age.

Concomitant with this, supplemental dietary L-arginine can also act on protein metabolism by increasing nitrogen retention and protein synthesis in broilers. A justification to explain these effects is that during the L-arginine metabolism, there is the biosynthesis of polyamines such as putrescine, spermidine and spermine and of other metabolic intermediates that displays growth-promoting effects (Khajali and Widerman, 2010). Therefore, in the present study, there was interest in also evaluating whether the supplementation of increasing levels of L-arginine in the diet of broilers has effect in the nitrogen balance and retention.

Therefore, two experiments were conducted with broilers of both sexes, from 29 to 42 days of age, to evaluate the effects of dietary L-arginine supplementation on performance, carcass and cuts yield, abdominal fat deposition, chemical composition of the breast, lipid profile, liver enzyme activity, nitrogen balance and retention, metabolizability coefficients and metabolizable energy of feeds.

Material and methods

The experiments were conducted in the Poultry Sector of the Federal University of Lavras (**UFLA**). All experimental procedures were approved by the UFLA Ethics Committee on Animal Use under protocol no. 057/14.

Experimental designs and diets

Experiment 1 was conducted in a randomized block design, considering the rearing site of the broilers (box or cage) for the formation of blocks. Experiment 2 was conducted in a completely randomized design, with the birds reared in metabolic cages. Both treatments were distributed in a 5×2 factorial design, with five concentrations of supplemental L-arginine in the diets (0, 3, 6, 9 and 12 g/kg) provided to male and female broilers, totalling 10 treatments. In experiment 1, each treatment was evaluated in 12 replicates, with six replicates of 23 broilers per box and six replicates of three broilers per cage, totalling 1 560 broilers. In experiment 2, each treatment was evaluated in six replicates of three broilers per cage, totalling 180 broilers. Each box and each cage consisted of one experimental unit. The mean initial weight of the broilers was similar (1.397 ± 39 g for males and 1.287 ± 32 g for females) among all experimental units.

The increasing levels of L-arginine in the experimental diets were obtained by replacing kaolin (inert), fixed at 20 g/kg in the basal diet without supplementary L-arginine (Table 1). The basal diet was formulated to meet the nutritional requirements of the broilers (Rostagno et al., 2011), with a CP level of 187.5 g/kg. The amino acid ratios were similar between the diets supplemented with L-arginine and the basal diet, except for the lysine:arginine ratio (Table 2). The concentrations of the main essential amino acids in the experimental diets were determined by HPLC, according to Association of Official Analytical Chemists (**AOAC**) method 982.30 (2005; Table 2). Experimental diets and water were provided *ad libitum* from 29 to 42 days of age.

Table 1
Ingredients and calculated nutritional composition of broiler diets.

	Pre-experimental diets		Experimental diet[3]
	1 to 7 days	8 to 28 days	29 to 42 days
Ingredients (g/kg)			
Corn	553.45	594.75	597.90
Soybean meal, 45%	381.30	340.60	302.30
Soybean oil	21.80	28.60	48.30
Salt	5.10	4.80	4.60
Limestone	9.10	9.10	8.00
Dicalcium phosphate	19.10	14.60	11.80
DL-methionine, 99%	3.60	2.70	2.50
L-Lysine HCl, 78%	3.00	2.20	2.00
L-Threonine, 98.5	1.10	0.70	0.50
L-Valine, 99%	–	–	0.20
Mineral supplement[1]	0.50	0.50	0.50
Vitamin supplement[2]	0.40	0.40	0.40
Choline chloride, 60%	0.50	0.50	0.40
Salinomycin, 66 mg/kg	0.50	0.50	0.50
Avilamycin, 100 mg/kg	0.05	0.05	–
Kaolin (inert)	–	–	20.0
Nutritional composition, calculated			
AME, MJ/kg	12.35	12.77	13.18
CP, g/kg	222.0	205.0	187.5
Calcium, g/kg	9.2	8.0	6.8
Sodium, g/kg	2.2	2.1	2.0
Available phosphorus, g/kg	4.7	3.8	3.2
Digestible methionine + cysteine, g/kg	9.4	8.2	7.6
Digestible arginine, g/kg	14.0	12.8	11.6
Digestible lysine, g/kg	13.1	11.5	10.4
Digestible arginine:lysine ratio	1.07	1.11	1.11
Digestible threonine, g/kg	8.5	7.6	6.8
Digestible valine, g/kg	9.3	8.7	8.1
Digestible isoleucine, g/kg	8.7	8.0	7.1
Digestible tryptophan, g/kg	2.5	2.3	2.0
Digestible leucine, g/kg	17.2	16.2	13.7
Total glycine + serine, g/kg	20.8	19.3	16.1

AME = apparent metabolizable energy.

