Re-evaluation of Arginine Requirements for Broilers Exposed to Hypobaric Condition during the 3-to 6-week Period

Hamid Basoo¹, Fariborz Khajali¹, Ebrahim Asadi Khoshouï¹, Mehrab Faraji¹ and Robert F. Wideman²

¹Department of Animal Science, Shahrekord University, Shahrekord, Postal code 88186–34141, Iran
²Department of Poultry Science, University of Arkansas, Fayetteville AR, 72703, USA

In order to estimate arginine (Arg) requirements of male broilers (Ross 308) during the 21-to 42-day period and exposed to hypobaric condition, six diets (200 g/kg CP and 13.4 MJ ME/kg) with graded levels of Arg (8.8 to 14.3 g/kg) were allocated to four pens of twelve birds each. Body weight gain, feed:gain, breast meat yield, plasma nitric oxide (NO) concentration, and right to total ventricular weight ratio (RV/TV) were determined as response criteria. Responses to Arg supply were nonlinear and attained plateau values (ymax) within the studied range of Arg supply. The estimated Arg requirements for maximal body weight gain and optimal feed:gain during the 21 days under study were 12.4, and 12.2 g/kg of diet, respectively. The estimated Arg requirement for maximizing breast meat yield was 12.6 g/kg of diet. Based on the response in plasma NO and RV/TV, the estimated requirements were 13.0, and 13.2 g/kg of diet, respectively. Data obtained for the individual factors clearly indicate that the NRC recommendations for Arg are not sufficient for maximizing body weight gain, optimizing feed:gain and preventing the onset of pulmonary hypertension in broiler chickens exposed to hypobaric condition. Data may be used for future modeling of broilers’ Arg requirements.

Key words: arginine, broiler, growing period, hypobaric condition, requirement


Introduction

Though most mature mammals are able to synthesize arginine (Arg) to meet their requirements, chickens fail to synthesize Arg because they lack the key enzyme carbamoyl phosphate synthase I (EC 6.3.5.5.) and have low activities of hepatic arginase (EC 3.5.3.1., 2) and ornithine transcarbamoylase (OTC, EC 4.4.4.17.). Therefore, chickens have an absolute need for Arg and are highly dependent on dietary sources for this amino acid. Hence, sufficient Arg must be available in the feed to support protein accretion and maintain the physiological and immunological functions (Khajali and Wideman, 2010).

Arginine requirements of broiler chickens advocated by the National Research Council are minimal requirements that established under ideal environmental conditions. These requirements recommend Arg at 12.5 g/kg of the diet to 3 wk, and 11.0 g/kg from 3 to 6 wk (NRC, 1994). In a review of literature, Khajali and Wideman (2010) 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. Arginine is the substrate 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 and endothelin-1 (Wideman et al., 2001; Khajali et al., 2011a). Diminished NO availability and production has been implicated in the pathogenesis of pulmonary hypertension and ascites in broilers grown at high altitude (Izadinia et al., 2010). Therefore, Arg requirements of birds reared at high altitude can be expected to exceed the NRC recommendation. 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 3 to 6 wk of age grown at high altitude (exposed to hypobaric hypoxia).

Materials and Methods

Birds and Experimental Facility

The experiment was conducted in the experimental facility of Shahrekord University, Shahrekord, Iran, an area with the altitude of 2,100 m above sea level. Rearing the birds at such
a high altitude region imposes severe hypobaric condition (The partial pressure of oxygen is estimated to be 145 mm Hg). The partial pressure of oxygen drops approximately 7 mmHg for each 1,000 m increase in altitude, reducing the amount of oxygen available to the hemoglobin in red blood cells as blood passes through the lungs. This is equivalent to a drop of approximately 2.5% in the air oxygen for every 1,000 m increase in altitude ( Julian, 2007). The experimental animals were kept, maintained and treated according to accepted standards for the human treatment of animals. The Institutional Animal Care and Use Committee of Shahrekord University approved all procedures used in this study.

