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Effect of Different Levels of Guanidinoacetic Acid and Lysine Supplementation on Performance, Blood Parameters, Carcass Characteristics and Myogenin Gene Expression of Arian Broilers

Research Article

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ABSTRACT

This study investigates the effects of guanidinoacetic acid (GAA) and high levels of lysine supplementation on performance, blood parameters, carcass characteristics, and myogenin gene expression (MYOG) in broiler chickens. A total of six hundred Arian day-old broiler chickens were arranged in a completely randomized design with a factorial arrangement, comprising 8 treatments, 5 replicates, and 15 chicks in each. Treatments included four levels of GAA (0, 0.3, 0.6, and 1.2 g/kg diet) and two levels of L-lysine (the National Research Council (NRC) recommended level and 20% higher than the NRC recommendation [Lys20]). The results showed that a significant effect on increasing body weight and improving the feed conversion ratio (FCR) by 0.3 g/kg GAA in diet ($P<0.05$). A significantly reduced feed intake and improved FCR by 20% increase lysine levels in the diet. The significant effect on body weight gain, reduced feed intake, and improved FCR ($P<0.05$) were observed by 0.3 g/kg GAA along with Lys20. In addition, significantly increased in breast muscle percentage ($P<0.05$). Blood parameters indicated that increased high-density lipoprotein (HDL) and decreased Low-density Lipoprotein (LDL) cholesterol levels by adding 1.2 g/kg GAA in diet, while increased HDL levels by Lys20 in this respect. The results showed that the highest level of MYOG gene expression was observed by interaction effect of 0.3 g/kg GAA and Lys20. Overall, the results indicated that improved the performance of broilers was pointed out by adding 0.3 g/kg diet of GAA along with Lys20.

KEY WORDS boilers, blood metabolites, guanodinoacetic acid, L-lysine.

INTRODUCTION

Creatine (Cr) plays an important role in animal metabolism energy, and guanidinoacetic acid (GAA) is the one of the precursor of Cr synthesis in animals Michiels *et al.* 2012). The endogenous synthesis of Cr provides about two-thirds of the animal's body needs, and the rest of that must be provided by the diet. Plant feed no contain Cr in diet and it should be added to the ration as an additive (Khajali *et al.* 2020). Guanidinoacetic acid, also called glycoamine or guanidinoacetate, is the natural precursor of Cr in vertebrates. Creatine is strongly involved in energy metabolism through the Cr and phosphocreatine (PCr) system (Abudabos *et al.* 2014). Creatine and PCr systems are not present in all cells. Rather, it is limited in cells with high but variable energy requirements, especially in muscle

cells. This system acts as a supporter of the Adenosine Diphosphate(ADP) / Adenosine Triphosphate (ATP) cycle to store and consume energy when needed in a short period of time (Heger *et al*. 2014). Overall, about 1.7% of stored Cr and PCr is irreversibly converted to creatinine and excreted in the urine (Khajali *et al*. 2020). Therefore, Cr must be continuously replaced. An animal's requirements for Cr can be met directly from animal protein (eg, fish meal) in the diet or via endogenous synthesis. This can occur in a two-step reaction (Brosnan and Brosnan, 2004; Westreicher-Kristen *et al*. 2025). According to current evidence, the first step occurs mainly in the kidneys and pancreas, while the second reaction occurs in the liver (Dilger *et al*. 2013). In the first step, which is catalyzed by the enzyme L-arginine glycine amidine transferase, arginine reacts with glycine and forms L-ornithine and GAA. Then, GAA is methylated at the amidino group by S-adenosyl-methionine (SAM) to form Cr in a reaction catalyzed by the enzyme S-adenosyl-methionine:N-guanidinoacetate. A sodium-bound transporter then transports Cr from the liver to various tissues. Farm animals fed diets containing low amounts of animal protein or no animal protein may be deficient in Cr. Therefore, due to the lack of protein in origin feed of animal (especially in the European Union), Cr supplementation or its precursor GAA may fulfill the need for Cr in tissues. Moreover, GAA is an immediate precursor. It is Cr that only requires the transfer of a methyl group from SAM, so Baker (2009) hypothesized that dietary GAA could saving dietary L-arginine intake. As a feed additive, Cr has disadvantages such as instability and high cost compared to GAA, which is more stable and cheaper (Zhang *et al*. 2014). Therefore, GAA may be more suitable in poultry feed. In some studies, Cr supplementation delayed the decline in Rigor mortis muscle pH, which has a possible positive effect on water holding capacity (WHC). In contrast, Nissen and Young (2006) have found a lower Rigor mortis pH and lighter color in the breast meat of Ross308 broilers after Cr supplementation. Stahl *et al*. (2003) have found similar results when broilers were fed Cr throughout the rearing period. Therefore, the effects of GAA on meat characteristics should also be considered.

Lysine is one of the most limiting amino acids in diets containing soybean meal-corn and soybean meal-sorghum for broiler chickens. The level of Lys diet required in the growing and finishing period to optimize breast meat may be higher than the level required for performance. It is desirable for birds (Nasr and Kheiri, 2011). Different recommended levels of dietary Lys have been determined in laboratories because there were several variations among experiments (genetic strain, environmental temperature, feed ingredients, protein source and quality, and sex) (Han and Baker, 1994; Zampiga *et al*. 2018). NRC (1994) recommendations for Lys up to 21 days of age have been shown to be too low for today's commercial broilers (Kidd and Fancher, 2001).

