Effects of guanidinoacetic acid supplementation on zootechnical performance and some biometric indices in broilers challenged with T₃-Hormone

Shady Khalil, Ahmed A. Al-Sagan, Hossam A. Abdellatif, Abdelbary Prince & Ramadan El-Banna


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Effects of guanidinoacetic acid supplementation on zootechnical performance and some biometric indices in broilers challenged with T3-Hormone

Shady Khalila, Ahmed A. Al-Saganb, Hossam A. Abdellatifa, Abdelbary Princec and Ramadan El-Banna

ABSTRACT

The objective was to elucidate the effects of dietary guanidinoacetic acid (GAA) supplementation on broiler performance, serum enzymes, oxidative biomarkers, mitochondrial activities, carcass traits, gross lesion of cardiac muscle and liver histopathology in broilers challenged with T3-hormone. A total-of-192 one-day-old mixed sexed broilers were randomly assigned in a two factorial design, including two dietary treatments; control diet supplemented with or without T3-hormone (1.5 ppm) and GAA diet (0.06%) supplemented with or without T3-hormone (1.5 ppm). Each group was subdivided into eight replicates. Results showed interactions between GAAxT3-hormone. GAA diet significantly mitigated the negative effect of T3-hormone on serum total creatine kinase (CK), cardiac muscle (CK-MB), liver malondialdehyde (MDA) and superoxide dismutase (SOD), mitochondrial activities of cardiac muscle and liver histopathological lesion. In conclusion, GAA at a rate of 0.06% may have the potential to mitigate the negative effect of dietary T3-hormone but could not reduce the ascites mortality at such inclusion rate.

HIGHLIGHTS

GAA protected heart muscle.
GAA mitigated the oxidative radicals in T3-hormone challenged birds.
GAA modulated the mitochondrial activities in T3-hormone challenged birds.

Introduction

Fast growth requires an adequate amount of oxygen to supply the accreted tissues with energy (Gupta 2011). In modern broilers, pulmonary and cardiac capacity are similar to the old broiler strains that force the cardiac muscle to work more while lung capacity does not meet the oxygen requirement to achieve rapid growth. Stressed cardiac muscle by time may lose its ability to sustain the work overload and may develop right ventricular heart failure (RVHF) or even sudden death syndrome (Baghbanzadeh and Decuyper 2008). Several factors can contribute to the development of ascites syndrome (AS) which include but not limited to high altitude, cold stress, moderate heat, high activity, hyperthyroidism, increased muscle mass, and high-density feed. Additionally, pathological conditions can contribute to AS such as pre-existing respiratory system pathology and anaemic hypoxaemia due to abnormal haemoglobin levels (Kaoud et al. 2016). The latter factors may increase the basal metabolic rate and oxygen requirements to produce adenosine-tri-phosphate (ATP). Failure to meet energy requirements may cause oxidative damages and AS (Ladmakhi et al. 1997; Baghbanzadeh and Decuyper 2008).

Creatine (Cre) is present in high concentration in skeletal muscle, cardiac muscle, and brain tissue. Therefore, it is confined to cells that have high-energy demand. The role of Cre is to store ATP in the form of phosphocreatine (PCre) in the cytoplasm. PCre can replenish ATP in the cytoplasm, instantly through the Cre-PCre shuttle system. Therefore, PCre build-up can...
reduce the need for oxygen to produce ATP from mitochondria (Wyss and Kaddurah-Daouk 2000). The latter may offer benefits for broiler subjected to a limitation in energy due to fast growth, high altitude, cold stress and heat stress. Cre can be de novo synthesised in the body via two enzymatic steps. The first enzymatic step, L-arginine (Arg) and glycine are required to form guanidinoacetic acid (GAA) that catalysed by arginine-glycine amidinotransferase in the kidney. The second enzymatic step, GAA is methylated in the liver by S-adenosylmethionine to form Cre in the reaction that is catalysed by S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase (Wyss and Kaddurah-Daouk 2000). Feed ingredients of animal origin are rich source of creatine and plant-based ingredients do not contain any metabolites of creatine (Khajali et al. 2020). Cre in these animal protein sources was found to be affected during rendering, which makes Cre a lost nutrient in poultry nutrition (Boney et al. 2020). GAA is a precursor source of creatine (EFSA 2016), showed better stability during feed processing and storage compared to Cre (Van der Poel et al. 2019). Furthermore, it was demonstrated that dietary supplementation of GAA could spare Arg in the broiler (Dilger et al. 2013; DeGroot et al. 2019). Arg plays a pivotal role, as it is the endogenous nitrogenous precursor for nitric oxide synthesis. The latter is a potent vasodilator that relaxes vascular smooth muscle; hence, it is found to reduce the incidence of ascites in broiler chicken raised in high-altitude and cold stress (Ahmadipour et al. 2018).