[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; vitamin A, 6 000 IU; vitamin B_1, 0.8 mg; vitamin E, 12.15 IU; vitamin B_{12}, 8.10 μg; vitamin B_2, 3.6 mg; vitamin B_6, 1.80 mg; vitamin D_3, 1 500 IU; vitamin K_3, 1.44 mg.

[3] Basal diet without supplementary L-arginine, formulated to meet the nutritional requirements of the broilers (Rostagno et al., 2011). The increasing levels of L-arginine in the experimental diets were obtained by replacing kaolin (inert), fixed at 20 g/kg in the basal diet.

Table 2
Concentration of the main essential amino acids in the experimental diets provided to broilers.[1]

Total amino acids (g/kg)	Supplemental dietary L-arginine (g/kg)				
	0	3	6	9	12
Arginine	12.0	14.9	17.7	20.4	22.9
Lysine	11.2	11.3	11.1	11.3	11.3
Total arginine:lysine ratio	1.07	1.32	1.59	1.80	2.03
Methionine + cystine	8.1	7.9	7.9	7.8	8.0
Threonine	7.6	7.7	7.5	7.6	7.8
Tryptophan	2.2	2.2	2.3	2.3	2.3
Valine	8.7	8.7	8.7	8.7	8.7

[1] Determined by HPLC according to AOAC method 982.30 (2005).

Animals, management and facilities

Male and female Cobb® chicks were acquired at 1 day of age from a commercial hatchery and reared until 28 days of age in a conventional broiler shed. During this period, the broilers received a standard diet based on corn and soybean meal, without supplemental L-arginine, formulated to meet their nutritional requirements (Rostagno et al., 2011; Table 1). On the 29th day of age, the broilers were individually weighed, separated according to their weight range and sex and subsequently distributed in the boxes and cages according to the designs described for experiments 1 and 2.

Each box, with dimensions of 1.5 × 2.0 m, was equipped with a tube feeder and a bell drinker, and the floor was covered with wood shavings. The galvanized wire cages (50 × 50 × 40 cm) contained a feeder, a drinker and a tray for collecting excreta. In the shed containing the boxes, minimum and maximum temperatures of 19.8 ± 1.7 °C and 30.7 ± 1.7 °C were recorded, respectively, with a minimum relative humidity of 46.4 ± 7.8% and a maximum of 73.3 ± 9.9%. In the metabolic room containing the cages, minimum and maximum temperatures of 21.3 ± 1.2 °C and 28.4 ± 1.7 °C were recorded, respectively, with a minimum relative humidity of 50.3 ± 7.2% and a maximum of 77.5 ± 5.4%. During the experimental period, the lighting was constant (24 h of natural and/or artificial light) in the shed and in the metabolic room.

Parameters evaluated in experiment 1

Feed intake, weight gain and feed conversion of broilers were evaluated in the period from 29 to 42 days of age. The mortality of the broilers was recorded daily and used to correct the results of the evaluated performance parameters.

On the 42nd day of age, after 12 h of fasting, two broilers were randomly selected from each experimental unit, totalling 24 broilers per treatment, and were slaughtered by cervical dislocation followed by bleeding. During the bleeding step, 6 ml of blood from each broiler was collected in tubes without anticoagulant (BD Vacutainer®, Juiz de Fora, Brazil). Next, the blood samples were centrifuged at 2 000 ×g for 6 min at room temperature to separate the serum. The serum levels of total cholesterol, triacylglycerol, high-density lipoprotein cholesterol (**HDL-cholesterol**) and low-density lipoprotein cholesterol (**LDL-cholesterol**) were determined colorimetrically (NUNC F spectrophotometer, Thermo Fischer Scientific Inc., Kamstrup, Denmark) using commercial serological kits (Labtest®, Lagoa Santa, Brazil). The serum very-low-density lipoprotein cholesterol (**VLDL-cholesterol**) level was calculated by the difference between the total cholesterol and the HDL-cholesterol and LDL-cholesterol fractions.