The NRC recommendation (NRC, 1984) for Lys in the diet of broiler chickens from 0 to 21 days of age was 1.2% in the diet, but this recommendation was reduced to 1.1% by NRC (1994). Amino acids are vital for muscle growth (Chrystal *et al*. 2020; Hilliar *et al*. 2020) and the effect of Lys in breast muscle is relatively higher than that of other amino acids (Zhai *et al*. 2016). It has been shown that insufficient Lys in diets reduces the performance of breast meat compared with other muscles (Cemin *et al*. 2017; Khwatenge *et al*. 2020).

Therefore, dietary amino acid requirements for optimal growth and performance is a great importance. Lysine is one of the key amino acids for protein synthesis and meat production, which also plays a vital role in the cytokines synthesis, the proliferation of lymphocytes and thus in the optimal the immune system function in response to infection. It is well known that protein, Lys and their interaction is priority factor in performance and carcass quality of growing chickens. The purpose of this experiment was the effect of different levels of GAA and Lys supplementation on performance, carcass characteristics and blood parameters as well as genetic status of Arian strain broiler chickens.

Increased muscle mass, function and prevent muscle mass loss in humans and animals by GAA as a dietary supplement (Michiels *et al*. 2012; Ostojic *et al*. 2013). The above effects are probably due to GAA cellular bioenergetic stimulation. Essential compound in the energy metabolism of muscle and nerve tissue was obtained by increasing the biosynthesis of creatine, however, the effect of GAA on skeletal muscle growth and myogenic differentiation is unclear. This study was conducted to investigate the effects of guanidinoacetic acid (GAA) and high levels of lysine supplementation on performance, blood parameters, carcass characteristics, and myogenin (MYOG) gene expression in broiler chickens.

MATERIALS AND METHODS

The experiment was conducted by 600 Arian strain broiler chickens in completely random design with a factorial arrangement consisting of eight treatments, five replicates and 15 chickens in each. The humidity was 50 to 60% and the lighting was 23 hours with one hour of darkness during the rearing period. Broilers were vaccinated against bronchitis and Newcastle disease (N.D) respectively at first and 7 days of life and then N.D was repeated at 16 days of age.

Experimental groups include treatment 1-basal diet containing Lys at the level recommended by NRC (1994) (starter=1.30%, grower=1.20%, finisher=1.05%) and without GAA supplement, treatments 2-3-4 - diet containing Lys at the level recommended by NRC (1994) and GAA supplement at the level of 0.3, 0.6 and 1.2 g/kg, respectively. Treatment 5- Diet containing Lys at a level 20% higher than NRC (1994) (starter=1.56%, grower=1.44%, finisher=1.26%). Recommendation and without GAA supplementation, Treatment 6-7-8- Diet containing Lys at a level 20% higher than NRC recommendation and GAA supplementation at 0.3, 0.6, 1.2 g/kg, respectively they were. Based on the requirements in the Arian strain catalog recommendation, the experimental diets were adjusted in the form of isocaloric and isonitrogenous in the three stages includes starter (0-14 days old), grower (15-28 days old) and finisher (29-42 days old) periods (Table 1). The amounts of GAA and amino acid lysine hydrochloride were added to the diets.

During the experimental period, body weight and feed consumption were recorded weekly and mortality was recorded daily. At the age of 35 days, blood was collected from 2 broilers in each replicate to determine blood parameters: cholesterol, triglyceride, creatine (Cr), phosphocreatine (CPK), creatine kinase (CK), hematocrit (HCT) , nitric oxide (NO).

After separating the serum from the blood samples, they were kept at -20 ˚C until the test.

At the end of the experiment, in order to determine the carcass characteristics (breast percentage, thigh percentage), visceral organs (heart, liver, abdominal fat) and meat quality, four broilers from each pen were selected and slaughtered according to their average weight. To measure the amount of GAA, Cr, breast tissue samples were prepared immediately after slaughter and kept at -80 ˚C until the test.

Blood parameters

To determine serum lipid parameters (cholesterol and triglycerides), an enzymatic-colorimetric method was used (Pars Azmoun kit and spectrophotometer with a wavelength of 546 nm). Briefly, 1000 µL of reagent was poured into 3 microtubes using a sampler. In the first microtube, 10 µL of double distilled water as a blank, in the second microtube 10 µL of standard, and in the third microtube 10 µL of serum were added and mixed, and the samples were placed at an ambient temperature of 20-25 ˚C for 20 minutes. Then, the optical absorption of the samples was measured within 60 minutes.

To measure creatine kinase (CK) in blood serum, Pars Azmoun kit and spectrophotometer with a wavelength of 340 nm were used.

To measure the blood hematocrit, the heparin capillary tube was filled to the extent of 3/4 of the whole blood sample and centrifuged at 10000 rpm for 5 minutes and the blood hematocrit percentage was obtained.

