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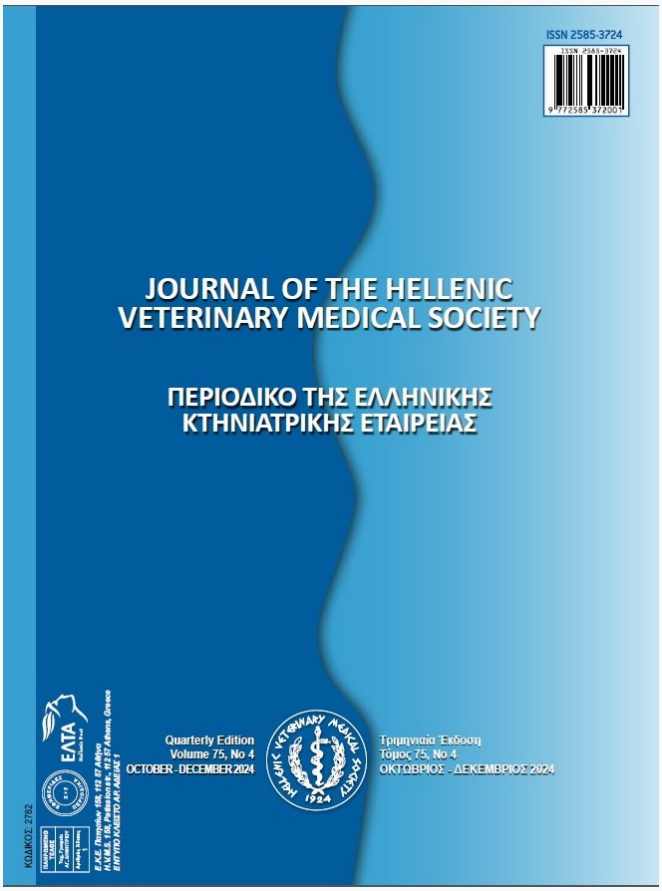

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Effects of guanidinoacetic acid on antioxidant status, inflammation and growth performance of broilers under cool temperature and excessive salt-induced ascites

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ABSTRACT: The study was carried out to investigate the effect of dietary guanidinoacetic acid (GAA) on growth, antioxidant capacity and cytokine responses in broiler chickens subjected to cold stress. Total of 200, day-old male Ross-308 broilers were randomly distributed into four dietary treatments with five replication pens having 10 birds each according to completely randomized design. All birds were investigated to cool temperatures environment in combination with excess salt in their drinking water to induce ascites and received basal diet supplemented with different levels of guanidinoacetic acid (GAA) at the levels of control; GAA1, 0.10%; GAA2, 0.20% and GAA3, 0.30%) from 16 to 42 days of age. On day 42, two birds per replicate were weighed and euthanized, and samples were collected. Results showed higher body weight gain & feed intake and lower Ascites mortality & RV/TV index in (GAA2 and GAA3) groups. Also, serum creatine kinase (CK) activity, cholesterol, triglycerides, nitric oxide and creatinine levels showed a marked significant ($P<0.05$) increase in (GAA2 and GAA3) groups supplemented groups compared to the other groups. Lipid peroxidation marker, Malondialdehyde (MDA) decreased with a significant increase of glutathione peroxidase (GPx) in serum and liver, and catalase (CAT) in serum in both (GAA2 and GAA3) compared to other groups. Similarly, GAA supplementation in both (GAA2 and GAA3) groups enhanced the interleukin (IL) -10 level and reduced the, tumor necrosis factor (TNF)-α and IL -1β serum and liver than broilers in the other groups. In conclusion, dietary GAA supplementation can alleviate ascites-induced oxidative stress also inflammation response by decreasing lipid peroxidation, TNF-α and IL-1β and increasing antioxidant function and IL-10 level in broiler chickens.

