



Digestible lysine levels obtained by two methods of formulation of diets for 22-to-42-day-old broilers

Will Pereira de Oliveira¹, Rita Flávia Miranda de Oliveira Donzele¹, Juarez Lopes Donzele¹, Luiz Fernando Teixeira Albino¹, Marcus Vinícius de Lima Antunes¹, Paulo Henrique Reis Furtado Campos¹, Matheus Faria de Souza¹, Silvana Marques Pastore¹

¹ Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, MG, Brasil.

ABSTRACT - The objective of this study was to evaluate the effect of digestible lysine levels in diets with or without supplementation of industrial amino acids on performance, carcass characteristics and nitrogen excretion in broilers of 22 to 42 days of age. Birds were distributed in a completely randomized experimental design in a 4 × 2 factorial arrangement, with four digestible lysine levels (9.0, 10.0, 11.0 and 12.0 g/kg) and two methods to obtain the lysine levels (variation in the proportion of corn and soybean meal, without supplementation; or supplementation of industrial amino acids), eight replicates and 20 birds per replicate. There was an interaction effect on the performance characteristics and on the weights and yields of prime cuts. In both diets, feed conversion improved linearly as the lysine levels were increased. Feed intake; weight gain; carcass, thigh and drumstick weights; and boneless breast yield increased and abdominal fat reduced linearly as the lysine levels were increased in the unsupplemented diet. The lysine levels of the supplemented diets linearly reduced the yield of drumstick and quadratically reduced the yields of bone-in and boneless breast up to the estimated levels of 10.4 and 10.7 g/kg, respectively. Diets without supplementation increased the excretion and retention of nitrogen. The levels of 9.0 and 12.0 g/kg digestible lysine obtained with supplementation of industrial amino acids and without it, respectively, provide the best performance and yield of prime cuts in the birds. Diets in which the digestible lysine levels are obtained without supplementation provide better performance responses and carcass characteristics compared with supplemented diets.

Key Words: crude protein, ideal protein, industrial amino acids, performance

Introduction

Over the years, animal nutritionists have developed alterations in the quality of diets intended for broilers aiming to increasingly adapt these diets to meet the nutritional requirements of these birds. Studies on the protein nutrition of broilers have considered the use of digestible amino acids in the formulation of diets, which makes it possible to reduce the amount of dietary protein and improve the adjustment between amino acids, thus contributing to decreasing the nitrogen excretion by the bird (Vasconcellos et al., 2011). However, to apply this practice, it is necessary to properly determine the requirements of amino acids by birds so that this supply of nutrients in the diet is adequate to ensure good performance rates.

To obtain an ideal balance between the dietary amino acids, lysine has been adopted as the standard amino acid,

based on which the amounts of the other essential amino acids are expressed as percentage (Baker and Han, 1994). Thus, the lysine requirements must be carefully determined to avoid inaccuracy in the results, which would lead to imbalance between the other essential amino acids of the diets and a consequent decrease in broiler performance (Rostagno et al., 1999).

Reduction of the dietary crude protein and supplementation of industrial amino acids has been commonly practiced in most of the studies whose objective was to determine the lysine requirements of broilers (Martinez et al., 2002; Lana et al., 2005; Garcia et al., 2006). In addition, this technique enables greater flexibility in adjusting the amino acid levels of the diet. However, several researchers have reported that birds fed diets with lower crude protein levels presented inferior performance to birds fed higher crude protein contents even when the ratios between the essential amino acids were kept constant (Assis et al., 2008; Oliveira et al., 2009), demonstrating that this method may not be suitable to determine the lysine requirement of broilers.

Therefore, the objective of this study was to determine the digestible lysine requirements for 22-to-42-day-old broilers using two methodologies: diets with

Received February 21, 2014 and accepted July 25, 2014.

Corresponding author: willpdeoliveira@yahoo.com.br

<http://dx.doi.org/10.1590/S1516-35982014001100004>

Copyright © 2014 Sociedade Brasileira de Zootecnia. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

supplementation of industrial amino acids, and diets in which the proportions of corn and soybean meal varied.

Material and Methods

This experiment was conducted at the Poultry Sector of the Department of Animal Science of Universidade Federal de Viçosa (UFV), located in Viçosa, MG, Brazil. It was conducted in accordance with applicable laws governing the care and use of animals. All procedures were performed in compliance with relevant laws and institutional guidelines.

A total of 1,280 male Cobb broiler chickens at 22 days of age and with an average initial weight of 787.5 g were distributed into pens provided with a bed, a tubular trough and a nipple drinker. The experiment was conducted in a completely randomized design in a 4 × 2 factorial arrangement (four lysine levels and two methods to obtain lysine), eight replicates and 20 birds per replicate.

Table 1 - Ingredients of the experimental diets whose lysine levels were obtained by modifying the proportions of corn and soybean meal

Ingredient (g/kg)	Digestible lysine level (g/kg)			
	9.0	10.0	11.0	12.0
Corn	639.68	592.92	546.16	499.37
Soybean meal	286.55	325.79	365.07	404.35
Vegetable oil	36.62	44.13	51.61	59.11
Dicalcium phosphate	16.58	16.34	16.09	15.85
Limestone	8.41	8.29	8.16	8.03
Common salt	4.78	4.78	4.78	4.79
Vitamin mix ¹	3.00	3.00	3.00	3.00
Mineral mix ²	1.00	1.00	1.00	1.00
Anticoccidial ³	0.55	0.55	0.55	0.55
Antioxidant ⁴	0.10	0.10	0.10	0.10
Choline chloride ⁵	1.25	1.25	1.25	1.25
Antibiotic ⁶	0.05	0.05	0.05	0.05
DL-methionine	1.43	1.80	2.18	2.55
Total	1,000.00	1,000.00	1,000.00	1,000.00
Calculated composition				
Crude protein (g/kg)	189.00	204.50	220.00	236.00
Metabolizable energy (kcal/kg)	3150	3150	3150	3150
Digestible lysine (g/kg)	9.00	10.00	11.00	12.00
Digestible methionine + cystine (g/kg)	6.48	7.21	7.93	8.46
Digestible threonine (g/kg)	6.42	6.96	7.50	8.05
Digestible tryptophan (g/kg)	2.06	2.28	2.49	2.70
Digestible valine (g/kg)	8.00	8.66	9.32	9.98
Digestible isoleucine (g/kg)	7.41	8.11	8.81	9.51
Digestible arginine (g/kg)	11.90	13.10	14.30	15.51
Sodium (g/kg)	2.08	2.08	2.08	2.08
Calcium (g/kg)	8.37	8.37	8.37	8.37
Available phosphorus (g/kg)	4.18	4.18	4.18	4.18

¹ Vitamin supplement - guaranteed levels per kg of diet: vitamin A - 10,000 IU; vitamin D3 - 2,000 IU; vitamin E - 30 IU; vitamin B1 - 2 mg; vitamin B6 - 4 mg; pantothenic acid - 12 mg; biotin - 0.1 mg; vitamin K - 3 mg; folic acid - 1 mg; nicotinic acid - 50 mg; vitamin B12 - 15 mcg; selenium - 0.25 mg.