Measurement of MYOG gene expression

After killing, 40 samples were taken from a similar place in the left side of the chest muscle (pectoralis major muscle), a sample of approximately 20 grams was taken and placed in sterile and RNase-free cryotubes. The samples taken were immediately transferred to the liquid nitrogen tank and then kept in the laboratory in a freezer at -80 degrees Celsius until RNA extraction. Gene expression was measured at Zanjan University Research Center. The RNA was extracted from tissues by the RNX-plus kit of CinnaGen Co. After RNA extraction, spectrophotometric evaluations were performed by NanoDrop and 1% agarose gel. Determination of concentrations and optical density (OD) of 260 to 280 and 260 to 230 as seen in the figure which was determined by nanotrap. One microgram of RNA obtained and of suitable quality was used to make cDNA (copy DNA or complementary DNA) and then in the Real Time PCR reaction according to the program: a cycle of 95 degrees Celsius for 15 minutes (as primary sequencing). And 95 ˚C for 10 seconds, 56 ˚C for 10 seconds, 72 ˚C for 20 seconds for 40 cycles in a Rotorgen 6000 machine. Melting analysis was also done to ensure the specificity of the reactions. The kit used for Real Time PCR Master Mixes was from Salice Biodyne. The statistical analysis of the results was done in GenEx software.

The statistical model

To analyze the data, a completely randomized design (CRD) with factorial arrangement and the GLM procedure of the software (SAS, 2004) was used, and Duncan's multi-range test was used to compare the means at a significance level of five percent. The statistical model of the experimental design was as follows:

$$Y_{ijk} = A_i + B_j + AB_{ij} + E_{ijk}$$

Where:
Y_{ijk}: value of each observation.
A_i: main effect of Lys.
B_j: main effect of GAA.
AB_{ij}: interactions of the main factors.
E_{ijk}: experimental error.

RESULTS AND DISCUSSION

The performance results of broiler chickens are presented in Table 2.

Table 1 Ingredient and chemical composition of basal diets in Arian broilers

Ingredient%	Starter	Grower	Finisher
Corn grain	53.65	58.23	62.54
Soybean meal, 44%	34.24	32.09	29.89
Corn gluten meal	4.00	2.00	0.00
Soybean oil	2.73	2.73	2.73
Dicalcium phosphate	2.00	2.00	2.06
Calcium carbonate	1.73	1.73	1.73
L-threonine	0.38	0.00	0.00
Common salt	0.32	0.32	0.29
Mineral premix[1]	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25
L-lysine-HCl, 75%	0.17	0.13	0.03
DL-methionine 99%	0.15	0.15	0.13
$NaHCO_3$	0.10	0.07	0.07
Total	100	100	100
Nutrient			
Metabolizable energy (kcal/kg)	2.95	2.98	3.00
Crude protein, %	22.51	20.70	18.76
Calcium, %	1.05	1.05	1.05
P (available), %	0.52	0.52	0.52
Na, %	0.18	0.17	0.16
Arginine, %	1.46	1.38	1.29
Lysin, %	1.30	1.20	1.05
Methionine, %	0.56	0.52	0.45
Met+Cys, %	0.92	0.58	0.75
Threonine, %	0.91	0.84	0.78
DCAB (mEq/kg)	235	222	218

[1] Each kilogram of feed supply provides the following substances: vitamin A: 8700 IU; vitamin D3: 2300 IU; vitamin E: 16 IU; vitamin K3: 1.5 mg; Thiamine: 3 mg; Riboflavin: 6.6 mg; Prodoxin: 2.5mg; Vitamin B12: 0.31 mg; Biotin: 30 mg; Niacin: 28 mg; Folic Acid: 0.8 mg; Ethoxyquin: 125 mg and Calcium Pantothenate: 35 mg.
[2] Each kilogram of ration contains: Inorganic manganese: 60 mg; Iron: 80 mg; Zinc: 40 mg; Copper: 8 mg; Iodine: 0.35 mg and Selenium: 0.2 mg.

Table 2 The effects of guanidinoacetic acid (GAA) and L-lysine (L-Lys) on the growth performance of Arian broilers (1-42 day)

		Feed intake (g/bird)	Body weight gain (g/bird)	Feed conversion ratio
Main effects GAA				
0		3797	2009^{b}	1.894^{a}
300		3736	2076^{a}	1.803^{b}
600		3712	2096^{a}	1.772^{b}
1200		3761	2068^{ab}	1.819^{b}
SEM		34.47	21.48	0.018
P-value		0.36	0.043	0.0006
Main effects L-Lys				
Level recommended by NRC		3805^{a}	2043	1.865^{a}
20% over NRC recommendation		3697^{b}	2081	1.778^{b}
SEM		24.37	15.19	0.013
P-value		0.04	0.83	0.0001
Interaction effects				
GAA**	Lys***			
G0	L0	3828^{a}	1959^{c}	1.96^{a}
G1	L0	3859^{a}	2013^{bc}	1.92^{a}
G2	L0	3718^{ab}	2096^{ab}	1.78^{b}
G3	L0	3815^{a}	2106^{ab}	1.81^{b}
G0	L1	3765^{ab}	2060^{ab}	1.83^{b}
G1	L1	3614^{b}	2140^{a}	1.70^{c}
G2	L1	3705^{ab}	2096^{ab}	1.77^{b}
G3	L1	3707^{ab}	2031^{bc}	1.83^{b}
SEM		48.75	30.38	0.27
P-value		0.023	0.004	0.0001

** G factor: different levels of GAA; G0= 0%, G1= 0.3 g/kg, G2=0.6 g/kg diet and G3= 1.2 g/kg diet.
*** L factor: different levels of L-laysin; L0= NRC (starter=1.30%, grower=1.20%, finisher=1.05%) and L1= 20% over NRC recommendation (starter=1.56%, grower=1.44%, finisher=1.26%).
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.

