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Influence of dietary guanidinoacetic acid on nutrient retention, carcass traits and breast muscle creatine in broilers fed animal protein diets

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Abstract
This study evaluated the effects of guanidinoacetic acid (GAA) supplementation at the rate of 600 g/tonne on nutrient retention, carcass characteristics, serum biochemical profile, and breast muscle creatine in commercial broiler chickens fed meat and bone meal (MBM) based diets. A total of 96 day-old Vencobb 430Y chicks were randomly assigned into three treatments with four replicates of eight birds each: Control T_1 (Corn-Soya MBM diet); T_2 (Corn-Soya MBM diet + GAA), and T_3 (Corn-Soya MBM diet with 50 kcal/kg reduced ME + GAA). At the end of 42 days, nutrient digestibility and nitrogen and calcium balance remained statistically comparable among treatments ($p>0.05$), whereas phosphorus excretion increased in reduced metabolizable energy (ME) with GAA (T_3) fed broilers ($p<0.05$). Carcass traits revealed significantly higher dressing percentage in GAA-supplemented birds (T_2), while abdominal fat, giblet percentage, and breast muscle yield showed no significant variation. Serum creatinine, SGPT, SGOT, and total protein remained within normal physiological ranges, though glucose was significantly affected ($p<0.05$). Breast muscle creatine concentration was significantly higher in both GAA supplemented treatments groups with or without reduced energy level than the control ($p<0.05$). Overall, GAA supplementation at 600 g/tonne improved dressing yield and breast muscle creatine deposition without compromising nutrient utilization or serum parameters in broilers fed MBM-based diets.

Keywords: Guanidinoacetic acid (GAA), Metabolizable Energy (ME), Meat and Bone Meal (MBM), broilers

Introduction
Guanidinoacetic acid (GAA) is the immediate biochemical precursor of creatine and has emerged as an efficient feed additive capable of increasing intramuscular creatine pools (Michiels *et al.*, 2012) [17]. Meeting the high metabolic energy demand of modern broilers is crucial, as rapid muscle growth increases the requirement for adenosine triphosphate (ATP) turnover in skeletal tissues (Wyss & Kaddurah-Daouk, 2000) [25]. GAA supplementation improves ATP availability, growth performance and breast muscle accretion in broilers by supporting muscle energy metabolism and sparing dietary arginine for protein synthesis (Lemme *et al.*, 2007; Ostojic, 2015) [15, 20],

Feed ingredients of plant origin, such as maize and soybean meal, are nearly devoid of creatine or its precursors, while animal-based ingredients like meat and bone meal (MBM) naturally contain creatine and phosphocreatine (Krueger *et al.*, 2010) [14]. Thus, MBM inclusion can improve muscle energy metabolism and carcass yield (Bozkurt *et al.*, 2004; Swe *et al.*, 2022) [5, 23]. However, the reduced usage of MBM in some regions due to regulatory and consumer concerns has renewed interest in alternative bioavailable creatine sources. Some European poultry producers observed a certain drop in performance (European community, 2000) [11]. This could have been partially caused by insufficient supply of creatine or its pre cursors which are absent in plant-based proteins.

Experimental evidence shows that 0.06% GAA enhances carcass traits and breast muscle creatine concentration under varying dietary energy and protein sources, including those containing animal by-products (Cordova-Noboa *et al.*, 2018; Michiels *et al.*, 2012) [7, 17]. Additionally, GAA supplementation has been shown to maintain stable serum biochemical

profiles, indicating physiological safety at recommended inclusion levels (EFSA, 2009; He *et al.*, 2019) [9, 12]. Therefore, the present study was designed to evaluate the effects of GAA supplementation (600 g/tonne) to corn-soya MBM-based diets, with or without reduced metabolizable energy, on nutrient utilization, carcass traits, serum biochemical parameters and breast muscle creatine concentration in broiler chickens.

2. Materials and Methods

2.1 Animal ethics statement

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

2.2 Experimental design, treatments and diets

Total of 96 day-old commercial broiler chicks (Vencobb 430Y strain) were procured from a commercial hatchery and randomly assigned to 3 treatment groups, each containing four replicates of eight chicks. The day-old body weight of chicks was found to be statistically similar across all treatments. Three different rations were formulated (T_1, T_2, and T_3). The supplementation level 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 manufactured with US patented technology. A reduction in 50 kcal/kg Metabolizable energy (ME) in each phase (starter, grower, and finisher) done by decreasing the amount of vegetable oil in the feed formulation. The treatment rations were given for a period of 6 weeks. The treatments included T_1 (Corn-Soya MBM diet), T_2 (Corn-Soya MBM diet + GAA), and T_3 (Corn-Soya MBM diet with 50 kcal/kg less ME than control + GAA).

