Arginine : Lysine digestible ratio in diets of broiler chickens fed with protein reduced

Carlos Henrique de Oliveira*; Romário Duarte Bernardes*; Kelly Morais Maia Dias*; Horacio Santiago Rostagno*; Luiz Fernando Teixeira Albino*

* Department of Animal Science, Federal University of Viçosa, Brazil.

Introduction

For many years diets have been formulated to meet broiler requirements according to crude protein level which results in some amino acid levels above the recommendation. New technologies such as crystalline amino acids allow diets to be formulated according to ideal protein concept. Emmert & Baker (1997) determine that ideal protein concept is an exact balance of amino acids, without excess or deficiency. Researchers of Agricultural Research Council (ARC) established in 1981 that all essential amino acid requirements must be express as proportion of lysine. However, the ratio of the amino acids to lysine in diets is not permanent and could be influenced by various factors.

Arginine is an essential amino acid for broilers, since these animals are not able to synthetize it efficiently due to the absence of a functional urea cycle (Bender, 2012). Once the formation of acid uric is not arginine dependent, the dietary arginine in chicks is primarily used for synthesis of important molecules such as creatine, nitric oxide, glutamate, and polyamines which contributes to growth performance and feed efficiency (Castro et al., 2019). Several studies in different species have demonstrated that arginine supplementation can positively influence body weight gain (BWG), carcass yield and reduced fat deposition by its versatile role in animal organism (Wu et al., 2011; Fouad et al., 2013). Arginine also plays a crucial role in collagen synthesis by proline pathway, in other words, arginine supplementation can improve broilers skin quality reducing carcass penalty in slaughterhouses.

Although the exact mechanism has not yet been fully elucidated, it is known that there is an antagonism between arginine and lysine (Fernandes & Murakami, 2010). Therefore, raising the level of arginine in feed can alter the ideal metabolic balance between these both amino acids. For this reason, it is crucial establish the best ratio between arginine: lysine digestible ratio.

The aim of this study was to evaluate the effect of different arginine: lysine digestible ratio in diets with lower crude protein on the performance parameters, skin quality and creatine content in muscle of broilers during the period from 01 to 21 days old.

Material and mathods

All procedures adopted in this trial were previously approved by the UFV Ethics Commission on the Use of Farming Animals (CEUAP-UFV), protocol nº 01/2021, in agreement with regulation of the Council for Animal Experimentation Control (CONCEA, 2008). This research was carried out between January and February of 2021 in the Research Facilities of Poultry Production and Nutrition at Federal University of Viçosa, Viçosa city, Minas Gerais State, Brazil. A total of 1,540 one-day-old Cobb 500 male chicks were used, with initial body weight 37.25 ± 0.014 g. The male chicks were assigned in a completely randomized design with seven treatments and 10 replicates with 22 birds each.

The treatments with different arginine: lysine digestible ratio (Arg:Lys SID) met or exceed the nutritional requirement of broilers according to Rostagno et al (2017), except for arginine, lysine and crude protein level. The Arg:Lys SID were: 94; 100; 106; 112; 118 and 124%. Also a positive control (PC) diet with 107% of Arg:Lys SID completed the experimental design. The PC diet was formulated to meet or exceed the nutritional requirement of broilers during 1 to 21 days of age according to Rostagno et al. (2017). To reach 94% of Arg:Lys SID (Basal Diet), it was necessary to reduce the crude protein and lysine in 7.48% and 8.44%, respectively, when compared to PC diet (Table 1).

Table 1. Basal and positive control diets for male broilers from 01 to 21 days of age (%).

	Basal Diet	Positive Control
Corn	61.913	58.125
Soybean meal	19.296	24.128
Corn, gluten meal	6.000	6.000
Fish meal	3.000	3.000
Blood plasma porcine	2.000	2.000
Dicalcium phosphate	1.533	1.496
Limestone	0.898	0.881
Salt	0.133	0.132
Soybean oil	1.896	2.760
L- Glutamic acid, 99%	0.850	0.000
Choline chloride 60%	0.100	0.100
Starch	0.400	0.000
L- Lysine HCl, 79%	0.362	0.350
L-Methionine, 99%	0.285	0.241
Potassium carbonate	0.250	0.000
Sodium bicarbonate	0.250	0.250
L- Glycine, 98,5%	0.181	0.017
L- Threonine, 98,5%	0.107	0.041
L- Isoleucine, 98,5%	0.091	0.008
L- Valine, 96,5%	0.085	0.000
L- Tryptophan, 98%	0.025	0.000
L- Arginine, 98,5%	-	0.126
Vitamin premix[2]	0.150	0.150
Mineral premix[1]	0.130	0.130
Salinomycin3	0.055	0.055
BHT4	0.010	0.010
Total	**100.00**	**100.00**
Crude protein, %	21.38	23.11
AME, kcal/kg	3150	3150
Calcium, %	0.970	0.970
Phosphorus, %	0.460	0.460
Lysine SID, %	1.150	1.256
Methionine SID, %	0.590	0.590
Met +Cys SID, %	0.929	0.929
Arginine SID, %	1.081	1.344
Threonine SID, %	0.829	0.829
Tryptophan SID, %	0.226	0.226
Valine SID, %	0.967	0.967
Leucine SID, %	2.017	2.133
Isoleucine SID, %	0.842	0.842
Glycine + Serine SID, %	1.846	1.846