At the time of evisceration of the broilers, liver samples (5 g) were immediately frozen in liquid nitrogen and stored at −80 °C until measurement of glucose-6-phosphate dehydrogenase enzyme (**G6PD**; EC 1.1.1.49) activity and malic enzyme (EC 1.1.1.40) activity. For both enzymes, liver extracts were prepared according to the method described by Alvarez et al. (2000) using a ratio of 10:1 (ml of buffer: g of liver). The G6PD activity and malic enzyme activity in the liver extract were determined according to Bautista et al. (1988) and Spina et al. (1970), respectively. Enzyme kinetics were monitored *via* a microplate reader (Multiskan FC model, Thermo Fisher Scientific Inc., Vantaa, Finland). The soluble protein level in liver extracts was determined by the method described by Bradford (1976), using bovine serum albumin as a standard. Enzyme activities were defined as μmol of substrate converted into its respective product per minute (U) per mg of protein.

Additionally, the carcass, breast and leg (thigh + drumstick) yields were determined. For the carcass yield, the ratio between the weight of the clean carcass (without head and feet) and the BW of the broiler was considered. Breast and leg yields were calculated relative to the weight of the eviscerated carcass. To determine abdominal fat deposition, the fat present in the region between the bursa of Fabricius and the cloaca was collected, weighed and related to the carcass weight, as described by Gomide et al. (2014).

After the yield analysis, the boneless, skinless breasts were ground and aliquots were removed (80 g per breast) and stored at −20 °C until the determination of the moisture, CP, ether extract, mineral matter and protein contents using a FoodScan™ Meat Analyser (FOSS, Hillerod, Denmark).

Parameters evaluated in experiment 2

In this experiment, the broilers were adapted to the diets and facilities in the period of 29 to 35 days of age, followed by total excreta collection for three consecutive days, from days 36 to 38 of age. The feed intake during the period of excreta collection was calculated as the difference between the amount of feed provided and the amount of leftovers in the feeder. The excreta from each experimental unit were collected daily in a duly labelled plastic bag and stored at −5 °C until the last day of collection. Subsequently, the excreta were thawed, weighed, homogenized, pre-dried in a forced ventilation oven at 65 °C until constant weight, ground in a knife mill with a 1.0-mm sieve and stored at 4 °C until total nitrogen (N), DM and ether extract analyses (Association of Official Analytical Chemists AOAC, 2005; methods 954.01, 943.01 and 920.39, respectively). Furthermore, the gross energy was determined using a calorimeter (model C200, IKA ®, Stauten, Germany).

N balance was calculated as the difference between ingested and excreted N. The N retention coefficient for the chickens was calculated using the following equation:

$$\text{N retention coefficient } (\%) = \frac{\text{N ingested} - \text{N excreted}}{\text{N ingested}} \times 100$$

The DM (**DMMC**), ether extract (**EEMC**) and gross energy (**GEMC**) metabolizability coefficients (**MC**) were calculated considering that:

$$\text{MC of the nutrient } (\%) = \frac{\text{Nutrient ingested} - \text{Nutrient excreted}}{\text{Nutrient ingested}} \times 100$$

The apparent metabolizable energy (**AME**) of the feed was calculated using the equation proposed by Matterson et al. (1965). The apparent metabolizable energy corrected for N balance (**AMEn**) was calculated according to Hill and Anderson (1958):

$$\text{EMAn} = \frac{\text{GE ingested} - (\text{GE excreted} \pm 8.22 \times \text{NB})}{\text{DM ingested}}$$

where,
GE = gross energy and
NB = nitrogen balance (nitrogen ingested − excreted nitrogen).

Statistical analysis

Data were analyzed using Bartlett's and Shapiro–Wilk tests at a level of significance of $P < 0.05$ to evaluate the ANOVA assumptions (homogeneity and normality). If one of the assumptions was not met, the data were log-transformed for subsequent statistical analysis. If both assumptions were met, the data were analyzed by ANOVA using the statistical software R, version 3.2.5 (R Core Team, 2017). The significance of the effect of sex in broilers was evaluated using the F test at 5% probability. Linear or quadratic regression models ($P < 0.05$) were used to evaluate the effect of dietary L-arginine concentration. However, when the data did not fit the linear or quadratic regression models, the Scott–Knott test for comparison of means ($P < 0.05$) was used to evaluate the effect of dietary L-arginine concentration because biological interpretations are difficult to make when using third-order regression models or higher.

Table 3
Effects of dietary L-arginine supplementation and sex on the performance of broilers from 29 to 42 days old (Experiment 1).

	Supplemental dietary L-arginine (g/kg)					Sex		SEM	P value[1]	
	0	3	6	9	12	Males	Females		L-arginine	Sex
Feed intake (kg)	2.35	2.40	2.33	2.31	2.28	2.32	2.34	0.013	0.060	0.514
Weight gain (kg)	1.35	1.40	1.39	1.38	1.37	1.40^{a}	1.35^{b}	0.009	0.607	0.028
Feed conversion (kg/kg)[2]	1.74	1.71	1.68	1.68	1.67	1.68^{b}	1.71^{a}	0.007	0.042	0.014

[ab] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the F test.

