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Effects of dietary arginine supplementation on broiler breeder egg production and hatchability

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■Keywords

Amino acids, egg weight, embryo diagnosis, hatch.

ABSTRACT

This study investigated the effects of arginine (Arg) supplementation on broiler breeder egg production and egg quality. Male (30) and female (360) Ross® breeders, totaling 390 birds, were studied. A completely randomized design with five Arg levels (0.943%, 1.093%, 1.243%, 1.393%, 1.543% digestible Arg) and six replicates of 12 females and one male per experimental unit was applied. The following performance and egg quality were evaluated: lay percentage, albumen and yolk contents, average egg weight, egg specific gravity, and eggshell percentage and thickness. Hatchability and and embryo mortality were also determined. The applied dietary digestible Arg levels quadratically influenced egg production ($p<0.05$), with the highest production obtained when 1.262% digestible Arg was supplemented. Egg weight linearly increased ($p<0.05$) with digestible Arg dietary level; however, egg specific gravity linearly decreased ($p<0.05$). Hatchability was not affected ($p<0.05$) by digestible Arg level. The supplementation of broiler breeder diets with Arg improved egg production and egg weight without any effect on hatchability. Further research is needed to determine the effect of dietary Arg supplementation on the performance of the progeny.

INTRODUCTION

During the last few years, improvements in nutritional management, genetics, and breeding techniques have resulted in approximately three additional chicks per breeder per year. This outstanding result derives from the combined improvements obtained in livability, egg production, egg quality, fertility, hatchability, and chick quality. Broiler breeders must be fed to meet their performance requirements, as well as the needs of the embryos and newly-hatched chicks. Embryo development and vitality are completely dependent on the nutrients present inside the egg, which derive from the diet and metabolism of laying hens (Oviedo-Róndon & Murakami, 1998).

However, the biggest challenge of feeding broiler breeders is not the diet itself, but how it should be supplied to the birds. Due to their large appetite and high weight gain capacity, broiler breeders have to be submitted to feed restriction after the second week of life. This means that from this early age on, daily nutrient intake is completely controlled by the farmer, and not by the birds. Their weight gain and egg production must be continuously monitored, and feeding must be adjusted to meet their daily nutritional requirements.

During lay, it is especially important to supply adequate amino acid quantities and ratios in order to obtained maximum egg mass yield at egg production peak, because egg size has a significant effect on initial chick weight and on its subsequent development (Vieira & Moran

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Jr., 1999). Achieving maximum egg weight during the initial phase of lay is an important economic factor.

Furthermore, improvements in breeder nutrition have a significant multiplying effect. Considering a breeder produces 130-140 chicks during its life time, small variations in breeder performance resulting from better nutrient intake have an important impact on farm profitablity (Calini & Sirri, 2007).

Although the supplementation of synthetic amino acids allows feed formulation to be easily adjusted, the adequate supply of essential amino acids demands proper knowledge on their metabolic effects on egg production, quality, fertility, and hatchability. The excessive or unbalanced intake of essential and non-essential amino acids relative to the requirements for optimal use by tissues can be deleterious to breeders' metabolism, particularly when there are amino acid antagonisms. The antagonism between Arg and lysine (Lys) involves structurally similar amino acids. The excess of one of them increases the requirement of the other (Jones *et al.*, 1967; Austic & Scott, 1975). Literature on the effects of the antagonism between these two amino acids on broiler performance (Mendes *et al.*, 1997; Costa *et al.*, 2001; Balnave & Brake, 2002; Atencio *et al.*, 2004), muscle development (Corzo *et al.*, 2003; Fernandes *et al.*, 2009), immune system (Kidd, 2004; Deng *et al.*, 2005; Tayade *et al.*, 2006), and skeletal system (Sekine *et al.*, 1996; Conconi *et al.*, 2001; Gadelha, 2004) is extensive; however, few studies have approached this subject relative to broiler breeder productive and reproductive performance (Basiouni *et al.*, 2006; Basiouni, 2009).

