Effects of starter diet supplementation with arginine on broiler production performance and on small intestine morphometry[1]

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ABSTRACT.- Murakami A.E., Fernandes J.I.M., Hernandes L. & Santos T.C. 2012. **Effects of starter diet supplementation with arginine on broiler production performance and on small intestine morphometry.** *Pesquisa Veterinária Brasileira 32(3):259-266.* Departamento de Zootecnia, Universidade Estadual de Maringá, Av. Colombo 5790, Maringá, PR 87020-900, Brazil. E-mail: aemurakami@uem.br

The effects of starter diet (days 1 to 21) supplemented with arginine (Arg) on the production performance and duodenum and jejunum mucosa morphometry of broilers were studied. Male Cobb broiler chickens (990) were randomly assigned to one of five treatments in a complete random design. Measurements of 33 chicks per treatment were made in six repetitions. The treatments consisted of a basal diet with 1.390% digestible Arg (no supplementation) and four dietary levels (1.490%, 1.590%, 1.690%, and 1.790%), providing a relationship with lysine of 1.103; 1.183; 1.262; 1.341 and 1.421%, respectively. From the age of 22 days on, all birds received conventional grower diet. The data were submitted to regression analysis by polynomial decomposition of the degrees of freedom in relation to the levels of Arg. The Arg supplementation increased (P<0.05) the live weight and the feed conversion ratio without increasing the feed intake of the birds. However, no effect was observed (P>0.05) in the growth phase (days 22 to 42) in the absence of the Arg supplementation. The supplementation of Arg over of NRC recommendation during the starter phase may be necessary for the expression of the maximal weight gain potential in birds. No effect (P<0.05) of Arg dietary supplementation was observed either on small intestine weight and length at any age. However, the duodenum villus:crypt ratio increased and the crypt depth decreased in the first week in response to increasing dietary Arg. It is concluded that broiler Arg dietary supplementation in the starter diet improved production performance and small intestine morphometry, especially in the first week.

INDEX TERMS: Starter diet supplementation, amino acids, polyamines, arginine, duodenum, villus: crypt ratio, broilers.

RESUMO.- [**Efeito da suplementação de arginina na dieta de frangos na performance produtiva e na morfologia do intestino delgado.**] O efeito na dieta inicial (1-21 dias) da suplementação de arginina (Arg) foi estudada sobre o desempenho e a morfologia da mucosa do jejuno em frangos de corte. Pintos machos Cobb (990) foram utilizados em um delineamento inteiramente casualizado com 5 tratamentos e 6 repetições com 33 aves cada. Os tratamentos consistiram em uma dieta basal com 1,390% de Arg digestível (sem suplementação) e 4 dietas com adição (1,490%; 1,590%; 1,690% e 1,790%), fornecendo uma relação de lisina de 1,103; 1,183; 1,262; 1,341 e 1,421%, respectivamente. A partir de 22 dias todas as aves receberam ração convencional. Os dados foram submetidos à análise de regressão pela decomposição polinomial dos graus de liberdade, referentes aos níveis de Arg A suplementação de Arg melhorou (P<0,05) o peso vivo e a conversão alimentar sem aumentar o consumo de ração. No entanto, não houve

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efeito (P>0,05) na fase de crescimento (22 a 42 dias) na ausência de suplementação de Arg. A suplementação acima do recomendado pelo NRC na fase inicial pode ser necessária para a expressão máxima do potencial de ganho de peso em aves. Não houve efeito da suplementação de Arg na dieta no peso e comprimento do intestino delgado em nenhuma idade. No entanto, a relação vilo:cripta no duodeno aumentou e a profundidade da cripta diminuiu na primeira semana em resposta ao incremento de Arg na dieta. Concluiu-se que em frangos de corte a suplementação de Arg na dieta inicial melhorou o desempenho e a morfometria do intestino delgado, especialmente na primeira semana.

TERMOS DE INDEXAÇÃO: Suplementação, aminoácidos, poliaminas, arginina, duodeno, relação vilo:cripta, frangos de corte.

INTRODUCTION

Nutrition and health during this first week of life prepares the broiler chicks for rapid growth and excellent livability throughout rearing. The nutritional investigations of the broiler starter phase has been intensified and disseminated due to its great significance for the growth of modern broiler lineages in relation to its total growth period.

Arginine (Arg) is essential amino acids for birds, especially in the starter phase, since the urea cycle is not functional in birds (Austic & Nesheim 1971). Birds cannot synthesize Arg, de novo, and thus are dependent of dietary supply of this AA. Additionally, corn and soy meal-based diets, Arg is the fifth limiting amino acid, after methionine, lysine, threonine, and valine (Vieira & Berres 2007), may be particularly important in what concerns Arg availability to broilers. Birds have the highest requirement of Arg among the studied animals (Ball et al. 2007). This high Arg requirement is explained by the lack of endogenous Arg synthesis, the high protein deposition rate due to the fast growth of current broiler lineages, and the antagonistic metabolic interaction between lysine (Lys) and Arg (Edmonds & Baker 1987).

Besides their role as building blocks of proteins and polypeptides, some aminoacids regulate key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity. They are called functional AA, which include arginine, cysteine, glutamine, leucine, proline, and tryptophan (Wu 2009). These amino acids may maximize the feeding efficiency and protein aggregation, reduce adiposity, and improve the health of humans and animals.

