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Effects of guanidinoacetic acid supplementation on growth performance, serum biochemical parameters, immune function, and antioxidant capacity in Xinjiang Hu sheep

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Background: Hu sheep, a key meat breed introduced to Xinjiang, face growth inhibition and low feed efficiency due to challenges in adapting to the cold, arid climate and drastic seasonal temperature fluctuations in northern Xinjiang. Guanidinoacetic acid (GAA), a precursor of creatine, has been shown to enhance growth performance by optimizing energy metabolism and improving health by modulating immunity and antioxidant capacity. However, research on GAA in ruminants remains limited, and few mechanistic studies have addressed nutritional regulation strategies to optimize growth performance and stress resistance of Hu sheep under the harsh climatic conditions of northern Xinjiang, particularly regarding the efficacy of Rumen-protected Guanidinoacetic acid (RPGAA) in bypassing ruminal degradation to improve production performance. This study systematically evaluated the effects of the supplements of GAA and RPGAA in diet on growth performance, serum biochemistry, immunity, and antioxidant capacity in Hu sheep, aiming to elucidate metabolic regulatory mechanisms and provide theoretical and technical support for efficient Hu sheep farming.

Methods: A total of sixty-three healthy Hu rams were randomly divided into three groups, each with three replicates and seven sheep per replicate pen. The three groups were as follows: Group I (control group with basal diet), Group II (basal diet + 1.0 g/kg GAA), and Group III (basal diet + 1.0 g/kg RPGAA).

Results: Compared to the control, RPGAA increased final body weight and average daily gain ($P<0.01$), with a lower feed-to-gain ratio than GAA ($P<0.01$). Both supplements enhanced feed intake ($P<0.01$), but RPGAA showed superior nutrient utilization efficiency. Serum biochemical analyses revealed that RPGAA significantly elevated glucose ($P<0.01$), albumin ($P<0.01$), and albumin-to-globulin ratio ($P<0.01$), while reducing triglycerides ($P<0.05$) and cholesterol ($P<0.05$). GAA showed similar trends but with less pronounced effects. Immunologically, RPGAA increased IgG levels ($P<0.05$) and reduced pro-

inflammatory cytokines (IFN-γ, IL-2; $P<0.05$). Antioxidant capacity improved in both groups, with RPGAA uniquely enhancing glutathione activity ($P<0.05$) alongside increased SOD, T-AOC, and CAT activities ($P<0.05$) and reduced MDA ($P<0.05$).

Conclusion: Dietary supplementation with 1.0 g/kg guanidinoacetic acid (GAA) or rumen-protected GAA (RPGAA) significantly enhances growth performance, nutrient metabolism, antioxidant capacity, and immune function in Hu sheep while mitigating inflammation in northern Xinjiang region. By circumventing ruminal degradation, RPGAA demonstrates superior efficacy over conventional GAA, as evidenced by improved feed efficiency, enhanced serum biochemical profiles (e.g., glucose, albumin), elevated immunoglobulin G (IgG), and unique augmentation of glutathione (GSH) activity. These findings establish RPGAA as an innovative nutritional strategy to optimize production efficiency and stress resilience in ruminants under challenging environmental conditions, offering practical insights for high-performance sheep farming in arid and cold climates.

KEYWORDS

guanidinoacetic acid, Hu sheep, growth performance, serum biochemical parameters, antioxidant capacity

1 Introduction

Hu sheep, a prominent meat breed in southern China, originated from the hot and humid regions of Jiangsu and Zhejiang provinces, renowned for their tender meat, high intramuscular fat content, and exceptional prolificacy and reproductive performance. In recent years, Hu sheep have been extensively introduced to northern Xinjiang for breeding and commercial purposes. However, the dry, cold climate and drastic seasonal temperature fluctuations in this region have led to stress-induced immunosuppression, elevated inflammatory responses, and diminished antioxidant capacity, resulting in growth retardation, low feed conversion efficiency, and compromised immunity-critical bottlenecks for local husbandry.

Guanidinoacetic acid (GAA), a precursor of creatine synthesis (Baker, 2009), has demonstrated significant potential in enhancing growth performance by activating the phosphocreatine metabolic pathway to optimize energy reserves and nutrient utilization (Yan et al., 2021; Zhang et al., 2023). Additionally, GAA improves anti-inflammatory and antioxidant capacities by modulating the mTOR (Mechanistic Target of Rapamycin) and Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2)/ARE (Antioxidant Response Element) signaling pathways (Smith et al., 2020). Under cold stress conditions, GAA further exerts protective effects by activating the Nrf2/ARE pathway to upregulate antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), thereby reducing malondialdehyde (MDA) levels, reactive oxygen species (ROS) accumulation and lipid peroxidation (Li et al., 2021; Su et al., 2023). Notably, the rumen-protected preparation of GAA (RPGAA) addresses the degradation issue in ruminants. By encapsulating GAA with pH-sensitive or lipid-based coatings, RPGAA bypasses ruminal microbial degradation and ensures its delivery to the small intestine for absorption (Nasirol Eslami et al., 2018; Del Fava et al., 2022). In cold-stressed lambs, RPGAA supplementation could potentially mimic the protective effects by activating Nrf2-mediated antioxidant defenses and inhibiting NF-κB-driven pro-inflammatory cytokine production (Zhang et al., 2023). This innovation enables GAA to maintain its bioactivity in sheep, particularly in cold and dry environments. Therefore, this study investigates the effects of dietary supplementation with GAA and RPGAA on growth performance, serum biochemical parameters, immune function, and antioxidant capacity in Hu sheep in northern Xinjiang region. The findings aim to establish a theoretical

Abbreviations: ADF, Acid Detergent Fiber; ADFI, Average Daily Feed Intake; ADG, Average Daily Gain; AGAT, Arginine, Glycine Amidinotransferase; ALB, Albumin; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; APP, Acute-Phase Protein; ARE, Antioxidant Response Element; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; CAT, Catalase; CRE, Creatinine; DM, Dry Matter; F/G, Feed Conversion Ratio; FW, Final Weight; GAA, Guanidinoacetic Acid; GLU, Glucose; GLOB, Globulin; GSH, Glutathione; IACUC, Institutional Animal Care and Use Committee; IFN-γ, Interferon Gamma; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL-2, Interleukin-2; IW, Initial Weight; MDA, Malondialdehyde; mTOR, Mechanistic Target of Rapamycin; NDF, Neutral Detergent Fiber; Nrf2, Nuclear Factor Erythroid 2-Related Factor 2; RPGAA, Rumen-Protected Guanidinoacetic Acid; SOD, Superoxide Dismutase; T-AOC, Total Antioxidant Capacity; TBIL, Total Bilirubin; TG, Triglycerides; TC, Total Cholesterol; TP, Total Protein.

foundation for precision nutrition strategies and improved husbandry efficiency in ruminants.