A total of 288 day-old male broilers (Ross 308) were randomized across 24 floor pens measuring 1.5 m² (12 bird/pen). Each pen was equipped with a bell drinker and a feed trough. Birds received a commercial corn-soybean meal diet (230 g/kg CP and 13.4 MJ ME/kg) from 1 to 21 days of age. At 21 days of age following an overnight fast, birds were reallocated to pens so that all pens had equal body weights (657.5 g ± 3.5 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 hr light and 1 hr dark throughout the trial. Broilers had ad libitum access to feed and water.

Treatments

A basal diet deficit in Arg containing 13.4 MJ ME/kg and 200 g/kg CP from intact protein sources was formulated using 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 1.1 g/kg increments was fortified to the basal diet at the expense of washed builder’s sand. Treatments, therefore, consisted of six graded levels of Arg (8.8, 9.9, 11, 12.1, 13.2 and 14.3 g/kg, which met 80, 90, 100, 110, 120, and 130% of NRC recommendations, respectively) fed to 21 and 42 days of age. All other essential amino acids met the requirement forbroilers from 3 to 6 wk of age. All diets had 13.4 MJ/kg ME. Each treatment had four replications. Prior to the experiment, the samples of corn, soybean meal, and corn gluten meal as well as mixed diets were analyzed for CP and amino acid content. For the determination of amino acid content, duplicate samples of each diet were subjected to 6 N HCl and hydrolyzed for 24 h 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). Performic acid oxidation was done to determine sulfur amino acids as well (Moore and Stain, 1963).

Measurements

Pen weights were obtained at 21 and 42 days of age. Feed intake, body weight gain, and feed: gain were calculated for the 21- to 42-day period. Feed: gain data for this period were corrected for mortality body weights. At 42 days of age, four birds per pen (16 birds/treatment) were selected for blood collection and processing. The selected birds had body weights within ±5% of the average pen body weight. Blood samples (3 mL) were collected from the brachial vein in heparinized syringes and centrifuged at 2,500 × g for 10 min 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-naphthylthylenediamine, 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 Spectrophotometer; Corning Incorporated, New York City, NY, USA). All chemical reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, USA).

After the blood collection, the birds were killed. Data obtained at processing included live body weight, hot carcass weight, and breast weight (skinless and boneless, Pectoralis major and Pectoralis minor). The hearts were also removed and the ventricles were dissected and weighed to calculate the right-to-total ventricular weight ratio (RV: TV ratio), as an indication of pulmonary hypertension (Saedi and Khajati, 2010; Khajali and Fahimi, 2010).

---

**Table 1. Composition of the arginine-deficit basal diet to which L-arginine added for broilers during 21 to 42 days of age**

<table>
<thead>
<tr>
<th>Feed ingredient</th>
<th>Amount in the growing diet (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>640</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>112</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>155</td>
</tr>
<tr>
<td>Fish meal</td>
<td>3</td>
</tr>
<tr>
<td>Soy oil</td>
<td>23</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>14</td>
</tr>
<tr>
<td>Limestone</td>
<td>16</td>
</tr>
<tr>
<td>Salt</td>
<td>3</td>
</tr>
<tr>
<td>L-lysine. HCl</td>
<td>3.5</td>
</tr>
<tr>
<td>L-threonine</td>
<td>0.4</td>
</tr>
<tr>
<td>Vitamin Supplement* (%)</td>
<td>2.5</td>
</tr>
<tr>
<td>Mineral Supplement** (%)</td>
<td>2.5</td>
</tr>
<tr>
<td>Sand</td>
<td>25</td>
</tr>
<tr>
<td>AME (MJ/kg)</td>
<td>13.4</td>
</tr>
<tr>
<td>Crude protein (g/kg)</td>
<td>200</td>
</tr>
<tr>
<td>Arginine (g/kg)</td>
<td>8.8 (8.76)</td>
</tr>
<tr>
<td>Lysine (g/kg)</td>
<td>10.0 (10.3)</td>
</tr>
<tr>
<td>Met+Cys (g/kg)</td>
<td>8.0 (8.0)</td>
</tr>
<tr>
<td>Threonine (g/kg)</td>
<td>5.6 (5.55)</td>
</tr>
</tbody>
</table>

*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.

