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Effects of Dietary Guanidinoacetic Acid Supplementation on Performance, Blood Parameters and Meat Quality of Male Broilers with Cold-Induced Ascites

Research Article

A. Mohebbifar[1], M. Torki[1*] and A. Abdolmohammadi[1]

[1] Department of Animal Science, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

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*Correspondence E-mail: torki@razi.ac.ir

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ABSTRACT

The effects of dietary supplemental guanidinoacetic acid (GAA) on performance, biochemical indices and meat pH of broilers with cold-induced ascites were studied. A total of 640 day-old male broiler chicks (Cobb 500) were assigned to four dietary treatments including control diet; control diet supplemented with either 0.6, 1.2 or 1.8 g of GAA per kg of feed. Each treatment was replicated in 8 battery cages (n=20). At day 14, temperature was reduced to amplify the incidence of ascites. The birds fed the control, 1.2 and 1.8 g/kg GAA diets exhibited higher average daily gain (ADG) compared to those fed 0.6 g/kg GAA (P<0.05). Bird fed diet with 1.2 g/kg GAA also showed poorer feed conversion ratio (FCR) compared to birds in other dietary groups (P<0.05). Higher average daily feed intake (ADFI) were observed in birds fed the diet with 1.2 g/kg GAA (P<0.05). The mortality due to ascites was significantly lower in birds fed diet with 1.2 g/kg GAA compared to birds fed the control, 0.6 and 1.8 g/kg GAA diets. Moreover, there was a quadratic effect of GAA levels on mortality due to ascites. No significant effects of dietary treatments on leucocyte subsets and relative weights of lymphoid organs were observed. Lower meat pH was detected in chicks fed the GAA-supplemented diets compared to birds fed the control diet. Dietary GAA supplementation resulted in a linear (P=0.01) and quadratic (P=0.03) responses in meat pH. In conclusion, diet supplementation with GAA had reducing effects on the occurrence of ascites-related mortality in broiler chicks.

KEY WORDS ascites, blood parameters, cold stress, guanodinoacetic acid, performance, meat quality.

INTRODUCTION

In the poultry industry, broiler chick that weighs 40 g at hatch has the potential to reach more than 4000 g in 8 weeks. Growth to almost 100-fold of the initial weight in 8 weeks cannot be achieved without equally dramatic increases in the functional capacities of the heart and lungs (Wideman *et al.* 2013). Study has shown that rapid growth rate in fast-growing meat-type chickens (broilers) has increased the metabolic demand for oxygen due to higher metabolic rate (Baghbanzadeh and Decuypere, 2008). Ascites syndrome (AS) or pulmonary hypertension syndrome (PHs) is a condition that inflicts financial loss on poultry farmers around the world. It has been reported that the characteristic symptoms of the disease process are an enlarged heart, variable liver changes, and accumulation of water in the abdominal cavity (Riddell, 1991). Cold temperatures increase AS by increasing oxygen demand and those broilers that fail to fully supply oxygen may develop ascites (Cahaner, 2011). It has been shown that PHs can be prevented when a dietary L-arginine (Arg.) is provided as the main source of a potent endogenous endothelial vasodilatator–exposed to the cold by elevating plasma nitric oxide (NO) levels (Wilson *et al.* 1988). Arginine is an essential

amino acid for avian species because birds lack a urea cycle which aids in the conversion of ornithine to citrulline and Arg. (Khajali and Wideman, 2010). It is shown that ascites not only decreases overall flock feed efficiency but it also increases mortality of the heaviest birds during transport to the processing plant and higher condemnation of carcasses (Hasanpur *et al.* 2015). Study has shown a correlation between hematocrit values and ascites susceptibility (Wideman *et al.* 1998). Recently, Kamely *et al.* (2015) have reported cold temperature increases hemoglobin concentration, mean cell volume, and hematocrit.