The current study was designed to investigate the effect of dietary supplementation of GAA on growth performance, selected serum parameters, oxidative biomarkers, mitochondrial activities in cardiac muscle, gross lesions of the heart, carcass traits, and liver histopathology in broilers challenged with T3-hormone. Dietary T3-hormone was used as a challenging agent to increase AS, basal metabolic rate, oxygen, energy requirement, (Ladmakhi et al. 1997) and to induce mitochondrial-dependent reactive oxygen species (ROS) (Cano-Europa et al. 2012).

Materials and methods

The current study was conducted at King Abdulaziz City for Science and Technology (KACST) Riyadh, KSA (altitude of 400 m above the sea level) following the guidelines of the International Animal Care Institute Committee of Faculty of Veterinary Medicine Cairo University (IACUC) with approval number Vet-CU (23012020112).

Birds husbandry and experimental design

A total of 192 one-day-old mixed sexed broiler chicks were randomly divided into 4 groups, each subgroup had 8 replicates, 6 birds per replicate and fed on same basal diet. The first group received no supplementation; meanwhile, the second group was supplemented with 1.5 ppm T3-hormone (T2877, Sigma-Aldrich), the third group was supplemented with 0.06% guanidinoacetic acid (Creamino®, minimum 96% GAA; AlzChem Trostberg GmbH, Germany) and the fourth group supplemented with 0.06% GAA and 1.5 ppm T3-hormone. Each group was subdivided into eight replicates contains (six birds; three males and three females). Birds in the different experimental groups were fed on 3 phases corn-soybean based diets in a mash form to avoid possible heat instability of T3-hormone (starter 1–10 d, grower 11–21 d and finisher 22–32d). The stress model of T3-hormone was applied according to (Ladmakhi et al. 1997; Taghizadeh et al. 2012; Habibian et al. 2017). Chickens were floor reared in fully automated closed system houses, bedded by a layer of sawdust, kept under standard hygienic conditions. Birds were provided with clean water and fed ad-libitum, continuous light from 1–6 days of age, then to 23:1 light-dark cycle throughout the experiment and were not subjected to any prophylactic vaccination or pharmacological program during the entire experiment that lasted up to 32 days of age. The diets were formulated to meet the nutrient requirements of Ross 308 as recommended by the breed manual. The diets ingredients and the analysed nutrient compositions are illustrated in Table 1.

Slaughter and sampling

On day 32, all birds were weighed. One male and one female were selected from each pen to represent the average pen weight. Birds were killed by severing the jugular vein and immediately after slaughter blood samples were collected in experimental tubes. Heart samples were immediately collected and kept on ice during tissue preparation for mitochondrial isolation. Part of liver samples (5 grams) was fixed in 10% formol saline for histopathological examination, and the rest was kept at −80°C for oxidative biomarkers analysis.

