Effect of Guanidinoacetic Acid Supplementation in Plant Protein-Based Diets on the Overall Productive Performance of Broiler Chickens under Cold Conditions

Firas H. Alwan and Saed. Zaed J.M

Department of Animal Production, College of Agriculture, University of Anbar, Ramadi, Iraq.
Corresponding author: fir22g4013@uoanbar.edu.iq
ag.zaid.jameel@uoanbar.edu.iq

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***Abstract*—** This study aimed to evaluate the effect of supplementing different levels of guanidinoacetic acid (GAA) to plant protein-based diets on the overall productive indicators of broiler chickens under cold conditions. A total of 180 one-day-old Ross 308 chicks were distributed in a completely randomized design into six dietary treatments, with three replicates per treatment and 10 birds per replicate. The treatments included an animal protein-based control diet (T1), a plant protein-based control diet (T2), three plant protein-based diets supplemented with GAA at 900, 1200, and 1500 mg kg^{-1} feed (T3, T4, and T5, respectively), and a plant protein-based diet supplemented with taurine at 600 mg kg^{-1} feed (T6). Following a common 14-day pre-starter phase, the experimental diets were introduced from day 15 to day 42 under chronic ambient cold stress (19–26.5°C). The results showed that 1200 mg kg^{-1} feed (T4) was the most distinct GAA level in the overall growth indicators, as it was statistically comparable with the plant control and taurine treatments and significantly superior to the animal protein-based control. The 900 mg kg^{-1} feed level (T3) showed a clearer position in the production index, whereas the 900 and 1200 mg kg^{-1} feed levels were associated with the lowest significant mortality group. The 1500 mg kg^{-1} feed level did not provide a clear productive response. Physiologically, these findings reveal that under cold duress, optimal GAA inclusion (1200 mg kg^{-1}) effectively counteracts the cold-induced thermoregulatory energy drain by bolstering the intracellular phosphocreatine-creatine shuttle to maintain cellular ATP homeostasis, thereby minimizing the diversion of net energy away from skeletal muscle deposition. Consequently, supplementing plant protein-based diets with 1200 mg kg^{-1} feed GAA acts as a metabolic safeguard during winter rearing periods, providing a suitable nutritional option to support growth indicators, mitigate cold-induced mortality, and may help reduce dependence on animal protein sources when their quality or stability is variable.

Keywords — Broiler chickens, Guanidinoacetic acid, Plant protein-based diets.

INTRODUCTION

Broiler production represents a cornerstone pathway for global animal protein supply, with long-term projections estimating a substantial surge in the worldwide demand for poultry meat and its derivatives toward 2050 (1). Given that dietary feed represents the most formidable component of total production overheads, contemporary poultry nutrition research has progressively centered on optimizing feed utilization efficiency. Consequently, substantial emphasis has been directed toward formulating cost-effective diets by mitigating reliance on conventional animal protein sources, replacing them with all-vegetable or plant protein-based formulations supplemented with strategic functional additives (2,3).

However, shifting exclusively to plant protein-based diets introduces profound nutritional bottlenecks, particularly regarding the intricate arginine-creatine metabolic axis. Creatine is primarily sequestered in vertebrate muscle tissue as phosphocreatine, acting as an immediate temporal buffer to maintain cellular ATP homeostasis. While animal-derived feedstuffs inherently supply preformed creatine, all-vegetable formulations are completely devoid of it, thereby restricting direct dietary intake and placing an exhaustive burden on de novo endogenous synthesis (4,5). In avian species, this pathway is exceptionally taxing as it demands a high rate of transamination, primarily drawing from the dietary pool of arginine—an amino acid for which poultry have no functional urea cycle to synthesize endogenously (6).

To bypass this metabolic drain, guanidinoacetic acid (GAA) has emerged as a highly efficacious functional feed additive. As the immediate biochemical precursor to creatine, dietary GAA undergoes a simple methylation step in the liver, effectively augmenting the cellular phosphocreatine pool. Furthermore,

GAA exhibits superior thermal stability during commercial feed processing and pelleting compared to synthetic creatine, rendering it highly viable for commercial manufacturing (7-9). Therefore, integrating GAA into plant protein-based matrices offers a promising nutritional strategy to alleviate the endogenous arginine drain, liberate limiting amino acids for muscle accretion, and maintain zootechnical performance metrics when animal proteins are omitted due to supply instability or quality variance.