Key words: Ascites; antioxidant; broiler; performance; guanidinoacetic acid; inflammation

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During the past several years, the growth rate of broiler chickens has increased surprisingly as a result of intensive genetic selection, hence improving feed conversion ratio (FCR), mortality rate and carcass yield in broiler industry. Moreover, providing welfare is considered an essential factor in order to increase the animal production systems (Babaahmadi milani et al., 2020; Mohebbifar et al., 2022). Metabolic diseases increase compromised welfare and financial losses because of deterioration of production value on account of increased mortality rate, less efficient utilization of nutrients and condemnation of carcasses; also, both intensive, quantitative selection and improved diet and management have caused to improve the overall performance of broilers over the past 50 years (Kalmar et al. 2010). Ascites syndrome or pulmonary hypertension syndrome (PHS) is a metabolic disorder of broilers (meat-type lines/hybrids) which is accompanied by faulted FCR and rapid growth rate (Franciosini et al., 2012; Huchzermeyer, 2012; Wideman et al., 2013; Fathi et al., 2016).

PHS increase systemic venous pressure, valvar insufficiency and right ventricular hypertrophy (Wang et al., 2012). Stress is one of the basic factors in the aetiology of PHS. Animals daily face a variety of environmental stressors, such as cold stress which occurs in cold regions. Cold stress dramatically affects the health and welfare of animals in cold regions (Tsutsayeva and Sevryukova, 2000). Increased oxygen demand during cold stress, hypobaric hypoxia (high altitude) or sodium chloride toxicity predisposes the birds to the develop PHS. Recent research has shown that oxidative stress is one of the causes of ascites (Shao et al., 2022). Oxidative stress had negative effects on performance, health, and wellbeing of broilers. Moreover, oxidative stress, which, in turn, inducing adverse effects on DNA, protein, and lipids, and finally led to tissue damage, inflammatory disease, and some other diseases. Oxidative stress also induced inflammation response (Wang et al., 2020). Oxidative stress also inflammation response was closely related with previous studies reported that oxidative stress can induce inflammation stress in broilers (Song et al., 2017). Both inflammation response and oxidative stress can activate tissue apoptosis (Jiang et al., 2018), therefore, the preventing tissue damage under stress affecting or suppressing oxidative stress and inhibiting inflammation response. Liver disorder may be occurring by cause of the oxidative stress and excessive inflammation response because of liver was the primary detoxified and metabolic organ (Sun and Karin, 2013). Therefore, suppressing oxidative stress also inhibiting inflammation response were important for maintaining liver function under stress. Feed supplementation with coenzyme Q_{10}, l-carnitine, uric acid, selenium, enalapril, aspirin lycopene, glutamine, selenium and natural essential oils is a simple preslaughter strategy to reduce the negative effects of oxidative stress on broiler performance and to improve the meat quality (Fathi et al., 2015, 2022).

Guanidinoacetic acid (GAA) is a compound formed from arginine (Arg) and glycine (Gly), which is produced via chemical synthesis from glycine cyanamide. GAA is capable to spare Arg that would otherwise be used for creatine (Cre) synthesis (Savage and O'dell, 1960). Positive effects of supplemental GAA on broiler chicks and turkey performance have been demonstrated (Michiels et al. 2012; Dilger et al., 2013; Heger et al. 2014). Guanidinoacetic acid can spare Arg in Arg- deficient diets and also improve growth performance in Arg adequate diets (Michiels et al., 2012). It was hypothesized that supplemental GAA has a potential for being a feed additive for chicks not only to replace dietary arginine but also to support overall energy homeostasis of the bird. On the other hand, one of the causes of using GAA it is referred to lower price of GAA compared with creatine and arginine, it was lead to GAA is a more suitable for feed additive and It is more chemically stable than creatine (Dilger et al., 2013)**.** In the present study, we evaluated effects of GAA on oxidative status, inflammation response, biochemical parameters and growth performance of broilers subjected to ascites.

MATERIALS AND METHODS

Animal ethics statement

All animal experiments were performed in accordance with the protocol (Number: 31.03.188) of the Animal Use Committee of the Iranian Ministry of Science, Research and Technology were approved by the Animal Care Committee of the Department of Animal Science of Payam Noor University. All efforts were made to minimize animal suffering. The experiment was conducted at the animal husbandry station, Sanandaj, western region of Iran positioned between 35°18′41″N 46°59′46″E at a height of 1538 m from sea level.

