Article

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Dietary Guanidinoacetic Acid Improves Meat Tenderness and Antioxidant Capacity in Rabbits via Modulating Muscle Fiber Characteristics and Fat Metabolism

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Simple Summary

Rabbit meat is widely favored as a healthy protein source. Accordingly, this study was conducted to investigated the effects of dietary guanidinoacetic acid supplementation on meat quality, antioxidant capacity, myofiber characteristics and fatty acid metabolism in rabbits, aiming to provide a novel nutritional strategy for improving rabbit meat quality. Our findings indicate that dietary guanidinoacetic acid supplementation enhances meat tenderness and antioxidant capacity without compromising growth performance, highlighting guanidinoacetic acid as a promising feed additive for improving the quality of rabbit meat, in line with the growing consumer demand for healthier and more sustainably produced animal products.

Abstract

Guanidinoacetic acid (GAA) is a direct precursor of creatine and plays a key role in energy and protein metabolism. Rabbit meat is increasingly recognized as a healthy food source due to its high protein and low fat content, and improving its quality is of growing interest to consumers and producers alike. This study investigated the effects of dietary GAA supplementation on meat quality, antioxidant capacity, muscle fiber characteristics and fatty acid metabolism in rabbits. A total of 960 weaned male rabbits were assigned to two age groups (40 ± 2 days, 1.19 ± 0.09 kg; 60 ± 2 days, 1.82 ± 0.15 kg). Within each age group, rabbits were randomly allocated to a control diet or a diet supplemented with 100 mg/kg GAA (CON-40, GAA-40, CON-60, GAA-60). After a 45-day feeding period, GAA supplementation significantly improved meat tenderness, as evidenced by reduced shear force in 60-day-old rabbits ($p < 0.01$), and decreased muscle fiber area and density in 40-day-old rabbits ($p < 0.05$). Moreover, GAA enhanced systemic antioxidant capacity, increasing serum superoxide dismutase (SOD) activity and total antioxidant capacity (T-AOC) ($p < 0.05$), while reducing malondialdehyde (MDA) levels in 60-day-old rabbits ($p < 0.05$). GAA also modulated the expression of genes involved in lipid metabolism (FAS, HSL, ACC) in intramuscular and perirenal fat, suggesting a regulatory role in fatty acid metabolism. In conclusion, dietary GAA supplementation improves meat tenderness and antioxidant capacity in rabbits without compromising growth performance. These findings support the potential of GAA as a nutritional strategy to enhance the quality of rabbit meat as a functional food for human consumption.

Keywords: guanidinoacetic acid; rabbits; production performance; antioxidant capacity; muscle fiber properties; fatty acid metabolism

1. Introduction

The rising global demand for high-quality animal protein, coupled with challenges in sustainable food production, has intensified efforts to improve the nutritional value and quality of meat products [1]. In this context, rabbit meat has gained increasing attention as a healthy and sustainable protein source. It is characterized by high protein content, low fat, low cholesterol, and favorable amino acid and fatty acid profiles, making it particularly suitable for health-conscious consumers and individuals with dietary restrictions [2]. Consequently, improving the quality of rabbit meat has become a priority for both producers and researchers. However, challenges remain in consistently achieving optimal meat quality in commercial rabbit production, particularly in terms of tenderness, water-holding capacity, and oxidative stability, emphasizing the need for effective nutritional strategies.

Guanidinoacetic acid (GAA), the immediate metabolic precursor of creatine, is endogenously synthesized from arginine and glycine and plays a central role in cellular energy homeostasis [3–5]. As a feed additive, GAA offers several advantages over direct creatine supplementation, including greater chemical stability, lower production cost, and higher bioavailability [6,7]. Upon absorption, GAA is methylated to form creatine, which is then phosphorylated to phosphocreatine. Phosphocreatine serves as a key energy buffer, helping to maintain ATP levels in tissues with high and fluctuating energy demands, such as skeletal muscle [5,8]. Beyond its well-established role in energy metabolism, emerging evidence suggests that GAA may exert broader physiological effects, including modulation of muscle fiber characteristics, antioxidant defense, and lipid metabolism.

In recent years, the effects of dietary GAA on meat quality have been investigated across various livestock species. In ruminants, GAA supplementation improved carcass quality by reducing excessive fat deposition in subcutaneous and visceral adipose tissues [8–10]. In Hu sheep, dietary GAA increased muscle shear force and fiber diameter while reducing drip loss and muscle fiber density [11]. In pigs, dietary GAA at 0.045% enhanced meat quality by altering muscle fiber characteristics and reducing mandibular fat [12], while 0.12% GAA fed pre-slaughter improved lean meat yield and reduced backfat thickness [13]. In poultry, GAA supplementation improved broiler performance without adversely affecting meat quality [14,15]. Collectively, these findings indicate that GAA can modulate muscle fiber properties and lipid metabolism, thereby influencing meat quality across multiple species.

Although the efficacy of GAA has been well-documented in various livestock and poultry species, studies on the application of GAA in rabbit production remain limited, while its effects on meat quality have not been systematically evaluated. Therefore, this study aimed to investigate the effects of dietary GAA supplementation on meat quality, antioxidant capacity, muscle fiber characteristics and fatty acid metabolism in rabbits. The findings are expected to provide a scientific basis for using GAA as a nutritional strategy to enhance the quality of rabbit meat as a functional food for human consumption.

2. Materials and Methods

2.1. Moral Statement

The study was approved by the Fujian Agriculture and Forestry University Animal Care and Use Committee (Approval ID: PZCASFAFU24122) on January 15, 2024.

