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Estimation of Arginine Requirements for Male Broilers Grown at High Altitude from One to Twenty-one Days of Age

F. Khajali[1*], H. Basoo[1], and M. Faraji[2]

ABSTRACT

Arginine (Arg) requirements of male broilers (Ross 308) reared at high altitude (2100 m above sea level) during the 1-to 3-week period was estimated in a graded supplementation method, in which six diets (230 g kg^{-1} CP and 3200 Kcal ME kg^{-1}) with Arg increments of 1.25 g kg^{-1} were applied to four pens of fifteen birds each. Dietary Arg content of the experimental groups ranged from 10 g kg^{-1} (80% of the NRC recommendation) to 16.25 g kg^{-1} (130% of the NRC recommendation). Body weight gain, feed: gain ratio, and plasma nitric oxide (NO) concentration were determined as response criteria. Arginine requirements for maximal body weight gain and optimal feed: gain during the 21 days under study were estimated to be 15.3, and 15.1 g kg^{-1} of diet, respectively. The estimated Arg requirement based on the response in plasma NO was 15.6 g kg^{-1}. In conclusion, broilers receiving dietary Arg of 15 g kg^{-1} had the best performance. Data obtained for the individual factors are by far the highest values reported for Arg requirements.

Keywords: Amino acids, Chicken, Growth stage, Needs.

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INTRODUCTION

Arginine (Arg) is an essential amino acid for the chicken. Unlike most mature mammals, chickens are not able to synthesize Arg because they lack the key enzyme carbamoyl phosphate synthase I (EC 6.3.5.5.). Therefore, Arg must be adequately available in chicken diets to support protein accretion and maintain the physiological and immunological functions (Khajali and Wideman, 2010).

Arg requirements of broiler chickens advocated by the National Research Council (NRC) are minimal requirements established under ideal environmental conditions. These requirements recommend Arg at 12.5 g kg^{-1} of the diet to 3 weeks, and 11.0 g kg^{-1} from 3 to 6 weeks (NRC, 1994). Recent studies (Izadinia *et al.,* 2010; Basoo *et al.,* 2012) reported that the NRC recommendations for Arg may not be adequate for broilers grown at high altitudes where atmospheric oxygen availability is limiting for maximal growth and physiological functions. Arg is the key nutrient from which nitric oxide (NO) is generated. Nitric oxide is a potent vasodilator that directly relaxes vascular smooth muscle and modulates or inhibits the production and release of vasoconstrictors such as serotonin (Khajali *et al.,* 2011a). Therefore, Arg requirements of birds reared at high altitude can be expected to exceed the NRC recommendation. Recent reports (Bautista-Ortega and Ruiz-Feria, 2010; Khajali *et al.,* 2011b) reported that Arg supplementation at levels beyond the NRC recommendations for broiler chickens grown at high altitude significantly increased plasma level of NO and improved growth performance. The present study was conducted to establish the Arg requirements for broiler chickens during 1 to 3 weeks of age grown at high altitude (exposed to hypobaric hypoxia).

[1] Department of Animal Science, Faculty of Agriculture, Shahrekord University, Shahrekord, Islamic Republic of Iran.
[*] Corresponding author; e-mail: khajali@agr.sku.ac.ir or khajali@gmail.com
[2] Jihad-e-Agriculture Organization, Shahrekord, Islamic Republic of Iran.

MATERIALS AND METHODS

Birds and Experimental Facility

The experiment was conducted in Shahrekord, Iran (2,100 m above sea level). The partial pressure of oxygen at such a high altitude region is estimated to be 145 mm Hg, which imposes severe hypobaric condition. According to Julian (2007), the partial pressure of oxygen reduces about 7 mmHg for each 1,000 m increase in altitude from sea level. The experimental animals were kept, maintained, and treated according to accepted standards for the humane treatment of animals.

A total of 360 day-old male broilers (Ross 308) were randomized across 24 floor pens measuring 1.8 m^2 (15 bird pen^{-1}). Each pen was equipped with a bell drinker and a feed trough. At 1 day of age, chicks were allocated to pens so that the mean body weight of birds was equal among all pens (42±1.5 g). Temperature of the experimental facility was maintained at about 32°C for week 1, 29°C for week 2, 26°C for week 3, and 22°C thereafter. Birds were subjected to 23 hours light and 1 hour dark throughout the trial. Broilers had *ad libitum* access to feed and water.