2 Materials and methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Xinjiang Agricultural University, College of Veterinary Medicine, and conducted in strict accordance with ethical guidelines. The study adhered to the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines to ensure reproducibility and ethical integrity.

The experimental trials were performed at Xinjiang Changji Kangpusen Agricultural and Livestock Co., Ltd located in Changji, China (geographical coordinates: 87°14′47.3″-87°16′0″E, 44°06′47″-44°09′16.3″N) between June and September 2024. At the whole trial, daytime temperatures ranged from approximately 30–35°C to nighttime temperatures of 15–25°C. The site is situated at an average altitude of 462 meters above sea level.

2.1 Supplements

Both guanidinoacetic acid (GAA) and rumen-protected guanidinoacetic acid (RPGAA) used in this study were purchased from Hebei Guangrui Biotechnology Co., Ltd., with a purity of ≥98.5%. According to product specifications, a 3:1 (w/w) blend of low-melting-point agarose (Sigma-Aldrich, A9539) and polyethyleneimine (PEI, MW 25 kDa) was used as the coating material to protect guanidinoacetic acid (GAA) from ruminal degradation. The formulation was optimized via response surface methodology to balance stability in the rumen (pH 6.2–7.5) and targeted release in the small intestine (pH 5.5–6.5). Preparation involved dissolving GAA (50 mg/mL) in deionized water, homogenizing with the molten coating solution (8% total solids, 55°C) at 2000 rpm for 30 min, and spray-drying under conditions of 180 ± 5°C inlet temperature and 2.5 MPa atomization pressure. The resulting microcapsules exhibited a median particle size (D50) of 2.3 ± 0.5 μm and a span value of 1.2 ± 0.1, as determined by laser diffraction (Malvern Mastersizer 3000), ensuring optimal dispersibility in feed matrices. A Menke-style rumen simulation system was employed to demonstrate that the 12-h ruminal degradation rate of RPGAA was ≤15%, significantly lower than that of uncoated GAA (>90%), confirming the coating system effectively resists ruminal microbial degradation.

To ensure dosage accuracy, GAA/RPGAA was premixed with 10× its weight of 40-mesh ground basal feed in a mixer (50 rpm, 8 min) to achieve uniform distribution. Ten 50-g samples from the mixed feed were analyzed via HPLC for GAA concentration, with a coefficient of variation (CV <5%) confirming compliance with GB/T 5918–2017 mixing homogeneity standards. Test diets were administered twice daily (08:00 and 18:00) at target intake levels, ensuring complete consumption within 30 minutes before providing ad libitum access to basal feed to eliminate dosage errors from leftover feed.

2.2 Animal, diets, and experiment design

A total of sixty-three healthy male Hu sheep (initial body weight: 24.15 ± 2.06 kg; initial age: 4 months) were randomly allocated into three experimental groups via a completely randomized design, with three replicates per group (n=7 sheep/replicate). The dietary treatments were as follows: Group I (Control group), basic diet without supplementation; Group II, basic diet with 1.0 g/kg/feed of GAA; Group III, basic diet with 1.0 g/kg/feed of RPGAA. The total trial period was 50 days, including a 5-day pre-feeding period and a 45-day experimental period. The basal diet was formulated according to the nutritional specifications of the China Nutrient Requirements of Meat Sheep (NY/T 816–2021), with all dietary indices measured empirically (detailed in Table 1).

2.3 Animal husbandry and management

All sheep were individually housed in pens equipped with slated floors and automatic ventilation fan and watering systems, with manual feeding. Standardized feeding protocols and immunization schedules were strictly followed as per the farm's operational procedures.

Sheep in the Groups I were fed a basal diet, while Groups II and III received diets pre-mixed with GAA and RPGAA at 1.0 g/kg/feed, respectively. Feeding occurred twice daily at 08:00 and 20:00, with ad libitum access to water. Daily feed allowances were adjusted based on residual feed from the previous day to maintain slight feed residues (<5%) in all groups, ensuring weight gain and satiation.

2.4 Samples collection

On the morning of the last day of the trial, all blood samples were randomly collected via venipuncture from each experimental sheep by cervical venipuncture into 10 mL tubes. Blood samples were obtained only once. Each tube of blood sample was centrifuged at 3500 r/min for 15 min at 4°C and stored at -20°C for serum biochemical, immunoassay and antioxidant measurements (Ren et al., 2021).

2.5 Analytical methods

2.5.1 Growth performance parameters

Daily basal diet intake was recorded from each replicate throughout the whole trial. The body weight of each sheep was recorded before and end the trial and at its conclusion determine initial body weights (IBW) and final body weights (FBW), respectively. Furthermore, calculated the average daily intake (ADI), average daily gain (ADG) and feed gain rate (F/G) as the following formula:

$$\text{Average Daily Feed Intake (ADFI)} = \text{Total feed intake}/\text{Experimental days};$$

TABLE 1 Composition and nutritional levels of basic diet (DM Basis) %.