2.2. Experimental Materials

A total of 960 healthy male rabbits were selected from Fujian Chunlong Agriculture and Animal Husbandry Technology Co., Ltd. (Fuzhou, China). The animals comprised two age groups: 480 rabbits aged 40 ± 2 days (body weight 1.19 ± 0.09 kg) and 480 rabbits aged 60 ± 2 days (body weight 1.82 ± 0.15 kg). Guanidinoacetic acid (GAA, purity ≥ 99%) used in the trial was supplied by Beijing Gendone Biotechnology Co.,Ltd. (Beijing, China).

2.3. Housing Conditions and Feeding Management

The experiment was conducted at the Rabbit Farm of Fujian Agriculture and Forestry University (Fuzhou, China). Rabbits were individually housed in cages under a randomized block design. Prior to the trial, the rabbit house, flooring, cages, feeders, and drinking systems were thoroughly cleaned and disinfected. All rabbits were ear-tagged and vaccinated before the start of the study.

During the experimental period, weekly disinfection and cleaning of the housing and cages were performed routinely. Feeders were cleaned daily, and drinking equipment along with cooling pads were inspected to ensure proper function. The animals were kept in a clean, quiet environment with room temperature maintained at 23–28 °C and relative humidity at approximately 80%. Feeding was carried out at 7:00, 15:00, and 20:00 daily. Rabbits had ad libitum access to feed and water throughout the trial. All other husbandry practices, including immunization procedures, followed standard operating protocols.

The basal diet was formulated according to NRC (1994) guidelines, and the composition and nutritional levels of the basal diets of the test rabbits are shown in Table 1.

Table 1. Diet composition and nutrient levels (air-dried basis).

Item	Contents
Alfalfa hay meal	28.50
Corn	26.20
Rapeseed meal	5.00
Wheat bran	15.40
Soybean meal	8.40
Rice bran	6.00
Squeeze soybeans	5.00
$Ca(HCO_3)_2$	0.70
NaCl	0.50
Methionine	0.10
Lysine	0.20
Premix[1]	4.00
Total	100.00
Nutrition Level[2]	
Moisture (%)	12.80
Crude Protein (%)	15.48
Crude Fiber (%)	12.25
Ether Extract (%)	3.63
Calcium (%)	0.73
Phosphorus (%)	0.37
Lysine (%)	0.76
Methionine + Cystine (%)	0.45
Digestible energy (MJ/kg)	10.46

[1] The premix provided the following per kg of diets: Cu 5 mg，Fe·50 mg，Zn 50 mg，Mn 8 mg，Mg 0.03 mg，I 0.5 mg，Se 0.1 mg，Co 0.25 mg，VA 6000 IU，VD3 1000 IU，VK 1.0 IU，VE 50 mg，VB1 1 mg，VB2 3 mg，VB3 1.2 mg，VB12 0.01 mg， folic acid 0.2 mg，pantothenic acid 10 mg，niacin 30.0 mg，biotin 0.08 mg，choline 100 mg; [2] Moisture, Crude protein (CP), crude fiber (CF), ether extract (EE), calcium (Ca), phosphorus (P), Lysine (Lys) and Methionine + Cystine (Met + Cys) were measured and recorded. CP was determined by the Kjeldahl method; CF were measured using the filter bag technique; Ca was determined by the ethylenediaminetetraacetic acid (EDTA) complexometric titration method; P was measured by spectrophotometry at a wavelength of 400 nm with the molybdenum blue reaction. Amino Acid was measure by HPLC. The digestive energy is the calculated value.

2.4. Experimental Design

As illustrated in Figure 1, weaned male rabbits were selected and assigned to two age groups: 40 days old and 60 days old. Within each age group, rabbits were randomly divided into two dietary treatments: control groups (CON-40, CON-60) and groups supplemented with 100 mg/kg GAA (GAA-40, GAA-60). Each group contained 240 rabbits, with 10 replicates per group and 24 rabbits per replicate. The experiment consisted of a 7-day adaptation period followed by a 45-day formal feeding period. In a preliminary trial, graded levels of 0, 50, 100, and 150 mg/kg GAA were added to the basal diet. The inclusion of 100 mg/kg GAA showed the most pronounced effects and was therefore selected for the formal experiment. During the formal trial, the control groups (CON-40 and CON-60) received the basal diet, whereas the GAA-supplemented groups (GAA-40 and GAA-60) were fed the basal diet supplemented with 100 mg/kg GAA. The supplement was first mixed into a premix and then uniformly blended with other ingredients before pelleting. Throughout the study, all animals were raised under standardized management practices to ensure consistent experimental conditions and reliable data collection.

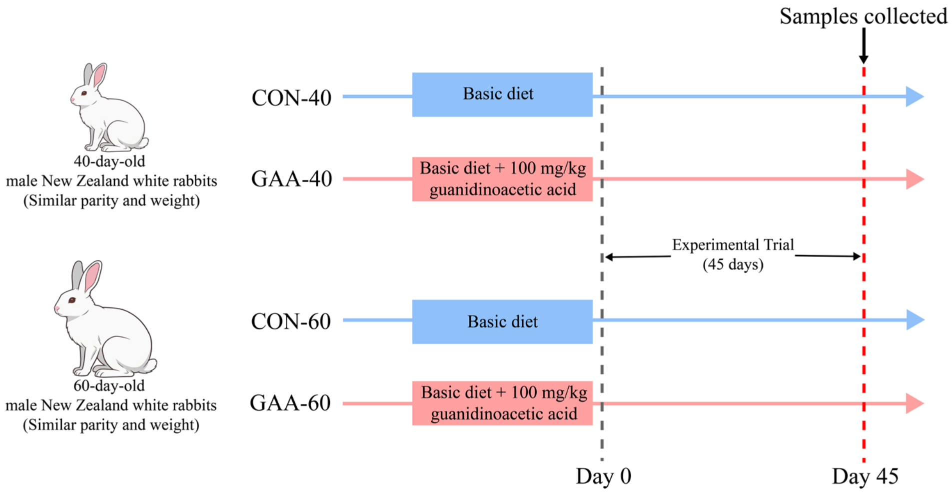

Figure 1. The schematic diagram of experimental design in this study.