Guanidino acetic acid (GAA) is formed from glycine (Gly) and Arg by the enzyme L-arginine:glycine amidinotransferase (AGAT) in the avian kidney and liver (Meister, 1965). It has been suggested that GAA could be used as an efficacious replacement for dietary Arg. (Dilger *et al.* 2013). The beneficial effects of GAA supplementation have been extensively studied (Heger *et al.* 2014; Michiels *et al.* 2012). In studies on other amino acids, Hasegawa *et al.* (2017) showed that the hepatic AGAT activity was reduced when supplementing levels of methionine and arginine were increased from deficient to adequate levels. It has been shown that the combined supplementation of valine, isoleucine, Arg and Gly is required to avoid the reduced performance of the birds during grower phase (Ospina-Rojas *et al.* 2014). Murakami *et al.* (2014) showed that dietary inclusion of GAA to meat-type quail breeders increased the availability of creatine in eggs and muscle of progeny, which finally caused to better reproductive parameters and better postnatal progeny performance. Arginine supplementation of the diet could significantly alleviate the negative effect of cold stress on performance, gut development and ascites syndrome (Khodambashi Emami *et al.* 2017). It was hypothesized that administration of GAA in the diet, as an Arg-sparing compound, may improve performance and reduce mortality due to ascites in broiler chickens. Therefore, this study was conducted to investigate the effects of dietary GAA supplementation on growth performance, blood biochemical parameters, meat pH and PHs susceptibility of broilers grown under a low-temperature condition.

MATERIALS AND METHODS

The experiment was conducted at the animal husbandry station, Razi University, Kermanshah located in western region of Iran positioned between 34 °18′N and 47 °03′E at height of 1350 m from sea level.

Bird source and management

A total of 640 newly hatched male chickens (Cobb) were obtained from a commercial hatchery and randomly assigned to 4 experimental groups, with eight replicates per treatment (20 birds per replicate). Birds were reared in battery cages (2.4×0.6×0.6 m) with a screen wired floor. Each cage was equipped with a tube feeder to be manually filled on a daily basis. The temperature in the house was 32 to 35°C in the first week, and was lowered by 1 °C every other day till 30 °C on d 10. From d 11, all the birds were exposed to a temperature cycle of 17 °C during the daytime and 14 °C at night in order to increase ascites susceptibility until the end of the experiment (Korte *et al.* 1999; Cahaner, 2011; Shlosberg *et al.* 1992).

Continuous light was provided 24 h for the first 3 days and then 23 L:1D light was adopted for the rest of the trial period. Except for the applied temperature schedule, the chickens were kept under conditions that closely resembled commercial practice.

Feeding and experimental diets

The basal diet was free of animal by-products to avoid contribution of creatine from an additional source. Prior to the experiments, the corn and soybean meal used for formulating the experimental diets were analyzed for dry matter (DM), crude protein (CP), and amino acids (AAs) by near-infrared spectroscopy (Paya Amin Mehr, Tehran, Iran).

Metabolizable energy contents of corn and soybean meal were estimated by using the regression models (NRC, 1994). All dietary nutrients met or exceeded (Cobb, 2008) recommendations (Table 1). The basal diet was supplemented with either 0.0, 0.6, 1.2 or 1.8 g GAA per kg of feed.

GAA was added in the form of CreAMINO® (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) and supplied at the expense of corn. The birds were allowed to access to feed and water *ad libitum* throughout the trial period. All birds received a pre-starter diet from 1 to 10 d. Starter and grower diets were provided from 11 to 21 d and 22 to 42d of age. The metabolic energy and CP contents of starter, grower and finisher diets were 2850, 3058 and 3130 kcal/kg; 250, 225 and 215 g/kg, respectively. No type of medication was administered during the entire experimental period.

Growth performance and ascites diagnosis

Initial weight gain was taken per replicate at the start of the experiment using salter weighing scale. The daily feed intake was calculated by subtracting feed left over from the quantity supplied daily. Feed conversion ratio (FCR) was calculated by dividing the average daily feed intake (ADFI) by the average daily gain (ADG). Dead birds were subjected to postmortem examinations and those with accumulation of abdominal or pericardial fluids were diagnosed as ascites mortality.

