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Effect of Guanidinoacetic Acid Supplementation on Growth Performance and Gut Morphology in Broiler Chickens

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Abstract

This study evaluates the effects of different levels of guanidinoacetic acid (GAA) supplement on growth performance and gut morphology in broilers (Ross 308 strain) raised at high altitude (2100 m). A total of 300 one-day-old male broiler chicks (Ross 308 strain) were used in a completely randomized design with five treatments and four replicate pens of 15 birds in each. Five dietary treatments were prepared by supplementing GAA at 0 (control), 0.5, 1.0, 1.5, and 2.0 g/kg to corn-soy based diet and fed to broilers from 1 to 42 days of age. Results indicated that weight gain and feed:gain ratio was significantly improved in the chickens when GAA was supplemented to control diet. Carcass and breast yields were significantly increased by GAA supplementation at 1 g/kg relative to the control. On the other hand, dietary inclusion of GAA significantly ($P < 0.05$) reduced the proportions of liver, heart and abdominal fat when compared to the control. The villus height, width and absorptive surface area in duodenum, jejunum, and ileum sections were significantly improved at GAA supplementation above 0.5 g/kg. However, the crypt depth showed a significant decrease in all parts of the small intestine compared to the control ($P < 0.05$). In conclusion, supplementing broiler diets with GAA could be an effective strategy to improve growth performance and gut function.

Introduction

Energy is the main limiting nutrient for growing chickens with enormous muscle growth and development. The energy supply to muscles dictates the maximal growth performance of broiler chickens. In cellular metabolism, energy transfers from adenosine-tri-phosphate (ATP) to various metabolic processes. In this context, a pool of phosphocreatine and creatine kinase are located in skeletal muscle keeping adenosine-di-phosphate (ADP) and ATP levels constant as a kind of buffering system, which is important for proper functioning of cellular energy metabolism (Wyss and Kaddurah-Daouk., 2000; Tossenberger *et al*., 2016). Guanidinoacetic acid (GAA) is formed from the amino acids glycine and arginine in the kidney or absorbed from the gut and transformed to creatine in the liver. Creatine in its phosphorylated form plays a crucial role as a high-energy carrier in muscles. The phosphocreatine/ creatine system buffers ATP/ADP ratio for all energy-demanding functions of the cell. To a considerable extent, GAA also spares arginine requirements (Ostogic, 2016).

The effect of arginine on improvement of intestinal absorption and gut function has been well documented. Foye *et al*. (2007) indicated

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that *in ovo* administration of arginine resulted in enhanced intestinal uptake in turkeys. Khajali *et al*. (2014) reported a significant improvement in intestinal mucosal development in broiler chickens fed arginine at 10 g/kg. Arginine is an indispensable amino acid for chickens due to the lack of functional urea cycle in birds (Khajali and Wideman, 2010). Researches have shown that dietary arginine requirement for broilers is inadequate to support maximal growth and immune function at high altitudes (Basoo *et al*., 2012; Khajali *et al*., 2014). Basoo *et al*. (2012) demonstrated that supplementation of arginine to commercial diets of broiler chickens may be necessary at high altitude regions. However, limited availability and expensive cost of arginine have forced researchers to find more competitive alternatives such as GAA, because arginine is not available as a feed grade amino acid in the market. GAA is altogether a suitable supplement to enhance the productivity of broilers grown at high altitude. Though research has indicated positive effects of supplemental GAA on broiler and turkey performance and carcass efficiency (Ringel *et al*., 2007; Lemme *et al*., 2007, 2010; Michiels *et al*., 2012; Dilger *et al*., 2013; Heger *et al*., 2014), there is scarcity of data on GAA impact on gut function. The objective of the current study was to evaluate the growth performance and gut function of broiler chickens in response to different levels of GAA.

Materials and Methods

Birds and experimental facility

The experiment was carried out in the experimental facility of Shahrekord University, Shahrekord, Iran (an altitude of 2100 m) according to the Institutional Animal Care and Use Committee. A total of 300 day-old male broiler chicks (Ross 308) were randomly distributed across 20 litter pens measuring 1.8 m^2 (15 birds per pen). Each pen was supplied with a bell drinker and a feed trough. One-day-old chicks were assigned to each pen in a way that all pens had equal initial body weights (630 ± 10g). Birds were allowed to 23 hrs light and 1 hr dark throughout the trial with free access to mash feed and water. The house temperature was set at 32±1°C on day one, and declined to 25±1°C on day seven, 20±1°C on day 14, and 15±1°C on day 21 onward (until 42 days of age) as previously described (Sharifi *et al*., 2015).

