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≪Research Note≫

Growth Performance and Right Ventricular Hypertrophy Responses of Broiler Chickens to Guanidinoacetic Acid Supplementation under Hypobaric Hypoxia

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Guanidinoacetic acid (GAA) has been shown to spare arginine (ARG) requirements in chickens. ARG plays a critical role in enhancing growth and preventing right ventricular hypertrophy (RVH) in broiler chickens subjected to hypobaric hypoxia. However, ARG is not available as a feed grade supplement in the market. Therefore, we evaluated the effects of commercially available GAA supplement as an alternative on growth performance and RVH in broilers raised at high altitude (2100 m). Five graded levels of GAA ranging from 0 (control) to 2 g/kg were provided in isoenergetic and isonitrogenous diets to broilers (Ross 308) from day 1 to 42, post-hatch. Results indicated that responses to GAA were nonlinear and attained plateau values within the studied range of GAA supply. While weight gain and feed intake were unaffected by GAA supply, feed conversion ratio was improved by GAA supplementation up to 1.5 g/kg. Similar trends were observed for the proportions in the liver and heart, as well as hematocrit. GAA supplementation at 1 and 1.5 g/kg resulted in reduced abdominal fat deposition as well as a decline in right-to-total ventricular weight ratio (RV:TV, an index of RVH). A significant ($P<0.05$) increase in serum nitric oxide concentration was observed at 1 and 1.5 g/kg GAA supplementation. However, GAA supply led to lower serum malondialdehyde and uric acid levels than in the control. In conclusion, GAA supplementation up to 1.5 g/kg had the potential to improve growth performance and RVH response. Meanwhile, GAA supply beyond 1.5 g/kg could deteriorate these responses.

Key words: altitude, ascites, chicken, guanidinoacetate

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Introduction

Hypoxia is defined as limited availability of atmospheric oxygen that occurs as altitude increases. Hypoxia results in hypoxaemia, which refers to a reduction in the oxygen content of arterial blood, reflecting reduced saturation of hemoglobin with oxygen (Julian, 2007; Khajali and Wideman, 2016). Beker *et al*. (2003) found a highly-correlated relationship between atmospheric oxygen concentration and growth performance of broiler chickens. They also indicated that reduced atmospheric oxygen availability effectively results in right ventricular hypertrophy and ascites.

Pulmonary vascular bed produces nitric oxide (NO), a potent vasodilator, which directly reduces pulmonary vascular resistance and subsequent right ventricular hypertrophy (Khajali and Wideman, 2010). NO is generated from arginine (ARG) through the action of endothelial NO synthase (eNOS, NOS-3; EC 1.14.13.39) located in the vascular bed. Research has shown that ARG supplementation of broiler diets significantly improved cardiopulmonary hemodynamics and reduced mortality from ascites (Wideman *et al*., 2013; Saki *et al*., 2013; Sharifi *et al*., 2015). It has been reported that ARG requirement for maximal growth and prevention of right ventricular hypertrophy was significantly greater than the requirement advocated by the National Research Council (NRC) (1994) (Basoo *et al*., 2012). In fact, dietary ARG requirements for broilers advocated by NRC may not be adequate to support maximal growth and immune function and to avoid pulmonary hypertension in broilers reared at high altitude (Izadinia *et al*., 2010). As a result, supplementation of ARG to commercial diets of broiler chickens may be necessary in high altitude regions. ARG is not commercially available as a feed-grade amino acid (see http: //www. ajiaminoacids. com/p-4-l-arginine. aspx). Limited availability and the expensive cost of reagent grade ARG (~180 USD per kg according to Ajinomoto Co. Inc.) compel researchers to find more competitive alterna-

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tives. Guanidinoacetic acid (GAA; also called glycociamine) is of high interest since it partially spares ARG requirements and also acts as an immediate precursor of creatine and its phosphorylated derivative, phosphocreatine. The latter, a high-energy molecule, can fuel cellular activities and moderate the accumulation of adenosine diphosphate (ADP) from adenosine triphosphate (ATP) during high rates of cellular metabolism. GAA has been reported to spare ARG requirements of broiler chickens (Dilger *et al*., 2013; Michiels *et al*., 2012). The objective of the present study was to investigate if GAA supplementation can boost energy status and improve growth performance of broiler chickens exposed to hypobaric hypoxia and simultaneously prevent right ventricular hypertrophy. In this regard, different doses of GAA were fed to hypoxic broilers to ascertain optimal doses for maximizing productivity and minimizing right ventricular hypertrophy.