Measurements

Growth performance parameters

Birds in different experimental groups were initially weighed. The cumulative (32 days) weight gain and
Feed intake were recorded. Feed conversion ratio (FCR) was corrected for mortality using the following equation

\[
\text{FCR corrected} = \frac{\text{Feed consumed}}{(\text{Weight gain of survivors} + \text{weight gain of mortalities})}
\]

**Serum parameters**

At the end of the experiment (day 32), serum samples were separated, refrigerated, and subsequently analysed. Serum samples were analysed for total creatine kinase (CK), creatine kinase of cardiac muscle (CK-MB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and creatinine using a colourimetric method as prescribed by the Chronolab® Barcelona, Spain commercial kits. Serum T3 hormone was determined using ELISA Kit (Calbiotech®, Spring Valley, CA, USA).

**Oxidative biomarkers**

Liver samples of birds were collected from all groups at the end of the experiment for oxidative biomarkers analysis. Reduced glutathione (GSH) was analysed according to the method described by (Beutler et al. 1963), glutathione peroxidase (GPx) was analysed according to the method described by (Paglia and Valentine 1967). Superoxide dismutase (SOD) was determined according to the procedure described by (Marklund and Marklund 1974) with some modification of (Nandi and Chatterjee 1988) and Malondialdehyde (MDA), an indicator of lipid peroxidation, was analysed according to (Kei 1978).

**Mitochondrial activities**

- Mitochondrial oxygen consumption (RCR) measurement was done according to (Hofhaus et al. 1996).
- Mitochondrial complex chains activities.
NADH ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), cytochrome c reductase (complex III), and cytochrome c oxidase (complex IV) were determined according to (Spinazzi et al. 2012).

**Carcass characteristics and relative organs weights**

At the end of the experimental period, two birds from each replicate (one male and one female) of different experimental groups, representing the average body weight of each pen, left overnight in the waiting yard where water was allowed but without diet. Each bird was weighed, hanged, slaughtered, scalded at 55–65°C, de-feathered, eviscerated, and washed with tap water. The dressing yield % (DY%), breast muscle yield (BMY %), and organ indices were recorded.

**Gross lesions of the heart and corresponding right ventricle/total ventricle ratio**

Mortalities in different experimental groups were recorded throughout the whole period. Dead birds along the whole experiment were macroscopically examined for the presence of any apparent lesions, especially those concerning heart failure syndrome and ascites as a result of the T3 challenge. Consequently, the heart was removed from dead birds; the atria, major vessels, and fat were trimmed off. The right ventricle (RV) was carefully cut away from the left ventricle and septum. The right ventricle was weighed, the left ventricle and septum were added, and the total ventricle (TV) weights were recorded accordingly. Birds having RV/TV ratio of over 0.299 were classified as suffering from right ventricular failure (Ladmakhi et al. 1997).

**Liver histopathology**

Autopsy samples (5 grams/sample) were taken from the liver from birds in different groups and fixed in 10% formal saline for twenty-four hours. Washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in a hot air oven for twenty-four hours. Paraffin beeswax tissue blocks were prepared for sectioning at four microns thickness by slides microtome. The obtained tissue sections were collected on glass slides, deparaffinised, and stained by haematoxylin & eosin stain for examination through the light microscope (Bancroft and Stevens 1990).

**Statistical analyses**

Statistical analyses of the obtained data were performed with IBM SPSS software (IBM SPSS Statistics 20, Chicago, IL). Results were expressed as treatment means with their pooled standard error of means (SEM) and replicate as an experimental unit. The data were analysed by two-way ANOVA with GAA and T3-hormone treatment as fixed factors. Main effects were considered when no significant interactions detected. When significant interactions between GAA and T3 observed, the mean of each treatment was individually compared. For multiple comparisons, Bonferroni’s post-hoc-test was carried out to compare the means. A probability value of \( p < .05 \) was described to be statistically significant, although \( p \)-values between .05 and .10 are shown and described as a trend.