² Mineral supplement - guaranteed levels per kg of diet: manganese - 80 mg; iron - 50 mg; zinc - 50 mg; copper - 10 mg; cobalt - 1 mg; iodine - 1 mg.

³ Salinomycin sodium, 60 ppm.

⁴ Butylated hydroxytoluene.

⁵ Choline chloride 60%.

⁶ Avilamycin 10%.

Birds were fed the experimental diets (Tables 1 and 2) formulated based on corn and soybean meal, supplemented with minerals and vitamins so as to contain 9.0, 10.0, 11.0 and 12.0 g/kg digestible lysine, with 3150 kcal of metabolizable energy. The lysine levels were obtained in two forms: supplementation with industrial amino acids, maintaining the same amounts of corn and soybean meal (supplemented diet), and by changing the proportion of corn and soybean meal (unsupplemented diet). The other essential amino acids were supplemented as their ratios with digestible lysine became lower than those recommended on the "ideal protein concept" established by Rostagno et al. (2005) (Table 3). Experimental diets and water were offered

Table 2 - Ingredients of the experimental diets whose lysine levels were obtained by supplementation with industrial amino acids

Ingredient (g/kg)	Digestible lysine level (g/kg)			
	9.0	10.0	11.0	12.0
Corn	619.79	619.79	619.79	619.79
Soybean meal	287.78	287.78	287.78	287.78
Vegetable oil	43.80	43.80	43.80	43.80
Dicalcium phosphate	16.65	16.65	16.65	16.65
Limestone	8.37	8.37	8.37	8.37
Common salt	4.79	4.79	4.79	4.79
Vitamin mix ¹	3.00	3.00	3.00	3.00
Mineral mix ²	1.00	1.00	1.00	1.00
Anticoccidial ³	0.55	0.55	0.55	0.55
Antioxidant ⁴	0.10	0.10	0.10	0.10
Choline chloride ⁵	1.25	1.25	1.25	1.25
Antibiotic ⁶	0.05	0.05	0.05	0.05
DL-methionine	1.47	2.20	2.94	3.67
Kaolin	11.40	9.37	5.85	0.70
L-lysine HCL	-	1.30	2.58	3.88
L-threonine	-	-	0.72	1.43
L-valine	-	-	0.64	1.43
L-isoleucine	-	-	0.14	0.82
L-arginine	-	-	-	0.94
Total	1,000.00	1,000.00	1,000.00	1,000.00
Calculated composition				
Crude protein (g/kg)	188.00	188.00	188.00	188.00
Metabolizable energy (kcal/kg)	3150	3150	3150	3150
Digestible lysine (g/kg)	9.00	10.00	11.00	12.00
Digestible methionine + cystine (g/kg)	6.49	7.20	7.93	8.64
Digestible threonine (g/kg)	6.39	6.50	7.15	7.80
Digestible tryptophan (g/kg)	2.06	2.06	2.06	2.06
Digestible valine (g/kg)	7.97	7.97	8.48	9.24
Digestible isoleucine (g/kg)	7.39	7.39	7.39	8.04
Digestible arginine (g/kg)	11.88	11.88	11.88	11.88
Sodium (g/kg)	2.08	2.08	2.08	2.08
Calcium (g/kg)	8.37	8.37	8.37	8.37
Available phosphorus (g/kg)	4.18	4.18	4.18	4.18

¹ Vitamin supplement - guaranteed levels per kg of diet: vitamin A - 10,000 IU; vitamin D3 - 2,000 IU; vitamin E - 30 IU; vitamin B1 - 2 mg; vitamin B6 - 4 mg; pantothenic acid - 12 mg; biotin - 0.1 mg; vitamin K - 3 mg; folic acid - 1 mg; nicotinic acid - 50 mg; vitamin B12 - 15 mcg; selenium - 0.25 mg.

² Mineral supplement - guaranteed levels per kg of diet: manganese - 80 mg; iron - 50 mg; zinc - 50 mg; copper - 10 mg; cobalt - 1 mg; iodine - 1 mg.

³ Salinomycin sodium, 60 ppm.

⁴ Butylated hydroxytoluene.

⁵ Choline chloride 60%.

⁶ Avilamycin 10%.

Table 3 - Composition in total and digestible amino acids, and dry matter and crude protein of the ingredients of the experimental diets

	Corn (g/kg)		Soybean meal (g/kg)	
	Total amino acids ¹	Digestible amino acids ²	Total amino acids ¹	Digestible amino acids ²
Lysine	2.03	1.84	29.73	26.96
Methionine + cystine	2.82	2.56	13.65	12.37
Threonine	2.48	2.15	20.28	17.57
Isoleucine	2.21	1.97	22.62	20.20
Arginine	3.23	3.02	36.36	33.95
Valine	3.17	2.84	23.15	20.72
Tryptophan	0.70	0.64	6.40	5.81
Leucine	7.50	6.80	37.88	34.35
Phenylalanine	2.79	2.53	25.19	22.82
Histidine	2.01	1.83	12.99	11.80
Dry matter	915.30		928.70	
Crude protein	70.80		476.40	

¹ Determined at the laboratory of AJINOMOTO - Biolatina.

² Calculated based on the digestibility coefficients recommended by Rostagno et al. (2005).

ad libitum. During the entire experimental period the birds were under a photoperiod of 24 hours of light daily.

The environmental conditions within the shed were monitored and recorded five times daily (at 7.30 h, 10.00 h, 12.30 h, 15.00 h and 17.30 h) by two sets of thermometers (dry bulb, wet bulb and black globe) kept in the center of the shed at the height of the birds. The collected data were converted to WBGT (Wet Bulb Globe Temperature Index) to characterize the environment, as proposed by Buffington et al. (1981).

The birds were weighed at the beginning and end of the experimental period to determine weight gain (WG). Feed intake (FI) was calculated as the difference between the total feed supplied and the feed left in the troughs. Based on the FI and WG, the feed conversion (FC) was calculated. At the end of the experimental period (42 days of age), three birds of each experimental unit with the closest weights to the average of the experimental unit (up to 10% above or below the average) were feed-deprived for 12 hours. After this period, they were subjected to the commercial slaughter procedures, where they were hung on a shackle, stunned by electronarcosis and manually slaughtered by sectioning their neck. Next, they were mechanically scalded and plucked and manually eviscerated. Carcasses were weighed

to evaluate the yield of the prime cuts (breast, drumstick and thigh) and abdominal fat.

The live weight after fasting was considered for the determination of carcass yield. Carcass weight was adopted to determine the yields of prime cuts and abdominal fats.