Wu & Morris (1998) described Arg as one of the most versatile amino acids in animal cells. It is required for the synthesis of several compounds, such as ornithine, polyamines (spermidine, spermine, and putrescine), proline, creatine, protein, nitric oxide (NO), and citrulline, besides glutamate and agmatine in mammals. Arg is also a powerful secretagogue, increasing the release of insulin, the growth hormone, and IGF-I in the blood stream (Newsholme et al. 2005).

The tissue polyamine concentrations in birds are particularly responsive to dietary manipulations of Arg levels. Due to the lack of a functional urea cycle, birds depend on an exogenous Arg source to form ornithine, which, in mammals, is derived from glutamic acid (Austic & Nesheim 1971). Putrescine is formed by the decarboxylation of ornithine, and spermine and spermidine are obtained from putrescine in the presence of decarboxylated S-adenosylmethionine, which is derived from methionine; thus both arginine and methionine are involved in their synthesis. These biogenic amines are also considered nutritionally important local factors for growth and the development of small intestinal and colonic mucosa (Löser et al. 1999). Ruemmele et al. (1999) had already reported that the lack of polyamines inhibits the proliferation, migration, and apoptosis of intestine cells. As a precursor of polyamines, Arg may be considered a trophic agent in the stimulation of the development of the intestine mucosa, accelerating the mitotic process in the villus-crypt region with a resulting increase in the number and size of villus cells.

According to Wu (2009), amino acids induce gene transcription mechanisms by activating important enzymes of the intestine mitotic process, such as glutamine in the induction of ornithine-decarboxylase, the enzyme responsible for the decarboxylation of ornithine in the synthesis of polyamines. They also regulate gene expression in highly specific processes that involve the transfer of coded information from a gene to its product (RNA and/or protein). In mammals, research has shown that dietary supplementation with Arg and glycine (Gln) can increase the expression of antioxidant genes and reduce the expression of proinflammatory genes in the small intestine and adipose tissue (Fu et al. 2005, Wang et al. 2008, Jobgen et al. 2009).

Despite the large growth of the small intestine in the first week post-hatching, it is not fully mature for the digestion and absorption processes (Noy & Sklan 1995). As a result, broiler chicks exhibit a negative correlation between feed intake and nutrient digestibility in the first week of life. However, after the second week of life, this correlation becomes positive due to the optimization of intestinal growth and enzyme activity (Nir 1998, Murakami et al. 2007).

This work aimed to evaluate the effects of starter diet supplementation with Arg on production performance and duodenum mucosa morphometry in broilers.

MATERIALS AND METHODS

The experiment was conducted in the poultry sector of the Iguatemi Experimental Farm with the approval of the Animal Experimentation Ethics Committee of the State University of Maringa. (Protocol 004/2006).

Male Cobb 500 chicks (n=990) produced by 39-week old broiler breeders were assigned in a complete randomized design to groups of 33 chicks per pen in a total of 30 experimental units submitted to five levels of Arg and six experimental repetitions. The open-sided house had thermostatically controlled heating and ventilation and each pen (0.173m^2/bird) had rice husk liter and was placed one feeder and one drinker. Birds had free access to feed and water during the experimental period. Continual artificial illumination was applied.

The nutritional program was divided in two phases, a starter phase (days 1 to 21) and a growth phase (days 22 to 42). Corn and soy meal-based diets were formulated in accordance with the chemical composition of the foods and the nutritional recommendations of Rostagno et al. (2005). Increasing levels of supplementary Arg were obtained by adding L-Arg HCl (0, 0.1, 0.2, 0.3, and 0.4%) to the starter basal feed as a replacement of the inert component. The digestible Arg dietary levels were 1.390, 1.490, 1.590, 1.690, and 1.790% (Table 1) with Arg:Lys ratios of 1.103, 1.183, 1.262, 1.341, and 1.421, respectively. From the age of 22 days on, all birds received conventional grower diet with 19.7% CP, 3,150 ME kcal/kg, 1.099% Lys and 1.249% Arg as advocated by Rostagno et al. (2005).

The chemical compositions of the experimental diets were calculated in accordance with AOAC Official Method 994.12 (AOAC 1995) by performic acid oxidation with acid hydrolysis-sodium metabisulfite method.

Table 1. Composition of experimental diets of broilers in the starter phase (1-21 days of age)