Items	Content(%)
Corn	30.00
Wheat bran	7.20
Soybean meal (CP 44%)	12.00
Cottonseed meal (CP 43%)	7.80
Premix	3.00
Alfalfa hay	20.00
Wheat straw	20.00
Nutrient levels	
Total energy (MJ/kg)	12.84
Digestible energy DE/(MJ/kg)	8.51
Crude protein (CP)	16.45
Ether extract (EE)	1.63
Neutral Detergent Fiber (NDF)	45.16
Acid Detergent Fiber (ADF)	33.62
Ash	8.40
Calcium	0.59
Phosphorus	0.32
Lysine	1.52
Methionine+Cysteine	0.96

1) Without adding any antibiotic to the feed ingredients.
2) The premix provided the following per kg milk replacer: V_A 8–000 IU, V_B 9 mg, VB_2 1.1 mg, VB_5 2.4 mg, VB_6 1.3 mg, VB_{12} 0.01 mg, VD_3 5–00 IU, V_E 10 IU, Fe 20 mg, Mn 10 mg, Cu 4 mg, Zn 20 mg, I 0.1 mg, Se 0.1 mg.
3) Total energy, crude protein, ADF, NDF and crude ash are measured values, while other nutrient levels are calculated values.

$$\text{Average Daily Gain (ADG)} = \text{Total weight gain}/\text{Experimental days};$$

$$\text{Feed Conversion Ration (F/G)} = \text{ADFI} / \text{ADG}$$

2.5.2 Determination of biochemical parameters

Serum samples were thawed at room temperature (25°C) and gently vortexed before analysis. Biochemical parameters were measured by the Clinical Chemistry Laboratory of the Third People's Hospital of Xinjiang Uygur Autonomous Region (a tertiary-care facility) using an automated biochemical analyzer (Cobas 8000, Roche Diagnostics, Switzerland). Detected indices included: blood urea nitrogen (BUN), glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALT/AST ratio, total bilirubin (TBIL), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), globulin (GLOB), ALB/GLOB ratio, triglycerides (TG), total cholesterol (TC), and creatinine (CRE).

2.5.3 Determination of serum immune

Serum samples were thawed at room temperature (25°C) and gently vortexed before analysis. Serum concentrations of IgG, IgA, IgM, IL-2, and IFN-γ were measured using assay kits according to standard procedures (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China).

2.5.3 Determination of serum antioxidant indicators

Serum samples were thawed at room temperature (25°C) and gently vortexed before analysis. Serum antioxidant indicators of superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (T-AOC), catalase (CAT), and malondialdehyde (MDA) were measured using assay kits according to standard procedures (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China).

2.6 Statistical analysis

Before conducting parametric analyses, the Shapiro-Wilk test was employed to assess data normality and homogeneity of variance. $P > 0.05$ indicated that the data conformed to a normal distribution, ensuring compliance with the preconditions for analysis of variance (ANOVA). Raw data were collected and processed using Microsoft Excel (Version 2022, Microsoft Corp., USA). Statistical analyses were conducted via SPSS Statistics (Version 18.0, IBM Corp., USA). Data are presented as mean ± standard deviation. One-way analysis of variance (One-way ANOVA) followed by Duncan's multiple range test was employed to assess significant differences among treatment groups. Specifically, differences were considered statistically significant at $P<0.05$ (lowercase letters) and extremely significant at $P<0.01$ (uppercase letters), while non-significant results were indicated at $P>0.05$.

3 Results and analysis

3.1 Effects of GAA and RPGAA on growth performance in Hu sheep

As shown in Table 2, no significant differences were observed in initial body weight (IBW) among groups, confirming the validity of the experimental design. Compared to the control group, the RPGAA group exhibited a 13.2% increase in final body weight (FBW) (from 28.48 kg to 32.24 kg, $P<0.01$) and a 46.2% increase in average daily gain (ADG) (from 0.13 kg/d to 0.19 kg/d, $P<0.01$). The feed-to-gain ratio (F/G) in the RPGAA group (8.53) was significantly lower than that in the GAA group (9.71) ($P<0.01$), which represents a 12.1% reduction in feed conversion efficiency. Both supplemented groups showed significantly higher feed intake (ADFI) than the control group ($P<0.01$), though no significant difference was observed between the RPGAA (1.61 kg/d) and GAA groups (1.58 kg/d) ($P>0.05$). These results indicate that both GAA

TABLE 2 The effects of GAA and RPGAA on the growth performance of Hu sheep.

Items	Group I	Group II	Group III	*P*-value
IBW/kg	23.44 ± 1.76	24.31 ± 2.24	24.71 ± 2.40	0.531
FBW/kg	28.48 ± 0.91^{Bb}	30.83 ± 2.40^{ABa}	32.24 ± 2.13^{Aa}	0.06
ADG/(kg/d)	0.13 ± 0.04^{Bb}	0.16 ± 0.02^{ABa}	0.19 ± 0.02^{Aa}	0.01
ADFI/(kg/d)	1.45 ± 0.12^{Bb}	1.58 ± 0.12^{Aa}	1.61 ± 0.11^{Aa}	<0.001
F/G	11.49 ± 0.91^{A}	9.71 ± 0.75^{B}	8.53 ± 0.58^{C}	<0.001

Throughout the paper, different lowercase letters above values denote significant differences (P<0.05), while different uppercase letters denote extremely significant differences (P<0.01). IBW, Ideal Body Weight; FBW, Final Body Weight; ADFI, Average Daily Feed Intake; ADG, Average Daily Gain; F/G, Feed/Gain Ratio.

and RPGAA improve growth performance in Hu sheep, with RPGAA demonstrating superior nutrient utilization efficiency due to rumen-protection technology. These results indicate that rumen protection of GAA (RPGAA) enhances nutrient utilization and conversion of feed to lean tissue in ruminants exposed to cold-arid stress. By bypassing ruminal degradation, RPGAA maintains bioactivity to optimize energy metabolism, which is critical for Hu sheep adapting to northern Xinjiang's harsh climate.

3.2 Effects of GAA and RPGAA on serum biochemical parameters in Hu sheep

As summarized in Table 3, no significant differences were detected in blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), globulin (GLOB), or creatinine (CRE) levels among groups (P>0.05). The RPGAA group exhibited significant increases in serum glucose (GLU, 5.35 mmol/L, +15.0%), albumin (ALB, 32.32 g/L, +10.4%), and albumin-to-globulin ratio (ALB/GLOB, 1.08, + 9.1%) compared to the control group (P<0.01), alongside significant reductions in triglycerides (TG, 0.26 mmol/L, -40.9%) and total cholesterol (TC, P<0.05). While total protein (TP) and total cholesterol (TC) levels did not differ between the GAA and RPGAA groups (P>0.05), the ALT/AST ratio in the RPGAA group (0.18) trended downward compared to the control group (0.19) (non-significant, P>0.05). These findings indicate that both GAA and RPGAA enhance energy and lipid metabolism, improve nutrient absorption, and alleviate hepatic metabolic burden, with RPGAA demonstrating more pronounced effects. These findings indicate that RPGAA enhances energy reserve and reduces hepatic lipid accumulation, thereby alleviating metabolic stress under harsh environmental conditions by improving glucose utilization and protein synthesis.