Birds, diets and experimental design

A total of 200, day-old male Ross-308 broilers were randomly distributed into four dietary treatments with 5 replication pens, each pen had 10 broilers according

to the completely randomized design. On day 16, all birds were exposed to cool environmental temperatures in combination with excess salt in their drinking water to induce ascites. From 16 to 42 days of age diet supplemented with 0, control; GAA1, 0.10%; GAA2, 0.20% and GAA3, 0.30%. The birds had ad libitum access to feed and water throughout the trial period. All birds received a starter diet from 1 to 10 d. Grower and finisher diets were provided from 11 to 21 d and 22 to 42d of age, respectively (Table 1). GAA was added in the form of CreAMINO (Evonik Degussa GmbH, Hanau-Wolfgang, Germany) and supplied at the expense of corn. Birds were reared in battery cages (2.4 × 0.6 × 0.6 m) with a screen-wired floor. Each cage was equipped with a feeder to be manually filled daily. Continuous light (L) was provided for 24 h for the first 3 days, and then, 23 L: 1D light was adopted for the rest of the trial period.

Ascites induction

As we have previously described (Tan et al., 2005) to induce ascites, on d 16, birds were exposed to cool environmental temperatures in combination with excess salt in their drinking water. Briefly, the brooding temperature was gradually decreased when starting on d 16 by 1°C per day until a final temperature of 17 °C was reached. Along with the exposure to cold stress, sodium chloride (0.3%, w/v) was given by drinking water to further accelerate the development of PHS. Daily bird mortality was recorded and necropsies were performed to identify PHS-related death from d 16 onward. Generally, the observation these following symptoms: cardiac muscle laxation; Swollen and stiff liver; Clear, yellowish, colloidal fluid in the abdominal activity causes of diagnosis of ascites (Fathi et al., 2022).

Table 1- Ingredients and chemical compositions of experimental diets (1-42 days old)

Finisher (25-42 d)	Grower (11-24 d)	Starter (0-10 d)	
			Ingredients (%)
57.56	51.63	47.53	Mize, 8% CP
32.35	37.99	42.35	Soybean meal, 44%CP
6.29	6.24	5.54	Soybean oil, 9000 kcal/kg
1.05	1.12	1.20	Limestone, 38% Ca
1.34	1.56	1.79	Di-calcium phosphate, 21%Ca
0.25	0.25	0.25	Vitamin premix[a]
0.25	0.25	0.25	Mineral premix[b]
0.40	0.40	0.40	NaCl
0.28	0.32	0.37	DL-Methionine, 99%
0.22	0.22	0.28	Lysine, 78%
0.00	0.02	0.05	Threonine, 98.5%
			Calculated values
3218	3082	2990	Metabolizable energy, kCal/kg
19.3	21.3	23	Crude protein, %
0.79	0.87	0.96	Calcium (Ca), %
0.361	0.409	0.456	Available phosphorus, %
0.16	0.16	0.16	Sodium (Na), %
0.58	0.64	0.71	Methionine, %
0.89	0.89	1.07	Methionine+cysteine, %
1.17	1.30	1.46	Lysine, %
1.30	1.45	1.56	Arginine, %
0.78	0.87	0.96	Threonine, %
0.29	0.32	0.35	Tryptophan, %

[a] Vitamin concentration per kilogram of diet: retinol, 13.50 mg; cholecalciferol, 4.15 mg; tocopherol acetate, 32.00 mg; vitamin K3, 2 mg; thiamin, 2 mg; riboflavin, 6.00 mg; biotin, 0.1 mg; cobalamin, 0.015 mg; pyroxidine, 3 mg; niacin, 11.00 mg; d-pantothenic acid, 25.0; menadione sodium bisulphate, 1.10; folic acid, 1.02; choline chloride, 250 mg; nicotinamide, 5 mg.