An additional group of 200 male chickens of the same strain, from 29 to 34 days of age, was used in an experiment to study the nitrogen balance. At 25 days of age, the birds were transferred to cages of 0.45 × 0.50 × 0.45 m (length, width and height, respectively) provided with nipple-type drinkers and gutter-type troughs, where they remained for an acclimation period of four days, on the site where the biological trial was conducted.

The experimental period began at the 29th day of age, lasting five days. Birds were distributed into a completely randomized experimental design with five treatments (lysine levels), eight replicates and five birds per replicate. Treatments consisted of a basal diet containing 9.0 g/kg digestible lysine (Table 2) and another four diets containing two levels of digestible lysine (10.0 and 12.0 g/kg) obtained by the two diet-formulation methods (Tables 1 and 2). Feed and water were provided *ad libitum* through the pre-experimental and experimental periods.

Excreta were collected twice daily, at 7.30 h and 17.30 h, stored in plastic bags and frozen at -5 °C.

Diets and excreta were chemically analyzed to determine the nitrogen content according to Silva and Queiroz (2002). The statistical analyses of feed intake, weight gain, feed conversion and absolute and relative weights of prime cuts and abdominal fats were performed using the SAEG (Sistema para Análises Estatísticas, version 7.0) computer software, developed at Universidade Federal de Viçosa. Performance and carcass characteristics were submitted to variance analysis at 5% probability. The digestible lysine requirement was estimated using polynomial regression models and/or Linear Response Plateau (LRP), according to the best fit of the data for each variable.

Results and Discussion

The Wet Bulb Globe Temperature index (WBGT) calculated for the experimental period (Table 4)

Table 4 - Climatic variables in the shed during the experimental period

Variable	Time of reading				
	07.00 h	10.00 h	12.30 h	15.00 h	17.30 h
Air temperature (°C)	22.1±1.09	26.0±1.62	28.2±2.44	27.4±3.57	25.8±2.23
Relative air humidity (%)	85.5±5.65	71.8±9.58	66.1±10.22	68.0±14.35	75.7±10.74
Wet Bulb Globe Temperature index	70.7±1.39	74.9±1.89	77.5±2.65	76.3±3.65	74.7±2.56

characterized, according to Medeiros et al. (2005), the thermal environment where the birds were kept as thermoneutral. However, the temperatures recorded at 12.30 h and 15.00 h were higher than those recommended in the manual of the strain (Cobb 500) to ensure thermal comfort (22 to 25 °C) to birds from 22 to 42 days of age (COBB, 2005). Thus, the heat stress periods that the birds underwent might have influenced the voluntary feed intake and interfered with the weight gain rate and their prime cuts yield (Furlan, 2006).

There was an interaction effect between lysine levels and diet-formulation methods on the results of feed intake, lysine intake, weight gain and feed conversion of the 22-to-42-day-old broilers (Table 5).

Table 5 - Effect of lysine levels and diet-formulation methods on the performance of 22-to-42-day-old broilers

Factor	Evaluated parameter			
	Feed intake (g)	Lysine intake (g)	Weight gain (g)	Feed conversion (g/g)
Lysine level (g/kg)				
9.0	2939.96	26.46	1212.35	2.44
10.0	3021.41	30.21	1315.90	2.30
11.0	3034.70	33.38	1404.88	2.19
12.0	3039.16	36.47	1462.84	2.10
Diet-formulation method				
CSMa	3049.60	32.12	1440.68	2.15
CSM + IAAb	2968.02	31.15	1257.30	2.40
Probability				
Lysine level	0.07	0.01	0.01	0.01
Diet-formulation method	0.01	0.01	0.01	0.01
Lysine level × DFM	0.01	0.01	0.01	0.01
CV (%)	3.90	3.76	6.18	5.62

CSM - corn + soybean meal; IAA - industrial amino acids; DFM - diet-formulation method.

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

No effect ($P > 0.05$) of lysine level was observed on the feed intake (FI) of the birds fed diets supplemented with industrial amino acids (Table 6). A similar result was obtained by Assis et al. (2008), who did not find variation in the FI of broilers kept in a thermoneutral environment from 22 to 42 days of age according to the lysine levels (9.0, 10.0, 11.0 and 12.0 g/kg) obtained by supplementation with industrial amino acids. Similarly, Viola et al. (2009a) did not observe differences in the FI of broilers evaluating increasing lysine levels (7.0 to 12.2 g/kg) in a diet supplemented with industrial amino acids from 19 to 40 days of age.

The feed intake of the broilers fed the unsupplemented diet increased linearly ($P < 0.01$) according to the equation $\hat{Y} = 2246.43 + 76.4919X$ ($r^2 = 0.64$), as the concentration of lysine in the diet was elevated. This result differs from that found by Assis et al. (2008), who did not observe effect of lysine levels on the FI of broilers fed a diet in which the lysine levels were obtained only by using different levels of corn and soybean meal in the diet. In the same way, it differs from the results found by Aletor et al. (2000), who did not observe influence of lysine levels on the FI of broilers in the same period when they were fed diets whose lysine levels were increased from 10.1 to 12.3 g/kg, without the use of industrial lysine.

The absence of effect of the increasing lysine levels on feed intake in the supplemented diet may be related to the industrial amino acid content of the diet. Since industrial amino acids need not be digested, they are more rapidly absorbed than the amino acids from the intact protein, and so they can result in an imbalanced amino acid mix, unsuitable for incorporation into the muscle protein (Colina et al., 2002; Yen et al., 2004; Nonis and Gous, 2006). In such cases, it is possible that both the levels of industrial amino acids and their metabolites (ammonia) levels in the blood

Table 6 - Performance of 22-to-42-day-old broilers according to the levels of digestible lysine in the diet

Parameters	Lysine levels (g/kg)				\bar{X}	CV (%)
	9.0	10.0	11.0	12.0		
CSMa						
Feed intake (g) ¹	2876	3062	3164	3097	3050	3.55
Lysine intake (g) ¹	25.88	30.62	34.80	37.16	32.12	3.34
Weight gain (g) ²	1190	1377	1584	1611	1441	5.67
Feed conversion ¹	2.42	2.23	2.00	1.93	2.15	4.56
CSM + IAAb						
Feed intake (g)	3004	2981	2905	2982	2968	4.24
Lysine intake (g) ¹	27.04	29.81	31.96	35.78	31.15	4.17
Weight gain (g)	1234	1255	1226	1314	1257	6.77
Feed conversion ³	2.45	2.38	2.38	2.27	2.40	6.35