Table 1 Ingredients and chemical composition of the basal experimental diets (GAA)

Ingredient, g/kg as fed basis unless noted	Growth phase		
	Pre starter (0-10 d)	Starter (11-21 d)	Grower (22-42 d)
Corn grain (75.1 g/kg crude protein)	47.665	53.348	53.197
Corn gluten (55.1 g/kg crude protein)	2.996	1.888	-
Soybean meal (436.9 g/kg crude protein)	40.280	35.696	36.623
Soybean oil (37.71 MJ/kg)	3.958	4.406	5.967
Lime stone ($CaCo_3$) (370 g/kg calcium)	1.213	1.002	0.954
Dicalcium phosphate[1]	2.301	2.039	1.855
Common salt	0.283	0.289	0.315
Sodium bicarbonate ($Na\ HCO_3$)	0.119	0.095	0.043
Mineral premix[2]	0.250	0.250	0.250
Multivitamin premix[3]	0.250	0.250	0.250
DL-Met, 990 g/kg[4]	0.307	0.284	0.268
L-Lys-HCl, 780 g/kg[5]	0.164	0.140	0.062
L-threonine, 985 g/kg[6]	0.034	0.133	0.033
Filler[7]	0.180	0.180	0.180
Calculated composition % (as fed basis)[8]			
Metabolisable energy (kcal/kg)	2985.000	3058.000	3130.000
Crude protein (%)	25.096	22.584	21.521
Calcium (%)	1.043	0.899	0.845
Available phosphorus (%)	0.497	0.449	0.418
Sodium (%)	0.169	0.164	0.160
Crude fiber (%)	3.800	3.560	3.574
DEB[5] (mEg/kg)	260.823	237.287	233.710
Choline, ppm	1.695	1.608	1.590
Lys (digestible) %	1.290	1.154	1.100
MET (digestible) %	0.645	0.589	0.552
M+C (digestible) %	0.970	0.885	0.834
THR (digestible) %	0.824	0.842	0.722
TRP (digestible) %	0.257	0.231	0.232
Arg (digestible) %	1.500	1.351	1.344
Iso (digestible) %	0.947	0.847	0.818
Leu (digestible) %	1.986	1.768	1.595
Val (digestible) %	1.019	0.917	0.883
Analysed nutrients (g/kg)			
Crude protein	24.43	23.29	20.5
Dry matter	91.28	92.50	91.19

[1] Contains 18.5% P and 21% Ca.
[2] Mineral premix provided per kilogram of diet: Mn (from $MnSO_4{\cdot}H_2O$): 65 mg; Zn (from ZnO): 55 mg; Fe (from $FeSO_4.7H_2O$): 50 mg; Cu (from $CuSO_4.5H_2O$): 8 mg; I [from Ca $(IO_3)2H_2O$]: 1.8 mg; Se: 0.30 mg; Co (from Co_2O_3): 0.20 mg and Mo: 0.16 mg.
[3] Vitamin premix provided per kilogram of diet: vitamin A (from vitamin A acetate): 11500 IU; Cholecalciferol: 2100 IU; vitamin E (from DL-α-tocopheryl acetate): 22 IU; vitamin B_{12}: 0.60 mg; Riboflavin: 4.4 mg; Nicotinamide: 40 mg; Calcium pantothenate: 35 mg; Menadione (from menadione dimethyl-pyrimidinol): 1.50 mg; Folic acid: 0.80 mg.
[4] MetAMINO, Evonik Degussa Gmbh, Essen, Germany.
[5] L-lysine HCl, AJINOMOTO EUROLYSINE S.A.S, Paris, France.
[6] ThreAMINO, Evonik Degussa Gmbh, Essen, Germany.
[7] Supplemental feed additive of CreAMINO (>96% Guanidinoacetic acid; Evonik Degussa GmbH, Hanau-Wolfgang, Germany) was added to the basal diets at two levels of 600 to 180 g/kg of diet at the expense of filler (sand).
[5] DEB= dietary electrolyte balance per milli-equivalent value for dietary electrolyte balance. Calculated from dietary sodium, potassium, and chloride concentration (DEB, mEq/kg of diet=Na+ K+ Cl−, mEq/kg of diet); calculated values.
Analysed by Evonik Industries AG animal nutrition analytical lab for crude protein (AMINOProx®), amino acids (AMINONIR®), ether extract (AMINOProx®), dry matter (AMINOLab®) and total and phytate phosphorous (AMINOProx®) contents. The amount of amino acids in the diets was calculated based on amino acid content of feed ingredients and the standardised ileal amino acid digestibility values reported by Lemme *et al.* (2004).