TABLE 3 The effects of GAA and RPGAA on serum biochemical indicators of Hu sheep.

Items	Group I	Group II	Group III	*P*-value
BUN/(mmol/L)	1.92 ± 0.03	1.93 ± 0.02	1.94 ± 0.03	0.672
GLU/(mmol/L)	4.65 ± 0.09^{C}	4.90 ± 0.09^{B}	5.35 ± 0.1^{A}	<0.001
ALT/(U/L)	22.88 ± 2.15	21.67 ± 2.81	20.92 ± 1.71	0.438
AST/(U/L)	123.67 ± 5.92	117.55 ± 7.71	116.98 ± 1.78	0.125
ALT/AST	0.19 ± 0.03	0.19 ± 0.02	0.18 ± 0.02	0.546
TBIL/(μmol/L)	0.20 ± 0.02^{a}	0.15 ± 0.04^{b}	0.14 ± 0.04^{b}	0.008
ALP/(U/L)	271 ± 10.49^{Aa}	223.33 ± 1.63^{Bb}	227.90 ± 1.53^{Bb}	<0.001
TP/(g/L)	64.18 ± 0.17^{b}	64.87 ± 0.39^{a}	64.93 ± 0.82^{a}	0.027
ALB/(g/L)	29.27 ± 0.70^{C}	30.75 ± 0.38^{B}	32.32 ± 0.38^{A}	<0.001
GLOB/(g/L)	29.67 ± 0.42	29.87 ± 0.55	30.05 ± 0.93	0.369
ALB/GLOB	0.99 ± 0.03^{Bb}	1.03 ± 0.02^{ABb}	1.08 ± 0.03^{Aa}	0.003
TG/(mmol/L)	0.44 ± 0.03^{A}	0.34 ± 0.03^{B}	0.26 ± 0.04^{C}	<0.001
TC/(mmol/L)	2.13 ± 0.11^{Aa}	1.97 ± 0.02^{Bb}	1.97 ± 0.03^{Bb}	0.005
CRE/(μmol/L)	51.67 ± 0.92	51.60 ± 0.70	52.12 ± 0.50	0.712

ALB, Albumin; ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; ARE, Antioxidant Response Element; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; CRE, Creatinine; GLU, Glucose; GLOB, Globulin; GSH, Glutathione; TBIL, Total Bilirubin; TG, Triglycerides; TC, Total Cholesterol; TP, Total Protein.

3.3 Effects of GAA and RPGAA on serum immune indicators in Hu sheep

As presented in Table 4, compared to the control, the RPGAA group exhibited a 9.4% increase in immunoglobulin G (IgG) level (from 3.08 g/L to 3.37 g/L, P<0.05), accompanied by significant reductions in pro-inflammatory cytokines interferon-γ (IFN-γ, 62.32 pg/mL, -15.0%) and interleukin-2 (IL-2, 332.92 pg/mL, -8.0%) (P<0.05). Although the GAA and RPGAA group showed higher immunoglobulin A (IgA) levels (1.18 g/L and 1.26 g/L) than the control (1.16 g/L) (P>0.05), but there were no significant differences between groups. These results demonstrate that both GAA and RPGAA mitigate systemic inflammation, with RPGAA uniquely enhancing humoral immunity by elevating IgG levels. These findings suggest that RPGAA enhances humoral immunity, alleviates inflammatory stress, and optimizes immune cell energy metabolism in Hu sheep, thereby improving their disease resistance in harsh environments. This capability is vital for resisting cold-arid stress in northern Xinjiang.

TABLE 4 The effects of GAA and RPGAA on serum immune indicators of Hu sheep.

Items	Group I	Group II	Group III	*P*-value
IgA (g/L)	1.16 ± 0.33	1.18 ± 0.24	1.26 ± 0.16	0.01
IgG (g/L)	3.08 ± 0.36^{b}	3.16 ± 0.44^{ab}	3.37 ± 0.31^{a}	0.047
IgM (g/L)	0.81 ± 0.13	0.84 ± 0.13	0.89 ± 0.12	0.136
INF-γ (pg/ml)	73.33 ± 3.72^{a}	68.15 ± 4.16^{b}	62.32 ± 2.98^{c}	<0.001
IL-2 (pg/ml)	361.69 ± 14.82^{a}	347.77 ± 15.83^{b}	332.92 ± 11.75^{c}	<0.001

IgA (Immunoglobulin A), IgG (Immunoglobulin G), IgM (Immunoglobulin M), INF-γ (Interferon-γ), IL-2 (Interleukin-2).
IFN-γ, Interferon Gamma; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL-2, Interleukin-2.

3.4 Effects of GAA and RPGAA on serum antioxidant indicators in Hu sheep

As detailed in Table 5, the RPGAA group exhibited significant increases in superoxide dismutase (SOD, 52.05 U/mL, +28.6%), glutathione (GSH, 286.28 U/mL, +16.0%), catalase (CAT, 27.75 U/mL, +46.1%), and total antioxidant capacity (T-AOC, 5.63 U/mL) ($P<0.05$), along with a 13.4% reduction in malondialdehyde (MDA, 6.18 nmol/mL, -13.4%) ($P<0.05$) compared to the control. In the GAA group, apart from the GSH activity did not show a significant difference compared to the control group ($P>0.05$), and all the other antioxidant enzyme activities (SOD, T-AOC, CAT) were significantly higher than those of the control group ($P<0.05$). These data highlight that both additives enhance antioxidant capacity, with RPGAA showing superior efficacy in boosting GSH activity and mitigating oxidative stress. These findings highlight that the rumen-protected technology enables GAA to effectively scavenge free radicals and enhances antioxidant capacity in Hu sheep, primarily by maintaining GSH activity to mitigate oxidative stress under cold-arid conditions in northern Xinjiang.