2.5. Sample Collection

At the end of the 45-day feeding period, five rabbits per group were randomly selected after fasting, weighed (live weight), and slaughtered. Throughout the trial, feed intake was recorded per replicate, and all rabbits were weighed on beginning and end of the trial to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). Blood samples were collected from the jugular vein, stored at 4 °C for 2 h, and centrifuged at 3,000× *g* for 15 min to obtain serum, which was stored at −20°C for subsequent biochemical and antioxidant analyses. Following slaughter, carcass weight was recorded, and samples of the longissimus dorsi muscle were collected. Muscle specimens intended for histological analysis were fixed immediately; all other tissue samples were snap-frozen in liquid nitrogen and stored at −80°C until further analysis.

2.6. Meat Quality and Histological Analysis

After slaughter, samples of the longissimus dorsi muscle were collected. Surrounding fat and connective tissues were removed for the determination of pH, drip loss, and shear force. Muscle pH was measured at three points using a hand-held pH meter (PHSJ-3F, Shanghai, China). Drip loss was determined by suspending samples in a sealed plastic bag at 4 °C for 24 h. For shear force measurement, samples were trimmed of fat and connective tissue, then sheared three times using a digital texture analyzer, and the average value was recorded. Intramuscular fat content was determined by petroleum ether extraction.

For histological analysis, longissimus dorsi samples were collected immediately after slaughter and fixed in universal tissue fixative, ensuring complete submersion. After fixation, samples were dehydrated, trimmed, embedded, sectioned, and stained following standard procedures. Image acquisition was then performed to determine muscle fiber diameter, cross-sectional area, and fiber density.

2.7. Serum Biochemical Indexes and Antioxidant Capacity

At end of the trial, five rabbits per group were randomly selected, and blood samples were collected from the jugular vein. The blood was allowed to stand for 1 h, then centrifuged at 3,500× *g* for 15 min. The resulting serum was divided into 1.5 mL centrifuge tubes and stored at −20 °C until analysis. Serum samples were used to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Antioxidant parameters, including superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and total antioxidant capacity (T-AOC), were measured in serum, longissimus dorsi muscle, and liver samples using commercial kits (Shanghai Liquid Quality Assay Technology Co., Ltd., Shanghai, China).

2.8. Real-Time Quantitative PCR of Gene Expression

After slaughter, samples of longissimus dorsi muscle and perirenal fat were collected, placed in 5 mL centrifuge tubes, immediately snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

Total RNA was isolated using NucleoZol reagent (Gene, Düren, Germany), and cDNA was synthesized using a reverse transcription kit (Servicebio, G3330-100, Wuhan, China). Quantitative real-time PCR (qPCR) was performed on an ABI 7300 system (Applied Biosystems, Foster City, CA, USA). Primer sequences for fatty acid synthase (FAS), hormone-sensitive lipase (HSL), acetyl-CoA carboxylase (ACC), and the endogenous reference gene β-actin (ACTIN) are listed in Table 2. All reactions were performed in triplicate. The relative expression levels of target genes were normalized to ACTIN and calculated using the $2^{-\Delta\Delta CT}$ method [16].

Table 2. Nucleotide sequences of primers used for quantitative real-time PCR assays.

Items	Primer Sequences (5′–3′）
FAS	F: GCTGGCTCACTGTCCACAAG R: CTGGTTTGCCCTCATTGCTT
HSL	F: CTCCTACGACCTGCGTGAAG R: CAGCTCTTGAGGTAGGGCTC
ACC	F: TGTCCGCACCGACTGTAATC R: AGTTGGTGTCAGGCGAATGT
ACTIN	F: GTGCTTCTAGGCGGACTGTT R: TCGGCCACATTGCAGAACTT

2.9. Meat Quality and Histological Analysis

Statistical analyses were undertaken using SPSS Statistics 27.0 software (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for data analysis, and Tukey’s multiple range tests were used for multiple comparisons. The results are presented as the mean ± standard error (SEM). Significance was declared at $p < 0.05$.

3. Results

3.1. Growth Performance and Carcass Traits

As shown in Table 3, dietary GAA supplementation had no significant effect on growth performance and carcass traits in either age group ($p > 0.05$).

Table 3. Growth performance and carcass traits of rabbits in different ages after addition of GAA.