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

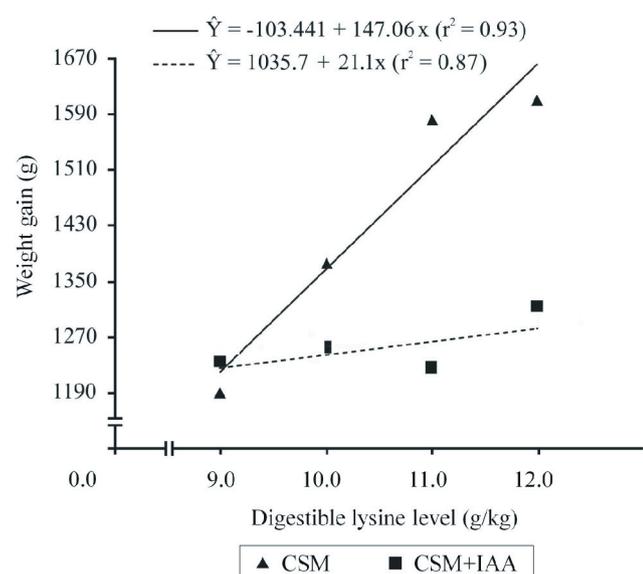
CSM - corn + soybean meal; IAA - industrial amino acids.

^{1, 2 and 3} Linear effect ($P < 0.01$), ($P < 0.02$) and ($P < 0.05$), respectively.

stream act as a sign to regulate feed intake (Namroud et al., 2008). Therefore, to determine the lysine requirement for broilers using high levels of industrial amino acids seems not to be appropriate since other sources of variation may interfere with the results.

Lysine intake increased ($P < 0.01$) linearly according to equations $\hat{Y} = 1.3519 + 2.83756X$ ($r^2 = 0.99$) and $\hat{Y} = -7.806 + 3.8022X$ ($r^2 = 0.98$), respectively, for the broilers fed supplemented and unsupplemented diets. The greater lysine intake shown by the chickens fed the unsupplemented diet is a reflection of the greater feed intake displayed by them compared with the birds fed the diet supplemented with industrial amino acids.

The lysine levels of the supplemented levels did not affect ($P > 0.05$) the weight gain (WG) of the broilers (Figure 1). On the other hand, the WG of the birds fed the unsupplemented diet increased linearly ($P < 0.03$). We could observe that the unsupplemented diet provided an increase of 35.4% in WG between the highest and lowest level of lysine assessed (9.0 and 12.0 g/kg), while the supplemented diet provided an increase of 6.5% in WG. This fact demonstrated that the unsupplemented diet was more effective in promoting WG in broilers. These results corroborate those found by Assis et al. (2008), who worked in a thermoneutral environment, and Balbino et al. (2008), who worked in a heat-stress environment with broilers in the period of 22 to 42 days of age and observed linear increase in WG as the lysine levels were increased. These results indicate that the methodology employed in the formulation of diets can interfere with the determination of the lysine



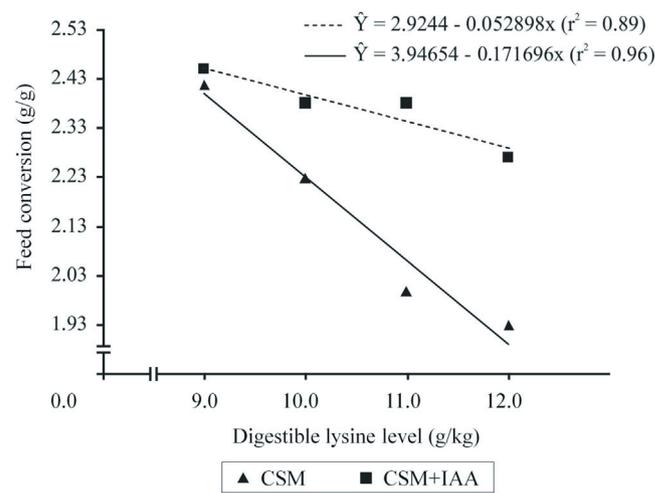
CSM - diet formulated with corn and soybean meal.
CSM + IAA - diet formulated with corn and soybean meal + industrial amino acids.

Figure 1 - Weight gain of 22-to-42-day-old broilers fed diets containing different lysine levels.

requirements of broilers. Besides, the level of 15.2 g/kg lysine estimated by Campestrini et al. (2008) for a greater response in WG makes it possible to infer, together with the results of the present study, that the lysine requirement of modern broilers may be higher than that recommended in the conventional tables of nutritional requirements (NRC, 1994; Rostagno et al., 2005).

Feed conversion (FC) was affected by the lysine levels of the supplemented ($P < 0.05$) and unsupplemented diets ($P < 0.01$), and improved linearly as the concentrations of lysine were elevated (Figure 2). Positive effect of increase in the dietary lysine levels, with or without supplementation of industrial amino acids, on the FC of growing broilers has been reported by several researchers (Leclercq et al., 1998; Rezaei et al., 2004; Lana et al., 2005; Costa et al., 2006; Assis et al., 2008; Balbino et al., 2008; Viola et al., 2009a; Ghahri et al., 2010).

Although the lysine levels positively affected the feed conversion of the birds, the intensity of the improvement observed for this parameter varied according to the type of diet. While the chickens fed the unsupplemented diets showed an improvement of 20.2% in FC, the broilers fed the supplemented diet demonstrated an improvement of 7.3% between the lowest and highest lysine level evaluated (9.0 and 12.0 g/kg). This result is in agreement with Assis et al. (2008) and Balbino et al. (2008), who also observed greater intensity in the improvement of this parameter in the birds fed diets whose lysine levels were obtained by varying the concentrations of corn and soybean meal in relation to the diet supplemented with industrial amino acids.



CSM - diet formulated with corn and soybean meal.
CSM + IAA - diet formulated with corn and soybean meal + industrial amino acids.

Figure 2 - Feed conversion of 22-to-42-day-old broilers fed diets containing different lysine levels.

Since the supplemented diet was formulated to meet the requirements of birds maintaining the ratios between amino acids, as proposed by Rostagno et al. (2005), the fact that it provided lower performance than the unsupplemented diet allows us to infer that there may be some imbalance in the ratios between the essential amino acids and the digestible lysine, or that there was deficiency of some amino acid deemed dispensable, especially when higher digestible lysine levels are used. Considering the productive potential of the current broilers, it is possible that the endogenous synthesis of amino acids once thought dispensable is not sufficient to meet the requirement for the growth of these birds.

There was an effect of interaction between the lysine levels and the diet-formulation methods on the absolute and relative weights of carcass, drumstick, thigh, bone-in breast and boneless breast in 22-to-42-day-old broilers (Tables 7 and 8). However, no interaction effect was observed on the absolute and relative weights of abdominal fat.