The antagonism between Arg and Lys significantly increases the activity of renal arginase, inducing Arg breakdown, and decreases the activity of glycine amidinotransferase, an enzyme that uses Arg for the synthesis of muscle creatine, an essential component of muscle energy metabolism. As a result, there is a metabolic requirement, but not necessarily a dietary requirement for Arg in this case.

Arginine participates in the synthesis of ornithine, a precursor of polyamines that have a key role in cell division, in DNA synthesis, and in cell cycle regulation. Arginine also participates in the synthesis of nitric oxide (NO) by the catalytic action of a group of isoenzymes called nitric oxide synthetases (NOS), which is the only physiological pathway for the production of NO. Nitric oxide is a highly reactive free radical; it crosses cell membranes and participates in several cell processes, including neurotransmission and immunity (Moncada *et al.*, 1991). Arginine is also considered a powerful secretagogue, increasing the release of insulin, growth hormone, and IGF-A in the blood stream. Arginine is also known for stimulating ovulation by increasing the release of luteinizing hormone (LH) (Basiouni *et al.*, 2006). It is part of the hormone vasotocin, which is involved in uterine contraction and oviposition.

This study aimed at evaluating the effects of dietary Arg supplementation on broiler breeder egg production and egg quality.

MATERIAL AND METHODS

This experiment was conducted at the experimental poultry farm of the Federal University of Paraná, Palotina campus. Ross broiler breeders were selected at 18 weeks of age from the breeder stock. Females weighing between 1,850 and 1,950 g and males weighing between 2,450 and 2,550 g were distributed into 30 pens, which were randomly assigned to five dietary treatments with six replicates each. Each pen housed 12 females and one male, totaling 390 birds. Pens were divided by wire mesh and were equipped with a bell drinker and two tube feeders: one had a male-restriction grid, while the other was placed higher to allow only males to feed.

The experimental period started in week 25, when birds reached 5% egg production, and ended when on week 56. Birds mated at 18 weeks of age in a ratio of one male for every 12 females.

The lighting program started when birds were 19 weeks old with 14 h of light per day, which was increased in 30 min per week until reaching 17 h of light per day, when birds were 25 weeks old.

Feed supply was restricted and daily controlled and water was provided ad litibum. The nutritional requirements used for the formulation of the male and female diets followed the recommendations of the genetic line manual.

After 23 weeks of age, females were fed the experimental diets, which consisted of the basal diet supplemented with five graded levels of L-Arg, ranging from 0 to 600 mg/kg. Arginine was added to the basal diet at the expense of the inert component, corresponding to 0.943%, 1.093%, 1.243%, 1.393%, and 1.543% digestible Arg (Table 1) and Arg/Lys ratios of 132, 153, 175, 196, and 217, respectively. Males were fed a single diet during the entire experimental period.

Egg production was determined by daily egg collection, which was recorded in one spreadsheet per replicate. Total weekly egg production and egg

production percentage per experimental unit were calculated. At 30, 34, and 38 weeks of age, all eggs produced on a single day were collected for quality evaluation (average weight, specific gravity, albumen and yolk percentages, and eggshell percentage and thickness).

Table 1 – Calculated percent composition of experimental diets of broiler breeders in the experimental period.