[1] There was no interaction ($P > 0.05$) between the dietary L-arginine concentration and broilers sex for the evaluated parameters.

[2] Linear response plateau (LRP) effect of dietary L-arginine concentration on feed conversion [$y = 1.671–0.011(x - 6.87)$, $R^2 = 0.985$] with plateau response starting at the inclusion level of 6.87 g/kg of L-arginine in the diet.

Results

There was no interaction ($P > 0.05$) of the dietary L-arginine concentration and the broiler sex for all parameters evaluated in experiments 1 and 2.

Experiment 1

The increase in the dietary L-arginine concentration did not affect ($P > 0.05$) feed intake and weight gain; however, it improved ($P < 0.05$) feed conversion, estimating a plateau starting at an inclusion level of 6.87 g/kg of L-arginine in the diet (LRP effect, $P = 0.014$) (Table 3). The broilers sex did not affect ($P > 0.05$) the feed intake. However, male broilers gained 3.17% more weight ($P < 0.05$) and had better ($P < 0.05$) feed conversion.

The serum triacylglycerol and VLDL-cholesterol levels were altered (quadratic effect, $P < 0.05$) by the dietary L-arginine concentration, and the lowest serum levels were estimated at concentrations of 6.69 and 6.63 L-arginine/kg of feed (Table 4). The serum levels of total cholesterol and LDL-cholesterol decreased linearly ($P < 0.05$) by up to 13.11 and 45.71%, respectively, with the increase in the dietary L-arginine concentration. The serum levels of triacylglycerols and cholesterol (total cholesterol and fractions) were not affected ($P > 0.05$) by the broilers sex.

The inclusion of L-arginine in the diet at concentrations ranging from 3 to 12 g/kg increased ($P < 0.05$) the mean G6PD activity in the liver by 36.18% relative to the mean G6PD activity in the liver of broilers fed diet without supplemental L-arginine (Table 4). On the other hand, the increase in the dietary L-arginine concentration reduced (linear effect, $P = 0.021$) the malic enzyme activity in the liver. The broilers sex did not affect ($P > 0.05$) the liver G6PD enzyme activity; however, female broilers had higher ($P < 0.05$) malic enzyme activity in the liver.

There was no effect ($P > 0.05$) of dietary L-arginine concentration on the carcass, breast and leg yields or on the levels of moisture, CP, ether extract and collagen measured in the breast of the broilers (Table 5). However, the increase in the dietary L-arginine concentration reduced abdominal fat deposition (linear effect, $P = 0.049$) by up to 16%. In addition, broilers fed diets containing L-arginine at concentrations of 6 and 12 g/kg showed the lowest ($P < 0.05$) mineral matter content in the breast. Regarding sex, males had higher ($P < 0.05$) breast and leg yields, lower ($P < 0.05$) abdominal fat deposition and higher ($P < 0.05$) levels of CP and mineral matter in the breast.

Experiment 2

The increase in the dietary L-arginine concentration linearly increased ($P < 0.05$) the N intake and balance without altering ($P > 0.05$) the amount of N excreted and the N retention coefficient for the broilers (Table 6). Regarding sex, males exhibited higher ($P < 0.05$) N intake, balance and retention than females.

Dietary L-arginine concentration did not influence ($P > 0.05$) the DMMC or the GEMC (Table 6). However, the inclusion of L-arginine in the diet at concentrations ranging from 3 to 12 g/kg increased

Table 4
Effects of dietary L-arginine supplementation and sex on serum lipid concentrations and activity of lipogenic enzymes in the liver of broilers (Experiment 1).

	Supplemental dietary L-arginine (g/kg)					Sex		SEM	P value[1]	
	0	3	6	9	12	Males	Females		L-arginine	Sex
Serum concentration (mmol/L)										
Triacylglycerols[2]	0.600	0.552	0.512	0.513	0.576	0.535	0.564	0.008	0.013	0.079
Total cholesterol[3]	3.535	3.314	3.228	3.102	3.071	3.257	3.242	0.035	0.001	0.833
High-density lipoprotein cholesterol	2.419	2.377	2.240	2.349	2.355	2.348	2.348	0.033	0.535	0.997
Low-density lipoprotein cholesterol[4]	0.844	0.684	0.752	0.517	0.453	0.664	0.636	0.021	0.011	0.709
Very-low-density lipoprotein cholesterol[5]	0.272	0.253	0.235	0.235	0.264	0.245	0.258	0.004	0.013	0.086
Enzyme activity (U/mg)[6]										
Glucose-6-phosphate dehydrogenase[7]	2.963^{b}	3.935^{a}	4.044^{a}	4.208^{a}	3.953^{a}	4.000	3.641	0.105	0.004	0.095
Malic enzyme[8]	41.437	35.142	33.380	29.952	30.313	32.194^{d}	35.896^{c}	0.533	0.001	0.001

[ab] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the Scott–Knott test.