Blood parameters

On 42 d of age, from one bird of each cage (eight birds per treatment) 3 mL of blood was collected via brachial vein. A portion of the blood sample was stored at 4 ˚C pending hematocrit and hemoglobin analysis, while the remainder was centrifuged to isolate serum by centrifugation at 3000 × g for 10 minutes. Serum concentrations of albumin, total protein and glucose were determined by spectrophotometric methods using commercially available kits (Pars Azmun, Tehran, Iran). Packed cell volume (PCV) percentage was determined in whole blood samples by centrifugation of micro hematocrit capillary tubes at 3000 × g for 5 min at room temperature (Jain, 1986). Red blood cells (RBC) were counted in a hemocytometer chamber using Natt and Herrick's solution to obtain a 1:200 blood dilution (Maxwell *et al.* 1986). Hemoglobin concentration was determined according to the cyanmethemoglobin method using a commercial kit (Pars Azmun, Tehran, Iran).

Mean corpuscular volume (MCV) was calculated according to the following formula: MCV (fL)= PCV × 10/RBC ($\times 10^{-6}$). The mean cell hemoglobin (MCH) was calculated according to the following formula: MCH (pg)= hemoglobin concentration (g/dL) / RBC ($\times 10^{-6}$). The mean corpuscular hemoglobin concentration (MCHC) was calculated according to the following formula: MCHC (g/L)= hemoglobin concentration (g/dL) / PCV (Clark *et al.* 2009). Erythrocyte osmotic fragility was determined in whole blood samples as a criterion of RBC membrane fluidity by using Dacie's method (Buffenstein *et al.* 2001) with minor modifications and a micro plate reader (Awareness Technology Inc., State Fax 3200, Palm City, Fla.). To determine blood leucocyte profiles, one hundred leucocytes per samples were counted by an optical microscope for heterophil to lymphocyte (H/L) separation according to the protocol described by Lucas and Jamroz (1961) and then H/L ratio was calculated.

Muscle pH

On 42 d of age, six chicks were selected per treatment, taking care to ensure that the mean and variance of their body weights were close to the corresponding values for the whole treatment groups. They were slaughtered sequentially with intervals of approximately 3 min between the slaughters of individuals. When the head, shanks and feathers were removed, the carcass was eviscerated by cutting around the vent to remove all of the viscera. Fifteen minutes post-harvest, the left breast muscle pH was measured on each specimen at three different locations at a depth of 2.5 cm below the muscle surface using a Model R. Matthaus PH-STAR meter (MATTHAUS Corporation, Germany) equipped with a spear electrode. Then the left breasts were placed in individual polyethylene bags and stored at 4 ˚C for 24 h. The 24 h pH was then measured in the same manner as the pH 30 min.

Statistical analysis

The data were analyzed based on a completely randomized design using the general linear method (GLM) procedure of SAS® (SAS, 2008). The results are reported as means. All data of experimental treatments were statistically analyzed by analysis of variance (ANOVA). The data were tested for linear and quadratic contrasts using the incremental dietary GAA treatments (0 or control diet, 0.6, 1.2 or 1.8 g GAA per kg of feed). Data were tested for distribution normality and homogeneity of variance. Variables with significant F-tests ($P<0.05$) were compared using Duncan's multiple range test. Results were considered significant when P-values were less than 0.05. Mortality data were subjected to Chi-square analysis.

RESULTS AND DISCUSSION

The effects of dietary inclusion of GAA on growth performance of broilers are summarized in Table 2. The birds fed the control, 1.2 and 1.8 g/kg GAA diets exhibited higher ADG compared to birds fed 0.6 g/kg GAA ($P<0.05$). Birds fed diet included 1.2 g/kg GAA had poorer FCR compared to birds in other dietary groups ($P<0.05$). In addition, quadratic responses to GAA levels were observed in terms of ADG and FCR. Higher ADFI were observed in birds fed diet 1.2 g/kg GAA than those of birds fed diets 0.6 and 1.8 g/kg GAA, whereas the birds fed the control diet had intermediate values with no significant different from other groups.

As indicated in Table 2, ascites-related mortality was significantly lower in the birds fed diet included 1.2 g/kg GAA compared to the birds fed the control, 0.6 and 1.8 g/kg GAA diets ($P<0.05$). Quadratic responses in ascites-related mortality to GAA levels were also detected.