Ingredients	Composition (%)
Corn	60.99
Wheat bran	4.20
Soy oil	2.00
Soybean meal	22.60
Common salt	0.32
Kaolin	1.00
Calcitic limestone	6.47
Dicalcium phosphate	1.31
Sodium bicarbonate	0.10
DL-Methionine	0.14
Choline chloride	0.16
Potassium carbonate	0.21
Vitamin and mineral premix1	0.50
Calculated Values	
Protein, %	15.51
ME, kcal/kg	2.815
Fat, %	4.77
Linoleic acid, %	2.48
Calcium, %	2.95
Available phosphorus, %	0.44
Digestible lysine, %	0.71
Digestible Met. + cystine, %	0.60
Digestible threonine, %	0.51
Digestible arginine, %	0.94
Mongin, Meq/100 g	213.88

1 Content per kg of vitamin premix. Vit. A 8,000 IU, Vit. D3, 2,200 ICU, Vit. E 6,200 ICU, Vit. K 3,2000 ICU, Vit. B 1 2.0 mg, Vit. B2 3.0 mg, Vit. B6 6.0 mg, Vit. B12 10 µg, Calcium pantothenate 6 mg, Niacin 25 mg, Folic acid 400 mg, Se 0.1 mg, Mn 65 mg, Fe 40 mg, Cu 10 mg, Zn 50 mg, I 1.0 mg,

The amount of feed supplied to the females was determined as a function of egg production and body weight, and to the males, as a function of body weight (according to the recommendations of the genetic company). Body weight was weekly monitored until week 35, and then every two weeks from week 36 until the end of the experiment.

Approximately 400 eggs (80 eggs per treatment) were collected in week 40. All eggs were incubated to determine hatchability. Eggs were weighed and incubated at each evaluated age, according to treatment and replicate, and evaluated for average hatched egg weight, average hatchling weight, progeny weight at hatch relative to egg weight, and hatchability. Non-hatched eggs from each replicate were analyzed at the end of the experimental period to determine embryo mortality cause and period, which was divided in early (1-7 days), intermediate (7-14 days), and late (14-21 days) mortality.

Specific egg gravity was determined by the method of immersing the eggs in saline solution. Six solutions of common salt dissolved in water with densities between 1,070 and 1,090, at 0.004 graded levels, were prepared. Egg specific gravity was determined using a petroleum densimeter.

Average albumen weight per replicate was determined as the difference between average egg weight and average yolk weight plus eggshell weight per replicate. In order to determine eggshell weight, eggs were broken and the shells were washed and dried at room temperature for 48h before weighing on an analytical balance. After weighing the shells, eggshell thickness was measured using a digital micrometer (Mitutoyo®) at four points at the equatorial region of each eggshell. .

Orthogonal polynomials were used for analysis of variance and analysis of regression according to data distribution using the statistical package software SAEG®.

The following statistical model was applied:

$$Yij = b0 + b1Ai + b2Ai + b3Ai + eij$$

where:

Yij: observation of the dependent variable in the experimental unit j submitted to level i of Arg, i: 1, 2, 3, 4, 5 (1 = 0.943, 2= 1.093, 3 = 1.243, 4 = 1.393, and 5 = 1.543%),

b0: constant;

b1, b2, and b3: are, respectively, linear, quadratic, and cubic coefficients of regression of the dependent variable as a function of the levels of Arg;

eij: random error associated with each observation Yij

Table 2 – Mean and standard error of egg production and egg quality parameters of broiler breeders fed diets supplemented with increasing Arg levels.

Arg (%)	Egg production (%)	Egg Weight (g)	Yolk (%)	Albumen (%)	Eggshell (%)	Eggshell thickness (mm)	Specific Gravity (g/ml)
0.943	77.41±4.60	60.46±0.48	29.98±0.35	59.55±0.45	8.76±0.15	0.577±0.004	1.082±0.001
1.093	81.01±5.44	60.81±0.52	29.72±0.42	59.74±0.40	9.02±0.14	0.584±0.005	1.082±0.001
1.243	82.40±3.70	60.86±0.51	29.71±0.43	60.35±0.31	8.75±0.11	0.569±0.004	1.081±0.001
1.393	81.58±3.68	61.33±0.57	29.86±0.41	59.84±0.43	8.80±0.09	0.571±0.003	1.080±0.001
1.543	78.55±6.00	61.67±0.46	29.64±0.30	60.15±0.38	8.76±0.10	0.573±0.004	1.081±0.001
CV(%)	6.64	1.83	1.95	1.16	3.51	1.98	0.133
Variance Analysis							
Effect	Quadratic1	Linear2	ns	ns	ns	ns	Linear3
R2	0.43	0.14	-----	-----	-----	-----	0.13