**Results**

**Growth performance**

Table 2 shows the effects of GAA on growth performance in broilers challenged with T3-hormone. Results confirmed that the distribution of the birds among individual treatments on the first day of the experiment was homogeneous so that bodyweight in all treatments was almost identical in all groups. No interaction between GAA x T3-hormone was observed on growth performance. T3-hormone in the challenge groups affected (\( p < .05 \)) all performance parameters, negatively. In contrast, the main effect of GAA on growth performance showed a tendency to improve the final body weight and weight gain; meanwhile, FCR was improved (\( p < .05 \)) compared to the control diet.

**Serum parameters**

Table 3 shows the effects of GAA on serum enzymes in broilers challenged with T3-hormone. Interaction between GAAxT3-hormone was noticed on serum CK and CK-MB. Serum CK was the lowest (\( p < .05 \)) in GAA diet devoid of dietary T3-hormone compared to other groups; meanwhile, CK-MB was the lowest (\( p < .05 \)) in GAA diets compared to control diets. Between GAA diet groups, GAA devoid of dietary T3-hormone was higher (\( p < .05 \)) than the GAA diet with T3-hormone supplementation. No interaction between GAAxT3 was noticed on serum AST, ALT and T3. Serum ALT and T3 were higher (\( p < .05 \)) in T3-hormone challenged groups than in the unchallenged groups. An unexpected decrease (\( p < .05 \)) in serum AST was noticed in T3-hormone challenged groups compared to T3-
hormone devoid groups. Serum GGT and creatinine levels were not differed \((p > 0.05)\) among groups.

### Oxidative biomarkers

Table 4 shows the effects of GAA on oxidative biomarkers in broiler’s liver challenged with T3-hormone. Interaction between GAAxT3-hormone was observed on liver MDA and SOD. In T3-hormone devoid groups, MDA and SOD in the liver were the same; however, control diet supplemented with T3-hormone had the highest MDA \((p < 0.05)\) and the lowest SOD activity \((p < 0.05)\) compared to other groups. Supplementation of GAA to T3-hormone supplemented diet mitigated \((p < 0.05)\) the negative impact of T3-hormone. The main effect of GAA diet on liver GSH was higher \((p < 0.05)\) than the control diet; meanwhile, GPx tended \((p = 0.06)\) to be improved. T3-hormone in the challenged groups negatively affected \((p < 0.05)\) liver MDA, GSH and GPx.

### Mitochondrial activities

Table 5 shows the effects of GAA on mitochondrial activities in broiler’s cardiac muscle challenged with T3-hormone. Interaction between GAAxT3-hormone was recorded on mitochondrial RCR, complex I, III and IV. The latter mitochondrial activities were higher \((p < 0.05)\) in T3-hormone devoid groups than T3-hormone supplemented groups. Supplementation of GAA to T3-hormone supplemented diet mitigated \((p < 0.05)\) the negative effect of T3-hormone on such measurements. Interestingly, in the groups that were not supplemented with T3-hormone, GAA diet improved \((p < 0.05)\) mitochondrial complex IV activity compared to the control diet. No interaction was noticed.
between GAAxT3-hormone on mitochondrial complex II. Nevertheless, the main effect of GAA diet on Complex II was higher \( (p < .05) \) compared to the control diet. T3-hormone groups were negatively affected \( (p < .05) \) compared to T3-hormone devoid groups.

### Carcase characteristics and relative organ weights

Table 6 shows the effects of GAA on carcase traits and relative organ weights in broiler challenged with T3-hormone. An interaction between GAA x T3-hormone was observed on the heart index (HI). T3-hormone supplementation increased HI \( (p < .05) \) compared to non-supplemented groups. Supplementation of T3-hormone to GAA diet increased HI \( (p < .05) \) compared to other groups. The main effect of GAA diet on CW, AFY, HI and WI were higher \( (p < .05) \) than broilers fed on the control diet, but feeding GAA had no impact on DY, BMY and LI. Carcass traits and relative organ weight were negatively \( (p < .05) \) affected in T3-hormone challenged groups.