Ingredients (%)	Digestible Arg %				
	1.390	1.490	1.590	1.690	1.790
Corn	54.00	54.00	54.00	54.00	54.00
Soybean oil	3.5	3.5	3.5	3.5	3.5
Soybean meal	37.94	37.94	37.94	37.94	37.94
Sodium chloride	0.284	0.284	0.284	0.284	0.284
Sodium bicarbonate	0.241	0.241	0.241	0.241	0.241
Calcitic limestone	0.796	0.796	0.796	0.796	0.796
Dicalcium phosphate	1.923	1.923	1.923	1.923	1.923
DL-Methionine	0.296	0.296	0.296	0.296	0.296
L-Lysine	0.246	0.246	0.246	0.246	0.246
L-Threonine	0.117	0.117	0.117	0.117	0.117
L-Arginine	0	0.101	0.202	0.303	0.404
Inert (kaolin)	0.500	0.399	0.298	0.197	0.096
Antioxidant (BHT)[a]	0.01	0.01	0.01	0.01	0.01
Vitamin and mineral premix[b,c]	0.150	0.150	0.150	0.150	0.150
Calculated values					
Crude protein (%)	22.4	22.4	22.4	22.4	22.4
ME kcal/kg	3,047	3,047	3,047	3,047	3,047
Calcium (%)	0.920	0.920	0.920	0.920	0.920
Nonphytate P (%)	0.471	0.471	0.471	0.471	0.471
Digestible TSAA (%)	0.890	0.890	0.890	0.890	0.890
Digestible Lys (%)	1.260	1.260	1.260	1.260	1.260
Digestible Trp %	0.252	0.252	0.252	0.252	0.252
Digestible Thr (%)	0.850	0.850	0.850	0.850	0.85
Total Arg %	1.479	1.579	1.679	1.779	1.879
Digestible Arg (%)	1.390	1.490	1.590	1.690	1.790
Sodium (%)	0.220	0.220	0.220	0.220	0.220
Chloride (%)	0.200	0.200	0.200	0.200	0.200

[a] BHT = butylated hydroxytoluene.

[b] Premix provided per kg of diet: retinyl acetate 3.5mg, cholecalciferol 82.5mg, dl-alfa-tocoferol 16.50mg, menadione 2.4mg, thiamin 3.00mg, riboflavin 7.50mg, vitamin B-12 18.00mg, niacin 52.50mg, pantothenic acid 19.50mg, folic acid 1.20mg.

[c] Mineral mix provided per kg of diet: Fe 15mg, Cu 24mg, iodine 3.60mg, Zn 150mg, Mn 210mg, Se 0.60mg.

At the ages of 7, 14, 21, and 42 days, all the birds and the feed remains were weighed in order to determine body weight gain, feed intake and feed conversion ratio. Each period, two birds per pen (12 birds/treatment) were randomly selected and euthanized by cervical dislocation and weighed individually. The carcasses were dissected in order to remove the small intestine, which was weighed and measured. Fragments of approximately 5cm length from the duodenum (from the pylorus until distal duodenal portion) and jejunum (from the distal duodenal portion until Meckel diverticulum) were obtained. Fragments were opened, washed with saline solution and fixed in Bouin's solution for 24 h for histological analysis. Each fragment was submitted to 5µm-thick semi-serials cuts and stained by Hematoxylin and eosin. To histological morphometric study the slides were analyzed by light microscopy, and digital images were captured (IMAGE PROPLUS 5.2 from Media Cybertechnics, São Paulo, Brazil). The height of villi and depth of crypts were measured in twenty different points in duodenum and jejunum in each bird.

The data were submitted to regression analysis by polynomial decomposition of the degrees of freedom in relation to the levels of Arg. The data were analyzed with software SAEG (1997) using the statistical model:

$$Y_{ij} = b_0 + b_1A_i + b_2A_i + b_3A_i + e_{ij}$$

where:

Y_{ij}: observation of the dependent variable in the experimental unit j submitted to level *i* of Arg, *i*: 1, 2, 3, 4, 5 (1= 1.390%, 2= 1.490%, 3= 1.590%, 4= 1.690% and 5= 1.790%);

b_0: constant;

b_1, b_2, *and* b_3: linear, quadratic, and cubic regression coefficients of the dependent variable as a function of the levels of Arg;

e_{ij}: random error associated with each Y_{ij} observation.

The determination coefficients were calculated as percentages of the sum of the squares of the model in relation to the total sum of squares.

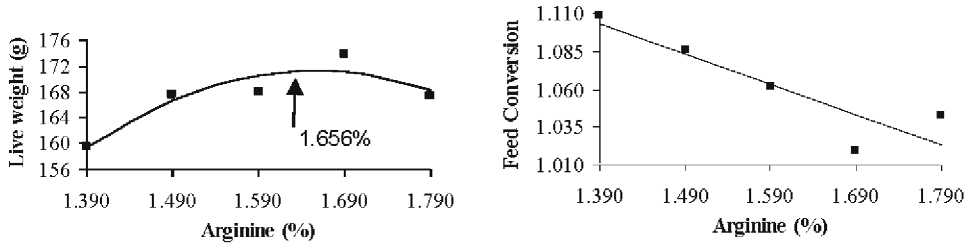
RESULTS

The analysis of the amino acid profile of the experimental feeds resulted in a total Arg content 10% over the calcu-

Table 2. Performance parameter means and estimates of broilers fed different amounts of Arg in diet in the 1 to 21 days of age