**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), 4 mg; I (from Ca(IO3)2·2H2O), 0.64 mg; Se (from sodium selenite), 0.08 mg.

Values in parenthesis are amino acid levels obtained by analysis.

---
Statistical Analysis

A completely randomized design was used to analyze data by means of ANOVA procedure of SAS (version 9, SAS Institute, Cary, NC). When there was sampling within pens, data were subjected to a nested design. The requirement for Arg was estimated with the single-slope broken-line regression model (Robbins et al., 2006) using the NLIN procedure of SAS (Version 9, SAS Institute, Cary, NC) with the Arg intake (g/kg) as the independent variable and performance criteria as dependent variables. The coefficients of a, b, and c were calculated by Maple software, Version 12. The data were also analyzed for linear and quadratic regression to assess the curvilinear responses to dietary Arg level.

Results

Amino acid analysis of the basal diet (Table 1) indicates very close agreement with calculated levels. The basal diet was calculated to have 8.80 g/kg Arg and analyzed to contain 8.76 g/kg Arg, which indicated very close agreement. Birds fed diets containing 8.8 and 9.9 g/kg Arg (80 and 90% of the NRC recommendations), respectively, had weight gains (1,090.6 and 1,147.5 g/bird) and feed:gain ratios (2.54 and 2.38) that were poorer than responses of body weight gain (1,090.6 and 1,147.5 g/bird) and feed:gain ratios (2.54 and 2.38) of birds provided Arg at the NRC recommendation (11 g/kg Arg) (Table 2). Supplementing the basal diet with Arg at levels of 10 and 20% higher than the NRC recommendation (dietary Arg of 12.1 and 13.2 g/kg) created significant (P < 0.05) improvement in weight gain (1,370.6 and 1,268.6 g/bird, respectively) and feed:gain (2.15 and 2.18, respectively). Responses to Arg supply were nonlinear and attained plateau values (ymax) with those recently reported (Fernandez et al., 2009; Ruiz-

Table 2. Responses of 3- to 6-wk-old broilers grown at high altitude to dietary arginine

<table>
<thead>
<tr>
<th>Dietary arginine (g/kg)</th>
<th>Weight gain* (g/b)</th>
<th>Feed:gain* (g/g)</th>
<th>Breast yield** (%)</th>
<th>Plasma nitric oxide** (μmol)</th>
<th>RV:TV**</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.8</td>
<td>1090.6±29.2</td>
<td>2.54±0.09</td>
<td>29.0±1.92</td>
<td>36.0±0.53</td>
<td>0.23±0.036</td>
</tr>
<tr>
<td>9.9</td>
<td>1147.5±29.2</td>
<td>2.38±0.09</td>
<td>29.4±1.92</td>
<td>36.3±0.53</td>
<td>0.23±0.036</td>
</tr>
<tr>
<td>11***</td>
<td>1218.9±29.2</td>
<td>2.34±0.09</td>
<td>30.5±1.92</td>
<td>36.4±0.53</td>
<td>0.20±0.036</td>
</tr>
<tr>
<td>12.1</td>
<td>1370.6±29.2</td>
<td>2.15±0.09</td>
<td>31.1±1.92</td>
<td>37.3±0.53</td>
<td>0.20±0.036</td>
</tr>
<tr>
<td>13.2</td>
<td>1268.6±29.2</td>
<td>2.18±0.09</td>
<td>31.4±1.92</td>
<td>36.7±0.53</td>
<td>0.19±0.036</td>
</tr>
<tr>
<td>14.3</td>
<td>1177.8±29.2</td>
<td>2.42±0.09</td>
<td>29.7±1.92</td>
<td>36.7±0.53</td>
<td>0.21±0.036</td>
</tr>
</tbody>
</table>

abcd Means in the same column with different letters are significantly different (P < 0.05).
* Each mean±SE represents values from four replicates.
** Each mean±SE represents values from 16 replicates.
*** This is equal to the NRC value.
RV:TV, right to total ventricular weight ratio.