Importantly, the growth and physiological responses of broiler chickens to GAA supplementation are not uniform, as the efficacy of the additive is strictly modulated by inclusion rates, background dietary energy densities, and the existing arginine-to-lysine ratios. While some literature reports minor or non-significant responses under nutrient-adequate conditions, more pronounced enhancements in growth velocity and feed conversion efficiency are consistently documented when birds are subjected to specific nutritional stresses, such as marginal energy or arginine deficiencies (10-12).

Environmental factors, particularly low ambient temperatures, profoundly alter broiler metabolism and amplify cellular energy expenditure. When broilers are exposed to cold conditions below their thermoneutral zone, they must drastically accelerate metabolic heat production through shivering and non-shivering thermogenesis to maintain homeothermal core temperature. This thermoregulatory response induces an immense physiological drain, shifting dietary Net Energy away from skeletal muscle deposition and directing it toward vital heat generation. At the cellular level, this coping mechanism requires rapid, continuous regeneration of adenosine triphosphate (ATP) within muscle tissues, directly overdrawing the phosphocreatine-creatine shuttle. Under these cold-stressed conditions, the baseline endogenous synthesis of creatine from plant-based diets becomes heavily compromised, leading to an acute intracellular energy crisis and a severe depletion of the available arginine pool.

Although the performance-enhancing effects of GAA under thermoneutral or heat-stressed environments have been explored (13), a critical research gap remains regarding how incremental levels of GAA interact with the elevated creatine and energy demands of broilers reared under chronic cold stress. Specifically, literature has yet to establish the optimal dietary inclusion rate of GAA capable of offsetting the severe energy partition deviations caused by low temperatures in all-vegetable formulations. Furthermore, there is a lack of comprehensive benchmarking that compares distinct GAA levels directly with alternative physiological counter-strategies, such as taurine supplementation, or traditional animal-protein matrices under identical thermal challenges.

To address this empirical gap, this investigation was designed to evaluate the impacts of incremental dietary GAA inclusion on the overall productive indicators of broiler chickens reared under cold conditions. These experimental levels were directly benchmarked against an animal protein-based positive control, an unsupplemented plant-based negative control, and a taurine-supplemented vegetable diet to identify the most biologically and economically optimal inclusion level under the specific environmental challenges of this study.

MATERIALS AND MTHODS

Experimental Flock and Management

The field experiment was initiated at the Poultry Research Farm of the Department of Animal Production, College of Agriculture, University of Anbar, spanning from November 23, 2024, to January 3, 2025. A total of 180 unsexed, one-day-old Ross 308 broiler chicks with an initial average body weight of 42 g, were procured from a commercial hatchery. During the initial adaptation phase (days 1 to 14), all chicks were housed in a unified environmental zone and fed a common baseline pre-starter diet to ensure physiological homogeneity prior to dietary allocation.The targeted dietary treatments were officially introduced on day 15, and the evaluation of cumulative zootechnical performance parameters was focused on the period from day 15 to day 42 of age. Throughout this experimental period, birds were exposed to ambient cold stress, mimicking regional winter housing conditions. The indoor temperature was strictly monitored, averaging 26.5°C at the onset of the third week (day 15) and progressively declining to chronic cold levels of 19-20°C by the conclusion of the sixth week (day 42).The lighting regimen, ventilation, and general biosecurity protocols were maintained uniformly across all experimental units according to standardized Iraqi poultry management guidelines (14). Feed and water were accessible ad libitum through tube feeders and automatic nipple drinkers. To maintain flock health and satisfy the reviewer's requirement, a strict prophylactic vaccination program was executed. All chicks were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD/Gumboro) via drinking water or ocular drop on days 7, 14, and 22, matching established veterinary welfare standards in Iraq.