	Digestible Arg %					CV (%)	Effect
	1.390	1.490	1.590	1.690	1.790		
1 to 7-day age period							
Weight gain g	118.55	125.72	129.67	130.38	127.86	5.42	Quadratic
Feed intake, g	131.20	137.14	135.39	134.50	132.65	5.43	NS
Feed conversion, g/g	1.103	1.083	1.063	1.043	1.023	4.18	Linear
1 to 14-day age period							
Weight gain g	390.44	396.5	396.67	406.42	398.99	2.47	NS
Feed intake, g	473.71	480.50	474.18	483.26	473.14	2.51	NS
Feed conversion, g/g	1.215	1.207	1.199	1.192	1.184	2.47	Linear
1 to 21-day age period							
Weight gain g	820.17	824.80	829.43	834.06	838.69	2.19	Linear
Feed intake, g	1086.12	1093.46	1077.62	1099.76	1079.49	2.08	NS
Feed conversion, g/g	1.328	1.319	1.311	1.303	1.295	1.09	Linear
1 to 42-day age period							
Weight gain g	2445.40	2378.81	2417.08	2439.48	2325.31	3.73	NS
Feed intake, g	4028.40	3961.46	3928.15	4041.34	3838.88	5.16	NS
Feed conversion, g/g	1.660	1.636	1.638	1.636	1.643	4.31	NS

CV = coefficient of variation, NS = $P>0.05$.

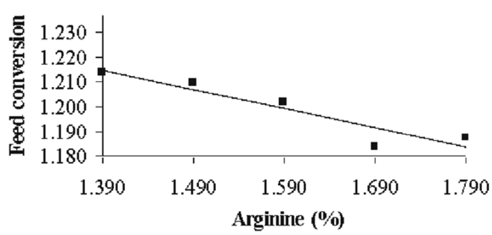


$\hat{Y}$=- 289.914 + 556.958X - 168.142X^2, R^2: 0.85 $\hat{Y}$=1.37953-0.19903X, R^2: 0.30

Fig.1. Live weight and feed conversion of broilers at 7 days of age fed different amounts of Arg in diet from 1 to 21 days of age.

lated values. The production performance results of broilers in periods 1 to 7, 1 to 14, 1 to 21, and 1 to 42 days of age are given in Table 2. The mortality ratio recorded during the whole experimental period was about 1.5% and it was not influenced (P>0.05) by the experimental treatments.

The live weight of the birds in the first week presented quadratic effect (P<0.05) for Arg supplementation, peaking at 1.560% Arg. The Arg supplementation increased (P<0.05) feed conversion linearly, but did not affect (P>0.05) feed intake (Fig.1).



$\hat{Y}$= 1.3233-0.078004X, R^2: 0.35

Fig.2. Feed conversion of broilers at 14 days of age fed different levels of Arg in diet in the 1 to 21-day age period.

At 1 to 14 day period, the feed conversion increased linearly (P < 0.05) with the Arg level in diet an effect was observed an effect (P<0.05) on feed conversion, which improved with the increase in Arg levels (Fig.2). From days 1-21, live weight and feed conversion improved linearly (P<0.05) with Arg supplementation, whereas feed intake did not vary (P>0.05) among the treatments (Fig.3).

In the total period, including the period of days 22-42, when the birds were not given Arg-supplemented feed, no effect (P>0.05) was observed on any production parameter despite the positive effect observed in the 1 to 21-day period.

The small intestine weight and length, villus height, crypt depth, and villus:crypt ratio of the duodenum and jejunum of broilers fed diets supplemented with different levels of Arg at 7, 14, 21, and 42 days of age are given in Tables 3 and 4.

Table 3. Mean small intestine weight and length of broilers fed different amounts of Arg in diet in the 1 to 21 days of age

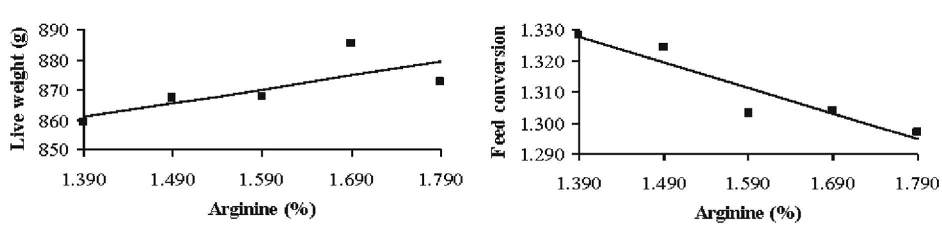
Small intestine	Digestible Arg, %					CV (%)	Effect
	1.390	1.490	1.590	1.690	1.790		
			7 days				
Weight, g	16.43	16.48	16.37	16.95	16.80	8.74	NS
Length, cm	92.83	96.00	96.50	93.83	93.42	7.31	NS
			14 days				
Weight, g	35.07	31.54	32.87	33.79	30.65	14.34	NS
Length, cm	121.42	121.83	121.00	122.58	121.92	5.56	NS
			21 days				
Weight, g	53.75	50.92	55.06	56.62	49.19	18.17	NS
Length, cm	140.75	145.83	135.50	143.00	141.00	11.63	NS
			42 days				
Weight, g	118.17	122.85	122.31	137.95	122.03	20.42	NS
Length, cm	191.17	189.25	187.33	198.08	187.00	8.23	NS

CV = coefficient of variation, NS = *P>0.05*.

No effect (P>0.05) was observed for Arg supplementation on the small intestine weight and length at any of the ages evaluated. The morphometry of the duodenum mucosa of broilers aged 7 days was affected (P<0.05) by the dietary Arg levels. The duodenum crypt depth and the villus: crypt ratio there was quadratic effect (P<0.05), according to the fitted equations, and 1.595% Arg resulted in a higher villus:crypt ratio and shallower crypts in the duodenum (Fig.4).