As it is showed in Table 3, blood biochemical parameters including albumin, glucose and total protein were not significantly affected by dietary treatments. Hematological parameters, leukocyte profile and the weights of lymphoid organs are summarized in Table 4. MCV was significantly increased in the birds fed diets supplemented with 1.2 g/kg GAA compared to the birds fed the 0.6 and 1.8 g/kg GAA diets ($P<0.05$). The relative weight of thymus and spleen were significantly lower in birds fed diets included 0.6 and 1.8 g/kg GAA, respectively, compared to the birds fed the control diet ($P<0.05$). Effect of dietary treatments on meat pH is represented in Table 5. The pH values were slightly higher for the 1.8 g/kg GAA diets compared with those of the control, 0.6 and 1.2 g/kg ($P<0.05$ at 0.5 and 1 h postmortem).

Furthermore, at 4 h postmortem, the control-fed broilers had the highest pH values compared with those of others groups. Moreover, at 0.5 h and 1 h postmortem pH values showed linear ($P=0.01$) and quadratic ($P=0.03$) responses to dietary GAA levels.

Based on our knowledge, there is little report evaluating the effect of in-feed supplementation of GAA on performance of broilers with induced-ascites. Growth performance was significantly affected by dietary GAA supplementation. No significant differences in ADG, ADFI and FCR to the diet level of GAA has been previously shown (Abudabos *et al.* 2014). In the present study, the chickens fed the 0.6 and 1.8 g/kg GAA diets showed reduced ADFI compared to the birds fed the control diet. GAA is known to have sparing effect of Arg (Dilger *et al.* 2013) and Arg is also precursor for synthesis of NO. It was expected that GAA by NO improves ADFI.

Table 2 Effect of dietary guanidinoacetic acid (GAA) on growth performance and mortality associated with ascites in male broiler chicks up to the age of 42 days

Item	Treatments				SEM	P-value	Orthogonal polynomials contrasts	
	Control	$GAA_{0.6}$	$GAA_{1.2}$	$GAA_{1.8}$			Linear GAA	Quadratic GAA
ADFI (g/bird/d)[4]	92.15[ab]	87.44[b]	96.53[a]	90.25[b]	2.37	0.00	0.205	0.415
ADG (g/bird/d)[4]	68.49[a]	61.79[b]	66.66[a]	65.77[a]	2.81	0.02	0.657	0.649
FCR (g/g)[4]	1.35[b]	1.42[ab]	1.45[a]	1.37[ab]	0.03	0.03	0.343	0.008
PHS mortality (%)[4]	28.25[a]	27.50[a]	23.75[b]	27.25[a]	0.734	0.01	0.103	0.029

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
$GAA_{0.6}$: control group supplemented with 0.6 g/kg of GAA; $GAA_{1.2}$: control group supplemented with 1.2 g/kg of GAA and $GAA_{1.8}$: control group supplemented with 1.8 g/kg of GAA.
ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio and PHS mortality: mortality associated with ascites (% of total mortality).
SEM: standard error of the means.

Table 3 Biochemical parameters at day 42 of age in male broilers given diets supplemented with guanidinoacetic acid (GAA)

Item	Treatments				SEM	P-value	Orthogonal polynomials contrasts*	
	Control	$GAA_{0.6}$	$GAA_{1.2}$	$GAA_{1.8}$			Linear GAA	Quadratic GAA
Albumin (g/dL)								
Blood	359.50	364.00	360.25	364.50	5.73	0.94	0.661	0.965
Sera	1.42	1.42	1.42	1.55	0.09	0.74	0.372	0.502
Glucose (mg/dL)	242.00	211.00	216.75	228.00	13.19	0.38	0.695	0.367
Total protein (g/dL)	3.30	3.02	3.20	3.30	0.92	0.54	0.246	0.565

* Linear and quadratic orthogonal contrasts were tested using the incremental dietary GAA treatments (0 or control, 0.6, 1.2 and 1.8 g/kg).
$GAA_{0.6}$: control group supplemented with 0.6 g/kg of GAA; $GAA_{1.2}$: control group supplemented with 1.2 g/kg of GAA and $GAA_{1.8}$: control group supplemented with 1.8 g/kg of GAA.
SEM: standard error of the means.