Items	Groups		p-Value	Groups		*p*-Value
	CON-40	GAA-40		CON-60	GAA-60	
Growth performance						
Initial body weight (kg)	1.19±0.03	1.29±0.04	0.06	1.82±0.05	1.92±0.06	0.22
Final body weight (kg)	2.40±0.03	2.37±0.07	0.71	3.51±0.08	3.62±0.09	0.38
Average daily gain (g)	26.99±0.90	24.08±1.91	0.19	27.32±1.33	27.45±1.28	0.95
Average daily intake(g)	132.43±4.88	125.98±4.04	0.32	220.38±13.52	223.21±10.61	0.87
F/G	4.64±0.27	5.29±0.63	0.35	8.28±0.37	8.26±0.50	0.98
Carcass traits						
Live weight before slaughter (kg)	2.70±0.04	2.99±0.14	0.08	3.86±0.06	3.81±0.08	0.63
Carcass weight (kg)	1.35±0.04	1.43±0.06	0.29	1.93±0.04	1.87±0.05	0.36
Dressing percentage (%)	49.99±1.05	47.88±0.66	0.13	50.00±0.67	49.08±0.66	0.36

Note: F/G, feed/gain ratio. The results are presented as the mean ± SEM. For growth performance, n = 10. For carcass traits, n = 5. *, $p < 0.05$; **, $p < 0.01$.

3.2. Organ Indices

As shown in Figure 2, lung and kidney indices were significantly lower in the GAA-40 group than in the CON-40 group ($p < 0.05$). No significant differences were observed in heart, liver, or spleen indices between the GAA-40 and CON-40 groups ($p > 0.05$). Furthermore, no significant differences were found between the GAA-60 and CON-60 groups for any of the measured organ indices, including heart, liver, spleen, lung and kidney ($p > 0.05$).

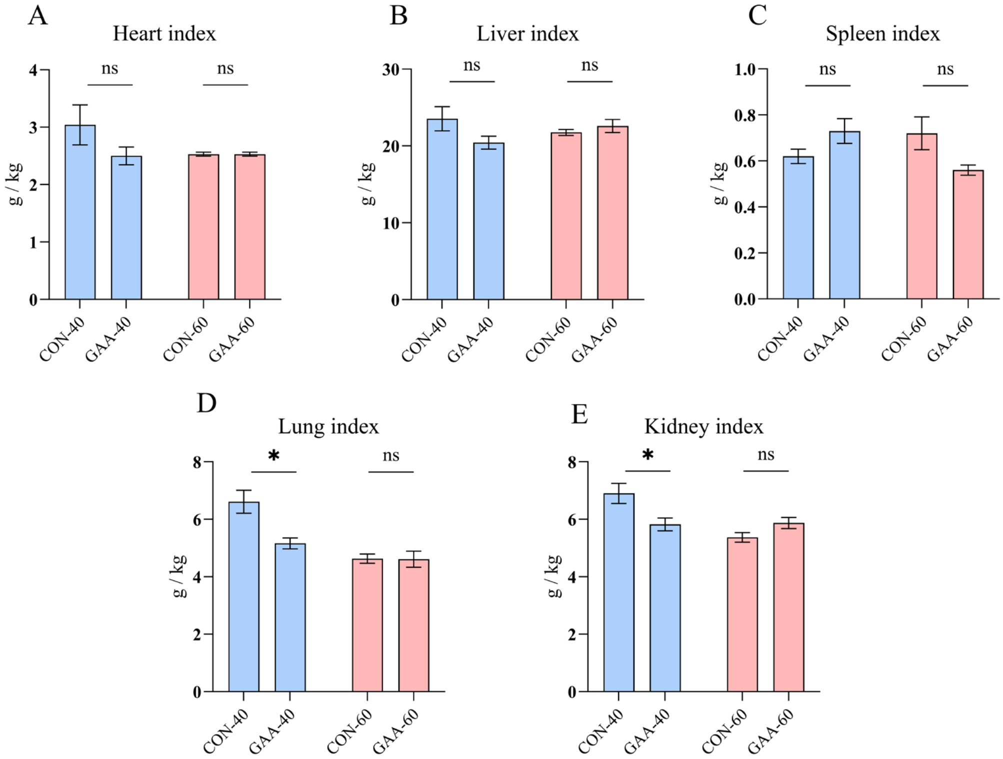

Figure 2. Effect of addition of GAA on the development of organs in rabbits (A-E: organ index of heart, liver, spleen, lung and kidney). The error bar represents the standard error of means (SEM). n = 3. *, $p < 0.05$; **, $p < 0.01$.

3.3. Meat Quality and Muscle Fiber Characteristics

Dietary GAA supplementation affected meat quality in an age-dependent manner (Table 4, Figure 3). In 40-day-old rabbits, GAA supplementation significantly reduced total muscle fiber area and muscle fiber density ($p < 0.05$), but had no effect on pH$_{45min}$, drip loss, shear force, or muscle fiber diameter ($p > 0.05$). In contrast, in 60-day-old rabbits, GAA significantly decreased shear force ($p < 0.01$), indicating improved tenderness, while no significant changes were observed in other meat quality parameters or muscle fiber characteristics ($p > 0.05$).

Table 4. Meat quality parameters and muscle fiber characteristics of rabbits fed diets with or without GAA.

Items	Groups		*p*-Value	Groups		*p*-Value
	CON-40	GAA-40		CON-60	GAA-60	
pH$_{45min}$	6.98±0.07	6.98±0.04	0.94	6.58±0.08	6.67±0.06	0.40
Drip loss (%)	2.45±0.16	2.48±0.15	0.89	2.82±0.19	2.90±0.21	0.80
Shear force (N)	19.24±1.80	17.56±1.94	0.54	37.99±1.67	28.56±1.11**	<0.01
Muscle fiber diameter (mm)	0.05±0.01	0.06±0.01	0.17	0.07±0.01	0.07±0.01	0.57
Total muscle fiber area (mm^2)	0.21±0.01	0.18±0.01*	0.04	0.17±0.01	0.18±0.01	0.44
Muscle fiber density (N/mm^2)	507.70±5.68	468.74±3.84**	<0.01	327.10±9.45	307.32±4.17	0.09

Note: Data are presented as mean ± SEM, n = 5. *, $p < 0.05$; **, $p < 0.01$.