Treatments

A basal diet deficit in Arg (230 g kg^{-1} CP and 3200 kcal. kg^{-1}) was formulated containing corn, soybean meal, and corn gluten meal (Table 1). To make dietary treatments, supplement of L-Arg (Ajinomoto AminoScience LLC, Raleigh, NC, USA) with 99% purity at 1.25 g kg^{-1} increments was fortified to the basal diet at the expense of washed builder's sand. Treatments, therefore, consisted of six graded levels of total Arg (10.00, 11.25, 12.50, 13.75, 15.00 and 16.25 g kg^{-1}, which met 80, 90, 100, 110, 120, and 130% of NRC recommendations, respectively) fed from 1 to 21 days of age. All other essential amino acids met the requirement for broilers from 1 to 3 weeks of age. All diets had 3200 kcal. kg^{-1}. Each treatment was replicated four times. The samples of corn, soybean meal, and corn gluten meal as well as mixed diets were analyzed for CP and amino acid content before the feeding trial. For the determination of amino acid content, duplicate samples of each diet were subjected to 6N HCl and hydrolyzed for 24 hours at 110°C (Andrew and Baldar, 1985). After acid hydrolysis, all samples were analyzed for amino acid content by using an ion-exchange chromatograph (LKB 4141 Amino Acid Analyzer; LKB Biochrom Ltd., Cambridge, UK).

Measurements

During 1 to 21 days of age, feed intake and body weight were weekly recorded to obtain weight gain and feed: gain. At 21 days of age, 10 birds per treatment were selected for blood collection. Blood samples (3 mL) were collected from the brachial vein in heparinized syringes and centrifuged at 2,500×g for 10 minutes to obtain plasma. Plasma samples were used for the determination of plasma NO (nitrate+nitrite) according to Hortelano *et al*. (1995) by adding 250 µl of plasma to 1 mL of Griess reagent. The Griess reagent was a mixture (1:1) of 1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine, giving a red-violet color in presence of nitrite, the stable form of NO. The absorbance was measured at 540 nm by means of a spectrophotometer (480 Specrophotometer; Corning Incorporated, New York City, NY, USA). All chemical reagents were obtained from Sigma–Aldrich Co. (Sigma–Aldrich Co., St. Louis, MO, USA).

Statistical Analysis

A completely randomized design was used to analyze data according to the

Table 1. Composition of the arginine-deficit basal diet fed to broilers from 1 to 21 days of age.

Feed ingredient	Amount in the starting diet (g kg^{-1})
Corn	620
Soybean meal (40% CP)	113
Corn gluten meal (62% CP)	200
Soy oil	11
Dicalcium phosphate	20
Limestone	16
Salt	4
L-lysine.HCl	4.2
L-threonine	4
Vitamin supplement[a]	2.5
Mineral supplement[b]	2.5
Sand	2.8
Total	100
Calculated composition	
AME (Kcal kg^{-1})	3200
Dry matter (g kg^{-1})	900
Crude protein (g kg^{-1})	230 (231)[*]
Ether extract (g kg^{-1})	40
Total arginine (g kg^{-1})	10.0 (9.96)
Total lysine (g kg^{-1})	11.0 (11.1)
Total Met+Cys (g kg^{-1})	9.0(8.9)
Total threonine (g kg^{-1})	7.6 (7.56)
Calcium (g kg^{-1})	10
Non-phytate phosphorous (g kg^{-1})	4.5

[a] Provided the following per kg of diet: Vitamin A (trans-retinyl acetate), 3600 IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-_-tocopheryl acetate), 7.2 mg; vitamin K3, 1.6 mg; vitamin B1, 0.72 mg; vitamin B2, 3.3 mg; vitamin B3, 0.4 mg; vitamin B6, 1.2 mg; vitamin B12, 0.6 mg; folic acid, 0.5 mg, choline chloride, 200 mg. [b] Provided the following per kg of diet: Mn (from MnSO4·H2O), 40 mg; Zn (from ZnO), 40 mg; Fe (from FeSO4·7H2O), 20 mg; Cu (from CuSO4·5H2O), 4mg; I (from Ca(IO3)2·H2O), 0.64 mg, Se (from sodium selenite), 0.08 mg.
[*] Values in parenthesis are levels obtained by analysis.

ANOVA procedure of SAS 2007 (version 9, SAS Institute, Cary, NC). In cases where there was sampling within pens, data were subjected to a nested design. The means were separated by Least Significant Difference (LSD) at $P<$ 0.05. The requirement for Arg was estimated with the single-slope broken-line regression model (Robbins *et al.,* 2006) using the NLIN procedure of SAS 2007 (Version 9, SAS Institute, Cary, NC) with the Arg intake (g kg^{-1}) as the independent variable and performance criteria as dependent variables. The data were also analyzed for linear and quadratic regression to assess the curvilinear responses to dietary Arg level.

RESULTS

As depicted in Table 1, calculated levels of amino acids in the basal diet showed very close agreement with their analysis values. Birds fed diets containing 13.75 and 15 g kg^{-1} Arg (110 and 120% of the NRC recommendations) had weight gains (520.7 and 587.5 g bird^{-1}) that were significantly higher than responses of body weight gain (488 g bird^{-1}) of birds provided with Arg at the NRC recommendation level (12.5 g kg^{-1} Arg) (Table 2). These significant (P< 0.05) improvements in weight gain occurred at dietary Arg levels of 10 and 20% higher

Table 2. Responses of 1- to 21-day-old broilers grown at high altitude to dietary arginine[a].