[b] Mineral concentrations per kilogram of diet:calcium pantothenate, 25 mg; Fe (from ferrous sulphate), 35 mg; Cu (from copper sulphate), 3.5 mg; Mn (from manganese sulphate), 60 mg; Zn (from zinc sulphate), 35 mg; I (from calcium iodate), 0.6 mg; Se (from sodium selenite), 0.3 mg.

Growth performance

Average BW gain (ABWG) and average feed intake (AFI) were measured weekly, and average feed conversion ratio (AFCR) was calculated.

Sample preparation

On day 42, after 12-hour fasting, 6 broilers per treatment (two chicks per from each replication pen) randomly selected, blood samples (2.5 mL) were collected from the vein and kept on ice until serum was separated by centrifugation for 10 min at 2,500 rpm. Serum samples were stored at -20°C until assayed (Fathi et al., 2022, 2023).

Serum biochemical parameters

At the age of 42 d, two chicks per replicate were randomly selected and then 5 ml of blood was collected from wing vein or jugular vein using sterile needles. Whole clotted blood was centrifuged to separate serum for determining the biochemical and antioxidant parameters. Blood samples (2.5 mL) were taken via wing vein and kept on ice until serum was separated by centrifugation for 10 min at 2,500 rpm. Serum samples were stored at -80°C until assayed. Cholesterol, triglyceride, uric acid and Creatinine were determined using an autoanalyser (Abbott alcyon 300, USA) by laboratory kits (Pars Azmoon, Tehran, Iran). Aspartate amino transferase (AST), alanine amino transferase (ALT), Creatine kinase (CK) and lactate dehydrogenase (LDH) were measured using appropriate laboratory kits (Pars Azmoon, Tehran, Iran).

Antioxidant Capacity

The determine of GPx activity by using a commercially enzyme kit which was available (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). Also, determined of CAT and SOD activity by using the commercially available enzyme kit (Ransod, RANDOX/SD-125 supplied by Randox Laboratories) and autoanalyzer (Alcyon 300, USA) according to the manufacturers' protocols. The level of MDA in serum was measured with the tiobarbituric-acid reaction by the method of Fathi et al (2022). This method evaluates oxidative stress by measuring MDA, the last product of lipid breakdown caused by oxidative stress.

Liver parameters

Approximately 2 g of chicken liver homogenized with 9 mL of 0.9% sodium chloride buffer (w/v, 1:9) on ice, and then centrifuged at 1,000 g at 4◦C for 10 min to obtain the supernatant. The supernatant was stored at -80◦C until further analysis. Total superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and Malondialdehyde (MDA) activities in the supernatant of the liver homogenate was determined using commercially available assay kits (Pars Azmoon, Tehran, Iran) via an automated spectrophotometric analyzer (Alcyon 300, USA). All procedures were performed according to the manufacturer's instruction.

Inflammatory tests

Inflammatory parameters such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin- 10 (IL-10) concentrations were determined by using Pars Azmoon, Tehran, Iran according to the manufacturer's instructions (Fathi et al., 2023).

Right-to-Total Ventricle Ratio (RV/TV) index

To calculate the index RV/TV, first Hearts were removed and carefully dissected. Weights of the free-wall of the right ventricle (RV) and the total ventricle (TV) were measured, and the ratio of RV/TV was calculated as the RV hypertrophy index.

Statistical Analysis

Data from all response variables were subjected to one-way analysis of variance by applying the SAS program (SAS, 2005) with four treatments and five replicates per treatment using a general liner model (GLM). Significant differences among treatment means were separated using Tukey's test at 5 % probability. The statistical model used for the analysis of dependent variables was: $Yij = \mu + Ai + eij$ Where Yij was the individual observation; μ the experimental mean; Ai the GAA effect, and eij the random error.