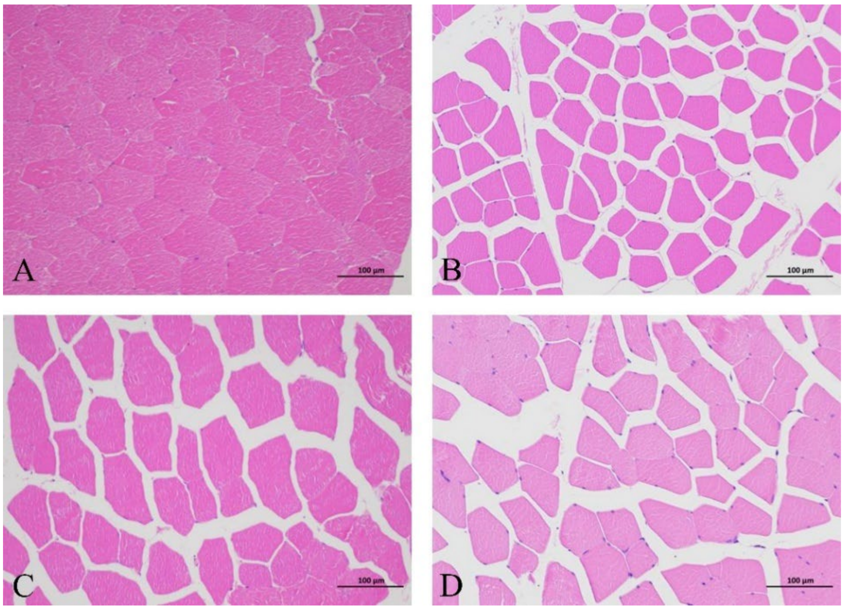

Figure 3. Microscopic images of the longissimus dorsi muscle cross-sections from the CON-40 (A), GAA-40 (B), CON-60 (C), and GAA-60 (D) groups. GAA was supplemented at 100 mg/kg in the GAA group.

3.4. Serum Biochemical Parameters

As shown in Figure 4, dietary GAA supplementation significantly affected serum biochemical parameters. In 40-day-old rabbits, the GAA-40 group exhibited significantly lower serum ALT and HDL levels compared with the CON-40 group ($p < 0.05$), while no significant differences were observed in AST or LDL levels ($p > 0.05$). In 60-day-old rabbits, HDL levels were significantly reduced in the GAA-60 group relative to the CON-60 group ($p < 0.05$), whereas AST, ALT, and LDL levels remained unchanged ($p > 0.05$).

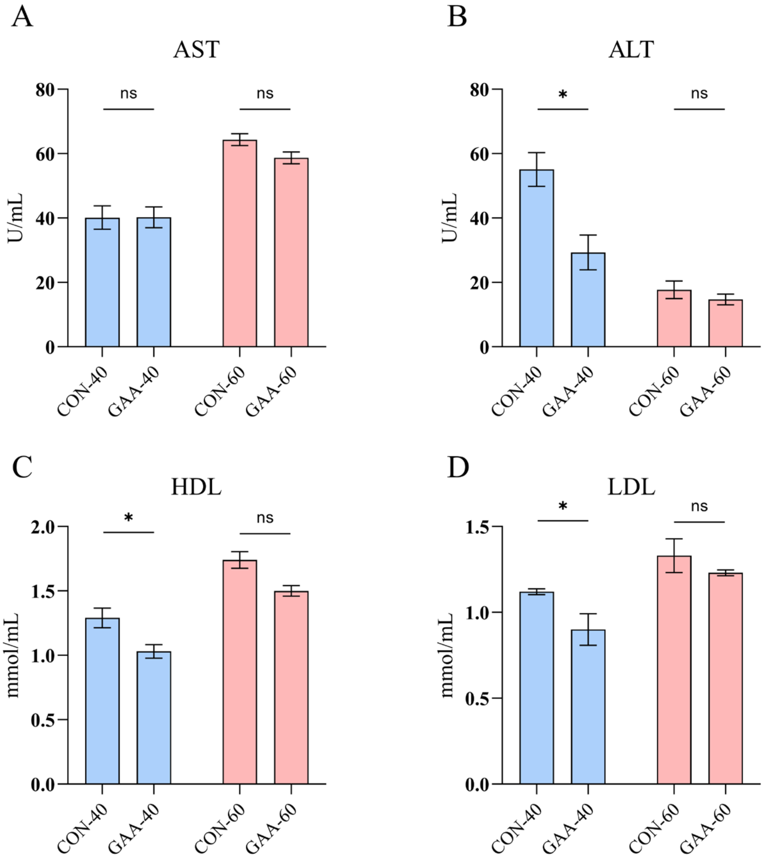

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Figure 4. Serum biochemical parameters of rabbits fed diets with or without GAA. (A) AST, (B) ALT, (C) HDL, (D) LDL. The error bar represents the standard error of means (SEM). n = 3. AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. *, $p < 0.05$; **, $p < 0.01$.

3.5. Antioxidant Capacity

As shown in Figure 5, dietary GAA supplementation enhanced serum antioxidant capacity in both age groups. In 40-day-old rabbits, GAA significantly increased SOD activity and T-AOC level ($p < 0.05$), but did not affect CAT activity or MDA level ($p > 0.05$). In 60-day-old rabbits, GAA significantly increased SOD activity and T-AOC level, and significantly decreased MDA level ($p < 0.05$), while CAT activity remained unchanged ($p > 0.05$).