Wang *et al.* (2014) showed that dietary Arg may regulate appetite in ducks by conversion to NO which was contrary to our results for birds receiving 0.6 and 1.8 g/kg GAA diet, with lower ADFI compared to the control.

In similarly with our results, Lemme *et al.* (2010) found lowered ADFI with dietary GAA supplementation in turkeys. No effect of GAA on FI of broilers was observed by Khodambashi Emami *et al.* (2017).

Furthermore, a similar results was also reported by Mousavi *et al.* (2013). This response might be due to the better energy utilization in chickens receiving the GAA-supplemented diets or a negative effect of GAA on ADFI (Heger *et al.* 2014).

The results of the current study for ADG and FCR indicated that feeding diets supplemented with 1.8 g/kg GAA had no significant difference with the control group. This outcome is inconsistent with the previous reports in which positive effects of GAA on FCR were observed (Heger *et al.* 2014; Michiels *et al.* 2012). Significant response of ADG and FCR to GAA were also reported when 1.2 g/kg GAA was added to the Arg.-deficient basal diet (Dilger *et al.* 2013).

In contrast, no effect for GAA on ADG of broilers was observed by Heger *et al.* (2014). It has been documented that cold temperature not only increase demand for oxygen consumption, but also lead to decreased ventilation and oxygen availability and ADG at market age in broiler (Buys *et al.* 1999; Deaton *et al.* 1996).

Regarding to energy consumption, it has been shown that the increased creatine concentration and the creatine phosphate:ATP ratio in muscle tissue due to GAA inclusion could play major roles in energy metabolism (Lemme *et al.* 2010), which subsequently influence ADG and FCR.

On the basis previous study, the improved performance response to supplemental GAA could be attributed to support overall energy homeostasis of the bird; an impact which is beyond the Arg sparing effect of GAA (Dilger *et al.* 2013).

In the present study, it does not seem that GAA could spare Arg and resulting in GAA did not improve ADG compared than control group.

Other reason is that the study is conducted under cold stress condition. On the other hand, GAA might reduce the nonenzymatic antioxidant capacity of cells under stress condition (Zugno *et al.* 2008), thus GAA may not improve performance and it may decrease performance under stress condition. Similar to our findings that 0.6 g/kg GAA lowered performance.

GAA based on orthogonal analysis, there were quadratic response for both ADG and FCR with incremental GAA levels, so that the highest level of GAA resulted in the best ADG and FCR. It may be attributed to sparing effect of GAA and higher dose may spare more Arg. Therefore, the potential effect of GAA in broiler chicks may depend on the usage dosage. The mortality rate was high in the current study.

Table 4 Hematological parameters, leukocyte profile and the weight of lymphoid organs at d 42 in male broilers given diet included supplemental guanidinoacetic acid (GAA)

Item	Treatments				SEM	P-value	Orthogonal polynomials contrasts	
	Control	GAA0.6	GAA1.2	GAA1.8			Linear GAA[1]	Quadratic GAA
Hematological characters								
Hb (g/dL)[5]	12.28	12.16	12.63	12.18	0.51	0.90	0.939	0.754
MCV (urn^3) (fL)	146.37^{ab}	145.00^{b}	149.25^{a}	144.37^{b}	1.25	0.04	0.757	0.174
MCH (Pg)	44.35	44.11	44.82	43.73	0.52	0.52	0.635	0.424
MCHC (g/dL)	30.52	30.41	30.03	30.42	0.31	0.71	0.637	0.437
WBC (×10 3/µL)	46.23	40.74	27.43	29.03	19.98	0.82	0.452	0.795
RBC (×10 6/µL)	2.77	2.75	2.81	2.79	0.11	0.98	0.827	0.983
Leukocyte profile								
Heterophil (H), %	14.75	15.25	12.87	16.62	2.26	0.70	0.751	0.479
Lymphocyte (L), %	84.87	84.25	86.25	83.62	2.39	0.88	0.871	0.679
H/L	18.01	18.34	15.48	21.07	3.45	0.72	0.685	0.452
Lymphoid organs weight								
Bursa of fabricius (%)	0.15	0.19	0.16	0.14	0.01	0.21	0.408	0.104
Thymus (%)	0.31^{a}	0.21^{b}	0.33^{a}	0.27^{ab}	0.45	0.04	1.000	0.412
Spleen (%)	0.18^{a}	0.16^{ab}	0.18^{a}	0.14^{b}	0.01	0.03	0.033	0.265