Treatments

A commercial broiler diet was prepared according to the NRC (1994) recommendations for the starter (1 to 21 days of age) and grower (21 to 42 days of age) stages and considered as control (Table 1). 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.

Table1. Ingredients and composition of the control diet

Item (% unless noted)	Starter (1–21days)	Grower (21–42 days)
Corn	51.10	60.5
Soybean meal (44% CP)	39.85	31.9
Soy oil	5.00	4.00
Dicalcium phosphate	1.50	1.30
Oyster shell	1.50	1.40
Salt	0.35	0.30
DL-Methionine	0.20	0.10
Mineral supplement†	0.25	0.25
Vitamin supplement#	0.25	0.25
Nutrient composition		
ME (Kcal/kg)	3050	3100
CP	21.95	19.20
Met + Cys	0.95	0.72
Lys	1.20	1.03
Thr	0.90	0.88
Arg	1.30	1.20
Ca	0.95	0.85
Available P	0.43	0.35

†Provided the following per kg of diet: vitamin A (trans retinyl acetate), 3600 IU; vitamin D3 (cholecalciferol), 800 IU; vitamin E (dl-α-tocopheryl acetate), 7.2 mg; vitamin K3, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; pyridoxine, 1.2 mg; cobalamin, 0.6 mg; folic acid, 0.5 mg; choline chloride, 200 mg.

#Provided the following per kg of diet: Mn (from $MnSO_4$-H_2O), 40 mg; Zn (from ZnO), 40 mg; Fe (from $FeSO_4$-$7H_2O$), 20 mg; Cu (from $CuSO_4$-$5H_2O$), 4 mg; I [from Ca $(IO_3)2$-H_2O], 0.64 mg; Se (from sodium selenite), 0.08 mg.

Measurements

Feed intake and body weight were recorded during the starter (1- 21days of age), and grower (21- 42 days of age) stages. Feed:gain ratio for each period was also calculated and corrected for mortality body weights. At the end of experiment (42 days of age), two birds per pen (eight birds per treatment) were euthanized for carcass processing to obtain the weights of hot eviscerated carcass, breast, liver, heart, and abdominal fat.

Assessment of intestinal morphology

At 42 days of age, eight additional birds per treatment were euthanized to measure intestinal morphology including villus height, villus width, crypt depth, and absorptive surface area in duodenum, jejunum, and ileum sections. Segments of about 2 cm from duodenum, jejunum, and ileum were diced, rinsed with phosphate buffered saline (PBS, pH=7), and fixed in Clark fixative solution for 45 min. Tissue samples were then transferred to ethyl alcohol for longer storage. Each segment was periodically put in acid-Schiff reagent for 2 to 3 min for staining. Muscle layers were trimmed from mucosa, and rows of villi cut, positioned on glass slides and covered with a coverslip. These samples were observed by an optical microscope (Hassanpour *et al*., 2013). The absorptive surface area was calculated utilizing the formula = π× (VW) × (VH); where π=3.14, VW is villus width and VH is villus height. Villus height was measured from the top of the villus to the top of the lamina propria. Villus width was taken from the average of villus width at one-third and two-third of each villus. Crypt depth was determined as the distance from the base of the villus to the sub mucosa.

Statistical analysis

Data were analyzed by GLM procedure of SAS (2002) software in a completely randomized design and the means were separated by the Duncan's multiple range test.

Results

The effect of GAA supplement on growth performance of broiler chickens is shown in table 2. There were no significant differences among dietary treatments for feed intake during feeding stages. Weight gain and feed:gain ratio were significantly improved in all feeding stages when GAA was supplemented to control diet ($P < 0.01$). However, the best weight gain and feed:gain ratio belonged to broilers received GAA at 1.5 g/kg of diet.