A significant increase in body weight were observed by 0.3 and 0.6 g/kg of GAA compared to the control group. In addition, improved 6.8% feed conversion ratio, was indicated by addition 0.6 g/kg GAA compared to the control group. No significant response was found in feed consumed by the GAA. A significant decreased in the feed consumption and improved the feed conversion ratio was shown by 20% increased Lys in diet. But no reaction was found on the increase in body weight in this respect. A significant effect on increasing body weight, reducing feed consumption and improving feed conversion ratio were observed by only 0.3 g/kg GAA and 20% Lys more than NRC recommendation ($P<0.05$). Improved weight gain and feed conversion ratio by adding GAA in broiler diets have been reported by researchers (Ringel *et al*. 2007; Michiels *et al*. 2012; Mousavi *et al*. 2013; DeGroot, 2014). Otherwise, Lemme *et al*. (2011) and Córdova-Noboa *et al*. (2018) have shown improves feed conversion ratio and body weight by 0.06% GAA in diets containing fish meal. The European Food Safety Authority (EFSA, 2009) has reported that reduced feed intake and weight gain in broilers by 0.6 g/kg GAA and higher in diet. They described body weight and feed consumption were as non-linear by GAA effect, therefore a decrease in body weight and feed consumption of broiler chickens were observed in this terms.

In the present experiment, decrease in feed consumption was observed by GAA addition, which is probably due to the improvement of energy metabolism and reduction of feed consumption per kilogram of weight gain.

The response of broilers to Lys level depends on the strain, sex, protein level and amino acids in the diet (Corzo *et al*. 2004; Sterling *et al*. 2006). The terms of, the improvement in performance by adding Lys to the diet in different rearing periods has led to different results. Kidd and Fancher (2001) have shown that improved performance by adding extra Lys to the diet of male broiler chickens in the early period. In the present experiment, improvement was observed in the early growth period (un published), but no such effect was observed in the entire rearing period. Nasr and Kheiri (2012) have reported that increased body weight by 20% lysine higher than the NRC recommendations in feeding broilers initial and growing periods. They have observed improved bird performance due to increased feed intake as a result of increased dietary Lys levels. In the present study, the improvement of efficiency and feed conversion ratio has been achieved by reducing the amount of feed consumed. It has been shown that excessive consumption and imbalance of dietary amino acids leads to a decrease in feed intake (EFSA, 2009). Considering the differences in strains and the differences in amino acid requirement, in the present experiment, the increase in Lys level probably led to the amino acid imbalance in the diet and the decrease in feed consumption. It has been shown that the increase of Lys in low protein diets has a positive effect on the feed consumed (Lee *et al*. 2020).

The results (Table 3) showed that the use of different levels of GAA or lysine had a significant effect on breast muscle percentage ($P<0.05$) however, the percentage of carcass, thigh, abdominal fat, heart, and liver was not affected by the addition of GAA or lysine. The highest percentage of breast muscle was appeared by 0.3 g/kg GAA, which was 8.4% higher than the control group. The addition of lysine led to a 9% improvement in the breast muscle. Breast weight improvement by adding GAA to the diet and this has been reported by a number of researchers (Ringel *et al*. 2007; Michiels *et al*. 2012; Esser *et al*. 2018). Heger *et al*. (2014) have shown that among carcass parameters, only breast meat was significantly affected by GAA supplementation. In contrast, no effect on carcass parameters was stated by GAA (Mousavi *et al*. 2013).

Unfortunately, the mechanism of GAA effects on broiler performance is not fully understood. Guanidinoacetic acid is a natural precursor of Cr, which is involved in cellular metabolism energy, especially in tissues with high energy requirements such as skeletal muscle (Michiels *et al*. 2012). In rapidly growing animals fed CREA-deficient plant diets, GAA may restore Cr source, thus improving metabolism energy and tissue growth. Another mode of action of GAA may be related to amino acid metabolism. *In vivo*, GAA is synthesized from glycine and arginine and therefore GAA supplementation may prevent arginine wasting in broilers (Baker, 2009).

Breast meat is a large part of carcass meat, which is affected by sex, age, strain and high concentration of lysine. Diets containing low levels of Lys can limit breast meat production due to reduced proliferation of breast muscle cells early in development (Han and Baker, 1994; Gorman and Balnave, 1995). Significantly the highest weight gain and feed consumption was fond by broilers fed with 1.37% high lysine (Garcia and Batal, 2005; Dozier *et al*. 2008). The improvement in breast percentage by increasing the level of lysine in the diet may be attributed to the improvement in feed efficiency (Han and Baker, 1994; Gorman and Balnave, 1995), which was match with the results of the present study. The effects of Lys and GAA supplementation on some blood metabolites of Arian broilers are shown in Table 4.

Increased level of HDL and decreased LDL level in the blood were found by adding GAA supplement to the diet. The highest level of HDL and the lowest level of LDL were related to the treatment of 1.2 g/kg of GAA. Cholesterol, triglyceride, CK enzyme and blood hematocrit were not affected by GAA supplementation. Adding lysine to the diet led to a significant increase in HDL.