[cd] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the F test.

[1] There was no interaction ($P > 0.05$) between the dietary L-arginine concentration and broilers sex for the evaluated parameters.

[2] Quadratic effect of dietary L-arginine concentration on the serum concentration of triacylglycerols ($y = 0.002x^2–0.028x + 0.605$, $R^2 = 0.951$) with a minimum estimated concentration of 6.69 g/kg L-arginine in the diet.

[3] Linear effect of dietary L-arginine concentration on serum total cholesterol level ($y = 3.478–0.038x$, $R^2 = 0.929$).

[4] Linear effect of dietary L-arginine concentration on the serum low-density lipoprotein cholesterol level ($y = 0.840–0.032x$, $R^2 = 0.852$).

[5] Quadratic effect of dietary L-arginine concentration on the serum very-low-density lipoprotein cholesterol level ($y = 0.001x^2–0.0120x + 0.275$, $R^2 = 0.930$) with a minimum estimated concentration of 6.63 g/kg L-arginine in the diet.

[6] Enzyme activities were defined as μmol of substrate converted into its respective product per minute (U) per mg of protein.

[7] As the data did not fit linear or quadratic models, the Scott–Knott test for comparison of means ($P < 0.05$) was used to evaluate the effect of dietary L-arginine concentration on glucose-6-phosphate dehydrogenase activity.

[8] Linear effect of dietary L-arginine concentration on malic enzyme activity ($y = 39.532–0.915x$, $R^2 = 0.866$).

Table 5
Effects of dietary L-arginine supplementation and sex on carcass and cuts yield, abdominal fat deposition and chemical composition of the breast of broilers (Experiment 1).

	Supplemental dietary L-arginine (g/kg)					Sex		SEM	*P* value[1]	
	0	3	6	9	12	Males	Females		L-arginine	Sex
Yield										
Carcass (g/kg live weight)	766.05	762.80	770.58	774.20	766.62	769.78	766.31	1.497	0.155	0.251
Breast (g/kg of carcass)	310.32	308.81	308.75	309.20	305.75	340.32^{a}	276.53^{b}	2.341	0.980	0.001
Leg (g/kg of carcass)	248.85	245.62	254.60	251.69	254.83	292.00^{a}	210.25^{b}	1.549	0.289	0.001
Abdominal fat (g/kg of carcass)[2]	15.307	16.033	14.682	14.310	12.96	13.49^{b}	15.82^{a}	0.318	0.046	0.001
Chemical composition of the breast (g/kg)										
Moisture	720.13	726.86	727.05	725.05	729.25	727.17	724.16	3.186	0.100	0.151
CP	229.24	229.34	229.63	231.05	228.15	231.03^{a}	227.94^{b}	0.532	0.562	0.008
Ether extract	15.00	11.75	13.20	12.21	13.78	13.49	12.88	0.495	0.282	0.541
Mineral matter[3]	29.91^{c}	27.15^{d}	25.07^{e}	26.75^{d}	23.37^{e}	27.10^{a}	25.81^{b}	0.302	0.001	0.044
Collagen	5.70	4.89	5.05	4.93	5.45	5.51	4.90	0.190	0.607	0.128

[ab] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the F test.
[cde] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the Scott–Knott test.
[1] There was no interaction ($P > 0.05$) between the dietary L-arginine concentration and broilers sex for the evaluated parameters.
[2] Linear effect of dietary L-arginine concentration on abdominal fat deposition ($y = 15.936–0.213x$, $R^2 = 0.773$).
[3] As the data did not fit linear or quadratic models, the Scott–Knott test for comparison of means ($P < 0.05$) was used to evaluate the effect of dietary L-arginine concentration on the mineral matter content in the breast.

($P < 0.05$) the EEMC by approximately 2.1% compared to the diet without supplemental L-arginine. For the energy value, the AME and AMEn were lower ($P < 0.05$) when the diets contained 9 and 12 g/kg of supplemental L-arginine. The DMMC, EEMC, GEMC, AME and AMEn were not affected ($P > 0.05$) by the broilers sex.