Dietary arginine ($g\ kg^{-1}$)	Weight gain* ($g\ b^{-1}$)	Feed:gain[b] (g:g)	Plasma nitric oxide[c] (µmol)	Initial weight ($g\ b^{-1}$)	Final weight ($g\ b^{-1}$)
10.00	481[c]	2.05 [b]	39.6[c]	45.0	526[c]
11.25	487[c]	2.01[ab]	39.7[c]	45.1	532[c]
12.50	488[c]	1.96[ab]	40.1[b]	45.0	534[c]
13.75	521[b]	1.94 [a]	40.1[b]	45.2	566[b]
15.00	579[a]	1.85 [a]	40.8[a]	45.0	624[a]
16.25	469[c]	2.10 [b]	39.9[b]	45.2	514[c]
LSD	28.8	0.11	0.30	0.75	30.1

[a] Means in the same column with different letters are significantly different (P< 0.05); [b] Each mean represents values from four replicates, [c] Each mean represents values from 10 replicates.

than the NRC recommendation clearly demonstrate that broilers reared at high altitude have Arg requirements that are remarkably higher than the NRC recommendation. Responses to Arg supply were nonlinear and attained plateau values (y_{max}) within the studied range of Arg supply. Regression analysis showed a significant quadratic response to Arg for weight gain ($Y= -600.8+166.2x- 6.07x^2$; R^2= 0.49; P= 0.0033; n= 4 pens). Broken-line analysis indicated the level of Arg needed for maximizing body weight gain was 15.3 g kg^{-1} of diet. Broilers on the diet including Arg at 20% higher than the NRC value had the best feed: gain. Response of feed: gain to dietary Arg content was curvilinear ($Y= 4.76-0.44x+0.016x^2$; R^2= 0.52; P= 0.0004; n= 4 pens). Feed : gain response was improved up to the level of 15 g kg^{-1} Arg in the diet. Broken-line analysis indicated that Arg requirement for optimal feed: gain response was 15.1 g kg^{-1} of diet. Table 3 shows weekly responses of broilers to dietary arginine during 1- to 21 days of age in terms of body weight gain, feed intake, and feed conversion ratio.

Plasma NO level exhibited a significant (P< 0.05) elevation trend when Arg was supplemented to the basal diet (Table 2). Regression analysis showed linear and significant quadratic responses to Arg for plasma NO ($Y= -89.5+18x-0.6x^2$; R^2= 0.66; P= 0.0001). Based on the responses of plasma NO, the estimated requirement was 15.6 g kg^{-1} of dietary Arg.

Table 3. Weekly responses of broilers grown at high altitude to dietary arginine during 1- to 21-day-old.

Weeks	Dietary arginine ($g\ kg^{-1}$)						LSD
	10.00	11.25	12.50	13.75	15.00	16.25	
	Cumulative feed intakes (g per bird)						
1	110[abc]	106[bc]	112[ab]	112[ab]	116[a]	104[c]	6.5
2	295	292	297	308	311	290	26.5
3	516[b]	526[b]	519[b]	528[ab]	570[a]	519[b]	43.2
	Cumulative body gains (g per bird)						
1	70d	70d	73cd	79b	83a	74c	3.5
2	158[bc]	160[b]	156[bc]	168[b]	187[a]	147[c]	12.8
3	253[c]	257[bc]	259[bc]	274[b]	309[a]	248[c]	18.5
	Feed conversion ratios (g feed: g gain)						
1	1.56[a]	1.52[a]	1.54[a]	1.42[b]	1.40[b]	1.42[b]	0.06
2	1.88[ab]	1.82[b]	1.91[ab]	1.83[b]	1.65[c]	1.98[a]	0.11
3	2.05[ab]	2.05[ab]	2.01[abc]	1.94[bc]	1.85[c]	2.10[a]	0.17

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DISCUSSION

The Arg estimates for performance and physiological responses reported herein are higher than those previously reported by Labadan *et al.* (2001) as well as published standards (NRC, 1994). These findings imply the direct role of Arg as a key amino acid to maintain growth (protein synthesis and accretion) and physiological functions through the biosynthesis of NO. It has been shown that Arg is the precursor of several growth factors including putrescine, spermine and spermidine. Through the formation of glutamate, Arg also increases the synthesis of proline and hydroxyproline, which are required for the production of connective tissue (Khajali and Wideman, 2010). Additionally, the production of insulin-like-growth-factor (IGF) and the release of anti-insulinemic hormones like glucagon, somatostatin, pancreatic polypeptides and catecholamines are enhanced by Arg (Barbul, 1986). In fairness to our study, there are some reports indicating that Arg supplementation beyond the NRC recommendations improved broiler growth performance (Kidd *et al.*, 2001; Fernandez *et al.*, 2009; Ruiz-Feria, 2009). Kidd *et al.* (2001) reported that supplementing broiler diets with 2 g kg^{-1} Arg beyond the NRC requirements resulted in improved growth performance, even under normal conditions.