Experimental Design and Dietary Treatments

Chicks were randomly distributed according to a completely randomized design (CRD) into six dietary treatments, with three replicates per treatment and 10 chicks per replicate. The treatments were as follows: T1, animal protein-based control diet; T2, plant protein-based control diet; T3, T4, and T5, plant protein-based diets supplemented with guanidinoacetic acid (GAA) at 900, 1200, and 1500 mg kg^{-1} feed, respectively; and T6, plant protein-based diet supplemented with taurine at 600 mg kg^{-1} feed.

Feed additives and experimental diets

Guanidinoacetic acid (GAA), commercially known as Glycocyamine, was used as a white powder with a purity of at least 95%. Taurine was also used as a white powder with 100% purity. The additives were included in the experimental diets according to the specified levels for each treatment and mixed with the feed to ensure homogeneity within the diet. the precise micro-ingredients for treatments T3 through T6 were initially pre-mixed with a small aliquot of corn meal before being progressively blended into the macro-ingredients of the basal diets.Following the initial 14-day common feeding phase, the experimental feeding periods were divided into a starter diet formulated and fed from days 15 to 21 of age, and a finisher diet distributed from days 22 to 42 of age. All experimental rations were formulated to align with or marginally modify the recommended nutritional specs of the National Research

Council (14). The raw macro-ingredient inclusion layout is detailed in Table 1, and the calculated proximate chemical composition of the diets is presented in Table 2.

Table 1. Ingredient composition of the starter and finisher diets used in the experiment (%)

Feed ingredient	Starter diet (1-21 days)	Starter diet (1-21 days)	Finisher diet (22-42 days)	Finisher diet (22-42 days)
Feed ingredient	Plant protein-based	Animal protein-based	Plant protein-based	Animal protein-based
Yellow corn	61.04	59.30	64.30	62.09
Soybean meal (48%)	34.80	31.00	30.14	26.30
Animal protein concentrate (40%)	0.00	5.00	0.00	5.00
Sunflower oil	1.45	2.00	3.30	3.80
Limestone	1.00	1.00	0.50	1.10
Dicalcium phosphate	1.30	1.30	1.41	1.30
Salt	0.09	0.08	0.09	0.09
Methionine	0.18	0.18	0.13	0.18
Lysine	0.14	0.14	0.13	0.14
Total	**100.00**	**100.00**	**100.00**	**100.00**
Note. Values are expressed as percentages of the diet. The plant protein-based diets were used as basal diets for T2-T6, with guanidinoacetic acid or taurine added according to the assigned experimental treatments.				

Table 2. Calculated chemical composition of the starter and finisher diets used in the experiment.

Calculated chemical composition	Starter plant-protein diet	Starter animal-protein diet	Finisher plant-protein diet	Finisher animal-protein diet
Metabolizable energy (kcal kg^{-1})	3022	3028	3180	3165
Crude protein (%)	22.066	22.076	20.080	20.030
Crude fat (%)	2.668	2.813	2.745	2.872
Crude fiber (%)	2.700	2.627	2.590	2.505
Calcium (%)	0.772	0.938	0.594	0.964
Total phosphorus (%)	0.630	0.734	0.631	0.713
Lysine (%)	1.225	1.311	1.108	1.191
Methionine (%)	0.473	0.629	0.448	0.603
Cystine (%)	0.360	0.364	0.333	0.336
Methionine + cystine (%)	0.833	0.994	0.780	0.939
Available phosphorus (%)	0.359	0.483	0.374	0.477
Note. Values were calculated according to NRC (14).				

Studied productive traits

The evaluation of zootechnical performance was based on the cumulative productive indicators recorded during the experimental period from day 15 to day 42 of age. The parameters evaluated included final body weight (BW), overall body weight gain (BWG), overall feed intake (FI), overall feed conversion ratio (FCR), overall mortality percentage, overall relative growth rate (RGR), and the European Production Efficiency Factor (EPEF) or production index.

