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Role of guanidinoacetic acid in optimizing growth performance and feed efficiency of broilers fed animal protein based diets

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Abstract

The present study was conducted to evaluate the influence of dietary guanidinoacetic acid (GAA) supplementation at 600 g/tonne on growth performance and return over feed cost in commercial broilers fed animal-based protein diets. A total of 96 day-old Vencobb 430Y broiler chicks were randomly distributed into three dietary treatments with four replicates of eight birds each. The experimental treatments consisted of control T₁ (Corn-Soya MBM diet), T₂ (Corn-Soya MBM diet + GAA) and T₃ (Corn-Soya MBM diet with reduced ME by 50 kcal/kg + GAA). All diets were formulated as per BIS (2024) nutritional specifications for starter (1-10 days), grower (11-21 days), and finisher (22-42 days) phases. In experimental period of 42 days, feed intake was not significantly influenced by GAA supplementation across dietary treatments. Final body weight, body weight gain and feed conversion ratio also found statistically non-significant ($p > 0.05$) but numerical improvements were observed, GAA supplementation in MBM-based diet with normal energy level enhanced body weight and body weight gain while reduced metabolizable energy group with GAA provided the highest return over feed cost, making it a practical nutritional intervention for commercial broiler production.

Keywords: Guanidinoacetic acid (GAA), Creatine, Meat and Bone Meal (MBM), Metabolizable energy (ME)

1. Introduction

Guanidinoacetic acid also called glycocyamine is a naturally occurring amino acid derivative which serves as a direct precursor of creatine, a crucial compound in the energy metabolism of muscle (Wyss and Kaddurah-Daouk, 2000) [21]. In rapidly growing modern broilers, endogenous creatine synthesis is insufficient to meet the high energy demands associated with accelerated muscle accretion (Brosnan *et al.*, 2009) [4]. Therefore, an exogenous supply of creatine becomes essential and dietary guanidinoacetic acid (GAA), as a direct creatine precursor, serves as an effective nutritional strategy to support enhanced energy metabolism and performance (Tossenberger *et al.*, 2016) [19]. Creatine is stored as phosphocreatine in muscles during rest and with rapid increase in energy demand, the high-energy storage molecule phosphocreatine is broken down, supplying phosphate to ADP and restoring ATP (Balsom *et al.*, 1994; Nabuurs *et al.*, 2013) [2, 17].

The demand for creatine or its precursors may be supplied either directly from animal protein in the diet or by endogenous synthesis (Wyss and Kaddurah-Daouk, 2000) [21]. Nowadays, poultry diets are mainly composed of ingredients from plant origin, mainly corn and soybean meal. Creatine is only found in feed ingredients of animal origin, while plant-based ingredients do not contain any metabolites of creatine (Krueger *et al.*, 2010) [13]. The most common animal by-products used in broiler diets are meat and bone meal, blood meal, feather meal, and poultry offal meal. (Caires *et al.*, 2010) [5]. Animal byproducts, such as Meat and bone meal (MBM), contain 207.7 ± 107 mg/kg of natural or preformed creatine (Krueger *et al.*, 2010) [13]. It is a potential feed supplement for poultry which has widely used as a protein source in to improve feed quality (Parsons *et al.*, 1997) [18]. However, Meat and bone Meal is usually incorporated in poultry diets at levels not exceeding 5 to 10% which meet phosphorus requirements (Drewyor and Waldroup, 2000) [20].

Besides, the increased levels of MBM incorporated in poultry diets might reduce costs relatively as cheaper protein, calcium and phosphorus source than those conventional feedstuffs (Waldroup, 2002) ^[20]. In energy-deficient diets, supplementation such as GAA has the potential to compensate for inadequate dietary energy supply and thus improve broiler growth (Cao *et al.* 2024) ^[6]. Optimal GAA supplementation level for broiler growth and FCR ranges from 0.6-1.2 g/kg (Lemme *et al.*, 2007a) ^[14, 15]. Considering the additional creatine contribution from MBM, a synergistic response between MBM and GAA is biologically important in enhancing growth performance. However, limited research exists regarding the combined effect of GAA supplementation and MBM inclusion under reduced ME conditions. Therefore, the present study was undertaken to evaluate the effect of enzyme-activated GAA supplementation in corn-soya MBM-based broiler diets under normal and reduced energy conditions on feed consumption, growth performance and return over feed cost (ROFC).