The feeding was carried out using 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. Table 1 shows the phase-wise formulation of feeds for treatments T_1, T_2 and T_3. The feeds were designed to meet 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, were thoroughly cleaned and disinfected prior to the experiment beginning. All treatment groups were raised in deep litter housing systems. The chicks were kept in a pen. Each pen contained four different compartments. Each compartment included eight chicks. To attain a brooding temperature of 95°F during the first week, the brooder's bulbs were turned on 12 hours before the chicks were placed under it. The brooding temperature was gradually reduced by 5°F every week until it reached the optimal level of 75°F. The birds were provided with a treatment-specific prepared feed. The birds were fed twice a day, and the amount of feed given each time was recorded. The vaccination schedule comprises new castle disease vaccine (Lasota) on day 7, Infectious Bursal Disease vaccine on day 14, and booster for new castle disease 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; T_1 = Corn-Soya MBM diet; T_2 = Corn-Soya MBM diet + GAA; T_3 = Corn-Soya MBM diet with 50 kcal/kg less ME than control + GAA

2.4 Measurements

2.4.1 Nutrient digestibility (retention) and balance studies

A metabolic trial was carried out during the sixth week on one bird per replicate, with a two-day adaption period followed by a three-day collection period. During the collecting time, data on the amount of feed supplied, left over, and excreta voided were recorded to measure nutrient utilisation. The samples obtained over a three-day period for each bird were pooled, ground, and stored for future study. Samples of offered feed, leftovers, and excreta were examined for proximate principles. Nutrient retention was calculated by subtracting the nutrient values of the excreta from the intake values. One-fifth of the excreta sample collected was kept in concentrated H_2SO_4 for nitrogen analysis. The nitrogen content of preserved acid excreta collected during the metabolic trial was determined using Kjeldahl's technique (AOAC, 2000) [3]. The average daily balance values for nitrogen, calcium, and phosphorus were calculated using data obtained during the metabolic trial (AOAC, 2000) [3].

2.4.2 Carcass characteristics

On day 42 of the experiment, four chickens from each treatment were randomly selected and slaughtered using the scientific technique to calculate carcass parameters. Giblet weight was determined by combining the weights of the liver, heart, and gizzard. The breast muscle was also removed from the carcass and weighed. The dressing % was determined above the pre-slaughter weights, whereas the giblet percentage, abdominal fat percentage, and breast muscle yield were estimated above the dressed weight.

2.4.3 Serum biochemical parameters

On the final day of the experiment, blood samples were taken aseptically from each individual bird from right jugular vein with a needle and syringe. The serum was obtained from the blood by centrifuging the clot-activated vacutainers at 2000 rpm for 15 minutes. Biochemical parameters were estimated with standard biochemical kits and a biochemical auto-analyzer.

2.4.4 Breast muscle creatine concentration

Breast muscle creatine was detected using Reverse Phase-High Performance Liquid Chromatography. About 300 mg of breast muscle was homogenised with 0.5 M Perchloric acid, centrifuged, and the supernatant was neutralised with 2 M KOH before filtration through a 0.22 µm syringe filter. The samples were analysed using a C18 column (250 × 4.6 mm, 5 µm) on an HPLC system with a UV detector set to 220 nm. The mobile phase was prepared by combining HPLC-grade methanol and water in a 20:80 (v/v) ratio and was supplied at a flow rate of 1.0 mL/min. The creatine concentration was determined using standard calibration curve obtained from creatine analytical standard.

2.4.5 Statistical Analysis

The trial data was evaluated using one-way ANOVA to determine significant differences ($p<0.05$) in treatment means. All statistical analyses were performed with SPSS (statistical software for social sciences) version 27.0. Duncan's Multiple Range Test (DMRT) showed significant changes across treatments at ($p<0.05$) (Duncan, 1955). All values in the tables indicated with mean±standard error (SE).

3. Results and Discussion

3.1 Effect on Nutrient digestibility

Average nutrient retention (%) of experimental broilers during metabolic trial are shown in table 2. The retention of major nutrients such as dry matter, organic matter, crude fat and crude fiber did not differ significantly ($p>0.05$) among the dietary treatments. Dry matter retention remained comparable across groups, indicating that GAA supplementation and 50 kcal/kg reduction in metabolizable energy did not influence overall nutrient digestibility. Similarly, organic matter and crude fat retention values exhibited only marginal variation among treatments, demonstrating no measurable effect of GAA inclusion on nutrient utilization. Crude fiber digestibility also remained statistically similar, suggesting that dietary GAA and MBM inclusion did not alter fiber digestibility.