1. Y= 4.21330+0.1239X-0.0004908X2 R2: 0.53; 2. Y = 58.5813+0.001963X R2: 0.95; 3. Y = 1.08413-0.0025X R2: 0.66; ns – not significant

RESULTS AND DISCUSSION

Egg production and egg quality results of broiler breeders fed increasing Arg levels are presented in Table 2. Dietary digestible Arg levels quadratically influenced egg production percentage ($p<0.05$). The highest egg production percentage was obtained when 1.262% Arg was fed (Figure 1).

These results are consistent with those obtained by other authors, who also found increasing egg production percentage as a function of Arg dietary supplementation, and consequently, of increasing in Arg:Lys ratio. According to Najib & Basiouni (2004), breeder egg production increased from 52% to 67.86% when dietary Arg and Lys levels were 1.5 and 1.2%, respectively. Basiouni *et al.* (2006) also observed an increase in follicle F1 weight in breeders fed diets containing 2.05% Arg, when compared with those fed diets with 1.54% Arg. This effect suggests that the increase in egg production may have been due to the specific stimulating effect of Arg on the secretion of LH (luteinizing hormone), which acts directly on the ovary and the follicles (Basiouni, 2009).

The egg production curve (Figure 1) shows an increase up to 1.262% Arg and then a decrease until 1.543% Arg, which may be related to amino acid imbalance in the diets with extremely Arg high levels. High Arg levels may also affect egg production because of the energy spent for its metabolism and excretion. Because Arg is the amino acid with the greatest number of nitrogen molecules in its structure, it also depends the most on energy for its degradation (Summers & Leeson, 2001), mainly in uricotelic species.

A uric acid molecule is always excreted together with a glycine molecule. Although poultry synthesize glycine, this synthesis is not fast enough to meet the tissue needs and to eliminate all nitrogen excess (Corzo *et al.*, 2004). Under these conditions, methionine can become limiting due its participation in methylation reactions.

The use of amino acids in broiler and breeder diets depends on the breeding objectives. According to their metabolic demand, they may be redirected to the supply of energy or to the synthesis of protein or of other nitrogen compounds. According to Baker (2005), the issue of priority in the functional synthesis is an area of nutrition that is not fully understood yet for many amino acids that have important precursor functions: Arg in the synthesis of proteins, creatine, nitric acid, proline, and polyamines; tyrosine in the synthesis of proteins, catecholamines, tyrosine, and melanin; tryptophanin the synthesis of proteins, serotonin, and niacin, and glycine in the synthesis of proteins (contractible proteins and collagen), creatine, and uric acid.

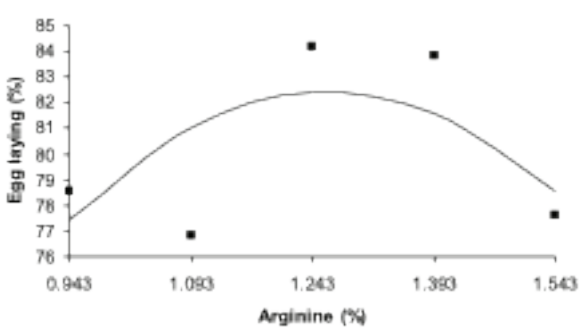

Figure 1 – Egg production percentage of broiler breeders fed diets supplemented with increasing digestible Arg levels.