RESULTS AND DISCUSSION

Growth performance and Ascites index

The growth performance parameters of broilers fed the experimental diets supplemented with different levels of GAA are presented in Table (2). The improvement in average feed intake (AFI), average body weight gain (ABWG), average feed conversion ratio (AFCR) and reduced ascites mortality & RV/TV index in GAA2 and GAA3 groups than the control and GAA1 groups ($P<0.05$); however, FCR showed no significant differences among all groups ($P>0.05$).

In this work, we challenged the birds with a combination of cold temperature and excessive salt in

drinking water. Under this condition, 25.53% of birds in the control group developed ascites (P=0.010). These results are consistent with Tan et al. (2005). Theses researchers reported that induction of ascites by a combination cold temperature and excessive salt significantly increased right ventricular hypertrophy and ascites mortality.

Consistent with the results of this study, Mohebbifar et al. 2019 reported that supplementation of the diet of broilers under induced ascites with 1.8 g of GAA significantly increased feed intake and weight gain. No significant differences in body weight gain, feed intake and FCR to the diet level of GAA has been previously shown (Khodambashi Emami et al., 2017).

There are several factors that could contribute to the growth improvement observed in our study, like the sparing of the endogenous synthesis of GAA from arginine and glycine, thereby providing more arginine and glycine available for body protein or endogenous amino acids synthesis, leading to growth enhancement. Besides, GAA is an immediate precursor of creatine and its phosphorylated derivative, phosphocreatine-a, a rapidly mobilizable reserve of high energy phosphates (Wangkahart et al., 2016). Since creatine acts as an intracellular signal-coupling enhanced muscle activity and enhanced muscle growth, it could subsequently lead to additional energy for cellular bioenergetics. Thus, the GAA growth promoting effect could be attributed to the enhanced creatine synthesis (Ostojic et al., 2015).

Furthermore, GAA favored the production of growth promoting polyamines (putrescine, spermidine and spermine), that have anabolic functions in synthesis of DNA, RNA, and proteins, along with its hydration effect on the muscle cells, bringing water into the cells, which promotes protein synthesis, reduces proteolysis, and enhances glycogen synthesis (Young et al., 2007). In addition, several studies reported that GAA supplementation has a role in increasing insulin-like growth factor 1 (IGF-1) in plasma, which led to the enhancement of protein synthesis and growth improvement (Young et al., 2007).

Moreover, similar results were also reported that might be due to the better energy utilization in chickens receiving the GAA supplemented diets (Heger et al., 2014). The improved performance response to supplemental GAA could be attributed to support overall energy homeostasis of the bird; an impact which is beyond the arginine sparing effect of GAA (Dilger et al., 2013). It is possible that GAA enhanced the enzymatic antioxidant capacity of cells under stress condition (Zugno et al. 2008), thus GAA by improving the antioxidant status may improve performance and it may improve performance under stress condition (Table 4).

Decreased RV/TV index as well as control of as-

Table 2: Effect of guanidine acetic acid (GAA) on growth performance, mortality and ascites index of broilers at 42 days of age

Treatments	Average FI (g)	Average BWG (g)	Average FCR	Ascites mortality (%)	RV/TV index
Control	3465^{b}	2100^{b}	1.65	25. 3^{a}	0.32^{a}
GAA1 (0.10%)	3465^{b}	2100^{b}	1.65	23.20 ^{a}	0.31^{a}
GAA2 (0.20%)	3755^{a}	2180^{a}	1.72	16.00 ^{b}	0.24^{b}
GAA3 (0.30%)	3723^{a}	2190^{a}	1.70	14.00^{b}	0.24^{b}
SEM	37	22	0.05	2.50	0.02
P -Value	0.001	0.011	0.010	0.010	0.001

$^{a, b, c}$ The same superscript alphabets in the same column indicate a non-significant different at P<0.05.
BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; RV/TV, right ventricle/ total ventricle

Table 3: Effect of guanidine acetic acid (GAA) on biochemical parameters of broilers at 42 days of age