The results of present study are in agreement with the finding of Pirgozliev *et al.* (2022) [21] and Borges *et al.* (2021) [4] who stated that dry matter digestibility and fat retention was not significantly influenced by GAA supplementation with normal energy levels as well as reduced energy levels.

Table 2: Average nutrient retention (%) of experimental broilers during metabolic trial

Nutrient	T_1	T_2	T_3	SEM	P value
Dry Matter	70.31±4.84	70.11±2.37	70.01±4.10	3.90	0.998
Organic Matter	71.65±4.42	71.39±2.26	70.22±4.58	3.90	0.963
Crude Fat	86.63±3.26	87.26±1.27	86.30±2.03	2.34	0.958
Crude Fiber	33.08±3.22	31.72±3.30	34.96±3.00	3.17	0.774

3.2 Effect on Balance Studies

Means of nitrogen, calcium and phosphorus balance (g/day/bird) during metabolic trial presented in Table 3. Nitrogen, calcium and phosphorus metabolism was not significantly influenced ($p>0.05$) by GAA supplementation or reduction of dietary ME by 50 kcal/kg in MBM based diets. Nitrogen intake, fecal excretion and nitrogen retention showed only slight numerical differences among treatments, indicating similar protein utilization across diets. Likewise, calcium and phosphorus intake as well as balance values remained statistically comparable among treatments, demonstrating that dietary GAA and ME reduction did not affect mineral metabolism in broilers fed MBM-based diets. Although statistically non-significant, birds fed GAA supplemented diets (normal and reduced ME) showed a marginal improvement in nitrogen and phosphorus retention, suggesting a possible positive trend in nutrient utilization efficiency linked to enhanced cellular energy metabolism (Michiels *et al.*, 2012; Bozkurt *et al.*, 2004) [17, 5]. Phosphorus excretion found significantly increased in GAA supplemented corn-soya MBM diet with reduced 50 kcal/kg ME as compared to normal energy and control diet**.** The higher phosphorus excretion in reduced ME diets may be linked to increased MBM inclusion and lower utilization efficiency of its mineral phosphorus under limited dietary energy availability, leading to excessive phosphorus being eliminated rather than retained (Mutucumarana *et al.* 2015) [19].

Table 3: Means of nitrogen, calcium and phosphorus balance (g/day/bird) during metabolic trial

Treatments	T_1	T_2	T_3	SEM	P value
Nitrogen					
Total Intake	3.51±0.15	3.74±0.20	3.23±0.11	0.16	0.125
Excreted in Faeces	0.06±0.02	0.08±0.03	0.05±0.01	0.03	0.299
Balance	3.45±0.15	3.62±0.16	3.17±0.11	0.14	0.127
Calcium					
Total Intake	1.63±0.10	1.80±0.06	1.62±0.16	0.114	0.469
Excreted in Faeces	0.61±0.10	0.65±0.04	0.82±0.20	0.131	0.519
Balance	1.03±0.14	1.15±0.10	0.81±0.10	0.118	0.172
Phosphorus					
Total Intake	0.99±0.04	1.05±0.03	1.11±0.06	0.04	0.222
Excreted in Faeces	0.22^b±0.03	0.17^b±0.01	0.32^a±0.04	0.03	0.015
Balance	0.77±0.05	0.88±0.04	0.79±0.07	0.05	0.331

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

3.3 Effect on carcass characteristics

Carcass characteristics of broilers at 42 days under different dietary treatments are presented in Table 4. No significant differences ($p>0.05$) were observed in pre-slaughter weight and dressed weight among dietary treatments. Dressing percentage differed significantly ($p<0.05$) among treatments, where the Corn-Soya MBM diet supplemented with GAA (T_2) recorded the highest dressing percent, followed by the control MBM diet (T_1), while the reduced ME + GAA diet (T_3) exhibited significantly lower dressing percentage. Weights of internal organs including liver, heart and gizzard did not show any significant treatment effect ($p>0.05$). Giblet weight and percentage were also found statistically similar among all treatment groups. Similarly, abdominal fat weight and abdominal fat percentage did not differ significantly ($p>0.05$). Breast muscle weight and breast muscle yield percentage also remained statistically unaffected ($p>0.05$) by the different dietary treatments.