Low Arg:Lys ratios in breeder diets reduce feed intake and egg production (Basiouni *et al.*, 2006). High Lys levels associated with low Arg levels promote a significant increase in the activity of renal arginase, and consequently, induce Arg degradation (Austic & Scott, 1975), causing symptoms of Arg deficiency, as poultry do not present a functional urea cycle (D' Mello, 2003). This antagonism also reduces the activity of glycine-amidinotransferase, the enzyme that uses Arg and glycine as substrates, together with methionine, for the synthesis of creatine in the muscles (Jones *et al.*, 1967).

The supplementation of breeder diets with Arg linearly increased ($p<0.05$) egg weight. In contrast, an opposite effect ($p<0.05$) was observed for specific egg gravity (Figure 2).

Figure 2 – Weight and specific gravity of eggs of broiler breeders fed diets supplemented with increasing Arg levels.

Egg weight and egg specific gravity are inversely correlated because, according to Basiouni *et al.* (2006), eggshell synthesis in the chicken is constant. As egg weight increased in the present experiment, calcium deposition on the shell surface was not sufficient to maintain eggshell thickness proportional to that egg weight increase.

Basiouni *et al.* (2006) observed that egg specific gravity was worse when breeder diets were supplemented with high Arg and Lys levels. These authors also observed that excessive Arg was more detrimental to eggshell quality than high Lys levels. Similar results were already reported by Najib & Basiouni (2004), who did not find any explanation for the negative effect of Arg on this parameter. In contrast, Lima & Silva (2007) did not observe any effect of different digestible Arg:Lys ratios on egg quality parameters.

Hatching results of broiler breeders fed increasing digestible Arg levels are shown in Table 3. Digestible Arg levels did not affect ($p>0.05$) any of the hatching parameters. Fertility and hatchabilty values found in present study agree with the values of the genetic strain manual. This indicates that Arg can be added

Table 3 – Mean reproductive values of broiler breeders fed diets supplemented with increasing levels of Arg.

	Variables				
Level of Arg (%)	Fertility (%)	Hatchability (%)	Egg Weight (g)	Chick Weight (g)	Egg Weight/Chick Weight
0.943	97.50±2.18	78.75±4.50	62.69±0.42	45.07±0.53	71.68±0.45
1.093	95.13±2.12	80.38±4.59	62.12±0.33	43.90±0.45	71.15±0.54
1.243	92.50±2.06	81.25±4.64	62.00±0.47	44.87±0.66	72.34±0.47
1.393	97.50±2.18	80.00±4.57	63.14±0.72	45.14±0.86	71.73±0.82
1.543	96.25±2.15	77.50±4.43	63.51±0.55	45.29±0.39	71.27±0.45
Mean	95.78	79.58	62.69	44.86	71.63
CV (%)	6.56	16.41	2.31	3.74	2.22
Analysis of variance					
% Arginine	ns	ns	ns	ns	ns

ns = not significant.

to breeder diets with no reproduction losses; in fact, it improves egg production and weight. Despite the worse egg specific gravity observed with increasing dietary Arg levels, neither hatchability nor embryo mortality were affected.

Embryo diagnosis data obtained were not influenced ($p>0.05$) by Arg levels. There was a numerical trend for increased late mortality rate (18 to 21 days of incubation). At this hatching age, mortality is more related to hatching environment factors, such as temperature, humidity, and CO^2 and oxygen levels, than to maternal nutrition (Oviedo-Róndon & Murakami, 1998).

The positive effects of Arg supplementation to the maternal diet on egg production and the lack of effect on hatching parameters suggest that advances may be achieved by nutritional programs and broiler genetic improvement. However, further studies should be conducted to investigate the possible effects of dietary Arg on progeny performance. Nutrition can significantly influence the phenotypical expression of specific genotypes through direct effects on genetic transcription, changing specific key metabolism pathways and consequently affecting the health and performance of the progeny.

CONCLUSION

The supplementation of broiler breeder diets with 1.262% and 1.543% arginine increased egg production and egg weight, respectively. Dietary arginine levels did not have any negative effect on the studied reproduction parameters, and therefore increasing levels may be used in the maternal diet to improve egg production.

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