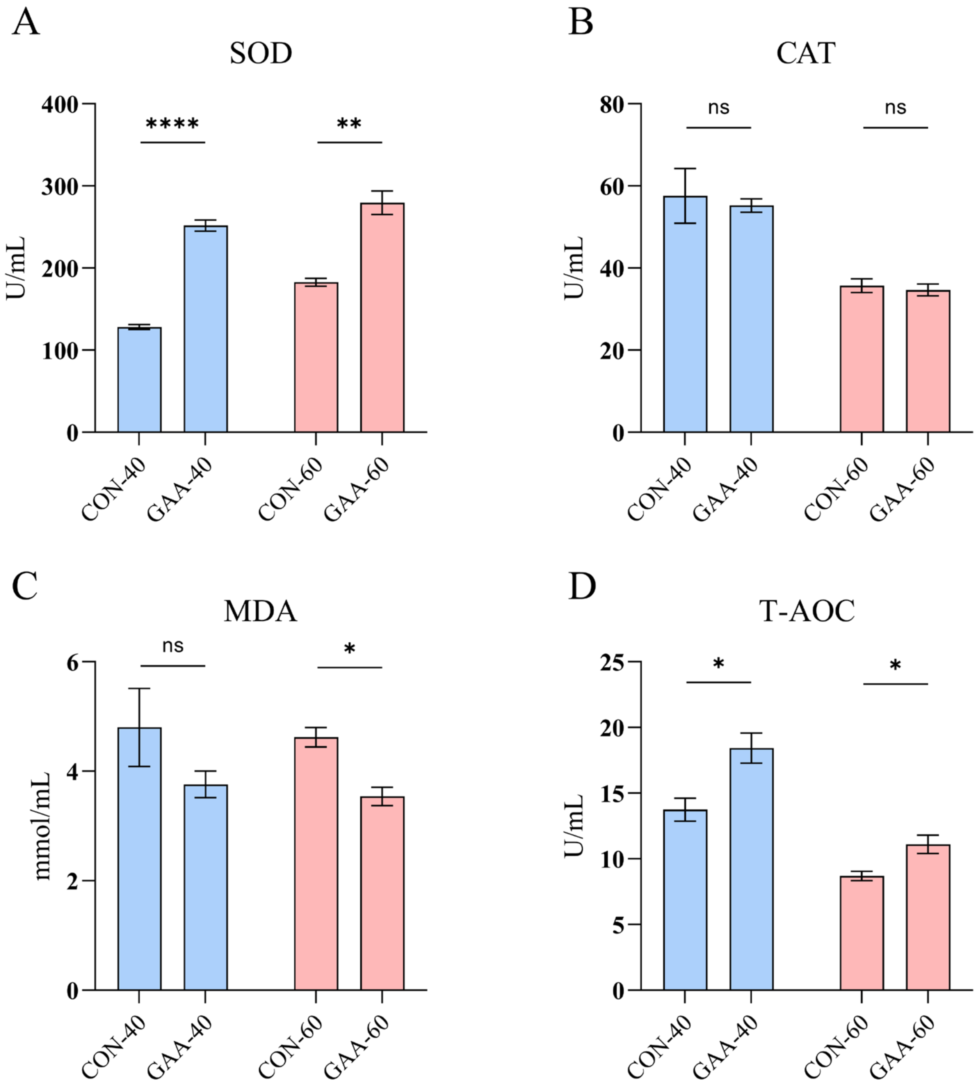

Figure 5. Serum antioxidant parameters of rabbits fed diets with or without GAA. (A) Superoxide dismutase (SOD), (B) Catalase (CAT), (C) Malondialdehyde (MDA), (D) Total antioxidant capacity (T-AOC). The error bar represents the standard error of means (SEM). n = 3. *, $p < 0.05$, **, $p < 0.01$.

3.6. Fatty Acid Metabolism Related Gene Expression

As can be seen from Figure 6, the relative expression of intramuscular fat *FAS* and *HSL* genes in the GAA-40 group was significantly higher than that in the CON-40 group ($p < 0.05$), and there was no significant difference in the relative expression of intramuscular fat *ACC* genes in the GAA-40 group when compared with that in the CON-40 group ($p > 0.05$); when compared with the CON-60 group, there was no significant difference in the relative expression levels of *FAS*, *HSL*, and *ACC* genes in intramuscular fat in the GAA-60 group ($p > 0.05$).

Meanwhile, the relative expression of *ACC* gene in perirenal fat in GAA-40 group was significantly lower than that in CON-40 group ($p < 0.05$); compared with the CON-40 group, there was no significant difference in the relative expression of *FAS* and *HSL* genes in perirenal fat in the GAA-40 group ($p > 0.05$); and compared with the CON-60 group, there was no significant difference in the relative expression of *FAS*, *HSL* and *ACC* genes in perirenal fat in the GAA-60 group ($p > 0.05$).