Discussion

Experiment 1

Dietary L-arginine supplementation did not alter feed intake or weight gain in broilers, similar to that reported by Fouad et al. (2013). However, dietary L-arginine improved feed conversion in the broilers, corroborating studies by Al-Daraji and Salih (2012), Ebrahimi et al. (2014) and Sharifi et al. (2015). The improvement in feed conversion of broilers fed diets containing L-arginine has been associated with reduced lipogenesis and increased protein synthesis (Jobgen et al., 2009; Ebrahimi et al., 2014; Yuan et al., 2016). In addition, it is important to consider that in experiment I, the broilers were submitted to mild heat stress because the maximal temperature recorded in the shed was around 30 °C. In the L-arginine metabolism, there is the biosynthesis of nitric oxide which has a vasodilating action (Fouad et al., 2013). Peripheral vasodilation is a mechanism that facilitates the loss of body heat to the environment. Therefore, supplemental L-arginine may have reduced heat stress in broilers, contributing to improved feed conversion.

On the other hand, regardless of the type of diet, there was no effect of sex on feed intake, similar to the results of the study by Salim et al. (2012). However, male broilers exhibited better feed conversion, corroborating the results reported by Benyi et al. (2015). In general, males can be slaughtered earlier than females because they exhibit faster growth, which in turn is associated with higher efficiency of the males in converting ingested ration into weight gain (Benyi et al., 2015; Mabelebele et al., 2017).

In an experiment with broilers fed diets supplemented with L-arginine, Fouad et al. (2013) reported reduced gene expression of the liver enzymes FAS and HMG-CoA reductase, which participate in the biosynthesis of triacylglycerol and cholesterol, respectively. Similarly, Ebrahimi et al. (2014) also observed reduced expression of the FAS-encoding gene when the broilers diet was supplemented with

Table 6
Effects of dietary L-arginine supplementation and sex on the utilization of nitrogen by broilers, metabolizable coefficients and metabolizable energy of feeds (Experiment 2).

	Supplemental dietary L-arginine (g/kg)					Sex		SEM	*P* value[2]	
	0	3	6	9	12	Males	Females		L-arginine	Sex
Feed intake (kg)[1]	0.591	0.573	0.583	0.582	0.568	0.607^{a}	0.551^{b}	0.006	0.811	<0.001
Utilization of nitrogen (N)										
N ingested (g)[3]	17.495	18.149	19.078	19.510	19.560	19.600^{a}	17.917^{b}	0.181	0.006	0.001
N excreted (g)	5.950	5.636	6.145	6.421	6.207	6.079	6.068	0.075	0.059	0.946
N balance (g)[4]	11.535	12.513	12.932	13.089	13.353	13.521^{a}	11.848^{b}	0.165	0.021	0.001
N retention (%)	67.933	70.945	69.784	69.088	70.260	68.895^{a}	66.115^{b}	0.392	0.203	0.002
Metabolizability coefficient (%)										
DM (DMMC)	72.211	72.790	71.706	70.982	72.224	71.587	72.337	0.220	0.158	0.271
Ether extract (EEMC)[5]	86.157^{d}	88.203^{c}	87.477^{c}	87.618^{c}	88.203^{c}	87.478	87.585	0.177	0.007	0.766
Gross energy (GEMC)	77.648	77.603	77.030	77.619	76.738	76.045	77.060	0.549	0.253	0.070
Metabolizable energy (MJ/kg)										
Apparent (AME)[5]	14.366^{c}	14.184^{c}	14.195^{c}	13.547^{d}	13.879^{d}	13.922	14.147	0.048	0.001	0.080
Apparent corrected for N (AMEn)[5]	13.597^{c}	13.352^{c}	13.441^{c}	12.677^{d}	12.971^{d}	13.109	13.307	0.049	0.001	0.091

[ab] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the F test.
[cd] Means followed by different superscript letters within a row are significantly different at $P < 0.05$ by the Scott–Knott test.
[1] Corresponding to the excreta collection period (36 to 38 days of age of the broilers).
[2] There was no interaction ($P > 0.05$) between the dietary L-arginine concentration and broilers sex for the evaluated parameters.
[3] Linear effect of dietary L-arginine concentration on ingested N ($y = 17.660 \pm 0.183x$, $R^2 = 0.920$).
[4] Linear effect of dietary L-arginine concentration on N balance ($y = 11.839 + 0.141x$, $R^2 = 0.876$).
[5] Because the data did not fit linear or quadratic models, the Scott–Knott test for comparison of means ($P < 0.05$) was used to evaluate the effect of the dietary L-arginine concentration on the DMMC, the AME and the AMEn.