Table 3 The effects of guanidinoacetic acid (GAA) and L-lysine (L-Lys) on Arian broiler carcass characteristics and visceral organs

		Liver %	Heart %	Fat %	Breast %	Thigh %	Carcass %
Main effects GAA							
0		2.211	0.510	0.625	25.53^{b}	20.52	75.58
300		2.039	0.516	0.605	27.69^{a}	20.08	75.59
600		2.088	0.505	0.457	26.33^{ab}	20.17	76.20
1200		2.040	0.532	0.421	26.57^{ab}	19.70	77.52
SEM		0.09	0.02	0.10	0.50	0.25	0.72
P-value		0.49	0.76	0.37	0.03	0.18	0.21
Main effects L-Lys							
Level recommended by NRC		2.120	0.518	0.600	25.39^{b}	20.32	75.95
20% over NRC recommendation		2.070	0.513	0.460	27.67^{a}	19.91	76.50
SEM		0. 06	0.01	0.07	0.35	0.18	0.51
P-value		0.60	0.77	0.16	0.001	0.11	0.45
Interaction effects							
GAA**	Lys***						
G0	L0	1.770	0.522	0.756	24.63^{c}	21.28^{a}	75.79
G1	L0	2.044	0.500	0.718	25.75^{bc}	19.52^{bc}	73.42
G2	L0	2.248	0.508	0.516	25.26^{bc}	20.45^{abc}	76.71
G3	L0	2.058	0.544	0.404	25.92^{bc}	20.07^{bc}	77.87
G0	L1	2.300	0.498	0.494	26.45^{bc}	19.77^{bc}	75.37
G1	L1	2.034	0.532	0.492	29.62^{a}	20.64^{ab}	77.76
G2	L1	1.924	0.502	0.398	27.40^{b}	19.89^{bc}	75.69
G3	L1	2.022	0.520	0.438	27.22^{b}	19.34^{c}	77.17
SEM		0.19	0. 02	0.14	1.07	0.36	1.08
P-value		0.60	0.69	0.72	0.31	0.008	0.12

** G factor: different levels of GAA; G0= 0%, G1= 0.3 g/kg, G2=0.6 g/kg diet and G3= 1.2 g/kg diet.
*** L factor: different levels of L-laysin; L0= NRC (starter=1.30%, grower=1.20%, finisher=1.05%) and L1= 20% over NRC recommendation (starter=1.56%, grower=1.44%, finisher=1.26%).
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 4 The effects of guanidinoacetic acid and L-lysine on blood parameters of Arian broilers

		HCT %	CK (U/L)	LDL (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/d)
Main effects GAA							
0		32.80	37.50	73.60^{ab}	17.20^{b}	56.40	154.3
300		31.70	41.30	79.20^{a}	19.60^{a}	61.40	164.4
600		34.00	40.00	72.30^{b}	20.00^{a}	54.10	162.9
1200		33.20	40.60	68.40^{b}	20.50^{a}	54.90	152.5
SEM		0.94	1.20	2.03	0.63	5.67	4.45
P-value		0.39	0.16	0.007	0.004	0.77	0.52
Main effects L-Ly							
Level recommended by NRC		32.95	39.30	72.95	16.00^{b}	59.60	148.6
20% over NRC recommendation		32.90	40.70	73.80	22.65^{a}	55.15	168.5
SEM		0.66	0.85	1.43	0.44	4.01	6.15
P-value		0.95	0.17	0.68	0.0001	0.44	0.20
Interaction effects							
GAA[1]	Lys[2]						
G0	L0	32.20	38.20	71.60	13.00	51.20	142.6
G1	L0	31.80	40.80	79.40	16.80	65.20	156.0
G2	L0	33.40	38.20	72.80	16.20	56.00	153.4
G3	L0	34.40	38.80	68.00	18.00	66.00	142.4
G0	L1	33.40	36.80	75.60	21.40	67.00	166.0
G1	L1	31.60	41.80	79.00	22.40	57.60	172.8
G2	L1	34.60	41.80	71.80	23.80	52.20	172.4
G3	L1	32.00	42.40	68.80	23.00	43.80	162.6
SEM		1.33	1.70	2.87	0.90	8.02	10.29
P-value		0.50	0.42	0.82	0.21	0.15	0.96

** G factor: different levels of GAA; G0= 0%, G1= 0.3 g/kg, G2=0.6 g/kg diet and G3= 1.2 g/kg diet.
*** L factor: different levels of L-laysin; L0= NRC (starter=1.30%, grower=1.20%, finisher=1.05%) and L1= 20% over NRC recommendation (starter=1.56%, grower=1.44%, finisher=1.26%).
HCT: hematocrit test; CK: creatine kinase; LDL: low-density lipoprotein and HDL: high-density lipoprotein.
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 The effects of guanidinoacetic acid (GAA) and L-lysine (L-Lys) on MYOG gene expression of Arian broilers

		MYOG gene expression
Main effects GAA		
0		8.862
300		7.751
600		6.777
1200		7.759
SEM		1.24
P-value		0.70
Main effects L-Lys		
		7.333
20% more than NRC recommendation		8.242
SEM		0.87
P-value		0.46
Interaction effects		
GAA**	Lys***	
G0	L0	6.932^{ab}
G1	L0	9.158^{ab}
G2	L0	4.372^{b}
G3	L0	8.869^{ab}
G0	L1	10.79^{a}
G1	L1	6.344^{ab}
G2	L1	9.182^{ab}
G3	L1	6.650^{ab}
SEM		1.75
P-value		0.07

** G factor: different levels of GAA; G0= 0%, G1= 0.3 g/kg, G2=0.6 g/kg diet and G3= 1.2 g/kg diet.
*** L factor: different levels of L-laysin; L0= NRC (starter=1.30%, grower=1.20%, finisher=1.05%) and L1= 20% over NRC recommendation (starter=1.56%, grower=1.44%, finisher=1.26%).
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.