### Gross lesions of the heart and right ventricle/total ventricle ratio in broiler challenged with T3-hormone

Table 7 shows the effect of GAA on gross lesions and right ventricle/total ventricle ratio in broiler’s heart challenged with T3-hormone. Control diet devoid of T3-hormone recorded two unspecific mortality. Dead birds did not show any signs of AS or heart affections. On the contrary, T3-hormone challenged groups...
showed signs of ventricular heart failure and AS mortality starting from week 2 onward with RV/TV ratio 0.31 and 0.29 on the control diet and GAA diet supplemented with T3-hormone, respectively. Total mortality from AS or SD were 25 and 26 in control diet and GAA diet supplemented with T3-hormone, respectively.

Liver histopathology

Figure 1 shows the effect of GAA on liver histopathology in broiler challenged with T3-hormone. Histological lesions were not recorded in the control diet and GAA diet devoid of T3-hormone. They showed the typical structure of hepatocyte and central vein. Control birds supplemented with T3-hormone showed thickness with collagen and oedema in the hepatic capsule. Moreover, cytoplasm showed vacuolation in some hepatocytes with coagulative necrosis underneath the thick capsule. In contrast, GAA birds supplemented with T3-hormone showed a thick vascular wall of the central vein and dilatation in a central vein,

Table 6. Effect of GAA on carcase traits and relative organ weights in broiler challenged with T3-hormone.

<table>
<thead>
<tr>
<th>T3-hormone</th>
<th>n</th>
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<th>Breast muscle yield, %</th>
<th>Abdominal fat yield, %</th>
<th>Liver index, %</th>
<th>Heart index, %</th>
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<td>32.62</td>
<td>0.21</td>
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Main effect means

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Source of variations

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Values within the same column with different superscripts are significantly different (p < .05).

GAA: guanidinoacetic acid; T3: triiodothyronine hormone; SEM: standard error of the means; n = sample size.

Table 7. Effect of GAA on gross lesions and right ventricle/total ventricle ratio in broiler’s heart challenged with T3-hormone.

<table>
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<th>mortality per week</th>
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<td>Without T3</td>
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</tr>
<tr>
<td>With T3</td>
<td></td>
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</table>

| Figure 1. Showed the effects of Guanidinoacetic acid (GAA) on liver histopathology in broiler challenged with T3-hormone. Livers in (a) and (c) groups not supplemented with dietary T3-hormone. Livers in (b) and (d) groups supplemented with dietary T3-hormone. Livers in (c) and (d) groups supplemented with GAA.

showed signs of ventricular heart failure and AS mortality starting from week 2 onward with RV/TV ratio 0.31 and 0.29 on the control diet and GAA diet supplemented with T3-hormone, respectively. Total mortality from AS or SD were 25 and 26 in control diet and GAA diet supplemented with T3-hormone, respectively.