Treatments	Control	GAA1 (0.10%)	GAA2 (0.20%)	GAA3 (0.30%)	SEM	P -Value
Triglyceride (mg/dL)	62.78^{c}	137.48^{b}	244.78^{a}	273.38^{a}	35.50	0.013
Cholesterol (mg/dL)	253.5^{a}	273.5^{ab}	297.5^{a}	305.5^{a}	19.50	0.011
Nitric oxide (μ*M*/L)	40.25^{b}	42.26^{b}	59.02^{a}	62.20^{a}	3.5	0.001
Uric acid (mg/dL)	3.56	3.25	3.20	3.18	0.25	0.230
Creatinine (g/dL)	0.60^{c}	0.65^{b}	0.69^{a}	0.71^{a}	0.03	0.010

$^{a, b, c}$ The same superscript alphabets in the same row indicate a non-significant different at P<0.05.

Table 4: Effect of guanidine acetic acid (GAA) on serum and liver antioxidant status of broilers at 42 days of age

Parameters		Treatments				SEM	P -Value
		Control	GAA1 (0.10%)	GAA2 (0.20%)	GAA3 (0.30%)		
Serum	GPx (Mu/mL)	1121.9[d]	1189.9[c]	1250.9[b]	1320.9[a]	19.50	0.013
	SOD (U /mL)	270.50	274.50	273.50	272.10	1.50	0.210
	CAT **(N mol/min/mL)**	26.64[c]	27.54[c]	33.25[b]	39.25[a]	2.50	0.010
	MDA (n mol/ml)	17.85[a]	16.89[a]	11.22[b]	9.08[c]	0.15	0.001
Liver	GPx (Mu/mL)	14.0[c]	15.1[c]	18.2[b]	21.1[a]	2.10	0.021
	SOD (U /mL)	700	708	709	711	15.10	0.193
	CAT **(N mol/min/mL)**	29.2	32.1	31.2	32.2	2.5	0.120
	MDA (n mol/ml)	0.50[a]	0.55[a]	0.43[b]	0.34[c]	0.02	0.010

[a, b, c] The same superscript alphabets in the same row indicate a non-significant different at P<0.05.
GPx, glutathione peroxidase; SOD, Superoxide dismutase; CAT, catalase; MDA, Malondialdehyde

cites mortality in GAA2 and GAA3 groups can be related to the positive effect of GAA on increasing plasma levels of nitric oxide (NO) because it has already been reported that nitric oxide can significantly reduce mortality. It is now well known that the most important factors produced by the vascular endothelium are the endothelium-derived relaxing and anti-proliferative factor NO**.** In particular, NO seems to be important for maintaining normal blood pressure and matching ventilation-perfusion within the lung in broilers. In general, the role of NO in the context of the pathogenesis of ascites may more probably be associated with its effects as a response factor affecting pulmonary vascular tone (Tan et al., 2007).

Serum biochemical analysis

Serum biochemical parameters are presented in Table 3. In this study, GAA2 and GAA3 groups, significantly increased triglycerides, cholesterol, NO and creatinine in broiler chickens under ascites-induced stress. High basal cholesterol levels and cholesterol have been observed in human males and females fed supplemental creatine (Earnest et al., 1996)**.** As observed, the creatinine level was increased in both GAA2 and GAA3 groups compared to others, which correlate to the increased creatine kinase (CK) level as well. This is consistent with the study conducted by Zeng et al. (2018) who observed the similar increased level of creatinine in bullfrog Rana (*Lithobates catesbeiana*) fed GAA supplemented soybean meal diet at 0.6 g/kg for 8 weeks. Similarly, Michiels et al. (2012) found that GAA supplementation increased the level of creatine deposition in muscles and subsequently increased the creatinine level.

In the current study, cholesterol and triglyceride level was elevated significantly in all GAA supplemented groups compared to control, being the higher significantly levels in GAA2 and GAA3 groups. However, triglycerides levels revealed a higher elevation in both GAA2 and GAA3 groups compared to the GAA1 group; and a significant increase in GAA3 compared to the control group as well. Our results could be associated with the increased lipid content of GAA1 and GAA2 in this study. Besides, it could be attributed to the increased creatine level due to GAA supplementation, which has a potential role in activating proteins of lipid metabolism such as [Apolipoprotein A (Apo-A) and 14 kDa Apo-A which could be involved in the cholesterol transport and thus lipid metabolism (De Castro Moreira et al., 2017). Similar to our results, Cobb broiler chicks fed creatine at 0.1% demonstrated a higher level of triglyceride after 21 and 42 days (Mahfouz et al., 2016).