Table 2. Effect of GAA supplementation on growth performance response in broiler chickens

Variables	Control	GAA (0.5 g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	*SEM*	*P*-value
Weight gain (g/bird)							
1–21 days of age	669^{c}	699^{ab}	693^{bc}	719^{a}	690^{bc}	7.86	0.007
21–42 days of age	1315^{b}	1447^{a}	1516^{a}	1539^{a}	1477^{a}	31.8	0.001
1–42 days of age	1985^{c}	2147^{b}	2210^{ab}	2259^{a}	2179^{ab}	34.1	0.005
Feed intake (g/bird)							
1–21 days of age	1057	1074	1036	1065	1069	13.54	0.364
21–42 days of age	2854	2934	2881	2922	2957	33.76	0.251
1–42 days of age	3912	4008	3918	3987	4027	39.04	0.18
Feed:gain (g:g)							
1–21 days of age	1.58^{a}	1.54^{ab}	1.49^{bc}	1.48^{c}	1.54^{a}	0.01	0.001
21–42 days of age	2.18^{a}	2.03^{b}	1.90^{b}	1.89^{b}	2.00^{b}	0.047	0.004
1–42 days of age	1.97^{a}	1.87^{b}	1.77^{c}	1.76^{c}	1.84^{bc}	0.03	0.001

$^{a\text{-}c}$Means in the same row with different letters are significantly different.
Each mean represents values from eight replicates.

Table 3 depicts the effects of GAA supplementation on carcass characteristics in broiler chickens measured at 42 days of age. There was a significant increase in yields of carcass ($P = 0.014$), and breast ($P = 0.0001$) at 1 and 1.5 g/kg GAA relative to the control. Our results indicated that proportional yields of the liver and abdominal fat were significantly reduced by GAA supplementation. The proportion of heart in birds fed with 1 and 1.5 g/kg GAA was significantly lower than the control ($P = 0.005$).

Table 3. Effect of GAA supplementation on carcass characteristics in broiler chickens (% of live body weight)

Item	Control	GAA (0.5 g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	*SEM*	*P*-value
Carcass yield	67.57^{b}	68.28^{ab}	68.97^{a}	69.12^{a}	68.32^{ab}	0.32	0.014
Breast yield	24.49^{d}	25.28^{c}	26.30^{a}	26.67^{a}	25.75^{b}	0.15	0.0001
Liver yield	2.4^{a}	2.16^{b}	2.04^{cd}	1.96^{d}	2.12^{bc}	0.03	0.0001
Heart yield	0.72^{a}	0.67^{ab}	0.65^{bc}	0.60^{c}	0.68^{ab}	0.01	0.005
Abdominal fat yield	2.26^{a}	1.91^{b}	1.64^{cd}	1.56^{d}	1.75^{c}	0.01	0.0001

$^{a-d}$Means in the same row with different letters are significantly different.
Each mean represents values from eight replicates.

Morphological measurements in different segments of the small intestine are presented in Table 4. Villus height and width in duodenum, jejunum, and ileum were significantly ($P < 0.01$) improved at GAA supplementation above 0.5 g/kg diet. All doses of GAA significantly increased absorptive surface area in duodenum, jejunum and ileum compared to the control ($P < 0.001$).

Table 4. Effect of GAA supplementation on intestinal morphology in broiler chickens

Variables	Control	GAA (0.5g/kg)	GAA (1 g/kg)	GAA (1.5 g/kg)	GAA (2 g/kg)	*SEM*	*P*-value
Duodenum							
Villus height (mm)	1.44^{b}	1.68^{ab}	1.85^{a}	1.84^{a}	1.80^{a}	0.09	0.014
Villus width (mm)	0.37^{b}	0.42^{ab}	0.47^{a}	0.48^{a}	0.47^{a}	0.021	0.026
Crypt depth (mm)	0.4^{a}	0.36^{ab}	0.31^{bc}	0.28^{c}	0.32^{bc}	0.01	0.0001
Surface area (mm^2)	1.68^{b}	2.27^{a}	2.71^{a}	2.74^{a}	2.66^{a}	0.18	0.001
Jejunum							
Villus height (mm)	1.01^{b}	1.23^{a}	1.35^{a}	1.39^{a}	1.38^{a}	0.06	0.003
Villus width (mm)	0.29^{c}	0.34^{bc}	0.39^{ab}	0.42^{a}	0.42^{a}	0.019	0.0003
Crypt depth (mm)	0.37^{a}	0.32^{b}	0.28^{bc}	0.27^{c}	0.28^{bc}	0.015	0.0002
Surface area (mm^2)	0.91^{c}	1.31^{b}	1.65^{ab}	1.83^{a}	1.82^{a}	0.011	0.0001
Ileum							
Villus height (mm)	0.86^{b}	1.09^{a}	1.14^{a}	1.16^{a}	1.12^{a}	0.04	0.006
Villus width (mm)	0.32^{b}	0.33^{b}	0.38^{a}	0.41^{a}	0.37^{ab}	0.01	0.004
Crypt depth (mm)	0.42^{a}	0.37^{b}	0.30^{c}	0.27^{c}	0.28^{c}	0.017	0.0001
Surface area (mm^2)	0.86^{c}	1.13^{b}	1.37^{ab}	1.49^{a}	1.30^{ab}	0.084	0.0001