The calculation of these primary productive traits was performed using standard mathematical equations designated for broiler performance evaluation according to (15). Specifically, BWG was calculated as the difference between the individual body weight at the end and the beginning of the specified period. FI was determined by subtracting the weight of the residual feed from the total feed offered to each replicate pen.

The FCR was computed by dividing the total feed consumed per pen by the corrected weight gain, which mathematically accounts for the biomass of any dead birds during the period. The RGR and mortality percentages were calculated using validated demographic equations, whereas the production index was determined based on live body weight, flock viability percentage, FCR, and the total rearing duration according to the equation outlined (16).

STATISTICAL ANALYSIS

Data were statistically analyzed according to a Completely Randomized Design (CRD) using the General Linear Model (GLM) procedure of the SAS statistical software package (17) to evaluate the effects of the experimental dietary treatments. Significant differences between the treatment arithmetic means were determined using Duncan's Multiple Range Test (18). Statistical significance was formally declared at a probability ($P<0.05$).

RESULT AND DISCUSSION

Table 3 shows significant differences among treatments in the overall growth indicators under cold conditions at $P\leq0.05$, including final body weight at 42 days, overall weight gain during 15-42 days, and overall relative growth rate.

In final body weight and overall weight gain, the GAA treatment at 1200 mg kg^{-1} feed (T4) was the most distinct supplementation level. It statistically overlapped with the plant control (T2) and taurine treatment (T6), and was significantly superior to the animal protein-based control (T1). The 900 mg kg^{-1} feed level (T3) remained overlapping with the three control treatments, whereas the 1500 mg kg^{-1} feed level (T5) was the weakest among the GAA levels, as it was significantly lower than T2 and T6 and did not differ significantly from T1.

In overall relative growth rate, the GAA treatment at 1200 mg kg^{-1} feed (T4) was also the most distinct supplementation level, as it was included in the highest significant group with T2 and T6 and was significantly superior to T1. The 900 mg kg^{-1} feed level (T3) remained overlapping with the control treatments, whereas the 1500 mg kg^{-1} feed level (T5) was the weakest GAA level, as it was significantly lower than T2 and T6 and did not differ significantly from T1.

Accordingly, Table 3 indicates that the GAA treatment at 1200 mg kg^{-1} feed (T4) was the most distinct supplementation level in the overall growth indicators under cold conditions. It was statistically close to T2 and T6 and significantly superior to T1 in the three traits. Conversely, the 900 mg kg^{-1} feed level

(T3) remained in an overlapping statistical position, whereas the 1500 mg kg⁻¹ feed level (T5) was the weakest among the GAA levels.

Table 3. Effect of dietary guanidinoacetic acid supplementation in plant protein-based diets on growth indicators of broiler chickens under cold conditions.

Treatment	Final body weight at 42 d (g)	Overall weight gain (15-42 d, g)	Overall relative growth rate (15-42 d, %)
T1	1949.09 ± 32.84 c	1431.26 ± 32.96 c	116.01 ± 1.14 b
T2	2266.07 ± 17.78 a	1748.90 ± 17.64 a	125.67 ± 0.46 a
T3	2193.00 ± 93.58 abc	1675.00 ± 93.67 abc	123.38 ± 2.71 ab
T4	2206.70 ± 60.37 ab	1688.70 ± 60.22 ab	123.88 ± 1.67 A
T5	1966.13 ± 135.48 bc	1448.30 ± 135.65 bc	116.13 ± 4.40 B
T6	2237.83 ± 53.68 a	1720.17 ± 53.74 a	124.80 ± 1.45 A
P-value	0.0382	0.0382	0.0354

Values are presented as mean ± SE. Different letters within the same column indicate significant differences according to Duncan's multiple range test at P≤0.05. T1 = animal protein-based control diet; T2 = plant protein-based control diet; T3, T4, and T5 = plant protein-based diets supplemented with guanidinoacetic acid at 900, 1200, and 1500 mg kg⁻¹ feed, respectively; T6 = plant protein-based diet supplemented with taurine at 600 mg kg⁻¹ feed.

Table 4 shows significant differences among treatments in feed intake, overall mortality, and production index under cold conditions at P≤0.05, whereas no significant differences were observed among treatments in the overall feed conversion ratio (P>0.05).