[1]Mineral Premix (per kg of diet): manganese – 77.0 mg, iron – 55.0 mg, zinc – 71.5 mg, copper – 11.0 mg, iodine – 1.10mg, selenium – 0.33 mg.
[2]Vitamin Premix (per kg of diet): vitamin A – 8,250 IU, vitamin D3 – 2,090 IU, vitamin E - 31.0 IU, vitamin B1 - 2.20 mg, vitamin B6 - 3.08 mg, pantothenic acid - 11.0 mg, biotin - 0.077 mg, vitamin K3 - 1.65 mg, folic acid - 0.77 mg, nicotinic acid - 33.0 mg, vitamin B12 - 0.013 mg, choline chloride (60%) - 1 g.
[3]Coccitax
[4]Butylated hydroxytoluene.
L-Arginine replaced starch in the basal diet
Treatments - Level of arginine SID: (1.08%; 1.150%; 1.219%; 1.288%; 1.357%; 1.426%)
Positive control: 1.344% of arginine SID

The birds and the diets were weighted, by pen, at day-21, to determine the feed intake (FI), body weight gain (BWG) and feed:gain ratio (FGR). At day-21, three birds/ pen were selected by average body weight with a range of ± 10 % for euthanasia, collect of skin samples according to Bilgili et al. (1993), to evaluate skin thickness (ST) by micrometer (mm). Additionally, samples of breast muscle (Pectoralis profundus) were collected to evaluate creatine in muscle (CM) using the Kit Bioassay Systems® Creatine Assay. This methodology is based on enzymatic reactions leading to formation of a pink colored product when mixed with the samples. The fluorescence intensity is directly proportional to the creatine concentration in the samples which were read by a reader, expressing the creatine level in muscle in ppm.

Statistical Analysis

Data were submitted to analysis of variance and regression applied to the six different Arg:Lys SID (94; 100; 106; 112; 118 and 124%). Dunnett Test was used to compare the six levels of Arg:Lys SID with the positive control (PC) treatment. The significance of the effects was tested by $P < 0.05$.

Results

The results of performance, skin thickness and creatine in muscle of broilers at 21 days of age, can be seen in Table 2. Broilers fed with diets containing 94, 100 and 106% of Arg:Lys SID had lower BWG than the ones fed with the PC diet ($P < 0.05$). Furthermore, birds fed with 94% of Arg:Lys SID showed worse FGR than the broilers fed with PC diet ($P < 0.05$). A linear effect ($P < 0.05$) of Arg:Lys SID was observed on BWG and FGR (Figure 1a and 1b). There was no effect of the treatments on the FI ($P > 0.05$).

ST was lower in broilers fed with 94% and 100% of Arg:Lys SID than the ones fed with PC diet ($P < 0.05$). On the other hand, broilers fed with 118% and 124% of Arg:Lys SID achieved higher ST than broilers fed with PC diet ($P < 0.05$). A linear increasing effect ($P < 0.05$) of Arg:Lys SID was found on ST (Figure 1c).

CM was lower in broilers that received the treatments 94; 100; 106 and 112% of Arg:Lys SID when compared PC treatment ($P < 0.05$), whereas the broilers fed with 124% of Arg:Lys SID showed higher CM than the ones from PC ($P < 0.05$). Only the treatment with 118% Arg:Lys SID showed same CM of broilers than PC. A linear increasing effect ($P < 0.05$) of Arg:Lys SID was observed on CM (Figure 1d).

Table 2. Means of body weight gain (BWG), feed intake (FI), feed: gain ratio (FGR), skin thickness (ST), and creatine in muscle (CM) of broilers at 21 days of age fed with different levels of Arg:Lys SID.

Traits	Arg:Lys SID (%)							p-value		SE
	94	100	106	112	118	124	PC	ANOVA	Lin	
FI (kg)	1.173	1.154	1.147	1.175	1.160	1.176	1.183	NS	NS	0.006
BWG (kg)	0.907*	0.918*	0.922*	0.945	0.946	0.961	0.963	<0.001	<0.001	0.004
FGR	1.294*	1.257	1.246	1.243	1.227	1.223	1.228	0.002	0.013	0.004
ST (mm)	0.434*	0.508*	0.576	0.639	0.812*	0.918*	0.592	<0.001	<0.001	0.020
CM (ppm)	0.151*	0.153*	0.160*	0.184*	0.234	0.265*	0.223	<0.001	<0.001	0.006

* Means in the same row differ from positive control (PC) by Dunnett Test ($P < 0.05$).
NS = not significant.
SE = standard error.