Serum and liver Oxidative and antioxidant biomarkers

The data in Table 4 show that (P<0.05). In addition, dietary GAA supplementation at GAA2 and GAA3 level alleviated the ascites induced effects on the activity catalase, & glutathione peroxidase and MDA concentration in serum and liver (except catalase) (Table 4). The state of oxidative damage results in over-production of ROS that promote lipid peroxidation responses, which are reflected by the induction of MDA content in plasma and tissues, besides, antioxidant enzymes are increased as a protective response against the highly produced ROS (Fathi et al., 2022). In the current study, GAA2 and GAA3 exhibited a decreased in the oxidative stress represented by the MDA level being the highest effect in GAA3. The antioxidant enzymes, GPx levels were in a similar pat-

tern, where they significantly increased in the higher supplemented doses of GAA compared to the lower and GAA1 groups. A well-known function of GAA is its ability to mitigate stress and improve antioxidant activity One possible mechanism that explains this potential could be that GAA and its derivatives react with free radicals due to the active hydroxyl and amino groups present on their chains (Sergej et al., 2015). Creatine, the end product of GAA utilization, is thought to have antioxidant capacity in some studies (Sestili et al., 2009).

According to the results of this study, Wang et al. (2012), reported that supplementation with GAA improved the ability to detoxify H_2O_2 and the superoxide anion in both plasma and muscle of pigs supplemented with up to 2.0 g/kg of GAA. Authors reported improved antioxidant status in growing-finishing pigs by elevating TAC and the activities of antioxidant enzymes (SOD and GPx). Since SOD is an important element of antioxidant defense and strong superoxide scavenger, GAA intervention (3g/kg) might be considered beneficial when used as a dietary supplement in broilers under stress condition. Raising the dosage of supplemental GAA even further (for up to 90 g/day) quadratically increased the activity of GPx, catalase and TAC in pig plasma (Wang *et al*., 2012). It is also, our results in accordance with previous studies reported that GAA dietary supplementation in ducks and growing-finishing pigs was able to improve the antioxidant enzymes and manipulate the oxidative stress through lowering MDA level (Wang et al., 2012, 2016).

Serum and liver analysis for cytokines

The results of Inflammatory parameters present in serum and liver as tumor necrosis factor-*α,* interleukin-1β (IL-1β), and interleukin- 10 (IL-10) are summarized in Table 5. Dietary GAA supplementation at levels GAA2 and GAA3, significantly decreased TNF-α & IL-1b levels, and significantly increased IL-10 level in serum and liver compared with other groups. Inflammation was induced by the innate immune system in response to tissue damage or invade pathogens. In response to stress, including heat stress and oxidative stress the levels of pro inflammatory cytokines increased, which may result in hemorrhage and necrosis in liver (Ohtsu et al., 2015; Azuma et al., 2015). Former studies indicated that the inflammatory cytokines production were associated with the overproduction of ROS under oxidative and heat stress in broilers (Ruixia et al., 2020).

It is postulated that creatine and arginine (the metabolites of GAA) have a potential role in improving immune response, particularly the inflammatory response and cytokine modulation (Andou et al., 2009). Therefore, increase the biosynthesis of both metabolites upon GAA supplementation further support our findings regarding the anti-inflammatory modulation of GAA. Similarly, Zhao et al. (2014) showed that Jian carp fed diets containing isoleucine at 7.0-11.9 g/kg was able to lower the intestinal TNF-α mRNA levels than those fed other dietary isoleucine levels. Consistent with our results, Hu et al. (2015) reported dietary glutamine supplementation has the capacity to significantly downregulate levels of TNF- α and IL-10 mRNA, whereas the level of TGF-β2 mRNA was upregulated in the head kidney of juvenile Jian carp.