Materials and Methods

Birds and Experimental Facility

The experiment was conducted in the Poultry Research Center of Shahrekord University, Shahrekord, Iran, at an altitude of 2100 m above sea level. The experimental animals were treated according to the Institutional Animal Care and Use Committee of Shahrekord University.

A total of 300 day-old male broilers (Ross 308) were randomized across 20 litter pens measuring 1.8 m^2 (15 birds per pen). Each pen was equipped with a bell drinker and a feed trough with free access. Day-old chicks were allocated to each pen so that all pens had equal initial body weights (630 g±10 g). Birds were subjected to 23 h light and 1 h dark throughout the trial.

Treatments

A commercial broiler diet was prepared according to the NRC (1994) recommendations for the starter/grower (12.75 MJ/kg AME and 220 g/kg crude protein) and finisher (12.96 MJ/kg AME and 192 g/kg crude protein) and considered as control. ARG content of the starter/grower and finisher diets was 13 and 12 g/kg, respectively. Four additional diets were prepared by supplementing 0.5, 1, 1.5 and 2 g/kg GAA to the control diet. GAA was provided by Evonik Degussa, Tehran, Iran. All diets had similar metabolizable energy and protein and offered in mash form.

Measurements

Records of body weight and feed intake were obtained throughout trial (1 to 42 days). Feed conversion ratio was also calculated. At 42 days of age, eight birds per treatment were selected for blood collection. Blood samples (3 m*l*) were collected from the brachial vein and centrifuged at 2500 g for 10 min to obtain sera. Serum samples were used for the determination of NO, uric acid (UA), and malondialdehyde (MDA). NO (nitrate + nitrite) was measured according to Behrooj *et al*. (2012). UA concentration was analyzed according to Fossati *et al.* (1980). MDA concentration, as biomarker of oxidative stress, was assayed by the method of Nair and Turner (1984).

Samples of blood were collected in microhematocrit tubes for measuring hematocrit. An aliquot of blood was also obtained on glass slides to prepare the blood smear for the determination of differential leukocyte count. The May-Grunwald and Giemsa stains were used for staining the smears 3 h after methyl alcohol fixation (Lucas and Jamroz, 1961). One hundred leukocytes, including granular (heterophils) and nongranular (lymphocytes) were enumerated and the heterophil to lymphocyte ratio (H:L) was calculated. All chemical reagents were obtained from Sigma–Aldrich Co. (Sigma–Aldrich Co., St. Louis, MO, USA).

The birds were then euthanized for carcass processing. Data obtained at processing included liver, abdominal fat, and heart. The hearts were further dissected to obtain the right-to-total ventricular weight ratio (RV:TV). Mortality from ascites was checked daily and whenever the RV:TV was greater than 0.25, considered as pulmonary hypertension (Ahmadipour *et al*., 2015; Khajali *et al*., 2011).

Statistical Analysis

Data were analyzed by ANOVA procedure of SAS (2007) software in a completely randomized design and the means were separated by Duncan's multiple range test. Data were also analyzed for linear and quadratic regression to assess curvilinear responses to GAA supply. Optimal levels of GAA were then estimated by broken-line regression model (Robbins *et al*., 2006) using the NLIN procedure of SAS (2007), with GAA dose (g/kg) as the independent variable and performance criteria as dependent variables.

Results

While body weight gain and feed intake were unaffected by GAA supply, feed conversion ratio (FCR) was significantly ($P<0.05$) improved at 1 and 1.5 g/kg GAA (Table 1). Regression analysis showed significant linear and quadratic responses to GAA for FCR ($y=1.98-0.033x+0.001x^2$; $P=0.0001$; $n=4$ pens). Broken-line regression analysis indicated that the level of GAA needed for optimal FCR was 1.2 g/kg of diet.

Table 2 depicts blood and serum variables of broiler chickens fed with different levels of GAA. GAA doses of 1 and 1.5 g/kg caused decreased hematocrit and H:L and increased concentration of serum NO, relative to the control. Serum MDA and UA concentrations were significantly ($P<0.05$) decreased in GAA-received groups. Broken-line regression analysis indicated that the levels of GAA needed for maximizing NO and minimizing MDA were 1.48 and 1.13 g/kg of diet, respectively.