[1] Linear (L) and quadratic (Q) orthogonal contrasts were tested using the incremental dietary GAA treatments (0 or control, 0.6, 1.2 and 1.8 g/kg).
Control PC: control group; GAA0.6: control group supplemented with 0.6 g/kg of GAA; GAA1.2: control group supplemented with 1.2 g/kg of GAA and GAA1.8: control group supplemented with 1.8 g/kg of GAA.
Hb: hemoglobin; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; WBC: white blood cells and RBC: red blood cell counts.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Meat characteristics at slaughter d 42 in male broilers given diet included supplemental guanidinoacetic acid (GAA)

Item	Treatments[1]				SEM	P-value	Orthogonal polynomials contrasts	
	Control[2]	GAA0.6	GAA1.2	GAA1.8			Linear GAA[1]	Quadratic GAA
Breast meat								
pH, 0.0 h	5.92	5.91	6.00	6.05	0.07	0.57	0.192	0.674
pH, 0.5 h	5.71^{b}	5.89^{ab}	5.84^{ab}	6.02^{a}	0.06	0.04	0.015	0.978
pH, 1 h	5.82^{ab}	5.77^{ab}	5.67^{b}	5.93^{a}	0.06	0.10	0.426	0.031
pH, 2 h	5.79	5.81	5.77	5.76	0.07	0.55	0.748	0.857
pH, 3 h	5.77	5.75	5.80	5.76	0.05	0.21	0.993	0.848
pH, 4 h	5.81^{a}	5.71^{bc}	5.78^{ab}	5.70^{c}	0.03	0.00	0.204	0.908

[1] Linear (L) and quadratic (Q) orthogonal contrasts were tested using the incremental dietary GAA treatments (0 or control, 0.6, 1.2 and 1.8 g/kg).
Control PC: control group; GAA0.6: control group supplemented with 0.6 g/kg of GAA; GAA1.2: control group supplemented with 1.2 g/kg of GAA and GAA1.8: control group supplemented with 1.8 g/kg of GAA.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Increased mortality is often accompanied by ascites (Daneshyar *et al.* 2009; Luger *et al.* 2002); therefore, mortality percentage (28%) of cold-induced birds in the present study indicated the ascites development.

Khodambashi Emami *et al.* (2017) reported that ascites-related mortality was lower in broiler chicks fed the diet supplemented with 0.86 g/kg Arg and reared under cold temperature than cold-stressed birds fed on control or GAA supplemented diets. They introduced the reduced hematocrit as reason for lower mortality in 0.86% Arg group. However, hematocrit was not influenced by GAA in this study. However, in the current study, 1.2 g/kg GAA lowered mortality. The reduced mortality in 1.2 g/kg GAA group shows beneficial effects of dietary GAA in alleviating detrimental effects of AS.

The experimental diets had no significant effect on serum albumin, glucose and total protein. In contrast to our findings, decreased concentrations of serum albumin were detected in broilers with ascites (Yersin *et al.* 1992; Biswas *et al.* 1995). The findings of the present study are also in agreement with Daneshyar *et al.* (2009), who found total protein was significantly influenced by cold-induced ascites, at week 6 (P>0.05). Similarly, Yang *et al.* (2016) showed that dietary inclusion of Arg could not improve the serum concentration of albumin and protein in laying hens. In this study, high mortality was criteria for ascite but unchanging blood biochemichal parameters may be attributed to adaptive physiological responses. Tankson *et al.* (2002) stated that reduction in serum protein may be adaptive physiological responses to the impending loss of extra cellular fluids via ascites.