Discussion

Feeding Arg-deficit diets (Arg levels at 80 or 90% of the NRC recommendation) resulted in poor performance in terms of body weight gain and feed: gain. Arg supplementation up to 20% greater than NRC values created significant (P < 0.05) improvement in weight gain and feed: gain. This observation implies the direct role of Arg in protein synthesis and accretion. In addition, arginine 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). There are reports indicating Arg supplementation beyond the NRC recommendations improved broiler growth performance. These findings are consistent with those recently reported (Fernandez et al., 2009; Ruiz-
Feria, 2009). Kidd et al. (2001) reported that supplementing broiler diets with 2 g/kg Arg beyond the NRC requirements even under normal conditions resulted in improved growth performance. While some reports (Labadan et al., 2001) on estimation of Arg requirements for of 3- to 6-wk-old broilers indicated that the requirements for maximum weight gain and feed efficiency were lower than the NRC (1994) recommendation, our findings clearly show that the NRC recommendations for Arg are not sufficient for maximizing BW gain and optimizing feed:gain for broilers grown at high altitude. Broken-line analysis indicated the levels of Arg needed for optimal body weight gain and feed:gain responses were 12.4 and 12.1 g/kg of the diet. Nutrient requirements advocated by NRC (1994) have been established under ideal environmental conditions and they are minimal requirements. Raising broilers at high altitudes 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 (Barbul, 1986).

Dietary supplementation with L-Arg increased plasma NO levels and decreased pulmonary hypertension as reflected in lower RV: TV. The RV: TV and plasma NO levels were inversely related. These observations indicate 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; 1996). NO is synthesized in broiler lungs by eNOS expressed in the pulmonary vascular endothelium (Wideman et al., 2006; Hamal et al., 2008; 2010). The competitive inhibitor L-NAME blocks NO synthesis in broilers, causes pulmonary arterial vasoconstriction, increases the baseline pulmonary vascular resistance and doubles the pulmonary hypertensive response and mortality imposed by partial occlusion of the pulmonary vasculature (Wideman et al., 2006). In agreement with our study, Ruiz-Feria and Abdulkalykova (2009) indicated that supplementing a broiler diet with 1% Arg plus vitamins E and C resulted in increased NO production and reduced pulmonary hypertension. Based on the response in plasma NO concentration and RV: TV, the estimated requirements for Arg were 13.0 and 13.2 g/kg of diet, which are approximately 18% and 20% greater than the NRC value (1.1% of diet). These are by far the highest values reported for Arg requirements of 3- to 6-week age broilers. The findings reported herein draw one's attention to the Arg priority in the bird's body. Arg is first used up to maintain the growth performance. Extra Arg is needed to support the maximum production of NO and to avoid pulmonary hypertension. Therefore, Arg levels sufficient to support maximal growth rates are not adequate for maximal NO production. 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 statement.

Conclusion

In conclusion, dietary requirements presented by the NRC (1994) provide minimal requirements that have been established in regions where the existing altitudes do 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.

Acknowledgments

Supported in part by Shahrekord University.

References

Izadinia M, Nobakht M, Khajali F, Faraji, M Zamani F, Qujeq D and Karimi I. Pulmonary hypertension and ascites as affected by


Wideman RF, Kirby YK, Tackett CD, Marson NE and McNew RW. Cardio-pulmonary function during acute unilateral occlusion of the pulmonary artery in broilers fed diets containing normal or high levels of arginine-HCL. Poultry Science, 75: 1587–1602. 1996.


Wideman RF, Fedde MR, Tackett CD and Weigle GW. Cardio-pulmonary function in preascitic (hypoxemic) or normal broilers inhaling ambient air or 100% oxygen. Poultry Science, 79: 415–425. 2000.