The absolute and relative weights of the carcass (Table 9) of the birds fed the diet supplemented with industrial amino acids were not influenced ($P>0.05$) by the dietary lysine levels. Contrary to this result, Assis et al. (2008) and Balbino et al. (2008) observed linear increase in the absolute carcass weight of broilers as the levels

Table 7 - Effect of lysine levels and diet-formulation method on the weights of carcass, prime cuts and abdominal fat of 22-to-42-day-old broilers

Factors	Evaluated parameters					
	Carcass	Bone-in breast	Boneless breast	Drumstick	Thigh	Abdominal fat
Lysine level (g/kg)						
9.0	1534.66	482.15	360.32	189.00	195.05	28.20
10.0	1623.09	510.93	378.67	199.84	201.84	29.70
11.0	1664.78	522.41	393.61	207.44	211.71	27.51
12.0	1726.10	550.37	409.73	215.61	216.93	24.90
Diet-formulation method						
CSMa	1702.88	541.25	406.68	212.68	216.93	26.15
CSM + IAAb	1575.68	493.28	365.80	193.87	196.47	28.95
Probability						
Lysine level	0.00001	0.00001	0.00001	0.00001	0.00001	0.21651
Diet-formulation method	0.00001	0.00001	0.00001	0.00001	0.00001	0.08997
Lysine level × DFM	0.00001	0.00001	0.00001	0.00001	0.00001	0.32460
CV (%)	5.61	7.78	9.15	9.14	10.80	38.12

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

CSM - corn + soybean meal; IAA - industrial amino acids; DFM - diet-formulation method.

Table 8 - Effect of the lysine levels and diet-formulation methods on the yields of carcass, prime cuts and abdominal fat of 22-to-42-day-old broilers

Factors	Evaluated parameters					
	Carcass	Bone-in breast	Boneless breast	Drumstick	Thigh	Abdominal fat
Lysine level (g/kg)						
9.0	79.13	31.87	1.74	12.51	12.91	1.88
10.0	79.78	31.89	2.05	12.48	12.62	1.86
11.0	79.94	31.79	1.60	12.61	12.87	1.70
12.0	79.85	32.35	1.65	12.65	12.72	1.48
Diet-formulation method						
CSMa	79.96	32.22	24.20	12.66	12.91	1.58
CSM + IAAb	79.41	31.74	23.54	12.47	12.66	1.87
Probability						
Lysine level	0.29411	0.20935	0.25013	0.35051	0.37737	0.02998
Diet-formulation method	0.07028	0.09087	0.01875	0.11148	0.17309	0.00001
Lysine level × DFM	0.09043	0.00016	0.01582	0.30006	0.04994	0.40139
CV (%)	2.64	5.24	7.15	6.24	9.21	39.17

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

CSM - corn + soybean meal; IAA - industrial amino acids; DFM - diet-formulation method.

of the lysine from the supplemented diet were elevated, although they did not observe variations in the relative weight.

The lysine levels had an impact ($P<0.01$) on the absolute weight of the carcass of the birds fed the unsupplemented diets; this variable increased linearly according to the equation $\hat{Y} = 495.703 + 115.796X$ ($r^2 = 0.92$). This result is in line with those found by Assis et al. (2008) and Balbino et al. (2008), who obtained linear increase in the absolute weight of the carcass as the lysine levels were increased in a corn- and soybean meal-based diet without supplementation of industrial amino acids.

The yield of carcasses from the birds fed the unsupplemented diet increased linearly ($P<0.01$) as a function of the lysine levels, according to the equation: $\hat{Y} = 73.6546 + 0.604873X$ ($r^2 = 0.83$). This result differs from those found by Assis et al. (2008) and Balbino et al. (2008), who did not find effect of lysine levels on the carcass yield of broilers fed a diet not supplemented with industrial amino acids.

The lysine levels of the diet supplemented with industrial amino acid had a quadratic effect ($P<0.01$)

on the absolute weights of bone-in and boneless breast (Table 9), which reduced up to the estimated levels of 10.3 and 10.5 g/kg, respectively, according to the equations $\hat{Y} = 1748.05 - 246.381X + 11.9557X^2$ ($r^2 = 0.93$) and $\hat{Y} = 1311.07 - 182.342X + 8.70052X^2$ ($r^2 = 0.98$). The yield of bone-in and boneless breast was also influenced ($P<0.01$) by the lysine levels in the supplemented diet, and reduced up to the estimated levels of 10.4 and 10.7 g/kg, respectively, according to the equations: $\hat{Y} = 88.9764 - 11.1077X + 0.533127X^2$ ($r^2 = 0.93$) and $\hat{Y} = 66.3906 - 8.10935X + 0.379717X^2$ ($r^2 = 0.80$).

In the birds fed the diets not supplemented with industrial amino acids, the lysine levels had a quadratic effect ($P<0.01$) on the absolute weights of bone-in and boneless breast, which increased up to the estimated levels of 11.7 and 11.8 g/kg, respectively, according to the equations: $\hat{Y} = -164.31 + 381.741X - 16.31X^2$ ($r^2 = 0.99$) and $\hat{Y} = -1335.69 + 302.723X - 12.8502X^2$ ($r^2 = 0.99$).

No effect ($P>0.05$) of the lysine levels was observed on the yields of bone-in and boneless breast of the birds fed the unsupplemented diets. This result corroborates Balbino et al. (2008), who also did not observe effect of

Table 9 - Absolute and relative weights of prime cuts of 22-to-42-day-old broilers according to the level of digestible lysine in the diet

Parameters	Lysine levels (g/kg)				\bar{X}	CV (%)
	9.0	10.0	11.0	12.0		
CSMa						
Carcass (g) ¹	1,512	1,672	1,831	1,844	1,703	5.53
Bone-in breast (g) ³	470	541	582	588	541	7.65
Boneless breast (g) ³	350	401	446	445	407	8.74
Drumstick (g) ¹	189	204	231	233	213	9.22
Thigh (g) ¹	192	205	236	241	217	11.16
Abdominal fat (g) ²	27.3	28.9	26.4	21.9	26.15	41.42
Carcass (%) ¹	78.81	80.09	80.58	80.63	79.96	2.73
Bone-in breast (%)	31.55	32.22	32.90	32.32	32.22	5.65
Boneless breast (%)	23.44	24.38	24.69	24.46	24.20	7.47
Drumstick (%)	12.71	12.38	12.76	12.80	12.66	6.52
Thigh (%)	12.86	12.49	13.05	13.27	12.91	9.55
Abdominal fat (%) ¹	1.85	1.75	1.47	1.20	1.58	43.46
CSM + IAAb						
Carcass (g)	1,564	1,580	1,547	1,613	1,576	5.69
Bone-in breast (g) ³	497	484	480	515	493	7.91
Boneless breast (g) ³	374	359	357	376	366	9.57
Drumstick (g)	189	196	191	199	194	9.04
Thigh (g)	199	199	195	194	196	10.36
Abdominal fat (g) ¹	29.4	30.4	28.3	27.8	28.9	35.33
Carcass (%)	79.55	79.51	79.48	79.11	79.41	2.56
Bone-in breast (%) ³	32.27	31.02	31.49	32.38	31.74	4.82
Boneless breast (%) ³	24.28	23.01	23.38	23.66	23.54	6.82
Drumstick (%)	12.26	12.56	12.51	12.51	12.47	5.96
Thigh (%) ¹	12.97	12.74	12.75	12.19	12.66	8.87
Abdominal fat (%)	1.92	1.95	1.87	1.75	1.87	35.80

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

CSM - corn + soybean meal; IAA - industrial amino acids.