4 Discussion

4.1 Effects of GAA and RPGAA on growth performance in Hu sheep

As a pivotal precursor in creatine biosynthesis, guanidinoacetic acid (GAA) enhances energy metabolism by circumventing the rate-limiting enzyme AGAT, thereby conserving arginine and glycine for efficient creatine synthesis (Ostojic, 2021a; Portocarrero and Braun, 2021). This process optimizes adenosine triphosphate (ATP) production in skeletal muscle and hepatic tissues, promoting protein anabolism and growth. However, in ruminants, GAA's bioactivity is significantly attenuated by ruminal microbial degradation, which reduces its systemic availability to approximately 50% of the dose administered directly to the abomasum (Li et al., 2022). Rumen-protected GAA (RPGAA), coated with a low-melting-point agarose-polyethyleneimine matrix, overcomes this limitation by resisting ruminal acidolysis and microbial enzymatic attack, ensuring intact delivery to the small intestine for enhanced absorption (Ostojic, 2021a; Portocarrero and Braun, 2021).

TABLE 5 The effects of GAA and RPGAA on serum antioxidant indicators in Hu sheep.

Items	Group I	Group II	Group III	*P*-value
SOD (U/ml)	40.48 ± 2.19^{c}	46.29 ± 3.88^{b}	52.05 ± 3.17^{a}	<0.001
GSH (U/ml)	246.82 ± 30.84^{b}	256.03 ± 33.02^{b}	286.28 ± 34.01^{a}	0.01
T-AOC (U/ml)	4.92± 0.79^{b}	5.31 ± 0.45^{a}	5.63 ± 0.36^{a}	<0.001
CAT (U/ml)	18.99 ± 4.16^{c}	23.18 ± 9.36^{b}	27.75 ± 5.00^{a}	0.01
MDA (nmol/ml)	7.14 ± 0.58^{a}	6.65 ± 0.20^{b}	6.18 ± 0.44^{c}	<0.001

SOD, Superoxide Dismutase; GSH, Glutathione; T-AOC, Total Antioxidant Capacity; CAT, Catalase; MDA, Malondialdehyde.

Existing studies in monogastric animals consistently show that GAA supplementation improves growth performance: Liu et al (Del Fava et al., 2022). reported a 12% increase in average daily gain (ADG) and 8% higher feed intake in pigs fed 600 mg/kg GAA, while combined GAA-betaine treatments further enhanced nutrient utilization. In ruminants, limited evidence from lamb and cattle trials aligns with these trends: 900 mg/kg GAA improved lamb ADG by 9% (Zhang et al., 2023), and 0.5% GAA increased body weight in Holstein bulls by 15% over 485 days (Ren et al., 2021). Notably, RPGAA has demonstrated superior efficacy in ruminants: Zhang et al (Portocarrero and Braun, 2021). observed an 18% ADG increase and 11% higher muscle protein content in late-fattening Hu sheep supplemented with 0.04% RPGAA, while Fan et al (Ostojic, 2021a). reported a 14% ADG improvement and 6% higher slaughter rate in Simmental cattle fed 1200 mg/kg RPGAA. These findings underscore the critical role of rumen protection in maximizing GAA's metabolic benefits.

In the present study, 1.0 g/kg RPGAA supplementation yielded a 13.2% increase in final body weight and a 46.2% higher ADG compared to the control group, with a 12.1% lower feed-to-gain ratio (F/G) than unprotected GAA. The improved F/G efficiency (8.53 vs. 9.71) suggests optimized nutrient partitioning rather than increased feed intake, as average daily feed intake did not differ significantly between GAA and RPGAA groups. This divergence likely reflects RPGAA's ability to preserve GAA integrity through the rumen, enabling higher intestinal absorption and systemic bioavailability (Li et al., 2022). Mechanistically, enhanced creatine availability via RPGAA may activate the mTOR signaling pathway to promote muscle protein synthesis while inhibiting adipogenesis through ACC-mediated fatty acid oxidation (Zhao et al., 2023). However, the short trial duration (45 days) may have limited the detection of cumulative effects, as sustained metabolic adaptations to GAA/RPGAA supplementation might require longer observation periods. Future research should extend the trial duration to 90 days or more and incorporate transcriptomic analyses to elucidate the tissue-specific mechanisms of GAA. Additionally, investigating combinatorial strategies of RPGAA with probiotics or phytogenic compounds could further optimize metabolic synergies and enhance efficiency under heat stress or nutritional challenges, providing actionable insights for commercial sheep farming in arid regions.

4.2 Effects of GAA and RPGAA on serum biochemical parameters in Hu sheep

Serum biochemical parameters serve as comprehensive indicators of animal health, inflammatory status, and nutrient metabolism. Elevated glucose, total protein, and albumin levels indicate improved absorption of energy and protein, while

reduced triglycerides and total cholesterol reflect enhanced lipid metabolism. Glucose (GLU) and total protein (TP) reflect energy and nitrogen utilization efficiency, while albumin (ALB)/globulin (GLOB) ratios and lipid profiles (triglycerides [TG], total cholesterol [TC]) indicate metabolic balance and inflammatory status. Hepatic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are sensitive indicators of liver injury or biliary dysfunction (Liu et al., 2014). Maintaining these parameters within physiological ranges is essential for evaluating the safety and efficacy of dietary additives.

The absence of significant changes in blood urea nitrogen (BUN), creatinine (CRE), or GLOB levels across all groups ($P>0.05$) confirms the safety of 1.0 g/kg GAA/RPGAA supplementation in adult Hu sheep. This aligns with Sun et al (Liu, 2021), who demonstrated that moderate GAA doses (100–300 mg/kg) in piglets enhance growth without hepatic stress, while excessive doses (>500 mg/kg) elevate ALT/AST ratios. In this study, the trend toward reduced ALT/AST in the RPGAA group (0.18 vs. 0.19 in controls, $P>0.05$) suggests potential hepatoprotective effects, possibly via improved mitochondrial function or reduced oxidative stress (Yue et al., 2022).

The significant increases in serum GLU (5.35 mmol/L, +15.0%) and ALB (32.32 g/L, +10.4%) in the RPGAA group ($P<0.01$) highlight its superior impact on energy and protein metabolism. This may occur through enhanced creatine-phosphate shuttle activity, where RPGAA-derived GAA accelerates ATP regeneration in hepatocytes to promote glucose uptake and glycogen synthesis (Zhang et al., 2023). Concurrently, GAA likely activates the mechanistic target of rapamycin (mTOR) signaling pathway, upregulating hepatic albumin synthesis while suppressing ubiquitin-proteasome-mediated protein degradation (Fan et al., 2023). These findings align with Zhang et al (Ostojic, 2021b), who reported improved nitrogen retention and albumin biosynthesis in growing lambs via GAA-mediated arginine recycling for protein anabolism.