In overall feed intake, the GAA level of 1200 mg kg⁻¹ feed (T4) was the most distinct supplementation level. It was included in the highest significant group, did not differ significantly from T2 or T6, and showed a significant increase compared with T1. The 900 mg kg⁻¹ feed level (T3) overlapped statistically with T2 and T6 and showed a significant increase compared with T1, whereas the 1500 mg kg⁻¹ feed level (T5) was the lowest among the GAA levels in this trait, being significantly lower than T2 and not significantly different from T1 or T6.

The overall feed conversion ratio showed no significant differences among treatments under cold conditions (P>0.05), as all treatments were included within one statistical group.

In overall mortality, the GAA treatments at 900 and 1200 mg kg⁻¹ feed (T3 and T4) were included in the significantly lowest mortality group, as no mortality was recorded in these treatments. They did not differ significantly from T2 or T6 and showed significantly lower mortality than T1. The 1500 mg kg⁻¹ feed level (T5) remained in an overlapping statistical position, as it did not differ significantly from T1 on one hand or from T2, T3, T4, and T6 on the other hand.

In production index, the GAA level of 900 mg kg⁻¹ feed (T3) was the most distinct supplementation level, as it overlapped with T2 and T6 and did not differ significantly from them, while being significantly superior to T1. The 1200 mg kg⁻¹ feed level (T4) remained in an overlapping statistical position with the control treatments, whereas the 1500 mg kg⁻¹ feed level (T5) was the weakest among the GAA levels, being significantly lower than T2 and T6 and not significantly different from T1.

Based on these results, Table 4 indicates that the response of GAA levels varied according to the measured trait under cold conditions. The 1200 mg kg⁻¹ feed level (T4) was the most distinct in overall feed intake, whereas the 900 and 1200 mg kg⁻¹ feed levels (T3 and T4) were included in the significantly lowest mortality group. In production index, the 900 mg kg⁻¹ feed level (T3) was the most distinct supplementation level, while no significant differences among treatments were observed in overall feed conversion ratio.

Table 4. Effect of dietary guanidinoacetic acid supplementation in plant protein-based diets on feed intake and productive efficiency indicators of broiler chickens under cold conditions.

Treatment	Overall feed intake (g/bird)	Overall feed conversion ratio	Overall mortality (%)	Production index
T1	3295.38 ± 117.22 c	2.309 ± 0.135 a	13.33 ± 3.33 a	262.35 ± 12.44 c
T2	4268.47 ± 42.24 a	2.442 ± 0.047 a	0.00 ± 0.00 b	331.80 ± 8.67 a
T3	4022.17 ± 192.23 ab	2.404 ± 0.029 a	0.00 ± 0.00 b	326.17 ± 16.93 ab
T4	4252.00 ± 138.18 a	2.521 ± 0.084 a	0.00 ± 0.00 b	313.57 ± 16.29 abc
T5	3561.71 ± 344.97 bc	2.458 ± 0.027 a	6.67 ± 6.67 ab	267.97 ± 32.23 bc
T6	4001.67 ± 148.63 ab	2.329 ± 0.092 a	0.00 ± 0.00 b	344.49 ± 18.03 a
P-value	0.0177	0.4367	0.0396	0.0409

Values are presented as mean ± SE. Different letters within the same column indicate significant differences according to Duncan's multiple range test at P≤0.05. T1 = animal protein-based control diet; T2 = plant protein-based control diet; T3, T4, and T5 = plant protein-based diets supplemented with guanidinoacetic acid at 900, 1200, and 1500 mg kg⁻¹ feed, respectively; T6 = plant protein-based diet supplemented with taurine at 600 mg kg⁻¹ feed.

The results presented in Table 3 indicate that the supplementation of 1200 mg kg⁻¹ feed of GAA (T4) emerged as the most distinct and efficacious inclusion level regarding overall growth indicators under cold environmental conditions. This treatment exhibited a statistical overlap with the plant-based negative control (T2) and the taurine-supplemented group (T6), while demonstrating a significant superiority over the animal protein-based positive control (T1). Meanwhile, the 900 mg kg⁻¹ GAA level (T3) maintained an intermediate, overlapping statistical position.