Table 3 depicts the effects of GAA supplementation on carcass characteristics in broiler chickens measured at 42 days of age. Proportions of the liver and heart were significantly ($P<0.05$) were lower in broilers fed with GAA at 1.5 g/kg than in the control. Abdominal fat pad and RV:TV were significantly ($P<0.05$) decreased when GAA was supplemented at 1 and 1.5 g/kg. Broken-line regression analysis revealed 1.25 g/kg as the optimal dose of GAA to reduce right ventricular hypertrophy (RV:TV).

Discussion

As shown in Table 1, FCR was significantly improved by GAA at 1 and 1.5 g/kg. Such improvement can be attributed to the function of GAA in boosting creatine/creatine kinase system, which plays a pivotal role in cellular bioenergetics (Wallimann *et al.*, 2011). This function constitutes the basis of GAA usage as a performance-enhancing agent (Ostogic, 2016). In addition, GAA contributes to the formation of ARG (Dilger *et al.*, 2013; Michiels *et al.*, 2012), which is a precursor of growth-promoting polyamines including putrescine, spermidine, and spermine. These polyamines have anabolic functions in the body such as synthesis of DNA, RNA, and proteins, as well as the cellular uptake of amino acids (Khajali and Wideman, 2010). Taken together, GAA-received birds seemingly utilized feed more efficiently than those on the control diet. In line with our findings, Mousavi *et al*. (2013) used GAA at 6 g/kg in broiler diets and observed a significant improvement in FCR.

Significant increase in serum NO when GAA supplied at 1 and 1.5 g/kg (Table 2) demonstrates its sparing action for ARG, as previously reported. Dilger *et al*. (2013) reported that GAA is an efficient replacement for ARG for young chicks. This research indicated that GAA could elicit greater response when added to ARG-deficit diets. ARG is the substrate from which NO is generated (Khajali and Wideman, 2010). The supplement of GAA decreased hematocrit. However, the link between GAA and hematocrit has yet to be determined. As shown in Table 2, GAA supplementation significantly ($P<0.05$) reduced serum UA concentration. In avian species, UA is the end-product of protein catabolism. Reduced UA production speculates improved dietary protein utilization in GAA-received groups. This observation is in line with improved FCR, as mentioned earlier. Reduced serum MDA in GAA-received birds (Table 2) suggests the antioxidative role of GAA. Research has shown that low doses of GAA boost the activity of superoxide dismutase (SOD). Ostogic *et al*. (2015) found that fasting plasma SOD activity increased by 35.4% from before to after administration in a GAA-supplemented group, when compared to the placebo. However, serum MDA level tended to increase when GAA was added at 2 g/kg. In the present study, the optimal level of GAA in broiler diets to render a minimal level of MDA was 1.13 g/kg. This observation suggests that dietary GAA has oxidant-antioxidant capacity (Ostogic *et al*., 2015). It has been shown that high levels of GAA could stimulate the generation of reactive oxygen species in the body (Mori *et al*., 1996), decrease non-enzymatic antioxidant capacity, and increase lipid peroxidation in rats (Zugno *et al*., 2008). This is observed as a higher heterophils to lymphocytes ratio (H:L) in birds fed GAA at 2 g/kg than in those fed with lower levels of GAA supplementation. In birds, H:L ratio is an index of stress. The higher the ratio of H:L, the more intensive the stress. This finding suggests that birds fed the highest level of GAA (2 g/kg) were under more oxidative stress than those fed with lower doses (particularly the significantly lower H:L in the 1.5 g/kg dose of GAA).

Table 3 indicates that liver weight as a percent of live body weight had a significant decrease in birds fed with GAA at 1 and 1.5 g/kg. Reduced proportion of the liver reflects reduced lipogenesis as the liver is the principal site of lipogenesis in avian species. This is speculated by the corresponding decrease in abdominal fat deposition. Amino acid supplements, particularly ARG, have been reported to sup-

Table 1. **Effects of guanidinoacetic acid (GAA) supplementation on growth performance of broiler chickens from 1 to 42 days of age**

Variable	Control	GAA (0.5 g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	LSD
Body weight gain	2101^{b}	2223^{ab}	2185^{ab}	2216^{ab}	2268^{a}	138.4
Feed intake	3912	4008	3918	3987	4027	111.7
Feed conversion ratio	1.91^{a}	1.87^{ab}	1.77^{b}	1.76^{b}	1.84^{ab}	0.118

Means in the same row with different letters are significantly different ($P<0.05$). The unit for body weight gain and feed intake values is grams per bird. Number of replicates=4.