No significant differences were found on cholesterol, triglyceride, creatine kinase enzyme and hematocrit (HCT) by different levels of GAA and Lys.

No change in blood parameters (cholesterol, triglyceride and hematocrit) and phosphocreatine kinase were observed by adding GAA in broiler diets (Michiels *et al*. 2012; DeGroot, 2014; Tossenberger *et al*. 2016; Córdova-Noboa *et al*. 2018) which was corresponding with the findings in the present study. Increased the level of HDL and decreased the level of LDL by GAA supplementation In Contrary to the report of Córdova-Noboa *et al*. (2018) in the present experiment increased the level of HDL and decreased the level of LDL.

It is hypothesized that GAA can act as an antioxidant in the blood to protect the red blood cell membrane structure and reduce the red blood cell number and subsequently the hematocrit. Mohebbifar *et al*. (2017) have shown that diets containing GAA had no significant effect on hematocrit, which is in line with the results of the present study. Nasiroleslami *et al*. (2018) have pointed out that the 1.2 g/kg of GAA in the broiler chickens diet under cold stress conditions had no effect on performance parameters (body weight, feed intake and feed conversion ratio) and blood parameters (hematocrit and LDH). In contrast, increased CK and LDL enzyme and decreased HDL level compared with the control group, which was contradicts the results of the present experiment. GAA may be able to support the production of creatine and increase CK activity. The increase in CK activity and serum creatinine could be due to the production of more than limit or decrease renal excretion or both (Hekimsoy and Oktem, 2005).

The results of the present experiment have indicated the effect of lysine on increasing the level of HDL and no effect on other blood serum lipid parameters of broiler chickens. A decrease in cholesterol and triglyceride levels (Chang *et al*. 2018) and an increase in blood triglyceride levels (Emamzadeh *et al*. 2022) have been shown as a result of increasing dietary lysine levels. Determining the optimal lysine requirement is economically important because feeding lysine-deficient or excessive rations results in poor performance or increased ration cost. Lysine requirement in poultry is influenced by many factors such as strain, sex, growth stage, production performance, etc. (Dozier and Payne, 2012).

Bouyeh and Gevorgyan (2011) have observed that increasing dietary lysine in 20% more than NRC recommend could decrease in lipid parameters, while increasing more lysine due to an imbalance of dietary amino acids causes an

increase in blood lipid parameters (Bouyeh and Gevorgyan, 2011). It has been suggested that increasing the level of lysine leads to a decrease in the expression of genes affecting fat synthesis, which can be effective in reducing fat storage in adipose tissue and increasing free triglycerides in the blood (Tian *et al*. 2019).

Lysine as a precursor of L-carnitine can play an important role in lipid and energy metabolism (Bouyeh and Gevorgyan, 2011). L-carnitine has the effects of lowering blood lipids, this reduces the concentration of cholesterol, triglycerides, free fatty acids, phospholipids and VLDL in circulation and increases the concentration of HDL and LDL. It has been monitored that when high levels of lysine are presented in the diet, plasma cholesterol, LDL and HDL levels increase significantly (Bouyeh and Gevorgyan, 2011). The present results have shown that increasing the level of lysine increased the concentration of cholesterol and HDL.

The results of the main and interaction effects in different levels of GAA and Lys in the diet on the expression of the MYOG gene growth performance in broiler chickens at the end of the period are presented in Table 5. The results of the current experiment showed that the main effects of GAA and Lys on MYOG gene expression were not significant (P>0.05), but the interaction effects tended to be significant (P>0.05). So that the highest expression of the MYOG gene was related to the treatment without GAA and lysin in 20% more than NRC value.

CONCLUSION

Increased body weight and improved feed conversion ratio by GAA. A significant decrease in feed intake and improved feed conversion ratio was shown by 20% more than NRC lysine recommendation in diet, but no response was found in body weight. Increased body weight, decreased feed consumption, an improved in feed conversion ratio and an increased percentage of breast muscle by 0.3 g/kg of GAA and 20% higher lysine than the NRC recommendation in diet. Adding GAA supplements to the diet increased HDL levels and decreased LDL levels in the blood serum. A significant increase in HDL by adding lysine to the diet. No significantly affect the expression of the MYOG gene was observed by GAA and lysine separately, but the significant reduction were found by the mutual effects of GAA and lysine on the expression of the MYOG gene. In general, the best performance has shown by 0.3 g/kg of GAA along with a more than 20% increase NRC lysine recommended.