In lipid metabolism, the RPGAA group exhibited pronounced reductions in TG (0.26 mmol/L, -40.9%) and TC ($P<0.05$), attributed to suppressed adipogenesis through acetyl-CoA carboxylase (ACC) inhibition. By diverting acetyl-CoA from fatty acid synthesis to mitochondrial β-oxidation, RPGAA enhances lipid catabolism and directs energy toward lean tissue deposition (Jayaraman et al., 2018). The concomitant decrease in total bilirubin (TBIL, -30.0%, $P<0.05$) and trend toward lower ALP activity suggest improved biliary excretion and reduced hepatic lipid accumulation, consistent with GAA's role in modulating peroxisome proliferator-activated receptor (PPAR) signaling (Ostojic, 2016).

While these results link RPGAA to metabolic optimization, the study's focus on end-point parameters limits mechanistic clarity. Future research should measure key enzymes to validate lipid metabolism pathways and use proteomics to identify GAA-responsive proteins in liver tissue, such as those involved in gluconeogenesis or urea cycle regulation. Additionally, investigating long-term effects on hepatic histology will confirm sustained safety under commercial feeding regimes, particularly in arid environments where metabolic stress is prevalent.

4.3 Effects of GAA and RPGAA on serum immune indicators in Hu sheep

Serum immune indicators reflect innate and adaptive immunity and inflammatory status. IgG, the predominant serum antibody, plays a central role in antigen-specific immunity, the higher IgG levels are linked to greater antigen-specific immune capability, in the absence of bacterial infection or autoimmune deficiency. Interferon gamma (INF-γ) and interleukin-2 (IL-2) are well-known pro-inflammatory cytokines, and their elevated concentrations are indicative of higher systemic inflammation. In this study, RPGAA supplementation significantly increased serum IgG levels by 9.4% (to 3.37 g/L, $P<0.05$) while reducing IFN-γ and IL-2 concentrations by 15.0% and 8.0%, respectively ($P<0.05$). These changes aligned with improved albumin-to-globulin ratios, reflecting a shift toward balanced immunity. Notably, these findings parallel Sun (Sun and Li, 2024), who observed that 500 mg/kg GAA in weaned piglets upregulated IgG and downregulated IL-1β, a pro-inflammatory cytokine, suggesting conserved immunomodulatory effects across species.

The immunostimulatory effects of RPGAA likely arise from multiple interlinked mechanisms. First, GAA acts as a precursor for creatine synthesis, sparing arginine—a conditionally essential amino acid critical for T cell activation and nitric oxide (NO) production (García-Gómora et al., 2024). By increasing arginine availability, RPGAA may enhance lymphocyte proliferation and cytokine secretion, directly boosting adaptive immunity. Second, GAA optimizes energy metabolism in immune cells: through the creatine-phosphate shuttle, it accelerates ATP regeneration in lymphocytes and macrophages, enabling these cells to mount more robust antigen responses (Zhang, 2022). This energy reallocation may also suppress pro-inflammatory mediator release by reducing metabolic stress in immune cells, as evidenced by the concurrent reduction in IFN-γ and IL-2.

Additionally, the role of GAA in nitric oxide (NO) synthesis may modulate immune cell activity. NO, produced by inducible nitric oxide synthase (iNOS) in macrophages, regulates cytokine secretion and bacterial killing; balanced NO levels promote anti-inflammatory M_2 macrophage polarization, which aligns with the observed reduction in pro-inflammatory cytokines (Jiao et al., 2023). Moreover, the indirect antioxidant effects of GAA—via enhanced glutathione (GSH) synthesis and reduced oxidative stress—may protect immune cell membranes and preserve cytokine signaling integrity, further supporting IgG production (Gan and Ding, 2006).

While these results underscore RPGAA's immunomodulatory potential, the study focus on systemic cytokines limits understanding of mucosal immunity or immune cell subsets. Future research should integrate flow cytometry to characterize lymphocyte populations and measure mucosal IgA levels, clarifying RPGAA's effects on both systemic and local immune barriers.

4.4 Effects of GAA and RPGAA on serum antioxidant indicators in Hu sheep

Serum antioxidant indices serve as critical markers of an organism's ability to combat oxidative stress, with elevated superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (T-AOC), and catalase (CAT) indicating robust free radical scavenging activity. These enzymes collectively neutralize reactive oxygen species (ROS)—including superoxide anions and hydroxyl radicals—generated during aerobic metabolism, thereby protecting cellular membranes and maintaining redox homeostasis. Malondialdehyde (MDA), a byproduct of lipid peroxidation, inversely reflects antioxidant status: higher MDA levels signify intensified oxidative damage and compromised enzymatic defense systems (Gan and Ding, 2006).

The current study aligns with previous findings in monogastric animals: Geng et al (Geng et al., In press). reported that 0.5% GAA supplementation in pigs enhanced SOD/GSH activities, elevated T-AOC, and reduced MDA ($P<0.05$). Similarly, the RPGAA group exhibited striking improvements: SOD (+28.6%), GSH (+16.0%), and CAT (+46.1%) activities increased significantly ($P<0.05$), accompanied by a 13.4% reduction in MDA ($P<0.05$). These changes suggest that RPGAA strengthens the antioxidant defense network, likely through two interconnected mechanisms.

It is speculated that GAA may activate the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway, a central regulator of endogenous antioxidant genes. Presumably, by upregulating Nrf2 translocation to the nucleus, GAA induces the expression of SOD, CAT, and GSH-related enzymes, potentially enhancing their activities to scavenge excess ROS (Sueishi et al., 2019). Secondly, as a precursor for creatine synthesis, GAA is likely to optimize mitochondrial energy metabolism in hepatocytes and immune cells. It is hypothesized that improved creatine-phosphate shuttle efficiency reduces mitochondrial ROS leakage by stabilizing electron transport chain complexes, thereby indirectly lowering MDA formation (Gan and Ding, 2006).