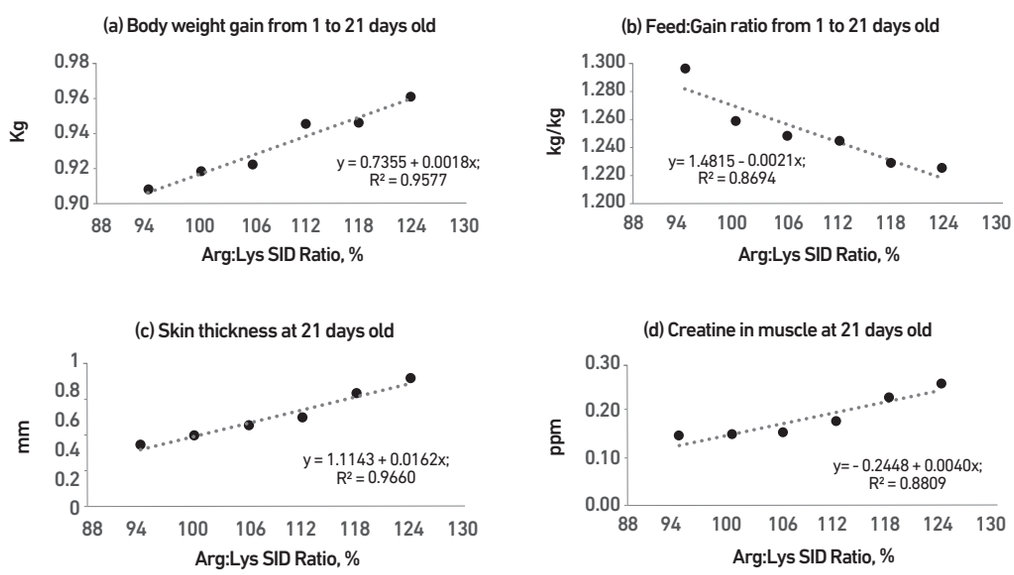

Figure 1. BWG (a) and FGR (b); ST (c) and CM (d) in broilers at 21 days fed with different levels of Arg:Lys SID.

Discussion

Arginine plays an important physiological role in broiler growth. On the other hand, the mechanism of arginine in muscle has not been fully elucidated, there is evidence that arginine influences muscle tissue by increasing growth hormone (GH) and insulin-like growth factor-1 (IGF-1) expression, besides activating some proteins of mTOR pathway (Oh et al., 2017; Castro et al., 2019). It is known that both GH and IGF-1 are key growth stimulating hormones in animals. In addition, mTOR pathway regulates numerous components involved in protein synthesis and its activation induces overall body growth. In the present study, BWG increased linearly with increasing Arg:Lys SID in the diets since arginine may have contributed to a positive protein turnover. Moreover, FGR improved linearly since there was an upward trend in the FI, but did not differ from the treatments.

To synthesize creatine in animal body, arginine and glycine are condensed to guanidinoacetic acid (GAA) and ornithine, in the kidney and pancreas by arginine: glycine amidinotransferase. Subsequently, GAA is methylated at the amidino group to form creatine (Majdeddin et al., 2020). Therefore, arginine plays an important role in creatine synthesis.

The creatine-phosphocreatine system functions as a rapidly mobilizable reserve of high-energy phosphate especially in skeletal muscle cells (Portocarero & Braun, 2021). Some fast-growing species such as broiler chickens demand high regeneration of ATP, therefore the demand of higher creatine seemed to be necessary to supply the muscle growth. In the present study, the CM in the Pectoralis profundus increased linearly with Arg:Lys SID.

Collagen is the major protein component of dermal connective tissue in broilers, and its main function is maintaining the structural integrity and elasticity of animal skin (Pines et al., 1996). Collagen contains specific amino acids such as glycine, proline and hydroxyproline. Proline can be synthesized in the animal organism from either glutamate or ornithine (Bender, 2012). Therefore, arginine, as a source of ornithine, contributes to proline synthesis and therefore in collagen synthesis. In the present study, the ST of broilers slaughtered at 21 days increased linearly with Arg:Lys SID. The ST is a variable directly proportional to the quality of the skin, offering better resistance and elasticity.

Animals from treatments with 94% and 100% of Arg:Lys SID showed thinner skin than the ones from PC, whereas birds from treatments with 118% and 124% of Arg:Lys SID showed thicker skin.

Conclusion

Broilers fed with 124% of Arg:Lys SID in diet with crude protein reduced linearly increases BWG, FGR, ST and CM of broilers at 21 days of age. From 112% of Arg:Lys SID seems to be possible to compensate for the reduction in crude protein and lysine at 7.48% and 8.44%, respectively in the starter phase of broilers. On the other hand, more studies must be carried out to better understand the mechanisms of arginine on the reduction of crude protein concept.

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