In present study, the differences between treatments in terms of hematological parameters were not significant. Azizian *et al.* (2013) indicated the higher hematocrit levels and RBC in cold-induced ascetic broilers. Huchzermeyer (2012) also observed an ascites-induced increase in blood

hematocrit value. The increased hematocrit values in ascitic chicks can be explained by increased oxygen demand of tissues due to enhanced metabolic rate and decreased oxygen saturation. Increased hematocrit value results increased number of RBC and decline in arterial blood oxygen saturation. Enhanced RBC accounts for increased blood viscosity and blood pressure which in turn contributes in enhanced cardiac work load in ascetic chicks (Druyan, 2012). However, the findings of Hafshejani *et al.* (2012) indicated no significant change in hemoglobin levels, which is in line with the findings in the present study. In the current study serum MCV counts also markedly increased but MCH and MCHC unchanged. These results suggest that the effects of dietary supplemental GAA are probably due to RBC counts because the increased erythrocyte osmotic fragility in birds exposed to the cold induced regimen suggests that the RBC counts decreased membrane's strength (Fathi *et al.* 2015).

This is parallel with the report by Kamely *et al.* (2015). It was hypothesized that GAA can act as an antioxidant in blood to protect the erythrocyte membrane structure and increase the deformability and transportation ability of erythrocytes, hence reducing RBC counts and subsequently hemoglobin. Khodambashi Emami *et al.* (2017) showed that Arg significantly lowered hematocrit rather than birds receiving the GAA. It seems that Arg can significantly improve hematological parameters than GAA. It may be attributed to GAA levels and sparing effect of GAA. We believed that GAA could not spare enough Arg. for affecting on hematological parameters.

There is an increasing interest in H/L ratio as a stress index in chickens (Scanes 2016; Davis *et al.* 2008). However, in the present experiment, no significant impact of treatment was detected on H/L values of the broilers. The H/L values observed in the current experiment is higher than the values measured in broilers in normal rearing conditions. So the high value of H/L in the present study is related to hypoxic condition (cold stress). Dietary inclusion of Arg could not improve H/L ratio in cold-stressed broiler chicks (Khajali *et al.* 2014). The lack of significant effect of treatment on H/L may be in part attributed to normal composition of the basal diet.

Bursa of fabricius and thymus are the sites of hematopoiesis, which are necessary for B cell and T-cell development in the chicken (Lechner *et al.* 2001). The lower relative weight of the thymus was observed in the chicks fed diet included 0.6 g/kg GAA, whereas the relative weight of bursa of fabricius was not significantly affected by experimental diets. Dietary inclusion of 1.72 g/kg Arg significantly lowered the weight of bursa of birds grown in either normal or cold temperature compared to birds fed on the control diet (Khodambashi Emami *et al.* 2017). Spleen, as a secondary lymphoid organ, plays important roles regarding to erythrocytes and immune system. It also synthesizes antibodies in its white pulp and removes antibody coated blood cells and antibody-coated bacteria by way of blood and lymph node circulation (Mebius and Kraal, 2005). In the present study, chicks fed the diet included 1.8 g/kg GAA exhibited lower spleen weights. As previously discussed by Grimble (2006), an adequate metabolic supply of dietary sulfur amino acids (SAA) is required for the synthesis of the myriad peptides and proteins involved in normal performance of the immune system. Recent study, Tossenberger *et al.* (2016) have discovered that an increase in plasma homocysteine at the highest level of dietary GAA. High plasma homocysteine is a consequence of a reduced methyl group transfer capacity, either by a relative methionine/choline/betaine deficiency or a lack of vitamin B_{12} and/or folic acid, or by both (Cuskelly *et al.* 2001). In this situation, it can be considered that the protein needs for optimum cell-mediated immune response may be reduced when diets contained optimum levels of GAA.

Dietary supplemental GAA significantly reduced pH of meat. GAA is a natural precursor of creatine in the vertebrate body. It was reported by James *et al.* (2002) that creatine supplementation in finisher diet of pigs was effective on energy status and meat quality. A lower postmortem pH and a lighter color of breast meat 48 h before slaughter were detected by Nissen and Young (2006) in Ross 308 broilers fed diet supplemented by creatine and glucose. Similar results were also observed by Stahl *et al.* (2003) in broilers fed diet supplemented with creatine during the entire rearing period.

CONCLUSION

The findings in the present study provide further insights into the diet GAA supplementation in chickens with rapid growth rate. Efficacy of GAA to decrease incidence of ascites due to cold exposure in broiler chickens can be confirmed by the results of the current study.

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