2. Materials and Methods

2.1 Animal ethics statement

The experiment was carried out from July to August 2025 at Poultry Research Station, Kamdhenu university, Anand, Gujarat. The Institutional Animal Ethics Committee approved the prior consent for the experiment (Approval Number 450/AN/24).

2.2 Experimental design, treatments and diets

A total of 96 day-old commercial straight run Vencobb 430Y broiler chicks were obtained from a commercial hatchery and randomly assigned to 3 treatment groups, each of which contained four replicates with eight chicks in each. Day-old body weight of chicks was found to be statistically comparable across all treatments. Three different rations (T₁, T₂, T₃) were prepared. The supplementation dosage of guanidinoacetic acid (GAA) in broiler starter, grower, and

finisher diets was 600 g/tonne (0.06%) of feed. The supplement used was an enzyme-activated guanidinoacetic acid product designed with U.S. patented technology. A decrease in 50 kcal/kg Metabolizable energy (ME) in each phase (starter, grower, and finisher) was achieved by reducing the proportion of vegetable oil in the feed formulation. The treatment rations were fed for a total of 6 weeks. The treatments included T₁ (Corn-Soya MBM diet); T₂ (Corn-Soya MBM diet + 0.06% GAA); T₃ (Corn-Soya MBM diet with 50 kcal/kg less ME than control + 0.06% GAA)

The feeding was done according to a three-phase feeding regime (BIS, 2024), which included a starter phase from 1 to 10 days, a grower phase from 11 to 21 days, and a finisher phase from 22 to 42 days. The phase-wise formulation of feeds for treatments T₁ to T₃ is presented in Table 1. The feeds were formulated to attain the calculated CP and ME values (BIS, 2024).

2.3 Housing, Feeding and Health Management

The broiler shed, as well as all brooding and rearing equipment, was completely cleaned and disinfected before the experiment started. All treatment groups were raised in a deep litter housing system. The chicks were kept in a pen. Each pen had four compartments. Each compartment had eight chicks. The floor space available was steadily expanded as the bird's age progressed. The bulbs in the brooder were turned on 12 hours before the chicks were placed under the brooder to achieve a brooding temperature of 95°F during the first week of age. The brooding temperature was gradually dropped by 5°F per week until it reached the ideal temperature of 75°F. The birds were fed using the treatment-specific prepared diet. Birds were fed twice per day, and the amount of feed supplied each time was recorded. The vaccination regimen includes the administration of Ranikhet Disease vaccine (Lasota) on day 7, Infectious Bursal Disease vaccine on day 14, and a Ranikhet Disease booster on day 21.

Table 1: Treatment wise proportion of feed ingredients (%) used in starter, grower and finisher diets

Sr. No	Ingredients	Broiler Starter			Broiler Grower			Broiler Finisher		
		T ₁	T ₂	T ₃	T ₁	T ₂	T ₃	T ₁	T ₂	T ₃
		Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg	Qty./100 kg
1	Maize	57.357	57.357	58.164	61.846	61.846	63.303	65.914	65.914	67.278
2	Soyabean DOC	35.466	35.466	35.097	30.954	30.954	30.52	26.909	26.909	26.573
3	MBM	3.617	3.617	3.613	3.466	3.466	3.462	3.253	3.253	3.263
4	Deoiled Rice Bran	0.000	0.000	0.486	0.000	0.000	0.000	0.000	0.000	0.000
5	Calcite Powder	0.818	0.818	0.801	0.886	0.886	0.888	0.989	0.989	0.988
6	Vitamins	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
7	Vitamin-B12	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
8	Trace Minerals	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
9	Choline Chloride 60%	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120
10	Lysine	0.367	0.367	0.371	0.354	0.354	0.398	0.204	0.204	0.209
11	Methionine	0.374	0.374	0.372	0.362	0.362	0.361	0.317	0.317	0.315
12	L-Threonine	0.137	0.137	0.138	0.108	0.108	0.110	0.114	0.114	0.115
13	Phytase-5000	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
14	Enzymes	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
15	Salt	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
16	Liver tonics	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
17	Immunomodulator	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
18	Toxin Binder	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
19	Emulsifier	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.048
20	Probiotic	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
21	Anticoccidial	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
22	Vegetable Oil	0.906	0.906	0.000	1.066	1.066	0.000	1.342	1.342	0.301
	Total	100.00	100.00	100.00	100.000	100.00	100.000	100.000	100.00	100.000
	Calculated Crude Protein	22.5	22.5	22.5	21.0	21.0	21.0	19.5	19.5	19.5
	Calculated (ME kcal/kg feed)	3000	3000	2950	3050	3050	3000	3100	3100	3050