At 14 days of age, was observed only a quadratic response (*P*<0.05) to dietary arginine levels in the height of the jejunal villi (Fig.5). In contrast, at 21 days of age, no difference was observed in the mucosa morphometry of any segment of the small intestine studied.

Although the birds did not receive Arg-supplemented diets after 21 days of age, a quadratic variation was observed (*P*<0.05) for the duodenum mucosa crypt depth at 42 days of age, similar to that observed at 7 days of age.



$\hat{Y}$=798.233+45.2646X, R^2: 0.12 $\hat{Y}$=1.44227-0.0825135X, R^2: 0.42

Fig.3. Live weight and feed conversion of broilers at 21 days of age fed different amounts of Arg in diet from 1 to 21 days of age.

Table 4. Means and estimated valued of morphometric measurements of the small intestine mucosa (duodenum and jejunum) of broilers fed different levels of Arg in diet in the 1 to 21 days of age

	Digestible Arg %						Effect
	1.390	1.490	1.590	1.690	1.790	CV (%)	
Duodenum			7 days				
Villus height, µm	629.26	581.23	638.18	641.93	599.66	13.70	NS
Crypt depth, µm	114.39	106.04	103.09	105.52	113.35	12.11	Quadratic
Villus:crypt ratio	5.40	5.95	6.14	5.96	5.42	13.39	Quadratic
Jejunum							
Villus height, µm	285.33	283.20	317.25	275.77	299.12	21.03	NS
Crypt depth, µm	74.33	79.05	78.87	81.69	72.36	15.08	NS
Villus:crypt ratio	3.87	3.65	4.06	3.46	3.74	18.13	NS
Duodenum			14 days				
Villus height, µm	746.25	732.71	728.57	754.17	760.33	11.22	NS
Crypt depth, µm	102.87	112.78	105.00	106.87	103.02	18.88	NS
Villus:crypt ratio	7.40	6.58	6.97	7.23	7.56	12.62	NS
Jejunum							
Villus height, µm	406.17	388.76	388.11	404.21	437.06	10.22	Quadratic
Crypt depth, µm	80.35	80.89	74.81	86.38	75.04	18.73	NS
Villus:crypt ratio	5.17	4.77	5.56	4.82	5.89	20.85	NS
Duodenum			21 days				
Villus height, µm	1016.78	934.98	1030.02	1038.10	1023.61	11.09	NS
Crypt depth, µm	117.70	114.45	118.44	118.75	126.17	11.75	NS
Villus:crypt ratio	8.67	8.32	8.67	8.77	8.17	8.67	NS
Jejunum							
Villus height, µm	606.78	565.21	600.10	574.56	639.81	13.69	NS
Crypt depth, µm	107.03	109.54	106.28	102.63	110.82	15.63	NS
Villus:crypt ratio	5.72	5.19	5.84	5.89	5.88	12.77	NS
Duodenum			42 days				
Villus height, µm	1046.16	1042.06	1078.65	1043.17	1125.33	10.36	NS
Crypt depth, µm	139.68	129.12	126.24	131.02	143.47	13.74	Quadratic
Villus:crypt ratio	7.42	8.68	8.66	7.73	8.02	15.82	NS
Jejunum							
Villus height, µm	700.97	737.22	693.44	736.87	711.27	13.27	NS
Crypt depth, µm	114.49	116.40	114.92	108.56	110.48	12.27	NS
Villus:crypt ratio	6.20	6.39	6.12	6.86	6.50	15.50	NS

CV = coefficient of variation, NS = P>0.05.

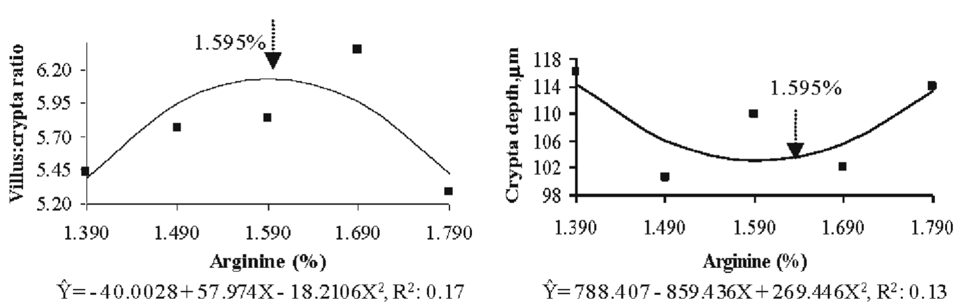


Fig. 4. Villus:crypt ratio and duodenum crypt depth of 7-day-old broilers fed different amounts of Arg in diet from 1 to 21 days of age.