Liver histopathology

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Histological lesions were not recorded in the control diet and GAA diet devoid of T3-hormone. They showed the typical structure of hepatocyte and central vein. Control birds supplemented with T3-hormone showed thickness with collagen and oedema in the hepatic capsule. Moreover, cytoplasm showed vacuolation in some hepatocytes with coagulative necrosis underneath the thick capsule. In contrast, GAA birds supplemented with T3-hormone showed a thick vascular wall of the central vein and dilatation in a central vein,
Dietary T₃ was used as a stress model to increase the AS mortality incidence and the discriminatory power for studying other factors involved in AS according to (Decuyper et al. 1994; Ladmakhi et al. 1997). Under physiological conditions, T₃-hormone plays an essential role in energy metabolism in skeletal muscle, heart, liver, and kidney. It can accelerate the basal metabolic rate by its direct influence on mitochondrial activities and ROS production (Lin et al. 2008). T₃-hormone is naturally increased when broilers are subjected to cold stress to increase the basal metabolic rate to warm the body. The latter may increase the cardiac output to provide sufficient oxygen to mitochondria to generate energy in the form of ATP or to be dissipated in the form of heat loss for warming purpose (May 1980). In our study, the decreased body weight gain, feed intake and higher FCR in T₃-hormone supplemented groups were consistent with previous studies under T₃-hormone challenge (Ladmakhi et al. 1997; Habibian et al. 2017). Our study showed no interaction between GAA and T₃-hormone on growth performance parameters. A previous study reported that GAA at an inclusion rate of 0.1% or 0.15% improved final body weight and FCR raised under high altitude and 15°C from day 21 onward (Ahmadipour et al. 2018). Therefore, a higher GAA inclusion rate needs to be considered in future studies for more accurate interpretation. In contrast, as a main effect, body weight and gain tended to be improved, but FCR was significantly improved in the GAA diet compared to the control diet. Boney et al. (2020) showed an improvement in FCR in birds fed on either plant protein or animal protein-based diets that were supplemented with GAA. Moreover, EFSA (2016); He et al. (2019) reported an improvement in both daily weight gain and gain per feed when supplemented with 0.06% or 0.12% GAA. The improvement in GAA diet can be explained on the basis that GAA was successfully metabolised to Cre that increased energy utilisation efficiency. Additionally, storage of energy in the form of PCre in the cytoplasm may have provided the muscle with ATP to support rapid growth. It may partially compensate for the low ATP as a result of T₃-hormone supplementation that might be dissipated in the form of heat loss or as a result of low feed intake. Although muscle Cre concentration was not measured under current study, previous studies showed an increase in muscle Cre level when supplemented with GAA either with or without fish meal supplementation (Lemme et al. 2011) or under the stressful condition of cyclic heat stress (Majededin et al. 2020).

CK and CK-MB are enzymes present in skeletal and cardiac muscles, respectively, playing a crucial role in energy metabolism and are indicators of muscle cell damage (Wyss and Kaddurah-Daouk 2000). Our study showed a significant interaction between GAA and T₃-hormone on CK and CK-MB. The GAA diet devoid of T₃-hormone was significantly the lowest in serum CK but did not decrease under T₃-hormone challenge. Noteworthy, the GAA diet showed a significant reduction in CK-MB compared to control diet. The latter may suggest the protective effect of Cre on cardiac muscle under both ideal and T₃-hormone challenge. Limited studies showed the effect of GAA on CK and CK-MB activity under ideal and stressful conditions. The low serum CK in the GAA diet devoid of T₃-hormone and the low serum CK-MB in GAA diet can be explained on the basis that PCre, the active form of Cre, may exert a protective effect on the cell membrane by its interaction with phospholipid bilayer (Tokarska-Schlattner et al. 2012). Under stress conditions, skeletal muscle may be broken down into glucose as a source of energy (Virden et al. 2009) that may explain the elevated CK in T₃-hormone supplemented groups as a result of skeletal muscle catabolism. Nevertheless, the effect of GAA supplementation was more pronounced on cardiac muscle by providing an immediate source of energy through Cre-PCre shuttle system. AST and ALT are indicators of liver function; meanwhile, GGT and creatinine are indicators of kindly function. In our study, no interactions were detected between GAA and T₃-hormone on the liver, kidney and T₃ hormone level in the serum. T₃-hormone, as a main effect on serum AST, showed an unexpected reduction compared to groups not supplemented with T₃-hormone. However, it is well established that excess T₃-hormone can cause liver injury (Yang et al. 2020). Nevertheless, an increase in the serum ALT level in T₃-hormone supplemented groups compared to T₃-hormone devoid group. T₃-hormone supplemented groups showed a significant increase in serum T₃-hormone level that confirmed its utilisation from the feed. Previous studies revealed that GAA had no deteriorative effect on the liver and kidney function (EFSA 2016) or T₃-hormone level in the blood of broiler chicken (Michiels et al. 2012; Amiri et al. 2019).