MBM = Meat and Bone Meal; DOC = De Oiled Cake; DCP = Dicalcium phosphate; ME = Metabolizable Energy; T₁ = Corn-Soya MBM diet; T₂ = Corn-Soya MBM diet + GAA; T₃ = Corn-Soya MBM diet with 50 kcal/kg less ME than control + GAA

2.4 Measurements

2.4.1 Growth performance

Birds of each replicate were offered weighed quantities of respective diets daily to ensure ad libitum feed intake. Birds were individually weighed weekly in the morning before adding feed using a digital balance to obtain their body weight (BW). The body weight gain (BWG), feed intake (FI), and FCR were calculated weekly as well as phase wise and for the entire period of the trial (1 to 42 days)

2.4.2 Livability

The percent livability was calculated based on the number of birds that remained alive after deducting mortality for each treatment diet during experimental periods.

2.4.3 Return Over Feed Cost (ROFC)

To calculate the cost of feeding per kilogram of live broiler bird, multiply the average feed intake for the starter, grower, and finisher phases by production cost each feed type. The total feed cost for each treatment was then divided by the average body weight of the birds to determine the cost per kilogram. The return over feed cost (ROFC) was computed by deducting the feed cost from the income procured by selling the birds at Rs. 91 per kilogram live weight.

2.4.4 Statistical Analysis

The trial data was analysed using a one-way ANOVA to identify significant differences ($p < 0.05$) in treatment means. All statistical analyses were carried out using the statistical software for social sciences SPSS 27.0 version. Duncan's Multiple Range Test (DMRT) revealed significant differences across treatments at $p < 0.05$ (Duncan, 1955) [11]. All values presented in the tables represent the means \pm standard error (SE).

3. Results and Discussion

3.1 Effect on Feed Intake

Phase wise and overall means of feed intake (g/bird) of experimental broilers under different dietary treatments is presented in Table 2. Feed intake did not differ significantly ($p > 0.05$) among the three dietary treatments throughout the experiment. During the starter phase (1-10 d), numerically higher feed intake was observed in the GAA-supplemented normal energy group (T₂) compared to control (T₁) followed by 50 kcal/kg less metabolizable energy with GAA group (T₃) compared to control (T₁). In the finisher phase (22-42 d), the normal energy GAA group showed numerically higher intake than both control and reduced energy diets. However, overall feed intake (1-42 d) and average daily feed intake remained statistically non-significant, suggesting that neither dietary GAA supplementation nor 50 kcal/kg ME reduction did not influence voluntary feed consumption in broilers receiving meat and bone meal (MBM)-based diets.

The present findings are in agreement with Cenesiz *et al.* (2020) [8], who fed 0.06% GAA to broilers under two dietary protein sources, maize-soya based diet and poultry by-product meal over three periods (starter 1-14, grower 15-28, and finisher 29-41 days) and found no significant effect of GAA or its interaction with energy level on feed intake.

Similar findings were previously reported by Cordova-Noboa *et al.* (2018) [9] and Esser *et al.* (2018) [12] and Lemme *et al.* (2007a) [14, 15] showing that animal-based protein diets supplemented with GAA did not influence feed consumption at normal energy level. This suggests that performance enhancements observed in the present study are driven by improved nutrient utilization and energy metabolism, rather than changes in feed intake.

3.2 Effect on body weight and body weight gain

Phase wise and overall means of body weight (BW) and body weight gain (BWG) of experimental broilers under different dietary treatments is presented in Table 2. Body weight at the end of starter phase showed no statistical variation ($p > 0.05$) among the treatments. However, significant improvement ($p < 0.05$) in body weight was recorded in the grower phase, where broilers fed GAA with normal ME exhibited significantly higher body weight compared to other groups ($p < 0.05$). A similar numerical trend continued up to the finisher phase, showing improved performance with GAA supplementation under normal ME conditions. During the starter and grower phase, Corn-soya MBM diet (control) showed significantly higher body weight gain as compared to reduced metabolizable energy diet by 50 kcal/kg. Corn-soya MBM diet supplemented with GAA at normal ME level had comparable body weight gain in starter phase while found significantly lower BWG ($p < 0.05$) in grower phase as compared to control diet without GAA supplementation. During the finisher phase, MBM diet with normal energy + GAA group (T₂) exhibited significantly higher BWG ($p < 0.05$) compared to the control group (T₁) and the reduced 50 kcal/kg ME + GAA group (T₃). In overall period (1-42 d), final body weight gain found numerically higher in the GAA-supplemented normal energy group, while reduced-energy supplemented with GAA remained comparable to control, indicating partial compensation of energy deficit. Overall, BWG showed a tendency for improvement ($p = 0.058$) with GAA supplementation at normal energy levels.