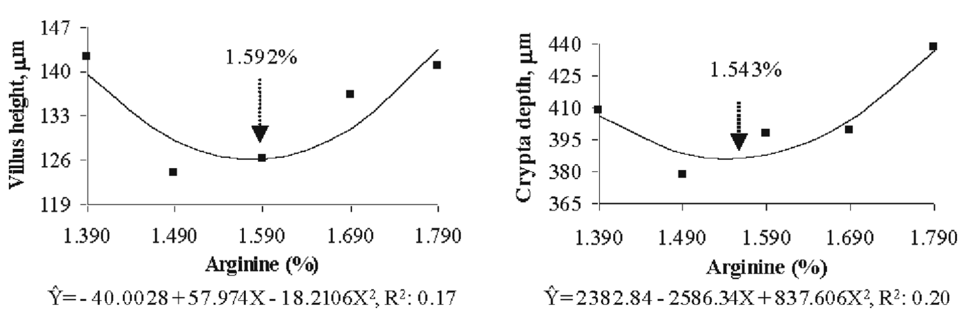


Fig. 5. Jejunum villous height (14 days of age) and duodenum crypt depth (42 days of age) of broilers fed different amounts of Arg in diet from 1 to 21 days of age.

DISCUSSION

The increase in the Arg:Lys ratio in the starter phase afforded a increased production performance.. At 1,25% digestible dietary lysine as is now common for young broiler chicks and if a lysine:arginine ratio 1.00:1.05 is to be maintained, as suggested by ideal protein (Dean & Scott 1965, Baker 1994, Rostagno et al. 2005) then the Arg level needs to be 1.31%. However, according to Marumatsu et al. (1991), the Arg use efficiency of birds falls linearly with the increase in the Lys level. The Arg requirement for modern broiler may be slightly below due of annual broiler genetic gains.

The present work confirmed this supposition. The Lys level in the starter diet was kept at 1.260% and the Arg level that maximized the live weight of birds was around 1.650%; however, higher levels were required to improve the feed conversion ratio.

Mendes, Watkins & England (1997) and Brake et al. (1998) also observe improved feed conversion with the addition of Arg to broiler diet. However, Labadan Jr et al. (2001) suggested that higher Arg amounts are needed for feed conversion than for live weight gain. Live weight was optimized at 1.24% Arg, whereas feed conversion was optimized at 1.31%. In contrast, the levels tested by these and other authors were lower than the ones used in this experiment, leading to some differences in the results obtained.

Burton & Waldroup (1997) reported a total Arg requirement of 1.40% in 1-28-day old broilers fed a 21% raw protein diet, and Costa et al. (2001) studied male Ross broilers aged 22-42 days submitted to seven Arg:Lys ratios ranging from 95 to 132.5% and did not observed an effect of Arg level on performance characteristics.

Chamruspollert et al. (2002) reported a total Arg requirement of 1.26% for weight gain and 1.27% for feed conversion. Corzo & Kidd (2003) recommended even lower levels, 1.15% for live weight gain and 1.28% for feed conversion at the 1 to 18-day age; however, the live weight gain of the birds was very low, around 50% the value obtained in the present result.

Atencio et al. (2004) did not observe any effect on feed intake; nevertheless, the live weight gain and feed conversion varied quadratically with digestible Arg levels between 1.10 and 1.39% in the 1 to 21-day period.

In the present experiment, the feed intake was not affected in any of the evaluated ages either, indicating that the differences in weight gain may be attributed to the efficient use of feeds by the birds and the balance between essential and non-essential amino acids. Growth consists, in part, of protein synthesis whereas efficiency of utilization is dependent not only on the protein synthesis but also on the amount of AA being catabolized (Sklan & Noy 2004). Different interrelationships between the dietary AA affect the amount of catabolism and these processes interact with an accompanying energy cost. Arg may be a limiting amino acid for the expression of maximal live weight gain in birds; therefore, its adjustment to optimal levels is fundamental. (Sklan & Noy 2004).

The positive effect of the increase in the Arg:Lys ratio on broiler performance may be related to the high temperature observed during the experimental period. The experiment was conducted between October and November and during this season the ambient temperature is high. Despite the facilities where the experiment was developed possess systems for temperature control it was not possible to maintain it constantly within the range of thermal comfort. The rearing temperature was maintained within the chicks comfort zone and the average temperature was 25±2°C, and relative humidity was 61 ± 10% throughout experimentation.. However, there were records of temperature above 32°C during the growth phase. Balnave & Brake (2002) reported that under this condition, besides reduced absorption of Arg in the intestine, it was more degraded, which demonstrated its higher demand. Furthermore, larger amounts of Arg are required for the synthesis of nitric oxide (NO), which is a powerful vasodilator, thus affecting the synthesis of other Arg-dependent metabolites negatively.

This proposition may be reinforced by Chamruspollert et al. (2004), who reported decreased creatine and creatinine levels in excreta of birds under heat stress, without concomitant increase in the concentrations of these substances in muscle, which implies that birds submitted to high temperatures had lower creatine biosynthesis. Arginine transfers a guanidino group to glycine for the formation of glycocyamine, and the synthesis of creatine is completed by methylation of glycocyamine by S-adenosylmethionine (Bloch & Schoenheimer 1941). Balnave & Brake (1999) also observed that heat stress reduces the synthesis of ornithine, and consequently of polyamines, due the lower activity of kidney arginase. Under those conditions, dietary manipulation of amino acids may alter the metabolism of polyamines in birds.