Dietary T₃-hormone as a stress factor can induce mitochondrial-dependent ROS production as a direct effect (Lin et al. 2008). Our results showed interactions
between GAA and T₃-hormone on liver MDA and SOD. T₃-hormone supplementation in the control diet resulted in a significant increase in liver MDA compared to other groups. Meanwhile, liver MDA was significantly lower in GAA diet supplemented with T₃-hormone than control diet supplemented with T₃-hormone. Liver SOD was significantly the lowest among the other groups in control diet supplemented with T₃-hormone. The negative effect of T₃-hormone on liver SOD was significantly mitigated in GAA diet. No interaction between GAA and T₃-hormone was recorded in GSH and GPx. GAA diet as a main effect showed significant improvement in liver MDA and GPx compared to control diets. Amiri et al. (2019) reported that GAA supplementation improved GPx and SOD when supplemented either at the rate of 0.06% or 0.12% compared to the control group under high and low crude protein diet. Furthermore, GAA supplementation at the rate of 0.12% under cold stress showed significant improvements in GPx in the liver and MDA in serum (Nasiroleslami et al. 2018). The same results were reported in Cherry valley ducks, where GAA supplementation reduced MDA in serum and increased GPx and GSH in both serum and liver (YaQiong et al. 2016). Lawler et al. (2002) concluded that Cre had a selective antioxidant effect against superoxide radicals and peroxynitrite. The improvement in the overall oxidative biomarkers in GAA diet supplemented with T₃-hormone can be attributed to Cre that may have interfered with superoxide radical and peroxynitrite formation that may result in a decrease in lipid peroxidation as indicated by low MDA value and higher SOD activity (Lawler et al. 2002).

Mitochondria have been blamed for being the primary source of ROS generation. Hyperthyroidism can cause mitochondrial damage and ROS production (Lin et al. 2008). Mitochondria contain respiratory chain complex I, II, III, and IV. Their role is to transfer electrons from electron bearing molecules along the complex chain until reaching the final electron acceptor, oxygen, to produce ATP (Liu et al. 2002). Complex I and III are considered the primary producers of superoxide radicals. Mitochondrial dependent ROS has been linked to pathological conditions, oxidative damage during ischaemia, and cardiac reperfusion injury (Blier and Dröse 2013). T₃-hormone under physiological condition has profound impacts on mitochondrial function by regulating aerobic respiration, proton leak, β-oxidation, and ROS production. T₃-hormone in excess can cause mitochondrial fatigue, increase ROS that may cause mitochondrial damage and cell death (Sinha et al. 2015). Our study provided novel results that showed the effect of dietary GAA supplementation as a precursor source of Cre on mitochondrial activities of cardiac muscle in diets supplemented with T₃-hormone. An earlier study demonstrated the relationship between pulmonary hypertension syndrome (PHS) and mitochondrial dysfunction. It was concluded that liver mitochondria obtained from broilers with PHS showed a functional defect characterised by a decrease in RCR (Cawthon et al. 1999). In our study, excluding complex II, data revealed an interaction effect between GAA and T₃-hormone on mitochondrial complex chain activity as well as respiratory control ratio (RCR) which in line with the aforementioned study. In mitochondrial RCR, complex I and III activities, the control diet supplemented with T₃-hormone was negatively affected; however, in the GAA diet, the negative effect of T₃-hormone was significantly mitigated. In control diet and GAA diet devoid of T₃-hormone supplementation, the mitochondrial activities were not affected except complex IV, which was better in GAA diet than the control diet. To our knowledge, no previous studies have investigated the effect of GAA on mitochondrial complex chain activities and RCR therefore; our data are considered as novel findings. Our data may highlight the role of Cre-PCre shuttle system in modulating mitochondrial activity. PCre is a potent regulator of mitochondrial ADP stimulated respiration by decreasing the mitochondrial sensitivity to ADP. Meanwhile, Cre has the opposite function. Therefore, a high PCre: Cre ratio may indicate a high energetic state of the cell that may reduce mitochondrial activity and damage (Walsh et al. 2001). Our results showed that GAA, had a protective effect on mitochondria integrity against T₃-hormone challenge as indicated by the improvement in mitochondrial respiration and complex chain activities.