L-arginine. Therefore, in the present study, the effects of dietary L-arginine on reducing the serum triacylglycerol and cholesterol (total and LDL and VLDL fractions) levels may be related to the reduced gene expression of FAS and HMG-CoA.

Lipogenesis occurs mainly in the liver of poultry, and the enzymes that produce NADPH + H^+ molecules are considered lipogenic because NADPH + H^+ is the coenzyme that provides the reducing equivalents for lipid biosynthesis (Alvarez et al., 2000). The malic enzyme produces NADPH + H^+ through the oxidation of malate to pyruvate. In the present study, the linear reduction in abdominal fat deposition observed with increased dietary L-arginine concentration may be correlated with a linear reduction in malic enzyme activity in the liver. The effect of dietary L-arginine on reducing the percentage of abdominal fat in broilers was also observed by Fouad et al. (2013), Ebrahimi et al. (2014) and Sharifi et al. (2015), indicating that dietary L-arginine supplementation results in less diversion of nutrients and energy to the synthesis and deposition of body fat. The reduction in lipogenesis by dietary L-arginine has been associated with increased nitric oxide synthesis (Fouad et al., 2013; Ebrahimi et al., 2014), but the intracellular regulation mechanisms by which nitric oxide modulates lipid metabolism are not yet fully understood. In addition, it is important to highlight that the peroxisome proliferator-activated receptor gamma (PPARγ) is the main regulator of adipogenesis in poultry (Wang et al., 2017) and its transcription is up-regulated by L-arginine supplementation (Khalaji et al., 2013).

G6PD can also be initially considered a lipogenic enzyme because it catalyzes the conversion of glucose-6-phosphate + $NADP^+$ into 6-phosphoglycololactone + NADPH + H^+, representing an important regulatory point of the phosphate pentose pathway. However, in addition to NADPH, the phosphate pentose pathway generates other important compounds, such as ribose 5-phosphate, which is a precursor of the pentoses present in RNA and DNA. In a study with laying hens, Yuan et al. (2016) showed that dietary L-arginine exerts a regulatory effect on protein metabolism because dietary L-arginine supplementation stimulated protein synthesis and reduced the intracellular proteolysis rate in the liver of broilers. Thus, in the present study, the increase in G6PD activity in the liver of broilers fed diets containing L-arginine seems not to be associated with increased lipogenesis but rather with increased DNA and RNA synthesis, which is directly related to protein synthesis.

Dietary supplementation with L-arginine at a concentration of up to 12 g/kg did not alter the levels of moisture, CP, ether extract or collagen in the breast of broilers. According to Fouad et al. (2013), the intramuscular fat content in the broiler's breast was also not affected by L-arginine supplementation in the diet. In turn, Ebrahimi et al. (2014) found that dietary L-arginine increased the level of ether extract in the breast of broilers. However, the authors provided the experimental diets for 42 days, a period longer than that assessed in the present study (29 to 42 days of age).

Although dietary L-arginine has been shown to reduce the mineral matter level in the breast, no scientific studies explaining how dietary L-arginine can reduce the deposition of minerals in the muscle tissue of broilers have been found. There are some studies that report the effects of dietary L-arginine on mineral concentrations in certain organs and bones of rats (Seaborn and Nielsen, 2002; Suliburska et al., 2014), but the results are still incipient, indicating the need for further research on this topic.

The greater accumulation of abdominal fat observed in female broilers corroborates the results reported by Benyi et al. (2015) and Marx et al. (2016) and can be explained by the fact that females usually have a higher number of adipocytes in the abdominal region. This higher number of adipocytes may be considered an evolutionary biological adaptation that increases the fat storage capacity of females in the period before reproduction, which is important considering that the reproductive activity in females generally demands greater energy expenditure than that in males (Langslow and Lewis, 1974). Furthermore, the higher malic enzyme activity observed in the liver of the females indicates an intensification of lipogenesis due to the greater supply of reducing power (NADPH + H^+) for lipid biosynthesis and is directly related to the greater abdominal fat deposition in females.

In broiler production, the breast has high commercial value and is one of the main targets of breeding programmes (Lorentz et al., 2011). In addition to performance, breast quality should also be studied. In the present study, male broilers showed higher deposition of protein and mineral matter in the breast, corroborating evidence reported by Evans et al. (1976). The higher breast protein content in males may be associated with the higher nitrogen retention exhibited by these broilers.

Correlating the results of feed conversion, breast protein content, dietary nitrogen retention, abdominal fat deposition and malic enzyme activity in the liver, it is possible to conclude that male broilers diverted less dietary nutrients to abdominal fat deposition and allocated more nutrients to the synthesis of muscle protein, consequently resulting in higher breast and leg yields.