The results obtained on intestine villus analysis demonstrates that the addition Arg in higher amounts than the current commercial feed formulation levels is required to maintain the intestine epithelium development. According to Maiorka et al. (2003), animals with ~~a~~ higher intestine mucosa cell turnover have deeper crypts as a result of high mitotic activity and hyperplasia. Considering that the intestine mucosa grows continually due to cell desquamation to the intestine lumen, cell turnover occurs at the expenses of the consumption of nutrients derived from the bird organism energy reserves and the feed intake. McBride & Kelly (1990) estimated that the maintenance of the intestine epithelium and its supporting structures corresponds to 20% of the total energy consumed by the animal. Therefore, the more extensive the intestine mucosa repairs, the smaller the net energy availability.

A positive effect similar to that with Arg supplementation on morphometry of the duodenum mucosa was observed on the live weight of 7-day-old birds, demonstration that Arg supplementation is involved in the maintenance of the duodenum mucosa and that this effect may be associated to the better performance observed at this age.

The villus height may be positively related to the body weight gain and feed intake (Kelly et al. 1991); nevertheless, such an effect has not been observed. Yet, the weight gain and feed conversion were optimized under Arg supplementation, which may be attributed to a lower mitotic activity of the duodenum mucosa crypt cells and the resulting lower maintenance energy expenditure of the birds.

Moreover, the mechanism involved in the intestine cell turnover by nutrients is little understood yet. There is evidence for the participation of adjustment mechanisms between intrinsic and primary extrinsic afferent neurons and the central nervous system in addition to enzyme gene transcription mechanisms, and even gene expression, as specifically reported for some amino acids, such as Arg & Gln (Fu et al. 2005, Wang et al. 2008, Jobgen et al. 2009, Wu 2009).

Arg is involved in the synthesis of polyamines, which in turn are associated with cell division, protein synthesis, and tissue growth (Pegg & McCann 1982) besides its nutritional importance for the growth and intestine development (Löser et al. 1999). However, for Arg to be used in the synthesis of polyamines, it needs to be hydrolyzed into urea and ornithine by kidney arginase (Meijer et al. 1990, Wu & Morris 1998), since ureotelic species do not synthesize ornithine. Unlike mammals, the arginase activity of the kidney (high rate of Arg degradation) is 30 times higher than that in the liver (Tamir & Ratner 1963). Consequences of arginase induction can include impaired NO synthesis as a result of reduced arginine bioavailability.. Furthermore, Ruiz-Feria et al. (2001) questioned if there may be a difference between broiler lineages or even within lineages in relation to kidney arginase activity. If so, birds with high kidney arginase activity may benefit from supplemental Arg that might result in a trophic effect on the intestine mucosa. On the other hand, birds with a low kidney arginase activity may not respond to increased levels of Arg.

Given the increasing commercial availability of food- and feed-grade amino acids for dietary supplementation, they will widely become cost-effective functional nutrients, exerting a regulatory action on the growth and development of tissues, thus optimizing production performance and meat production in broilers.

CONCLUSIONS

Arg supplementation of broiler starter diets over the recommended levels improved production performance and intestine mucosa development only during the starter phase (1-21 days).

Dietary supplementation with Arg at levels above the ones recommended for the growth phase may be necessary for improved performance productive in lines with fast growth broilers.

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REFERENCES

AOAC 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. Association of Official Analytical Chemists, Washington, DC.

Atencio A., Albino L.F.T., Rostagno H.S., Oliveira D.C., Vieites F.M. & Pupa J.M.R. 2004. Exigência de arginina digestível para frangos de corte machos em diferentes fases. Revta Bras. Zootec. 33:1456-1466.

Austic R.E. & Nesheim M.C. 1971. Arginine, ornithine and proline metabolism of chicks: Influence of diet and heredity. J. Nutr. 101:1403-1413.

Baker D.H. 1994. Ideal protein and amino acid requirement of broiler chicks. Proc. California Nutrition Conference, Fresno, p.21-24.

Ball R.O., Urschel K.L. & Pencharz P.B. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. J. Nutr. 137:1626-1641.

Balnave D. & Brake J. 1999. Responses of broilers to sodium bicarbonate supplementation of diets containing varying arginine: Lysine ratios. Aust. J. Agric. Res. 50:425-430.

Balnave D. & Brake J. 2002. Re-evaluation of the classical dietary arginine:lysine interaction for modern poultry diets: A review. World's Poult. Sci. J. 58:275-289.

Bloch K. & Schoenheimer R. 1941. The biological precursors of creatine. J. Biol. Chem. 138:167-169.

Brake J., Balnave D. & Dibner J.J. 1998. Optimum dietary arginine:lysine ratio for broiler chickens is altered during heat stress in association with changes in intestinal uptake and dietary sodium chloride. Brit. Poult. Sci. 39:639-647.

Burton E.M. & Waldroup P. 1997. Arginine and lysine needs of young broiler chicks. Nutr. Int. 19:607-614.

Chamruspollert M., Pesti G.M. & Bakalli R.I. 2002. Dietary interrelationships among arginine, methionine, and lysine in young broiler chicks. Brit. J. Nutr. 88:655-660.

Chamruspollert M., Pesti G.M. & Bakalli R.I. 2004. Chick responses to dietary Arg and met levels at different environmental temperatures. Brit. Poult. Sci. 45:93-100.

Corzo A. & Kidd M.T. 2003. Arginine needs for chick and growing broiler. Int. J. Poultry Sci. 2:379-382.