^{1,2} Linear effect ($P<0.01$) and ($P<0.09$), respectively.

³ Quadratic effect ($P<0.01$).

lysine levels of an unsupplemented diet on the breast yield of broilers kept in a heat-stress environment. However, our results differ from those found by Assis et al. (2008), who observed a quadratic effect of lysine levels on the breast yield, which increased up to the optimal level of 10.4 g/kg lysine in the unsupplemented diet.

Despite the results found in the present study for breast yield, many reports in the literature have demonstrated that increasing the digestible lysine level in the diet promotes increase in this variable (Kerr et al., 1999; Kidd et al., 2004; Rezaei et al., 2004; Mukhtar et al., 2007; Ghahri et al., 2010). Yet, the greater values obtained with the diet lacking supplementation of industrial amino acids in relation to the supplemented diet may be linked to the differences in the CP content of the tested diets (Viola et al., 2009b; Ghahri et al., 2010). Research has demonstrated that high CP levels promote increase in the plasma concentration of the growth factor similar to type-1 insulin (IGF-1) (Rosebrough et al., 1996; Caperna et al., 1999; Nagao et al., 2010), improving the meat content in the carcass, likely due to the increase in the size of the muscle fibers via modulation of the synthesis of degradation of body proteins (Dozier et al., 2008).

No effect ($P>0.05$) of the lysine levels from the diet supplemented with amino acids was observed on the absolute weights of drumstick and thigh (Table 9). This result differs from those found by Lana et al. (2005), Siqueira et al. (2007) and Viola et al. (2009b), who observed linear increase in the absolute weights of drumstick and thigh of broilers as the lysine levels of the diet were increased.

Drumstick yield was not affected ($P>0.05$) by the lysine levels of the supplemented diet. However, the thigh yield was influenced ($P<0.01$) by the lysine levels of the supplemented diet and reduced linearly according to the equation: $\hat{Y} = 15.0951 - 0.231023X$ ($r^2 = 0.80$). This result is in accordance with Siqueira et al. (2007), who observed a decrease in thigh yield as a consequence of increase in the dietary lysine levels. However, it differs from those obtained by Lana et al. (2005), who did not observe changes in the thigh yield according to the lysine levels, and also diverges from the results found by Mukhtar et al. (2007), who observed improvement in the thigh yield of chickens fed diets containing increasing lysine levels.

The weights of drumstick and thigh of the birds fed the unsupplemented diet increased ($P<0.01$) linearly along with the lysine levels, according to the equations $\hat{Y} = 48.5171 + 15.7466X$ ($r^2 = 0.93$) and $\hat{Y} = 31.4825 + 17.7883X$ ($r^2 = 0.95$), respectively. Still, the yields of thigh and drumstick were not affected ($P>0.05$) by the lysine levels of the unsupplemented diet. These results contradict

those reported by Assis et al. (2008) and Balbino et al. (2008), who verified improvement in the drumstick yield of birds fed an unsupplemented diet containing increasing lysine levels. These researchers, however, also did not find effects on thigh yield.

With regard to the abdominal fat (Table 9), the lysine levels of the diet without supplementation of industrial amino acids influenced ($P<0.09$) the absolute weight of this variable, which reduced linearly according to the equation $\hat{Y} = 45.0246 - 1.81052X$ ($r^2 = 0.64$). The abdominal-fat yield reduced ($P<0.01$) linearly as a function of the lysine levels, according to the equation $\hat{Y} = 3.86417 - 0.218862X$ ($r^2 = 0.96$). On the other hand, the absolute and relative weights of the abdominal fat of the broilers fed the diet supplemented with industrial amino acids was not affected ($P>0.05$) by the lysine levels. This result is in agreement with those found by Almeida et al. (2002) and Goulart et al. (2008), who also did not observe effects of the lysine levels on the abdominal fat of chickens fed a diet supplemented with industrial amino acids.

From the obtained data, we can infer that the crude protein level of the diet affects the response pattern of abdominal fat. According to Rosebrough et al. (2002, 2007 and 2011), increase in the dietary crude protein levels reduced lipogenesis *in vitro*, acting both on the regulation of the mRNA and on the post-transcriptional events involved in lipogenesis in birds. This fact may explain the reduction of 9.5 and 15.5% in the mean values of weight and yield of abdominal fat, respectively, in the birds fed the diet supplemented with industrial amino acids as compared with those observed in the broilers fed the unsupplemented diet.

The increase in the dietary lysine levels obtained by varying the proportion of corn of soybean meal and without supplementation of industrial amino acids provided a linear increase ($P<0.01$) in intake, excretion and retention of total nitrogen (Table 10), according to the equations $\hat{Y} = 4.01102 + 1.84391X$ ($r^2 = 0.98$); $\hat{Y} = 2.80961 + 0.688778X$ ($r^2 = 0.99$) and $\hat{Y} = 1.20142 + 1.15513X$ ($r^2 = 0.93$), respectively. However, the relative data (%) of excretion and retention of nitrogen were not influenced ($P>0.05$) by the dietary lysine levels.

The lysine levels of the diet supplemented with industrial amino acids, in turn, did not influence ($P>0.05$) in total or relative values the intake, retention and excretion of nitrogen (N), which is attributed to the lower CP content of this diet and to the better adjustment of the levels of amino acids to meet the nutritional requirements of the birds. Similarly, several researchers have demonstrated reduced excretion of N by birds due to lower ingestion

Table 10 - Nitrogen use efficiency of 22-to-42-day-old broilers according to the level of digestible lysine in the diet

Parameters	Lysine levels (g/kg)			\bar{X}	CV (%)
	9.0	10.0	12.0		
	CSMa				
Nitrogen intake (g) ¹	20.3	22.9	26.0	23.1	4.40
Nitrogen excretion (g) ¹	9.1	9.6	11.1	9.9	8.24
Nitrogen retention (g) ¹	11.2	13.3	14.9	13.1	6.10
Nitrogen excretion (%)	44.7	42.0	42.7	43.1	6.52
Nitrogen retention (%)	55.3	58.0	57.3	56.9	4.94
	CSM + IAAb				
Nitrogen intake (g)	20.3	20.7	20.0	20.3	5.57
Nitrogen excretion (g)	9.1	9.1	9.1	9.1	8.64
Nitrogen retention (g)	11.2	11.6	10.9	11.2	8.72
Nitrogen excretion (%)	44.7	43.9	45.5	44.7	7.50
Nitrogen retention (%)	55.3	56.1	54.5	55.3	6.06

a - Digestible lysine levels obtained by modifying the amounts of corn and soybean meal in the diet.

b - Digestible lysine levels obtained by including industrial amino acids in the diet.