It is worth to mention that the NRC (1994) recommendations for nutrient requirements are minimal requirements that have been established under ideal environmental conditions. Hypoxic condition at high altitude imposes an additional stress on Arg requirements due to the low availability of oxygen attributable to hypobaric hypoxia. Each 500 m increase in altitude above sea level reduces oxygen availability by about 1% from 20.95% at sea level (Julian, 2007). Hypoxic pulmonary vasoconstriction is counteracted by increased synthesis of the potent pulmonary vasodilator NO from Arg.

The remarkable decrease in growth performance of broilers at dietary Arg level of 16.25 g kg^{-1} was attributed to the antagonism between Arg and Lys. The antagonism between Arg and Lys has been well documented so that when there is an excess of one of these two amino acids, the utilization of the other will be reduced (Khajali and Wideman, 2010).

Dietary supplementation with L-Arg increased plasma NO levels. This observation indicates that an increase in endogenous NO synthesis may relax the tone of the resistance arterioles in the lungs of broilers. An important role for L-Arg as precursor of NO in attenuating the onset of broiler pulmonary hypertension was first demonstrated by Wideman *et al.* (1995). In agreement with our study, Ruiz-Feria and Abdukalykova (2009) indicated that supplementing a broiler diet with 1% Arg plus vitamins E and C resulted in increased NO production and reduced pulmonary hypertension. The estimate of Arg requirement for NO production is by far the highest value reported for broilers. Reports of Ruiz-Feria (2009) and Khajali *et al.* (2011b), who demonstrated that dietary Arg supplementation exceeding the NRC recommendation elicited increased NO production. further supports this observation. Most recently, Basoo *et al* (2012) re-evaluated the Arg requirements for broilers raised at high altitude during the 3- to 6-week period. They estimated that the Arg requirement for maximal growth and the prevention of pulmonary hypertension was 20% higher than that of the NRC (1994) recommendation (1.32 *vs.* 1.1% of diet). In line with the study of Basoo *et al* (2012), the present study reports the Arg requirement for maximizing growth and optimizing feed conversion ratio to be 22% higher than the NRC (1994) recommendation.

CONCLUSIONS

In conclusion, dietary requirements presented by the NRC (1994) provide minimal requirements that have been established in regions where altitude does not limit the availability of atmospheric oxygen. Dietary Arg requirements for broilers advocated by NRC are not adequate to support maximal growth and to avoid pulmonary hypertension at high altitudes.

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تعیین احتیاجات آرژنین جوجه خروس های گوشتی پرورش یافته در ارتفاع بالا در دوره ۱ تا ۲۱ روزگی

ف. خواجعلی، ح. باسو و م. فرجی

چکیده

در مطالعه حاضر، احتیاجات آرژنین جوجه خروس های گوشتی (راس ۳۰۸) پرورش یافته در منطقه ای مرتفع (۲۱۰۰ متر از سطح دریا) در دوره آغازین با استفاده از روش مکمل سازی درجه بندی شده تعیین گردید. بدین منظور، شش جیره غذایی (انرژی متابولیسمی 3200 کیلوکالری در هر کیلوگرم و پروتئین خام ۲۳۰ گرم در هر کیلوگرم) با سطوح ۱۰، ۱۱/۲۵، ۱۲/۵، ۱۳/۷۵، ۱۵ و ۱۶/۲۵ گرم در کیلوگرم آرژنین تهیه گردید. برای هر تیمار، چهار تکرار ۱۵ جوجه ای در نظر گرفته شد و خوراک ها از ۱ تا ۲۱ روزگی به صورت آزاد تغذیه شدند. پاسخ های مورد بررسی شامل اضافه وزن بدن، ضریب تبدیل خوراک و غلظت نیتریک اکساید پلاسما بود. احتیاجات آرژنین برای به حداکثر رساندن اضافه وزن بدن، بهینه نمودن ضریب تبدیل خوراک و به حداکثر رساندن تولید نیتریک اکساید به ترتیب ۱۵/۳، ۱۵/۱ و ۱۵/۶ گرم در کیلوگرم خوراک تعیین گردید. این مقادیر تاکنون بیشترین مقادیر احتیاجات آرژنین است که برای جوجه های گوشتی تجارتی تعیین می گردد. در مجموع، بهترین پاسخ جوجه های گوشتی به آرژنین در سطح ۱۵ گرم در کیلوگرم خوراک حاصل شد.

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