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REFERENCES

Abudabos A.M., Saleh F., Lemme A. and Zakaria H.A.H. (2014). The relationship between guanidino acetic acid and metabolisable energy level of diets on performance of broiler chickens. *Italian J. Anim. Sci.* **13,** 548-556.

Baker D.H. (2009). Advances in protein-amino acid nutrition of poultry. *Amino Acids*. **37,** 29-41.

Bouyeh M. and Gevorgyan O.K. (2011). Influence of excess lysine and methionine on cholesterol, fat and performance of broiler chicks. *J. Anim. Vet. Adv*. **10,** 1546-1550.

Brosnan M.E. and Brosnan J.T. (2004). Renal arginine metabolism. *J. Nutr*. **134,** 2791-2795.

Cemin H.S., Vieira S.L., Stefanello C., Kipper M., Kindlein L. and Helmbrecht A. (2017). Digestible lysine requirements of male broilers from 1 to 42 days of age reassessed. *PLoS One.* **12,** e0179665.

Chang L.L., Xie P., Bu Z., Wang Q., Fu S.Y. and Mu C.Y. (2018). Effect of dietary lysine level on performance, egg quality and serum biochemical indices of laying pigeons. *J. Appl. Poult. Res.* **27,** 152-158.

Chrystal P.V., Moss A.F., Khoddami A., Naranjo V.D., Selle P.H. and Liu S.Y. (2020). Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. *Poult Sci*. **99,** 1421-1431.

Córdova-Noboa H.A., Oviedo-Rondón E.O., Sarsour A.H., Barnes J., Sapcota D., López D., Gross L., Rademacher-Heilshorn M. and Braun U. (2018). Effect of guanidinoacetic acid supplementation on live performance, meat quality, pectoral myopathies and blood parameters of male broilers fed corn-based diets with or without poultry by-products. *Poult. Sci.* **97,** 2494-2505.

Corzo A., Mcdaniel C.D., Kidd M.T., Miller E.R., Boren B. and Fancher B.J. (2004). Impact of dietary amino acid concentration on growth, carcass yield, and uniformity of broilers. *Australian J. Agric. Res*. **55,** 1133-1138.

Degroot A.A. (2014). Efficacy of dietary guanidinoacetic acid in broilers chicks. Master Thesis. Illinois, India.

Dilger R.N., Bryant-Angeloni K., Payne R.L., Lemme A. and Parsons C.M. (2013). Dietary guanidino acetic acid is an efficacious replacement for arginine for young chicks. *Poult. Sci.* **92,** 171-177.

Dozier III W.A. and Payne R.L. (2012). Digestible lysine requirements of female broilers from 1 to 15 days of age. *J. Appl. Poult. Res.* **21,** 348-357.

Dozier III W.A., Corzo A., Kidd M.T. and Schilling M.W. (2008).

Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. *Poult. Sci*. **87,** 1385-1391.

EFSA (European Food Safety Authority). (2009). Safety and Efficiency of Guanidino Acetic Acid as Feed Additive for Chickens for Fattening. *The EFSA J*. **988,** 1-30.

Emamzadeh E.M., Mazhari M., Ziaei N. and Roudbari Z. (2022). Effect of lysine level on growth performance, carcass characteristics, blood metabolites and breast muscle tissue of Ross 308 broilers. *J. Anim. Environ.* **14,** 73-82.

Esser A.F.G., Taniguti T.L., da Silva A.L., Vanroo E., Kaneko I.N., Dos Santos T.C. and Fernandes J.L.M. (2018). Effects of guanidionoacetic acid and arginine supplementation to vegetable diets fed to broiler chickens on performance, carcass yield and meat quality. *Semin. Cienc. Agrar*. **39,** 1307-1318.

Garcia A. and Batal A.B. (2005). Changes in the digestible lysine and sulfur amino acid needs of broiler chicks during the first three weeks posthatching. *Poult. Sci.* **84,** 1350-1355.

Gorman I. and Balnav E.D. (1995). The effect of dietary lysine and methionine on the growth characteristics and breast meat yield of australian broiler chickens. *Australian J. Agric. Res*. **46,** 1569-1577.

Han Y. and Baker D.H. (1994). Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. *Poult. Sci*. **73,** 1739-1745.

Heger J., Zelenka J., Machander V., De La Cruz C., Lestak M. and Hampel D. (2014). Effects of guanidinoacetic acid supplementation to broiler diets with varying energy content. *Acta Univ. Agric. Silvic. Mendel. Brun.* **62,** 477-485.

Hekimsoy Z. and Kavalali Oktem I. (2005). Serum creatine kinase levels in overt and subclinical hypothyroidism. *Endocr. Res*. **31,** 171-175.

Hilliar M., Keerqin C., Girish C.K., Barekatain R., Wu S.B. and Swick R.A. (2020). Reducing protein and supplementing crystalline amino acids, to alter dietary amino acid profiles in birds challenged for subclinical necrotic enteritis. *Poult. Sci.* **99,** 2048-2060.

Khajali F., Lemme A. and Rademacher-Heilshorn M. (2020). Guanidinoacetic acid as a feed supplement for poultry. *Worlds Poult. Sci. J.* **76,** 270-291.

Khwatenge C.N., Kimathi B.M., Taylor-Bowden T. and Nahashon S.N. (2020). Expression of lysine-mediated neuropeptide hormones controlling satiety and appetite in broiler chickens. *Poult. Sci.* **99,** 1409-1420.