$^{a-c}$Means in the same row with different letters are significantly different.
Each mean represents values from eight replicates.

Discussion

While feed intake was not influenced by GAA supplementation, body weight gain and feed:gain ratio were significantly improved by GAA supplementation to control diet. Previous studies showed an improvement in feed:gain ratio (Lemme *et al*., 2007, 2010; Michiels *et al*., 2012; Dilger *et al*., 2013; Mousavi *et al*., 2013; Heger *et al*., 2014) or body weight gain (Lemme *et al*., 2007; Michiels *et al*., 2012; Dilger *et al*., 2013) by supplementation of GAA to broiler diets. These findings can be explained by the role of GAA in biosynthesis of creatine phosphate, the rapidly mobilizable reserve of energy in muscles. Improved FCR without a significant change in feed intake can be translated into boosting energy efficiency (which is logically expectable as GAA is immediate precursor of creatine and its phosphorylated derivative, phosphocreatine- a rapidly mobilizable reserve of high energy phosphates in bird's body-). Another presumable way is that GAA has favored production of growth promoting polyamines (putrescine, spermidine and spermine). These polyamines have anabolic functions in synthesis of DNA, RNA, and proteins (Smith, 1990).

GAA, as a precursor of creatine, plays a significant role in development of muscle tissues. In this regard, Stahl *et al*. (2003) reported a significant improvement in feed:gain ratio in broilers following creatine monohydrate supplementation. Supplemental GAA is highly digestible (98% to 99%) in broilers. True availability of GAA decreases as dietary concentration increases and may result in poor performance. Excess GAA (beyond 2 g/kg as

observed in the present study) may counterbalance its beneficial effects as suggested by Tossenberger *et al*. (2016).

In the present study, a significant increase in yields of carcass and breast was observed when GAA was added at 1 and 1.5 g/kg. Michiels *et al*. (2012) reported a significant effect on yield of breast when GAA was added at 0.6 and 1.2 g/kg to broiler diets. Mousavi *et al*. (2013) supplemented broiler diets with 0.6 g/kg GAA at different metabolizable energy levels and did not observe any difference for carcass components. The reason for the discrepancy is not clear.

Information on the effect of GAA supplement on intestinal morphology in poultry is limited. The vast majority of information has addressed the role of arginine on gut morphology and function. Murakami *et al*. (2014) reported benefits of arginine supplement on morphometry of the duodenum mucosa in broiler chickens. Khajali *et al*. (2014) reported increased villus height, width, and absorptive surface area in the jejunum as a consequence of arginine supplementation (10 g/kg). Increase in villus height increases total luminal villus absorptive area and subsequently results in satisfactory digestive enzyme action and higher transport of nutrients at the villus surface (Tufarelli *et al*., 2010).

A recent study suggested that addition of arginine to the culture medium stimulated the growth intestinal epithelial cells in the chicken (Yuan *et al*., 2015). The proposed action of arginine in improving intestinal health included upregulating gene expression of the target of rapamycin cell-signaling pathway that increased protein synthesis and reduced protein degradation (Yuan *et al*., 2015). Recently, Kodambashi Emami *et al*. (2017) showed that villus surface area in cold-stressed birds fed on a diet supplemented with 1.72 g/kg arginine was improved to the extent that was similar to those grown in normal temperature. By virtue of the fact that GAA is synthesized from arginine, beneficial effects of GAA were not far beyond expectation. In the present study, we observed a significant improvement in villus height and width and absorptive surface area.

Conclusion

There is a dose-response effect of in-feed GAA supplement on growth performance and morphometric indexes of broiler chickens. However, more research needs to be done to elucidate biological events that underlie response to GAA.

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