The significantly higher finisher body weight gain in broilers fed MBM diet supplemented with GAA under normal ME reflect improved energy utilization from dietary creatine synthesis and creatine-phosphocreatine recycling (Michiels *et al.*, 2012; Lemme *et al.*, 2007b) [14, 15]. Similar improvements in growth performance with GAA supplementation in animal-based diets were also confirmed by Córdova-Noboa *et al.* (2018) [9], Lemme *et al.* (2007a) [14, 15], and Esser *et al.* (2018) [12]. The ability of dietary GAA to support comparable growth under a 50 kcal/kg reduced ME diet further suggests that GAA compensated for the lowered dietary ME by improving cellular energy turnover (Michiels *et al.*, 2012) [16].

In contrast to the present study, Carvalho *et al.* (2013) [7] reported non-significant effects of GAA supplementation on body weight at the end of 42 days. Bozkurt *et al.* (2004) [3] reported performance depression in MBM diets at higher inclusion levels due to imbalance in digestible amino acids and higher ash content. However, in the present study MBM inclusion level was optimized, preventing such negative impacts and instead enhancing response to GAA.

Table 2: Phase wise and overall means of body weight (BW) and body weight gain (BWG) of experimental broilers

Parameters Phase (Period)	Treatments				
	T ₁	T ₂	T ₃	SEM	P value
Feed Intake (g)					
Starter (1-10 d)	285.66±2.60	294.67±4.36	293.44±2.57	3.28	0.165
Grower (11-21 d)	721.44±21.97	675.00±25.36	738.25±5.49	19.63	0.114
Finisher (22-42 d)	2553.25±140.41	2736.66±88.97	2342.63±25.63	97.10	0.054
Overall (1-42 d)	3560.63±148.05	3703.91±115.16	3370.25±31.65	109.82	0.154
Avg. Daily FI	84.78±3.53	88.19±2.74	80.25±0.75	2.61	0.154
Body weight (g)					
Starter (10 d)	259.11±4.83	252.41±4.85	242.54±4.80	4.83	0.056
Grower (21 d)	844.44 ^a ±20.47	782.23 ^b ±16.88	777.83 ^b ±10.85	16.55	0.008
Finisher (42 d)	2099.98±55.24	2190.08±46.41	2027.74±40.52	47.77	0.060
Body weight gain (g)					
Starter (1-10 d)	215.37 ^a ±4.76	209.40 ^{ab} ±4.71	198.76 ^b ±4.72	4.73	0.470
Grower (11-21 d)	585.33 ^a ±17.84	529.82 ^b ±13.87	535.29 ^b ±7.99	13.84	0.010
Finisher (22-42 d)	1255.54 ^b ±48.09	1407.85 ^a ±38.42	1249.91 ^b ±36.78	41.40	0.012
Overall (1-42 d)	2056.24±55.18	2147.06±46.31	1983.96±40.34	47.67	0.058
Avg. Daily Gain	48.96±1.31	51.12±1.10	47.24±0.96	1.13	0.058

The means bearing different superscripts in the same row differ significantly ($p < 0.05$)

3.3 Effect on Feed Conversion Ratio

Phase wise and overall means of feed conversion ratio of experimental broilers under different dietary treatments is presented in Table 3. Feed conversion ratio (FCR) remained statistically non-significant ($p > 0.05$) across all phases. Numerically, Corn-soya MBM diet with 50 kcal/kg reduced energy supplemented with GAA group (T₃) exhibited better overall FCR compared to the control group, indicating improved efficiency of feed utilization despite lower dietary ME levels. Control and normal ME diets with GAA had

similar feed efficiency values.

Although statistically non-significant, improvement in numerical FCR, particularly in the reduced ME + GAA group, reflects better metabolic efficiency of energy utilization. These findings align with results of Cenesiz *et al.* (2020) [8], Carvalho *et al.* (2013) [7], and Lemme *et al.* (2007a) [14, 15], where GAA supplementation improved feed efficiency without increasing feed intake. Therefore, reduced-energy diets combined with GAA offer a cost-effective feeding strategy without compromising growth.