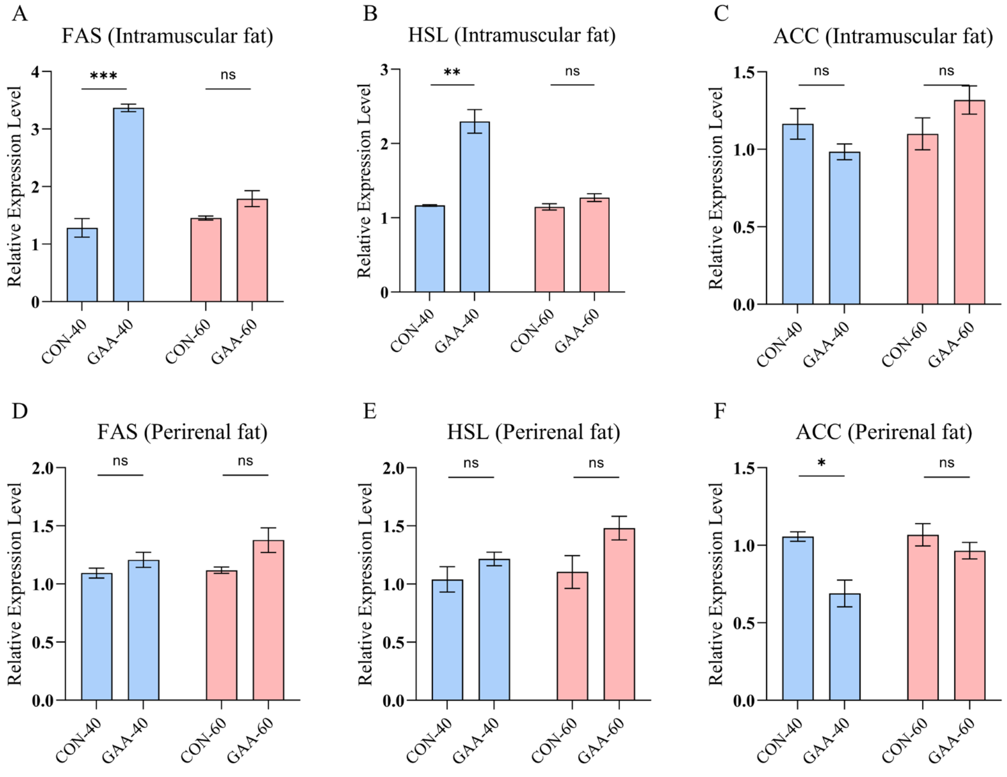

Figure 6. Expression of fatty acid metabolism-related genes in intramuscular and perirenal fat of rabbits fed diets with or without GAA. (A) FAS, (B) HSL, (C) ACC. The error bar represents the standard error of means (SEM). n = 3. *FAS*: *Fatty acid synthase*; *HSL*: *Sensitive lipase*; *ACC*: *Acetyl coenzyme A carboxylase*. *, $p < 0.05$, **, $p < 0.01$.

4. Discussion

GAA serves as the only direct metabolic precursor of creatine, and dietary GAA supplementation has been shown to increase endogenous creatine levels, thereby enhancing energy metabolism in animals [17]. However, the effects of GAA on growth performance remain inconsistent across published studies: dietary GAA inclusion was reported to improve weight gain and feed efficiency in broilers[18] and in pigs from weaning to finishing [13]. In contrast, other studies observed no significant growth response to GAA in chickens [19,20] or finishing pigs [21]. In rabbits, research on GAA application is still limited, with one previous study reporting improved weight gain in growing rabbits fed 0.04–0.12% GAA [22], which contrasts with the present findings that dietary supplementation with 100 mg/kg GAA exerted no significant effect on the growth performance or carcass traits of rabbits. These discrepancies may be attributed to differences in animal species, age, basal diet composition, GAA dosage, or experimental conditions, warranting further investigation. Beyond growth performance, organ indices provide critical insight into the physiological status and functional load of key organs in response to dietary interventions [23]. In the present study, 100 mg/kg dietary GAA supplementation reduced the lung and kidney indices in 40-day-old rabbits, suggesting a potential modulatory effect of GAA on these organs. Although creatinine and other GAA-derived metabolites are primarily excreted via the kidneys [24] , no increased renal functional burden was observed in this study; instead, the reduced kidney index may reflect enhanced renal efficiency, as indicated by slower relative organ weight gain. Notably, this effect was absent in the 60-day-old group, likely due to organ maturation, suggesting an age-dependent response. Furthermore, the absence of significant changes in heart, liver, and spleen indices implies that GAA supplementation did not impose additional metabolic stress on the experimental animals. Further investigations are warranted to elucidate the underlying mechanisms and long-term physiological implications of these observations.

Meat quality is primarily assessed by tenderness, water-holding capacity, and pH. Shear force is widely used to assess meat tenderness, while drip loss reflects the water-holding capacity of muscle proteins during processing and storage [25]. Dietary GAA has been shown to modulate meat quality traits across livestock species, with previous work reporting reduced shear force in fattening pigs [12] and bulls [26], as well as improved shear force and muscle fiber cross-sectional area in pigs, accompanied by reduced drip loss and muscle fiber density [27]. In the present study, GAA supplementation significantly improved meat tenderness in 60-day-old rabbits, as evidenced by reduced shear force, and decreased muscle fiber density in 40-day-old rabbits. These findings are consistent with previous reports linking GAA to improved tenderness and altered muscle fiber characteristics[12,26,27]. Muscle fiber properties are closely related to meat tenderness, as skeletal muscle consists of approximately 90% muscle fibers, with the remaining 10% comprising connective and adipose tissue [28,29]. Therefore, the observed reduction in muscle fiber density may contribute to the improved tenderness, although the effect was age-dependent and more pronounced in the older group. No significant changes were observed in pH or drip loss following GAA supplementation, which aligns with findings in pigs [27]. A possible explanation involves the role of GAA in energy metabolism. As a creatine precursor, GAA increases muscle creatine and phosphocreatine stores, providing a larger energy reserve that may reduce the reliance on glycogenolysis during post-mortem metabolism [17]. This could limit lactic acid accumulation and help stabilize pH, thereby preserving water-holding capacity. However, the exact mechanisms linking GAA to muscle fiber remodeling and tenderness, as well as the age-dependent responses, warrant further investigation.