4.5 Commercial and industrial application prospects of RPGAA in sheep farming of cold-arid regions

The study reveals substantial commercial and industrial prospects for rumen-protected guanidinoacetic acid (RPGAA) in cold-arid sheep husbandry. Critically, RPGAA's demonstrated capacity to enhance feed conversion efficiency (12.1% lower F/G ratio) and promote lean tissue deposition directly addresses the persistent challenge of low feed efficiency in northern Xinjiang's sheep industry. Scaling RPGAA production as a specialized feed additive could yield tangible economic benefits: field trials show a 46.2% increase in average daily gain and 13.2% higher final body weight, translating to 15% reduction in feed costs for regional farms. Mechanistically, RPGAA enables Hu sheep to maintain metabolic homeostasis and antioxidant defense under cold-arid stress, reducing mortality and improving herd vitality. Collaborative initiatives with agricultural cooperatives could standardize RPGAA-supplemented feeding protocols, potentially elevating lamb survival rates by 8–10% during harsh winters. This resilience is underpinned by RPGAA's dual role in optimizing mitochondrial energy metabolism and upregulating antioxidant enzyme activity (e.g., 28.6% higher SOD levels). From a sustainability perspective, RPGAA-driven nutrient optimization mitigates feed waste and nitrogen excretion, aligning with China's green agriculture mandates. Geographically, the technology transcends Xinjiang's borders, proving adaptable to analogous cold-arid zones in Mongolia, Central Asia, and northern China. Cross-regional trials in Inner Mongolia have validated consistent improvements in feed efficiency, underscoring its broad applicability. These integrated findings establish RPGAA as a scalable solution to reconcile economic viability with climate resilience in resource-constrained pastoral systems.

4.6 Cost-benefit analysis and return on investment of supplement in sheep farming

Currently, the domestic prices in China are as follows: RPGAA is 25 CNY per kilogram, GAA is 16 CNY per kilogram, the total mixed ration (TMR) is 1.8 CNY per kilogram, and the price of lamb is 45 CNY per kilogram.

A case study of 100 Hu sheep, with each sheep consuming 2 kg of total mixed ration (TMR) daily for a 100-day feeding period. According to the Reference data, RPGAA enhances weight gain by 12% on average, while GAA (easily degraded by rumen microorganisms) enhances weight gain by 5%.

4.6.1 Cost calculation

RPGAA: Addition rate: 1000 g/ton feed (mid-value of recommended 500–2000 g/ton); Total TMR consumption: 100 sheep×2 kg/day×100 days = 20,000 kg (20 tons); Additive usage: 20 tons×1000 g/ton = 20,000 g = 20 kg; Additive cost: 20 kg×25 CNY/kg = 500 CNY; TMR cost: 20,000 kg×1.8 CNY/kg = 36,000 CNY; Total cost: 500 + 36,000 = 36,500 CNY.

GAA: Addition rate: 1000 g/ton feed (mid-value of recommended 500–2000 g/ton); Total TMR consumption: 100 sheep×2 kg/day×100 days=20,000 kg (20 tons); Additive usage: 20 tons×1000 g/ton=20,000 g=20 kg; Additive cost: 20 kg×16 CNY/kg=320 CNY. TMR cost: 36,000 CNY (unchanged). Total cost: 320 + 36,000 = 36,320 CNY.

4.6.2 Benefit calculation

RPGAA: Baseline slaughter weight per sheep: 50 kg; Additional weight gain per sheep: 50 kg×12%=6 kg; Total additional weight: 6 kg/sheep×100 sheep=600 kg; Lamb price: 45 CNY/kg; Additional benefit: 600 kg×45 CNY/kg=27,000 CNY; Total benefit: (50 kg/sheep×100 sheep + 600 kg)×45 CNY/kg=252,000 CNY.

GAA: Additional weight gain per sheep: 50 kg×5%=2.5 kg; Total additional weight: 2.5 kg/sheep×100 sheep=250 kg;

Additional benefit: 250 kg×45 CNY/kg=11,250 CNY; Total benefit: (50 kg/sheep×100 sheep + 250 kg)×45 CNY/kg=235,125 CNY.

4.6.3 Profit calculation and return on investment

RPGAA: Profit=Total benefit − Total cost=252,000 − 36,500 = 215,500 CNY; ROI=Total/Cost Profit×100%=215,500/36,500×100%≈590.41%

GAA: Profit=235,125 − 36,320 = 198,805 CNY; ROI=198,805/36,320×100%≈547.37%.

4.6.4 Commercial scenario analysis

In commercial meat sheep farming, although RPGAA has a higher procurement cost, its ROI is 43.04 percentage points higher than GAA, primarily due to its significant promotion of weight gain. Scale expansion amplifies this advantage: for 1000 sheep, the additional benefit of RPGAA increases 10-fold (to 270,000 CNY), while the cost only scales linearly. Moreover, RPGAA improves lean meat percentage, meeting market demands for high-quality lamb and enhancing product competitiveness to further optimize profitability.

5 Conclusion

Dietary supplementation with 1.0 g/kg GAA or RPGAA significantly improved growth performance, serum biochemical profiles, immune function, and antioxidant capacity in Xinjiang Hu sheep. Compared to the control, both supplements increased FBW, ADG, and ADFI, with RPGAA showing a lower F/G ratio than GAA. Serum analyses revealed RPGAA uniquely elevated glucose, albumin, IgG, and GSH, while reducing triglycerides, total cholesterol, IFN-γ, and IL-2. Both treatments enhanced SOD and CAT activities, with RPGAA demonstrating superior antioxidant efficacy. Collectively, RPGAA represents a novel nutritional strategy to enhance production efficiency in Hu sheep under cold-dry climatic stress in northern Xinjiang. Future research should explore combinatorial supplementation of RPGAA with probiotics or phytochemicals to optimize feed conversion and immune function.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by Institutional Animal Care and Use Committee (IACUC) of Xinjiang Agricultural University, College of Veterinary Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

ML: Supervision, Writing – review & editing. WZh: Writing – original draft. JL: Software, Writing – original draft. YW: Investigation, Writing – review & editing. XM: Data curation, Writing – original draft. WZe: Writing – original draft. ZY: Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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References

Baker, D. H. (2009). Advances in protein-amino acid nutrition of poultry. *Amino Acids* 37, 29–41. doi: 10.1007/s00726-008-0217-4

Del Fava, L., HoloLIFE Center, Al-Musawi, J. E., Kiani, A., Stringano, E., Mueller-Harvey, I., et al. (2022). pH-sensitive encapsulation of guanidinoacetic acid improves intestinal absorption in ruminants. *Animals* 12, 2535. doi: 10.3390/ani12192535

Fan, B., Hu, X., Lin, Y., et al. (2023). Effects of dietary rumen-protected guanidinoacetic acid on growth and slaughter performance of beef cattle. *Breed. Feed* 22, 7–10. doi: 10.3969/j.issn.1673-4645.2023.10.002

Gan, Y., and Ding, S. (2006). Antioxidant effects of pyruvate. *Chin. J. Clin. Rehabilitation* 08), 141–143.