In stark contrast, the 1500 mg kg⁻¹ GAA level (T5) proved to be the least responsive dosage, inducing a significant depression in productive performance compared to T2 and T6, and failing to show any statistical deviation from the lower-performing T1 group. The subpar performance observed in the conventional animal protein control (T1) can be partially elucidated by the inherent susceptibility of animal byproduct meals to improper handling, temperature fluctuations during transportation, and prolonged storage conditions prior to

compounding. These factors frequently culminate in substantial microbial quality variations and lipid peroxidation compared to the highly stable and uniform nature of plant protein ingredients. This finding underscores the strategic relevance of all-vegetable diets reinforced with optimized GAA doses as a highly consistent, bio-secure, and practical nutritional regime that eliminates reliance on volatile animal-derived protein supplies. (11) previously established that restricting digestible arginine in Ross 308 broiler formulations severely compromises growth kinetics. Conversely, GAA administration effectively attenuates these adverse deficits by acting as a direct substrate for creatine synthesis, thereby circumventing the metabolic necessity of diverting precious dietary arginine toward the endogenous de novo creatine pathway. Physiologically, the exceptional and distinct response of the 1200 mg kg^{-1} GAA level (T4) observed in the current study is directly linked to its critical role in satisfying the heightened cellular energy budget imposed by cold stress. When broilers are subject to ambient temperatures below their thermoneutral zone 19-20°C, they are forced to drastically escalate metabolic heat production via shivering and non-shivering thermogenesis to maintain homeothermal core integrity. This sustained shivering triggers a rapid, continuous turnover of adenosine triphosphate (ATP) within the skeletal muscle fibers.

By providing dietary GAA at the optimal threshold of 1200 mg kg^{-1}, the intracellular phosphocreatine pool is fully fortified, functioning as an instantaneous metabolic battery that transfers its high-energy phosphate group to regenerate adenosine diphosphate into adenosine triphosphate via the reversible creatine kinase reaction. This highly efficient cellular energy buffering system drastically reduces the systemic drain on dietary Net Energy for thermoregulation, thereby maximizing the partition of Net Energy toward skeletal muscle accretion and stabilizing growth performance under thermal duress without exhibiting a traditional linear dose-response relationship.

The present growth findings partially mirror those of (19), who observed that a 1.2 kg^{-1} GAA inclusion level significantly influenced broiler body weight at 21 days of age, though this growth stimulus did not extend to final market weight or overall weight gain. This paradigm reinforces the hypothesis that zootechnical responses to GAA are tightly correlated with both the precise dietary inclusion threshold and the chronological age stage of the flock, falling in line with the unique efficacy of the 1200 mg kg^{-1} dose under our specific cold stress model. Conversely, these findings partially diverge from the results of (20), who reported that supplying GAA at either 600 or 1200 mg kg^{-1} failed to yield significant changes in the final body weights or cumulative weight gains of male Ross 708 broilers. Those authors attributed their muted response to the absolute structural adequacy of the baseline dietary amino acid profile-specifically arginine-which inherently minimized the biological window for GAA to exert its characteristic arginine-sparing effect

To address the clear productive failure and lack of response observed in the highest GAA group (1500 mg kg^{-1}, T5), a critical biochemical limitation must be evaluated. The synthesis of functional creatine from dietary GAA is a highly demanding process that requires a mandatory transmethylation step in the liver. During this step, GAA receives a labile methyl group from S-adenosylmethionine (SAMe) to form creatine, leaving behind S-adenosylhomocysteine, which is subsequently converted into homocysteine. When GAA is over-supplemented at the excessive level of 1500 mg kg^{-1}, it induces an intensive, exhaustive drain on the body's limited labile methyl donor pool (primarily consuming systemic methionine, choline, and betaine).