Kidd M.T. and Fancher B.I. (2001). Lysine needs of starting chicks and subsequent effect during the growing period. *J. Appl. Poultry Res.* **10,** 385-393.

Lee C.Y., Song A.A.L., Loh T.C. and Rahim R.A. (2020). Effects of lysine and methionine in a low crude protein diet on the growth performance and gene expression of immunity genes in broilers. *Poult. Sci*. **99,** 2916-2925.

Lemme A., Elwert C., Gobbi R. and Rademacher R. (2011). Application of the guanidino acetic acid as creatine source in broilers fed diets with or without fish meal. Pp. 453-455 in Proc. 18th European Symp. Poult. Nutr., Ceşme, Turkey.

Michiels J., Maertens L., Buyse J., Lemme A., Rademacher M., Dierick N.A. and De Smet S. (2012). Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. *Poult. Sci.* **91,** 402-412.

Mohebbifar A., Torki M. and Abdolmohammadi A. (2017). Effects of dietary guanidinoacetic acid supplementation on performance, blood parameters and meat quality of male broilers with coldinduced ascites. *Iranian J. Appl. Anim. Sci*. **9,** 125-133.

Mousavi S.N., Afsar A. and Lotfollahian H. (2013). Effects of guanidinoacetic acid supplementation to broiler diets with varying energy contents. *J. Appl. Poult. Res*. **22,** 47-54.

Nasiroleslami M., Torki M., Saki A.A. and Abdolmohammadi A.R. (2018). Effects of dietary guanidinoacetic acid and betaine supplementation on performance, blood biochemical parameters and antioxidant status of broilers subjected to cold stress. *J. Appl. Anim. Res.* **46,** 1016-1022.

Nasr J. and Kheiri F. (2011). Effect of different lysine levels on arian broiler performances. *Italian J. Anim. Sci.* **10,** 32-33.

Nasr J. and Kheiri F. (2012). Effects of lysine levels of diets formulated based on total or digestible amino acids on broiler carcass composition. *Rev. Bras. Cienc. Avic*. **14,** 249-258.

Nissen P.M. and Young J.F. (2006). Creatine monohydrate and glucose supplementation to slow- and fast-growing chickens changes the postmortem pH in pectoralis major. *Poult. Sci*. **85,** 1038-1044.

NRC. (1984). Nutrient Requirements of Poultry, 6th Rev. Ed. National Academy Press, Washington, DC., USA.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Ostojic S.M., Niess B., Stojanovic M. and Obrenovic M. (2013). Co-administration of methyl donors along with guanidinoacetic acid reduces the incidence of hyperhomocysteinaemia compared with guanidinoacetic acid administration alone. *British J. Nutr.* **110,** 865-870.

Ringel J., Lemme A., Knox A., Mc Nab J. and M.S. Redshaw. (2007). Effects of graded levels of creatine and guanidine acetic acid in vegetable based diets on performance and biochemical parameters in muscle tissue. Pp. 387-390 in Proc. 16th European Symp. Poult. Nutr., Strasbourg, France.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Stahl C.A., Greenwood M.W. and Berg E.P. (2003). Growth parameters and carcass quality of broilers fed a corn-soybean diet supplemented with creatine monohydrate. *Int. J. Poult. Sci*. **2,** 404-408.

Sterling K.G., Pesti G.M. and Bakalli R. (2006). Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. *Poult. Sci*. **85,** 1045-1054.

Tian D.L., Guo R.J., Li Y.M., Chen P.P., Zi B.B., Wang J.J. and Liu F.Z. (2019). Effects of lysine deficiency or excess on growth and the expression of lipid metabolism genes in slow-growing broilers. *Poult. Sci*. **98,** 2927-2932.

Tossenberger J., Rademacher M., Németh K., Halas V. and Lemme A.J.P.S. (2016). Digestibility and metabolism of dietary guanidino acetic acid fed to broilers. *Poult. Sci*. **95,** 2058-2067 .

Westreicher-Kristen E., Davin R., Agostini P. and Saremi B. (2025). Effect of different arginine-to-lysine ratios and guanidinoacetic acid supplementation on the growth performance,

carcass characteristics and breast myopathies in broiler chickens. *Livest. Sci.* **291,** 105624-105634.

Zampiga M., Laghi L., Petracci M., Zhu C., Meluzzi A., Dridi S. and Sirri F. (2018). Effect of dietary arginine to lysine ratios on productive performance, meat quality, plasma and muscle metabolomics profile in fast-growing broiler chickens. *J. Anim. Sci. Biotechnol.* **9,** 1-14.

Zhai W., Peebles E.D., Schilling M.W. and Mercier Y. (2016). Effects of dietary lysine and methionine supplementation on Ross 708 male broilers from 21 to 42 d of age (I): Growth performance, meat yield, and cost effectiveness. *J. Appl. Poult. Res.* **25,** 197-211.

Zhang L., Li J.L., Gao T., Lin M., Wang X.F., Zhu X.D., Gao F. and Zhou G.H. (2014). Effects of dietary supplementation with creatine monohydrate during the finishing period on growth performance, carcass traits, meat quality and muscle glycolytic potential of broilers subjected to transport stress. *Animal.* **8,** 1955-1962.