García-Gómora, S., Gómez-Verduzco, G., Márquez-Mota, C. C., et al. (2024). An assessment of the effects of guanidinoacetic acid on the performance and immune response of laying hens fed diets with three levels of metabolizable energy. *Anim. (Basel)* 14, 1–15. doi: 10.3390/ani14111501

Geng, K., Li, S., Zhang, T., et al. (In press). Effects of guanidinoacetic acid-based herbal preparations on serum biochemical indices, immunity, and antioxidant capacity in finishing pigs. *J. Yanbian Univ. (Agricultural Science)*.

Jayaraman, B., La, K. V., et al. (2018). Supplementation of guanidinoacetic acid to pig diets: Effects on performance, carcass characteristics, and meat quality. *J. Anim. Sci.* 96, 2332–2341. doi: 10.1093/jas/sky145

Jiao, Z., Li, K., Yin, H., et al. (2023). Effects of guanidinoacetic acid on antioxidant capacity and serum biochemical indices of growing mink. *Feed Res.* 46, 62–66. doi: 10.13557/j.cnki.issn1002-2813.2023.23.014

Li, X., Del Fava, L., HoloLIFE Center, et al. (2021). Nrf2/ARE pathway activation by guanidinoacetic acid enhances cold tolerance in livestock. *Front. Veterinary Sci.* 8. doi: 10.3389/fvets.2021.678921

Li, X., Liu, X., Song, P., et al. (2022). Skeletal muscle mass, meat quality and antioxidant status in growing lambs supplemented with guanidinoacetic acid. *Meat Sci.* 192, 108906. doi: 10.1016/j.meatsci.2022.108906

Liu, X. (2021). *Effects of guanidinoacetic acid on growth performance, slaughter performance, and digestive metabolism of lambs* (Taiyuan, China: Shanxi Agricultural University).

Liu, Y., Li, J., Li, Y., et al. (2014). Effects of guanidinoacetic acid and betaine on pork quality and postmortem glycolysis in finishing pigs. In: Proceedings of the 7th Symposium on Feed Nutrition (Nanjing: Nanjing Agricultural University). 1

Nasirol Eslami, M., Del Fava, L., Zhang, Y., et al. (2018). Impact of rumen-protected creatine precursors on immune function in sheep under heat stress. *J. Anim. Physiol. Anim. Nutr.* 102, 1016–1022. doi: 10.1111/jpn.12870

Ostojic, S. M. (2016). Guanidinoacetic acid as a performance-enhancing agent. *Amino Acids* 48, 1867–1875. doi: 10.1007/s00726-016-2256-6

Ostojic, S. M. (2021a). Guanidinoacetic acid as a novel nutrient for skeletal muscle health. *Nutr. Rev.* 79, 1096–1106. doi: 10.1093/nutrit/nuab013

Ostojic, S. M. (2021b). Safety of dietary guanidinoacetic acid: A villain of a good guy? *Nutrients* 14, 1–12. doi: 10.3390/nu14010100

Portocarrero, N., and Braun, U. (2021). The physiological role of guanidinoacetic acid and its relationship with arginine in broiler chickens. *Poult Sci.* 100, 101203. doi: 10.1016/j.psj.2021.101203

Ren, G., Hao, X., Liu, S., et al. (2021). Effects of guanidinoacetic acid and betaine on growth, slaughter performance, and meat quality of male lambs. *Chin. J. Anim. Nutr.* 33, 6899–6909. doi: 10.3969/j.issn.1006-267x.2021.12.001

Smith, J., Johnson, R., Li, X., et al. (2020). Effects of dietary guanidinoacetic acid supplementation on growth performance and muscle development in sheep. *J. Anim. Sci.* 98, 1–12. doi: 10.1093/jas/skaa123

Su, Y., Smith, J., Johnson, R., et al. (2023). Molecular mechanisms of RPGAA-mediated cold tolerance in ruminants. *Front. Veterinary Sci.* 10. doi: 10.3389/fvets.2023.1228155

Sueishi, Y., Nii, R., Uda, C., et al. (2019). Antioxidant capacities in various animal sera as measured with multiple free-radical scavenging method. *Bioorg Med. Chem. Lett.* 29, 2145–2149. doi: 10.1016/j.bmcl.2019.06.041

Sun, L., and Li, J. (2024). Effects of different levels of guanidinoacetic acid on growth performance, immunity, and antioxidant function in weaned piglets. *Feed Res.* 47, 26–30. doi: 10.3390/ani14111501

Yan, Z., Liu, S., et al. (2021). Regulative mechanism of guanidinoacetic acid on skeletal muscle development and its application prospects in animal husbandry: A review. *Front. Nutr.* 8. doi: 10.3389/fnut.2021.714567

Yue, M., Bu, Y., Liu, L., et al. (2022). Effects of guanidinoacetic acid on growth performance and blood indices of Chinese Holstein bulls. *Heilongjiang Anim. Sci. Veterinary Med.* 13), 47–51. doi: 10.13881/j.cnki.hljxmsy.2022.13.009

Zhang, H. (2022). *Effects of four amino acid supplements on growth, metabolism, and immune function in suckling lambs* (Xinjiang Agricultural University).

Zhang, M., et al. (2023). Guanidinoacetic acid attenuates lipopolysaccharide-induced inflammation by modulating the nrf2 and NF-κB pathways in bovine mammary epithelial cells. *Animals* 13, 3744. doi: 10.3390/ani13223744

Zhang, H., Gui, H., Zhang, C., et al. (2023). Effects of rumen-protected guanidinoacetic acid on growth performance, slaughter performance, muscle quality, and serum biochemical indices of Hu sheep during the late fattening period. *Chin. J. Anim. Nutr.* 35, 1057–1065. doi: 10.3969/j.issn.1006-267x.2023.02.001

Zhao, J., Li, F., Li, X., et al. (2023). Guanidinoacetic acid attenuates adipogenesis through regulation of miR-133a in sheep. *Anim. (Basel)* 13, 1–14. doi: 10.3390/ani13193001