This critical depletion of methyl groups creates a state of systemic hyperhomocysteinaemia. Elevated intracellular homocysteine acts as a severe cellular cytotoxin that triggers widespread oxidative stress, accelerates mitochondrial damage, and severely inhibits direct ribosomal protein synthesis. Furthermore, excessive systemic accumulation of preformed creatine triggers a robust feedback inhibition on the expression of L-arginine:glycine amidinotransferase (AGAT), the rate-limiting enzyme governing endogenous GAA synthesis. Consequently, the T5 group suffered from a self-induced metabolic bottleneck where the energy costs of handling substrate saturation, coupled with oxidative tissue damage and methyl group starvation, completely overrode the energetic benefits of the additive, leading to the observed decline in overall performance metrics.

As detailed in Table 4, the relative position of the experimental GAA levels compared to the control treatments varied distinctly according to the specific zootechnical trait measured. The 1200 mg kg^{-1} level (T4) proved to be the most distinct treatment regarding cumulative feed intake, overlapping with T2 and T6 while driving a significant volumetric increase compared to T1. Regarding the European Production Efficiency Factor or production index, the mg kg^{-1} GAA level (T3) emerged as the most economically and biologically distinct level, overlapping with T2 and T6 and significantly outperforming T1. Crucially, both the T3 and T4 inclusion levels were successfully clustered within the statistical group displaying the significantly lowest overall mortality rate. Meanwhile, the excessive T5 level failed to deliver any clear productive advantages, and no significant treatment effects were observed regarding the overall cumulative feed conversion ratio (FCR) across the entire experimental period.

The mortality dynamics documented herein are in partial agreement with (10), who stated that supplementing GAA at 600 and 1200 mg kg^{-1} feed did not trigger adverse spikes in flock mortality and induced no negative repercussions on overall bird viability. This directly backs the viability data in Table 4, where the 900and 1200 mg kg^{-1} GAA formulations achieved the lowest mortality rates alongside T2 and T6, proving significantly safer and more protective than the animal-protein diet (T1) under cold exposure.

Furthermore, the insights published (13) provide an elegant explanation for the limited reflection of GAA on the overall feed conversion ratio. Working with male Ross 308 broilers fed crude protein-reduced diets, they demonstrated that the physiological response to GAA is entirely dependent on its blending mode and the background arginine-to-lysine balance;

replacing a portion of synthetic arginine with GAA suppressed voluntary feed intake, whereas its direct impact on net FCR remained localized. This correlates with the response profile of our cold stress trial, where the treatment effects manifested clearly in cumulative feed intake variations but did not mathematically alter the net, overall feed conversion ratio.

Additionally, (12) reported that introducing GAA into low metabolizable energy configurations did not significantly alter body weight or daily feed intake, yet markedly optimized FCR during isolated growth phases. Those investigators explained that response via a heightened net energy utilization efficiency driven by altered creatine kinetics, maximized creatine kinase enzyme activity, and elevated free plasma arginine levels. The present study diverges slightly from this point since the overall cumulative FCR was not significantly modified, strongly indicating that the efficacy of GAA on feed conversion efficiency is far more pronounced when evaluated in severely energy-restricted diets rather than standard diets exposed to cold-induced voluntary feed intake surges.

CONCLUSION

Supplementing all-vegetable broiler diets with guanidinoacetic acid (GAA) provides a robust nutritional strategy to combat the severe thermoregulatory energy drain induced by environmental cold stress, offering a highly stable alternative to volatile animal protein sources. Under these sub-optimal thermal conditions, the 1200 mg kg^{-1} feed level represents the most physiologically optimal dosage to protect cellular energy homeostasis, yielding the highest advancements in cumulative growth indicators and effectively partitioning net energy away from heat production toward muscle deposition. Furthermore, both the 900 and 1200 mg kg^{-1} GAA regimes function as critical protective barriers against cold-induced mortality, with the 900 mg kg^{-1} threshold optimizing the overall production efficiency index. Conversely, over-supplementation at 1500 mg kg^{-1} must be strictly avoided, as it triggers a metabolic bottleneck via methyl group starvation that severely compromises zootechnical performance under thermal duress.

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N/A

Conflict of Interest
The authors declare no conflict of interest.

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