Table 3: Feed Conversion Ratio and Livability of experimental broilers under different dietary treatments

Feed Conversion Ratio					
Phase (Period)	T ₁	T ₂	T ₃	SEM	P value
Starter (1-10 d)	1.13±0.03	1.17±0.04	1.21±0.03	0.03	0.269
Grower (11-21 d)	1.26±0.09	1.28±0.05	1.38±0.02	0.06	0.366
Finisher (21-42 d)	2.02±0.14	1.95±0.10	1.88±0.06	0.11	0.651
Overall (1-42 d)	1.70±0.09	1.70±0.08	1.66±0.04	0.07	0.931
Livability %					
Overall (1-42 d)	93.75±3.61	96.88±3.13	90.63±3.13	3.29	0.440

The means bearing different superscripts in the same row differ significantly ($p < 0.05$)

3.4 Effect on Livability

Livability percentage of experimental broilers under different dietary treatments is presented in Table 3. Livability percentage remained high and statistically non-significant ($p > 0.05$) among dietary treatments throughout the experiment, indicating that GAA supplementation, MBM inclusion, or moderate dietary energy reduction did not adversely affect broiler survivability under standard management.

Livability remained high in all dietary treatments, confirming that dietary GAA at 600 g/tonne and moderate ME reduction are safe in animal-based protein diets. This observation is supported by Cenesiz *et al.* (2020) [8], Córdova-Noboa *et al.* (2018) [9] and Lemme *et al.* (2007a) [14, 15], who reported no adverse effects on health or mortality in broilers fed similar diets.

3.5 Effect on Return over feed cost (ROFC)

Cost of feeding and return over feed cost of experimental broilers under different dietary treatments is presented in Table 4. Total feed cost per bird was significantly influenced by dietary treatments ($p < 0.05$). The lowest total

feed cost was recorded in birds fed Corn-Soya MBM diet with reduced 50 kcal/kg ME + GAA followed by control. Income generated per bird showed a numerical improvement in the normal ME + GAA group compared to control, whereas the reduced ME + GAA group recorded slightly lower revenue due to comparatively lower body weights. Cost of feed per kg broiler bird and ROFC (Rs/bird and Rs/kg bird) remained statistically non-significant ($p > 0.05$) among dietary treatments. However, numerically higher ROFC was observed in the reduced ME + GAA group followed by the normal ME + GAA group, clearly indicating improved profitability with GAA supplementation along with reduced ME level.

The reduction in total feed cost observed in broilers fed MBM diet supplemented with GAA under reduced ME conditions indicates improved dietary energy utilization and feed conversion efficiency, which helped maintain growth performance despite lower ME input. Similar economic benefits of GAA addition in broiler diets were also stated by Arafa *et al.* (2017) [1] reported where supplementation of 600 mg/kg GAA improved net return and economic efficiency by 15.7% compared to control diets.

Table 4: Cost of feeding and return over feed cost (ROFC) of experimental broilers under different dietary treatments

Treatments	T ₁	T ₂	T ₃	SEM	P value
Total Feed Cost (Rs./Bird)	132.46 ^{ab} ±5.51	139.43 ^a ±4.25	121.19 ^b ±1.12	4.07	0.033
Body weight (g)	2099.98±40.52	2190.08±46.87	2027.74±39.80	42.51	0.069
Cost of Feed (Rs./kg broiler bird)	63.22±3.40	63.83±2.92	59.84±1.41	2.72	0.557
Income from selling of birds (91 Rs./kg bird)	191.10±3.69	199.30±4.27	184.53±3.62	3.87	0.069
ROFC (Rs./bird)	58.65±8.03	59.87±7.66	63.34±4.01	6.81	0.882
ROFC (Rs./kg broiler bird)	27.78±3.40	27.18±2.92	31.16±1.41	2.71	0.557

The means bearing different superscripts in the same row differ significantly ($p < 0.05$)

4. Conclusions

Supplementation of guanidinoacetic acid (GAA) at the rate of 600 g/tonne in Meat and Bone Meal (MBM) based broiler diets with normal metabolizable energy (ME) improved body weight and body weight gain, whereas inclusion of GAA in 50 kcal/kg metabolizable reduced energy MBM diets provided the highest return over feed cost, without compromising broiler health or survivability. Thus, GAA supplementation is recommended in MBM diets to support performance and maximum profitability under reduced dietary ME conditions.

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