Serum enzymes activity

As shown in Table 6, CK activity was increased significantly in GAA2 and GAA3, compared to other groups with no statistical difference between them (P<0.05), However, GAA supplementation did not significantly affect the serum activity of ALT, AST and LDH (P> 0.05).

Table 5: Effect of guanidine acetic acid (GAA) on serum and liver cytokines of broilers at 42 days of age

Parameters		Treatments				SEM	P -Value
		Control	GAA1 (0.10%)	GAA2 (0.20%)	GAA3 (0.30%)		
Serum	IL-10 (ug/mL)	12.20[b]	15.10[b]	31.90[a]	30.20[a]	2.90	0.010
	IL-1*β* (ug/mL)	15.10[a]	15.50[a]	7.10[b]	6.90[b]	2.17	0.012
	TNF-*α* (ug/mL)	15.20[a]	14.50[a]	11.30[b]	10.20[b]	3.60	0.011
Liver	IL-10 (ug/mL)	6.20[b]	8.10[b]	15.10[a]	16.30[a]	3.01	0.010
	IL-1*β* (ug/mL)	1.30[a]	1.25[a]	0.63[b]	0.69[b]	0.05	0.013
	TNF-*α* (ug/mL)	32.20[a]	29.20[a]	17.50[b]	18.20[b]	5.20	0.017

[a, b, c] The same superscript alphabets in the same row indicate a non-significant different at P<0.05.
IL-10, interleukin-10; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor- α

Table 6: Effect of guanidine acetic acid (GAA) on serum enzymes activity of broilers at 42 days of age

Treatments	ALT (U/L)	AST (U/L)	LDH (U/L)	CK (U/L)
Control	7.80	195.98	1658	4580[b]
GAA1 (0.10%)	7.40	200.50	1780	5021[b]
GAA2 (0.20%)	7.30	188.08	1935	8520[a]
GAA3 (0.30%)	7.00	173.98	1980	9085[a]
SEM	2.50	24.50	350	650
P- Value	0.211	0.351	0.250	0.010

[a, b, c] The same superscript alphabets in the same row indicate a non-significant different at P<0.05.
Aspartate aminotransferase, AST; Alanine aminotransferase, ALT; Lactate dehydrogenase, LDH; CK, creatine kinase

Various studies have reported that stress significantly increased serum enzymes activity including ALT, AST in broilers (Fathi et al., 2022), but few studies assessed the effect of dietary supplementation GAA on the mentioned parameters in broiler chicks reared in ascites-induced stress condition. In the current study, addition of 2 and 3 mg/kg GAA yielded increases in the activity of CK. The phosphocreatine/creatine system buffers ATP/ADP ratio for all energy consuming functions of the cell. CK catalyzes the conversion of creatine and utilizes ATP to create phosphocreatine and ADP. The rise in CK activity and serum creatinine could be either due to reduced clearance or due to overproduction or both (Hekimsoy and Oktem, 2005).

GAA might be able to support creatine production and increase CK activity. In the present study, the increased level of circulating CK indicates the previously reported potential role in maintaining high ATP turnover at low temperature (Jayasundara et al., 2015). Similar to this study, a significant increase in CK activity was seen in the group supplemented with creatine. Also, a significant increase in CK activity is observed in the male rats kept in induced hypothermia condition, and it is concluded that CK played a role in energy supply of muscle tissue during the hypothermia condition due in part to the increased plasma level of CK (Dobgenski et al. 2016).

CONCLUSION

It can be concluded that dietary supplemental 3.0 g/kg GAA would be a beneficial way to improve growth performance, decrease the mortality rate of broiler reared under cold stress conditions. In addition, the increased blood level of Nitric oxide and the decreased pulmonary hypertension and relative weight of the right ventricle was seen in broilers fed the 3.0 g/kg GAA-included diet. Moreover, it had showed the potential roles of GAA as an antioxidant and anti-inflammatory agent

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors

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