Table 2. **Effects of guanidinoacetic acid (GAA) supplementation on blood and serum variables in broiler chickens measured at 42 days of age**

Variable	Control	GAA (0.5 g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	LSD
Hematocrit (%)	43.1^{a}	39.7^{ab}	35.62^{cd}	33.8^{d}	37.6^{bc}	3.38
Heterophil/lymphocyte	1.30^{a}	0.90^{bc}	0.72^{cd}	0.62^{d}	0.95^{b}	0.22
Nitric oxide (μmol/L)	10.24^{c}	12.22^{c}	17.94^{ab}	19.47^{a}	14.07^{bc}	5.29
Malondialdehyde(μm/L)	4.88^{a}	3.58^{b}	2.86^{b}	2.78^{b}	3.14^{b}	1.22
Uric acid (mg/dL)	6.71^{a}	5.71^{b}	4.85^{b}	4.88^{b}	5.51^{b}	0.99

Means in the same row with different letters are significantly different ($P<0.05$). Number of replicates=8.

Table 3. **Effects of guanidinoacetic acid (GAA) supplementation on carcass characteristics and ascites mortality in broiler chickens measured at 42 days of age**

Variable	Control	GAA (0.5 g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	LSD
Liver (% of BW)	2.32[a]	2.16[b]	2.03[cd]	1.95[d]	2.12[bc]	0.119
Abdominal fat	2.26[a]	1.91[b]	1.64[cd]	1.55[d]	1.75[c]	0.149
Heart (% of BW)	0.72[a]	0.67[ab]	0.65[bc]	0.60[c]	0.68[ab]	0.062
RV: TV ratio	0.33[a]	0.33[a]	0.24[bc]	0.22[c]	0.28[ab]	0.049
Ascites mortality (%)	27.5	27.3	20.0	17.0	32.5	15.5

Means in the same row with different letters are significantly different ($P<0.05$). Number of replicates=8.

press lipogenesis in poultry (Wu *et al*., 2011) by down-regulation of lipogenic gene expression in the liver. Mousavi *et al*. (2013) also reported that liver percentage declined with GAA supplementation to chicken diets. The liver to abdominal fat weight ratio for the control, 0.5, 1, 1.5, and 2 g/kg GAA was 1.02, 1.13, 1.23, 1.26, and 1.21, respectively.

Feeding GAA significantly decreased right ventricular hypertrophy as reflected in lower RV:TV with GAA added at 1 and 1.5 g/kg levels (Table 3). RV: TV is indicative of pulmonary hypertension, so that values greater than 0.25 are considered as pulmonary hypertension in broiler chickens (Ahmadipour *et al*., 2015). This finding is in accordance with the significantly higher concentration of serum NO in GAA-received groups. NO is a potent vasodilator that inhibits pulmonary hypertension (Khajali and Wideman, 2016). Basoo *et al*. (2012) re-evaluated the ARG requirements for broilers raised at high altitude (2100 m) during a 3-to-6-week period. They estimated that the ARG requirement for maximal growth and prevention of pulmonary hypertension was 20% higher than that of NRC (1994) recommendation (1.32 vs. 1.1% of diet). In fact, dietary requirements advocated by NRC (1994) provide minimal requirements that have been established under thermoneutral conditions in regions where the existing altitudes do not limit the availability of atmospheric oxygen (Khajali and Wideman, 2016). This is the reason why GAA could elicit reduced pulmonary hypertensive in the present study, which was conducted at high altitude. GAA endogenously formed from ARG and glycine and has the potential to spare dietary ARG (Dilger *et al*., 2013; Michiels *et al*. 2012). In this experiment, improved RV:TV may explain better nutritional fulfilment for ARG in GAA groups.

Conclusion

In summary, the present study suggests that GAA supplementation acts as an alternative to ARG to improve growth performance and counteract right ventricular hypertrophy in broiler chickens reared at high altitude. However, it seems that higher doses of GAA deteriorate the response.

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