Experiment 2

The increase in N intake by broilers due to the inclusion of increasing dietary L-arginine concentrations is justified by the presence of four N atoms in each L-arginine molecule ($C_6H_{14}N_4O_2$). There was an increase in N balance because the increase in N intake was not accompanied by changes in the amount of N excreted. Therefore, there was a directly proportional relationship between the dietary L-arginine concentration and the use of absolute amounts of N by broilers. According to Khajali and Widerman (2010), the higher dietary L-arginine intake results in higher ornithine concentration, which subsequently can be used for biosynthesis of polyamines and of other metabolic intermediates with anabolic functions in the broilers. Increased N balance is indicative of greater synthesis and deposition of body protein (Wu, 2013). In this sense, the results of N balance associated with improved feed conversion and increased G6PD activity suggest that there was an increase in protein synthesis when the broiler diet was supplemented with L-arginine.

In turkeys fed diets supplemented with L-arginine, Oso et al. (2017) observed an increase in the height of the intestinal villus, explaining this result by the participation of L-arginine and its metabolic intermediates in several processes related to cell division, protein synthesis, intestinal development and tissue growth. As the increase in the intestinal villus promotes greater absorption of nutrients, this may be a possible explanation for the increase in the EEMC observed in the present study when the diet was supplemented with L-arginine at concentrations ranging from 3 to 12 g/kg. Results from broiler chickens also indicated that supplemental dietary L-arginine increased villus height and absorptive surface area in jejunum and ileum (Khajali et al., 2014). In addition, the beneficial effects of the supplemental L-arginine on the gut morphology and functionality have also been reported in broiler chickens challenged with pathogenic *Eimeria* species (Tan et al., 2014) and *Clostridium perfringens* (Zhang et al., 2018). These results are relevant because coccidiosis and necrotic enteritis are diseases that cause economic losses in the poultry industry.

There was lower energy utilization (AME and AMEn) of diets containing 9 and 12 g/kg of supplemental L-arginine. One hypothesis to explain these results is that high arginine supplementation may have reduced the lysine absorption rate because both compete for the same cation binding sites in the intestine (Closs et al., 2004). It is also known that L-arginine has a lower AME for poultry (2 863 kcal/kg) than does L-lysine (3 762 kcal/kg, Rostagno et al., 2011). In this sense, the lower utilization of lysine may have resulted in a higher gross energy in the excreta, consequently reducing AME and AMEn.

Therefore, it is concluded that male broilers have intrinsically better production outcomes than females. However, supplementing the diet of male or female broilers from 29 to 42 days of age with L-arginine at a concentration of 6.87 g/kg represents a nutritional strategy to improve feed conversion and reduce circulating levels of triacylglycerol and cholesterol, NADPH + H^+ synthesis by liver enzymes and abdominal fat deposition, without negatively affecting the carcass and noble cuts yield, the amount of nitrogen excreted by the chickens and the energy value of the feed.

Ethics approval

All experimental procedures were approved by the UFLA Ethics Committee on Animal Use under protocol no. 057/14.

Data and model availability statement

This article is part of a thesis, which is deposited in an official repository with open access: http://repositorio.ufla.br/jspui/handle/1/29082.

Author ORCIDs

S. T. Sobrane Filho: https://orcid.org/0000-0002-2849-6170.
E. M. da C. Lima: https://orcid.org/0000-0002-9278-5831.
D. H. de Oliveira: https://orcid.org/0000-0003-3669-4967.
M. L. T. de Abreu: https://orcid.org/0000-0001-5272-3233.
P. V. Rosa: https://orcid.org/0000-0002-0052-079X.
A. C. de Laurentiz: https://orcid.org/0000-0001-9553-730X.
L. de P. Naves: https://orcid.org/0000-0001-8067-829X.
P. B. Rodrigues: https://orcid.org/0000-0001-7188-709X.

Author contributions

S. T. Sobrane Filho defined the experimental design, reviewed the literature, coordinated and helped with the field experiment, performed the laboratory analysis, helped with the statistical analysis and helped with the write and format of the manuscript. E. M. da C. Lima and D. H. de Oliveira helped with the field experiment and laboratory analysis. M. L. T. de Abreu, P. V. Rosa and A. C. de Laurentiz provided the laboratories and equipment for analysis and helped with technical support. L. de P. Naves reviewed the literature and helped with technical support, writing and formatting the manuscript. P. B. Rodrigues defined the experimental design, obtained financial support, helped with the statistical analysis, supervised this study and reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no conflicts of interest.

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