Costa F.G.P., Rostagno H.S., Toledo R.S. & Albino L.F.T. 2001. Efeito da relação arginina:lisina sobre o desempenho e qualidade de carcaça de frangos de corte de 3 a 6 semanas de idade, em condições de alta temperatura. Revta Bras. Zootec. 30:2021-2025.

Dean W.F. & Scott, H.M. 1965. The development of an amino acid reference diet for the early growth of chicks. Poult. Sci. 44:803-808.

Edmonds M.S. & Baker D.H. 1987. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: effects on growth and dietary choice in the chick. J. Anim. Sci. 65:699-705.

Fu W.J., Haynes T.E., Kohli R., Hu J., Shi W., Spencer T.E., Carroll R.J., Meininger C.J. & Wu G. 2005. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J. Nutr. 135:714-721.

Jobgen W., Fu J., Gao H., Li P., Stephen B.S., Spencer T.E. & Wu G. 2009. High fat feeding and dietary L arginine supplementation differentially regulate gene expression in rat white adipose tissue. Amino Acids 37:187-198.

Kelly D., Smith J.A. & McCracken K.J. 1991. Digestive development of the early-weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. Brit. J. Nutr. 65:169-180.

Labadan Jr M.C., Hsu K.N. & Austic R.E. 2001. Lysine and arginine requirements of broiler chickens at two to three weeks intervals to eight weeks of age. Poult. Sci. 80:599-606.

Löser C., Eisel A., Harms D. & Foëlsch U.R. 1999. Dietary polyamines are essential luminal growth factors for small intestinal and colonic mucosal growth and development. Gut 44:12-16.

Maiorka A., Santin E., Dahlke F., Boleli I.C. Furlan R.L. & Macari M. 2003. Post hatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. J. Appl. Poult. Res. 12:483-492.

Marumatsu T.H., Ohshima H., Goto M., Mori S. & Okumura J. 1991. Growth prediction of young chicks. Do equal deficiencies of different essential amino acids produce equal responses? Brit. Poult. Sci. 32:139-149.

Mcbride B.W. & Kelly J.M. 1990. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J. Ani. Sci. 68:2997-3010.

Meijer A.J., Lamers W.H. & Chamuleau A.F.M. 1990. Nitrogen metabolism and ornithine cycle function. Physiol. Rev. 70:701-748.

Mendes A.A., Watkins S.E. & England J.A. 1997. Influence of dietary lysine levels and arginine:lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. Poult. Sci. 76:472-481.

Murakami A.E., Sakamoto M.I., Natali M.R.M., Souza L.M.G. & Franco J.R.G. 2007. Supplementation of glutamine and vitamin E on the morphometry of the intestinal mucosa in broiler chickens. Poult. Sci. 86:488-495.

Newsholme P., Brennnan L., Rubi B. & Maechler P. 2005. New insights into amino acid metabolism, beta-cell function and diabetes. Clin. Sci. 108:185-194.

Nir I. 1998. Mecanismos de digestão e absorção de nutrientes durante a primeira semana. Annais da Conferencia APINCO Ciencia e Tecnologia Avícolas. FACTA, Campinas, p.81-91.

Noy Y. & Sklan D. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366-373.

Pegg A.E. & Mccann P.P. 1982. Polyamine metabolism and function. Am. J. Physiol., Cell Physiol. 243:212-221.

Rostagno H.S., Albino L.F.T., Donzele J.L., Gomes P.C., Oliveira R.F., Lopes D.C., Ferreira A.S. & Barreto S.L.T. 2005. Tabelas Brasileiras para Aves e Suínos: composição de alimentos e exigências nutricionais. 2ª ed. Universidade Federal de Viçosa, Viçosa. 185p.

Ruemmele F.M., Ruemmele C., Levy E. & Seidman E. 1999. Les mécanismes moléculaires de la régulation du renouvellement de cellules épithéliales intestinales par des nutriments. Gastroenterol. Clin. Biol. 23:47-55.

Ruiz-Feria C.A., Kidd M.T. & Wideman R.F. 2001. Plasma levels of arginine, ornithine, and urea and growth performance of broilers fed supplemental L-Arginine during cool temperature exposure. Poult. Sci. 80:358-369.

SAEG 1997. Sistema de Análises Estatísticas e Genéticas: manual do usuário. Universidade Federal de Viçosa, Viçosa. Versão 7.1.

Sklan D. & Noy Y. 2004. Catabolism and deposition of amino acids in growing chicks: effect of dietary supply. Poult. Sci. 83:952-961.

Tamir H. & Ratner S. 1963. Enzymes of arginine metabolism in chicks. Arch. Biochem. Biophys. 102:249-58.

Vieira S.L. & Berres J. 2007. El cuarto aminoácido limitante para pollos de engorde. Anais XX Congreso Latinoamericano de Avicultura, Porto Alegre, p.143-152.

Wang J.J., Chen L.X., Li P., Li X.L., Zhou H.J., Wang F.L., Li D.F., Yin Y.L. & Wu G. 2008. Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J. Nutr. 138:1025-1032.

Wu G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1-17.

Wu G. & Morris JR S.M. 1998. Arginine metabolism: Nitric oxide and beyond. Biochem. J. 336:1-17.