CSM - corn + soybean meal; IAA - industrial amino acids.

¹ Linear effect (P<0.01).

of this element when low-CP diets and supplementation of industrial amino acids are used (Waldroup et al., 1976; Aletor et al., 2000; Corzo et al., 2005; Faria Filho et al., 2006; Kamran et al., 2010; Vasconcellos et al., 2011).

It is known that an increase in N excretion is desirable from both the economic and environmental standpoints, which makes the unsupplemented diet not very attractive from this perspective. However, the unsupplemented diet is notable for presenting increases of 14.6 and 36.7% in the absolute retention of N as compared with the diet supplemented with industrial amino acids for the levels of 10.0 and 12.0 g/kg of lysine in the diet, respectively. These results explain the greater values for the absolute and relative weights of bone-in and boneless breast, drumstick and thigh observed in the birds that were fed the unsupplemented diet.

Thus, it is clear that although the diets in which the digestible lysine levels were obtained by supplementation with industrial amino acids allow for reduction in nitrogen excretion, performance, carcass yield and prime cuts yield of the birds are compromised. This indicates that this method may be having some deficiency that prevents the birds from expressing their maximum genetic potential for production, which renders it inappropriate for the determination of the nutritional requirements of broilers in the studied period.

Conclusions

The levels of 9.0 and 12.0 g/kg of digestible lysine obtained with supplementation of industrial amino acids and without supplementation, respectively, provide the best performance and yield of prime cuts in the birds. Diets whose digestible lysine levels are achieved without

supplementation of industrial amino acids provide the best responses in performance and carcass characteristics compared with supplemented diets.

References

- Aletor, V. A.; Hamid, I. I.; Niess, E. and Pfeffer, E. 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass, characteristics, whole-body, composition and efficiencies of nutrient utilization. *Journal of the Science of Food and Agriculture* 80:547-554.
- Almeida, I. C. L.; Mendes, A. A.; Oliveira, E. G.; Garcia, R. G. and Garcia, E. A. 2002. Efeito de dois níveis de lisina e do sexo sobre o rendimento e a qualidade da carne de peito de frangos de corte. *Revista Brasileira de Zootecnia* 31:1744-1752.
- Assis, A. P.; Balbino, E. M.; Campos, P. H. R. F.; Oliveira, W. P.; Souza, M. G.; Soares, F. P.; Oliveira, R. F. M. and Donzele, J. L. 2008. Níveis de lisina em rações para frangos de corte machos mantidos em termoneutralidade dos 22 aos 42 dias de idade. In: *Anais do Zootec 2008*. Zootec 2008, João Pessoa.
- Baker, D. H. and Han, Y. 1994. Ideal amino acid profile for chickens during the first three weeks posthatching. *Poultry Science* 73:1441-1447.
- Balbino, E. M.; Assis, A. P.; Souza, M. G.; Campos, P. H. R. F.; Oliveira, W. P.; Lima, A. L.; Oliveira, R. F. M. and Donzele, J. L. 2008. Níveis de lisina em rações para frangos de corte machos mantidos em ambiente de calor dos 22 aos 42 dias de idade. In: *Anais do Zootec 2008*. Zootec 2008, João Pessoa.
- Buffington, D. E.; Collazo-Arocho, A.; Canton, G. H.; Pitt, D.; Thatcher, W. W. and Collier, R. J. 1981. Black globe humidity index (BGHI) as a comfort equation for dairy cows. *Transactions of the American Society of Agricultural Engineers* 24:711-714.
- Campestrini, E.; Barbosa, M. J. B.; Nunes, R. V.; Bruno, L. D. G.; Silva, W. T. M. and Appelt, M. D. 2008. Níveis de lisina com dois balanços eletrolíticos para frangos de corte na fase de crescimento (22 a 40 dias). *Revista Brasileira de Zootecnia* 37:1405-1411.
- Caperna, T. J.; Rosebrough, R. W.; McMurtry, J. P. and Vasilatos-Younken, R. 1999. Influence of dietary protein on insulin-like growth factor binding proteins in the chicken. *Comparative biochemistry and physiology - Parte B: Biochemistry Molecular Biology* 124:417-421.