Serum ALT and AST are widely used biomarkers of hepatocellular integrity, with elevated levels typically indicating hepatocyte membrane damage or impaired liver function [30]. In the present study, dietary GAA supplementation significantly reduced serum ALT levels in 40-day-old rabbits, suggesting a potential hepatoprotective effect. The absence of a corresponding change in AST activity may reflect its lower tissue specificity, as AST is also abundant in cardiac and skeletal muscle. Regarding lipid metabolism, HDL facilitates the reverse transport of cholesterol from peripheral tissues to the liver, while LDL is associated with increased cardiovascular risk [31]. In both age groups, GAA supplementation significantly reduced serum HDL levels without affecting LDL, consistent with findings in broilers fed 1200 mg/kg GAA [32]. This parallel suggests that GAA may play a regulatory role in lipid metabolism, potentially influencing cholesterol transport and redistribution.

The antioxidant defense system, comprising enzymes such as superoxide dismutase (SOD) and catalase (CAT) along with non-enzymatic antioxidants, plays a crucial role in protecting cells from oxidative damage, with malondialdehyde (MDA) serving as a key marker of lipid peroxidation [33]. In the present study, GAA supplementation significantly enhanced serum antioxidant capacity, as evidenced by increased SOD activity and total antioxidant capacity (T-AOC) in both age groups, and decreased MDA levels in 60-day-old rabbits. These findings are consistent with a study in broilers, where GAA has been reported to elevate T-AOC and decrease reactive oxygen species (ROS) and MDA levels [34]. Similarly, a previous study has demonstrated that GAA could increase hepatic SOD and glutathione peroxidase (GSH-Px) activities while reducing MDA levels in tilapia [35].

Collectively, these results indicate that GAA enhances systemic antioxidant defense, which may contribute to improved meat quality by reducing oxidative stress-induced damage.

Fatty acid synthase (FAS), hormone-sensitive lipase (HSL), and acetyl-CoA carboxylase (ACC) are key enzymes regulating lipid metabolism. FAS catalyzes the synthesis of long-chain saturated fatty acids, and its expression level is positively correlated with fat deposition in animals [36–38]. HSL hydrolyzes triglycerides into free fatty acids and glycerol, playing a central role in lipolysis [39,40]. ACC is the rate-limiting enzyme in de novo fatty acid synthesis, providing malonyl-CoA for subsequent chain elongation by FAS [41]. Dietary GAA has been shown to improve carcass quality by reducing excessive fat deposition in livestock [9]. In the present study, GAA upregulated FAS and HSL expression in the intramuscular fat of 40-day-old rabbits, suggesting a balanced regulation of lipid synthesis and hydrolysis that may help prevent excessive fat accumulation. In contrast, ACC expression was downregulated in perirenal fat, indicating reduced de novo lipogenic capacity in this depot. This tissue-specific response may reflect a metabolic adaptation to energy demands: under conditions of enhanced energy turnover, suppression of ACC could shift metabolism toward fatty acid oxidation, thereby supporting energy homeostasis [9]. These findings provide new insights into the regulatory role of GAA in lipid metabolism, although the long-term implications for fat deposition and meat quality warrant further investigation.

Collectively, the findings of this study demonstrate that dietary GAA supplementation exerts multiple beneficial effects on rabbit meat quality, antioxidant capacity, and lipid metabolism, despite having no significant impact on growth performance. The improved tenderness observed in 60-day-old rabbits, together with enhanced systemic antioxidant defense and modulated expression of lipid metabolism-related genes, suggests that GAA acts through integrated mechanisms involving energy metabolism, oxidative stress reduction, and tissue-specific regulation of fat deposition. These effects are age-dependent, highlighting the complexity of the biological functions of GAA in rabbits. While the present study provides a comprehensive evaluation of GAA in rabbits, further research is needed to elucidate the molecular pathways underlying these observations, particularly the crosstalk between muscle and adipose tissues. Additionally, long-term studies assessing the persistence of these effects and their translation to consumer-relevant outcomes, such as sensory attributes and nutritional value of rabbit meat, would be valuable. Overall, GAA represents a promising feed additive for improving the functional quality of rabbit meat, aligning with the growing demand for healthier and sustainably produced animal products.

5. Conclusions

In summary, dietary GAA supplementation did not significantly affect growth performance or carcass traits in rabbits. However, it improved the tenderness of the longissimus dorsi muscle and enhanced systemic antioxidant capacity. Furthermore, GAA modulated the expression of key genes involved in fat deposition. Collectively, these findings provide a scientific basis for the application of GAA as a nutritional strategy to improve meat quality in rabbit production.

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Abbreviations

The following abbreviations are used in this manuscript:

ACC	Acetyl-CoA carboxylase
ACTIN	Endogenous reference gene β-actin
ADFI	Average daily feed intake
ADG	Average daily gain
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine Triphosphate
CAT	Catalase
CF	Crude fiber
CP	Crude protein
EDTA	Ethylenediaminetetraacetic acid
EE	Ether extract
F/G	Feed-to-gain ratio
FAS	Fatty acid synthase
GAA	Guanidinoacetic acid
GSH-Px	Glutathione peroxidase
HDL	High-density lipoprotein
HSL	Hormone-sensitive lipase
LDL	Low-density lipoprotein
MDA	Malondialdehyde
NRC	National Research Council
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity

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