It was expected that dietary T₃-hormone could have a negative effect on carcass traits as well as relative organ weights, which were confirmed under the current study. Our results showed an interaction between GAA and T₃-hormone on heart index and showed a trend on BMY. HI was higher in T₃-hormone supplemented groups compared to the counterparts and was significantly the greatest in GAA diet. An earlier study revealed that under cold stress, serum T₃-hormone increased significantly compared to the control group and resulted in an increase in heart weight in both male and female broiler chicken (Blahová et al. 2007). Our results may highlight the importance of Cre in providing an immediate source of energy to support the heart pump and increasing cardiac muscle mass. As a main effect, CW, AFY, HI and WI were
significantly improved in GAA diet compared to control diet. Limited studies investigated the effect of dietary T3-hormone supplementation or cold stress on carcase traits. However, under normal condition, earlier studies showed that GAA had a pronounced effect on breast meat yield (Michiels et al. 2012) and improved carcase dressing, breast muscle, and reduced abdominal fat (Heger et al. 2014; Metwally et al. 2015; EFSA 2016). The overall improvement in carcase traits in GAA diet can be attributed to the improvement in energy utilisation efficiency in response to GAA supplementation.

It was proven that dietary T3-hormone supplementation in broiler diet could cause RVHF and AS mortality. Earlier studies showed an increase in RV: TV (0.33–0.37) starting from the third week onward as a result of dietary T3-hormone supplementation (Ladmakhi et al. 1997; Habibian et al. 2017), or dietary supplementation of T4 (Taghizadeh et al. 2012) and when broilers were subjected to cold stress (Ahmadipour et al. 2018). A previous study revealed a reduction in RV: TV ratio from 0.30 to 0.27 and 0.27, as actes mortality from 26% to 22% and 18%, and an increase in serum nitric oxide level from 4.7 μM to 6.1 μM and 10.1 μM when the diets supplemented with 0.075% and 0.15% GAA, respectively (Faraji et al. 2019). Our study showed that T3- hormone supplemented group resulted in AS starting from the second week onward, which was one week earlier than these previous studies. The gross lesion of the dead birds showed dilatation of the right ventricle. Interestingly, dietary T3-hormone in GAA diet showed less dilated right ventricle and showed more muscle mass (69 g) compared to the control group supplemented with T3-hormone (60 g). T3-hormone supplementation in the control diet showed greater RV: TV (0.31) than when supplemented in GAA diet (0.29). Supplementation of GAA to T3-hormone supplemented diet did not decrease the asctes mortality under this current study but showed a potentiality as indicated by lower RV: TV and more muscle mass. The latter can be explained on the ground that serum nitric oxide level was not high enough to reduce the asctes mortality. Although nitric oxide was not measured in our study, a previous study showed that high inclusion level of GAA (0.075–0.15%) increased serum nitric oxide level and decreased the ascites mortality in broiler chicken raised at high altitude (Faraji et al. 2019). Therefore, more investigations are required to elucidate if higher inclusion rate of GAA can reduce the ascites mortality under T3-hormone challenge.

Dietary T3-hormone supplementation not only affected cardiac muscle but also caused a liver injury. A previous study confirmed the negative impact of hyperthyroidism on the rat liver (Yang et al. 2020). Our results showed that liver injury as a result of dietary supplementation of T3-hormone in GAA diet was less severe compared to the control diet. The improvement in oxidative biomarkers, mitochondrial activities and energy utilisation efficiency in our study may have contributed to lowering the liver injury. Further studies are needed to confirm our results.

Conclusions

GAA supplementation at the rate of 0.06% was able to mitigate the negative effect of dietary T3-hormone supplementation on oxidative biomarkers, mitochondrial activities and liver injury. Still, it was not able to reduce the incidence of AS at such inclusion rate. Further studies are needed to elucidate the effect of GAA at higher inclusion rate for more data accuracy with regards to AS.

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Ethical approval

The protocol used in this experiment was approved by the Veterinary Medicine Cairo University Institutional Animal Care and Use Committee.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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