- COBB. 2005. Manual de manejo de frangos Cobb 500: guia de manejo. Cobb-Vantress Brasil, São Paulo.
- Colina, J. J.; Lewis, A. and Miller, P. S. 2002. Dietary amino acid utilization for body protein deposition: current and future research. Nebraska Swine Reports No. 75. University of Nebraska, Lincoln, USA.
- Corzo, A.; Fritts, C. A.; Kidd, M. T. and Kerr, B. J. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. *Animal Feed Science and Technology* 118:319-327.
- Costa, F. G. P.; Amarante Júnior, V. S.; Nascimento, G. A. J.; Brandão, P. A.; Barros, L. R.; Silva, J. H. V. and Costa, J. S. 2006. Níveis de lisina para frangos de corte nos períodos de 22 a 42 e de 43 a 49 dias de idade. *Revista Brasileira de Zootecnia* 30:759-766.
- Dozier, W. A.; Kidd, M. T. and Corzo, A. 2008. Dietary amino acid responses of broiler chickens. *Journal of Applied Poultry Research* 17:157-167.
- Faria Filho, D. E.; Rosa, P. S.; Figueiredo, D. F.; Dahlke, F.; Macari, M. and Furlan, R. L. 2006. Dietas de baixa proteína no desempenho de frangos criados em diferentes temperaturas. *Pesquisa Agropecuária Brasileira* 41:101-106.
- Furlan, R. L. 2006. Influência da temperatura na produção de frangos de corte. p.104-135. In: Anais do 7^o Simpósio Brasil Sul de Avicultura, Chapecó.
- Garcia, A. R.; Batal, A. B. and Baker, D. H. 2006. Variations in the digestible lysine requirement of broiler chickens due to sex, performance parameters, rearing environment, and processing yield characteristics. *Poultry Science* 85:498-504.
- Ghahri, H.; Gaykani, R. and Toloie, T. 2010. Effect of dietary crude protein level on performance and Lysine requirements of male broiler chickens. *African Journal of Agricultural Research* 5:1228-1234.
- Goulart, C. C.; Costa, F. G. P.; Lima Neto, R. C.; Souza, J. G.; Silva, J. H. V. and Givisiez, P. E. N. 2008. Exigência de lisina digestível para frangos de corte machos de 1 a 42 dias de idade. *Revista Brasileira de Zootecnia* 37:876-882.
- Kamran, Z.; Sarwar, M.; Mahr-Un-Nisa; Mukhtar, A. N. and Mahmood, S. 2010. Effect of low levels of dietary crude protein with constant metabolizable energy on nitrogen excretion, litter composition and blood parameters of broilers. *International Journal of Agriculture & Biology* 12:401-405.
- Kerr, B. J.; Kidd, M. T.; Halpin, K. M.; McWard, G. W. and Quarles, C. L. 1999. Lysine level increases live and performance and breast yield in male broilers. *The Journal of Applied Poultry Research* 8:381-390.
- Kidd, M. T.; McDaniel, C. D.; Branton, S. L.; Miller, E. R.; Boren, B. B. and Fancher, B. I. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. *Journal Applied Poultry Research* 13:593-604.
- Lana, S. R. V.; Oliveira, R. F. M.; Donzele, J. L.; Gomes, P. C.; Vaz, R. G. M. V. and Rezende, W. O. 2005. Níveis de lisina digestível em rações para frangos de corte de 22 a 42 dias de idade, mantidos em ambiente de termoneutralidade. *Revista Brasileira de Zootecnia* 34:1624-1632.
- Leclercq, B. 1998. Specific effects of lysine on broiler production: comparison with threonine and valine. *Poultry Science* 77:118-123.
- Martinez, K. L. A.; Pezzato, A. C.; Gonçalves, J. C.; Sartori, J. R.; Cruz, V. C. and Pinheiro, D. F. 2002. Níveis de lisina em rações formuladas a partir de aminoácidos totais e digestíveis para frangos de corte submetidos a diferentes temperaturas. In: Anais da 39^a Reunião Anual da Sociedade Brasileira de Zootecnia. Sociedade Brasileira de Zootecnia, Recife.
- Medeiros, C. M.; Baeta, F. C.; Oliveira, R. F. M.; Tinoco, I. F. F.; Albino, L. F. T. and Cecon, P. R. 2005. Efeitos da temperatura, umidade relativa e velocidade do ar em frangos de corte. *Engenharia na Agricultura* 13:277-286.
- Mukhtar, M. A.; Mekkawi, A. and Eltigani, M. 2007. The effect of feeding increasing levels of synthetic lysine and methionine in broiler chicks. *Research Journal of Animal and Veterinary Sciences* 2:18-20.
- Nagao, K.; Hiramatsu, K.; Tsukada, A. and Kita, K. 2010. Effects of insufficient level of dietary protein on IGF-I and IGF-BPs in young chickens. *Journal of Poultry Science* 47:236-239.
- Namroud, N. F.; Shivazad, M. and Zaghari, M. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poultry Science* 87:2250-2258.
- Nonis, M. K. and Gous, R. M. 2006. Utilisation of synthetic amino acids by broiler breeder hens. *South African Journal of Animal Science* 36:126-134.
- NRC - National Research Council. 1994. Nutrient requirements of poultry. Nutrient requirements of domestic animals. 9th ed. National Academy Press, Washington, D.C.
- Oliveira, W. P.; Souza, M. F.; Antunes, M. V. L.; Campos, P. H. R. F.; Soares, F. P.; Oliveira, R. F. M. and Donzele, J. L. 2009. Níveis de lisina para frangos de corte no período de 08 a 21 dias de idade. In: Anais do Zootec 2009. Zootec 2009, Águas de Lindóia.
- Rezaei, M.; Moghaddam, H. N.; Reza, J. P. and Kermanshahi, H. 2004. The effects of dietary protein and lysine levels on broiler performance, carcass characteristics and N excretion. *International Journal of Poultry Science* 3:148-152.
- Rosebrough, R. W.; Mitchell, A. D. and McMurtry, J. P. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor I concentrations in broiler chickens. *The Journal of Nutrition* 126:2888-2898.
- Rosebrough, R. W.; Poch, S. M.; Russell, B. A. and Richards, M. P. 2002. Dietary protein regulates in vitro lipogenesis and lipogenic gene expression in broilers. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* 32:423-432.
- Rosebrough, R. W.; Russell, B. A.; Poch, S. M. and Richards, M. P. 2007. Expression of lipogenic enzymes in chickens. *Comparative Biochemistry and Physiology - Parte A: Molecular & Integrative Physiology* 147:215-222.
- Rosebrough, R. W.; Russell, B. A. and Richards, M. P. 2011. Further studies on short-term adaptations in the expression of lipogenic genes in broilers. *Comparative Biochemistry and Physiology - Parte A: Molecular & Integrative Physiology* 159:1-6.
- Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F. M.; Lopes, D. C.; Ferreira, A. S. and Barreto, S. L. T. 2005. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 2.ed. Universidade Federal de Viçosa, Viçosa, MG.
- Rostagno, H. S.; Nascimento, A. H. and Albino, L. F. T. 1999. Aminoácidos totais e digestíveis para aves. p.65-83. In: Anais do Simpósio Internacional sobre Nutrição de Aves. Fundação Apinco de Ciência e Tecnologias Avícolas, Campinas.
- Silva, D. J. and Queiroz, A. C. 2002. Análise de alimentos: métodos químicos e biológicos. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.
- Siqueira, J. C.; Oliveira, R. F. M.; Donzele, J. L.; Cecon, P. R.; Balbino, E. M. and Oliveira, W. P. 2007. Níveis de lisina digestível da ração e temperatura ambiente para frangos de corte em crescimento. *Revista Brasileira de Zootecnia* 36:2054-2062.
- Vasconcellos, C. H. F.; Fontes, D. O.; Lara, L. J. C.; Vidal, T. Z. B.; Silva, M. A. and Silva, P. C. 2011. Determinação da energia metabolizável e balanço de nitrogênio de dietas com diferentes teores de proteína bruta para frangos de corte. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 63:659-669.
- Viola, T. H.; Kessler, A. M.; Ribeiro, A. M. L.; Silva, I. C. M. and Krás, R. 2009a. The influence of crude protein level in the basal

- diet on the determination of lysine requirements for broiler performance and part yields. *Revista Brasileira de Ciência Avícola* 11:155-160.
- Viola, T. H.; Kessler, A. M.; Ribeiro, A. M. L.; Viola, E. S.; Trevizan, L. and Gonçalves, T. A. 2009b. Desempenho e peso de frações corporais, na suplementação crescente de lisina, dos 19 aos 40 dias de idade em frangos de corte. *Ciência Rural* 39:515-521.
- Waldroup, P. W.; Mitchell, R. J.; Payne, J. R. and Hazen, K. R. 1976. Performance of chicks fed diets formulated to minimize excess levels of essential amino acids. *Poultry Science* 55:243-253.
- Yen, J. T.; Kerr, B. J.; Easter, R. A. and Parkhurst, A. M. 2004. Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. *Journal Animal Science* 82:1079-1090.