The improvement in dressing percentage observed in broilers fed MBM diet supplemented with GAA at normal ME level aligns with the role of GAA in enhancing ATP resynthesis efficiency through the creatine-phosphocreatine system, supporting improved muscle accretion (Lemme *et al.*, 2007; Esser *et al.*, 2018) [15, 10]. Cordova-Noboa *et al.* (2018) [7] similarly reported enhanced carcass yield and breast muscle deposition when GAA was combined with animal protein sources such as poultry by-product meal. Although breast muscle did not show significant improvement in the present study, a numerical increase was noted in the reduced energy + GAA group, indicating that performance benefits might be more pronounced under energy-deficient conditions. These findings are in agreement with Cenesiz *et al.* (2020) [6], who demonstrated better carcass traits and breast muscle yield only when GAA was supplemented under diets with lower metabolizable energy fed poultry by product meal.

Al-Abdullatif *et al.* (2024) [2] found similar non-significant responses, with the percentage of breast, liver, and gizzard not affected by dietary GAA (0, 0.06%, and 0.12%) or sex. In contrast to the current findings, Ahmadipour *et al.* (2018) [1] showed decreased heart and liver yield with 1.0-1.5 g/kg GAA supplementation. The current findings are consistent with previous studies published by Yen *et al.* (2025) [27], Valentini *et al.* (2025) [24], and Mohebbifar *et al.* (2022) [18], who found no significant effect of GAA supplementation on abdominal fat percentage in broiler chickens. Overall, the findings reveal that GAA supplementation in MBM-based diets improves dressing percentage without negatively affecting organ weights or fat deposition under normal energy diets.

Table 4: Carcass characteristics of broilers at 42 days under different dietary treatments

Treatments	T_1	T_2	T_3	SEM	P value
Pre Slaughter Wt (g)	2300.50±82.99	2309.00±70.29	2178.75±104.12	86.92	0.520
Dressed Wt (g)	1537.35±57.38	1567.53±39.04	1394.75±49.63	49.26	0.075
Dressing %	66.82^a±0.46	67.92^a±0.38	64.15^b±1.16	0.75	0.017
Liver Wt (g)	53.80±5.59	52.73±2.49	55.10±3.51	4.07	0.919
Heart Wt (g)	12.03±1.25	14.63±1.44	13.73±0.50	1.14	0.310
Gizzard Wt (g)	45.48±0.86	50.80±3.94	44.58±3.60	3.12	0.356
Giblet Wt (g)	109.97±6.31	120.36±6.86	113.40±5.53	6.25	0.515
Giblet %	7.66±0.72	7.85±0.35	8.18±0.59	0.57	0.816
Abdominal Fat Wt (g)	47.33±5.07	46.75±4.03	40.45±7.40	5.68	0.651
Abdominal Fat %	3.08±0.30	2.99±0.29	2.88±0.49	0.37	0.935
Breast muscle (g)	298.60±22.57	292.75±10.32	301.03±18.26	17.78	0.945
Breast muscle yield %	19.41±1.31	18.72±0.86	21.61±1.25	1.16	0.237

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

3.4 Effect on Serum biochemical parameters

Serum biochemical parameters of broilers fed different MBM-based diets are presented in Table 5. Serum glucose levels differed significantly ($p<0.05$) among treatments. Birds fed reduced ME + GAA diet (T_3) showed the highest glucose levels, whereas those supplemented with GAA at normal ME level (T_2) recorded the lowest glucose concentration, with the control group (T_1) remaining intermediate. Serum creatinine, SGPT (ALT), SGOT (AST), and total protein levels did not show any significant differences ($p>0.05$) among dietary treatments. All values remained within normal physiological ranges for broilers, indicating absence of renal or hepatic stress across the treatments.

The decreased serum glucose concentrations seen in GAA-supplemented corn-soya MBM diet with normal energy levels could possibly be attributed to enhanced cellular energy status and stress resilience, decreasing cortisol-driven gluconeogenesis (Michiels *et al.*, 2012; Sapolsky *et al.*, 2000) [17, 22]. However, Xiao *et al.* (2025) [26], Esser *et al.* (2018) [10], and Cordova-Noboa *et al.* (2018) [7] found that GAA supplementation had no significant effect on glucose levels during the experimental period. The absence of significant variations in Serun creatinine, SGPT, SGOT, and total protein suggests that dietary GAA at 600 g/tonne is physiologically safe and does not compromise kidney or liver function.

Table 5: Serum biochemical parameters of experimental broilers at the end of 42 day

Parameters	Treatments			SEM	P value
	T_1	T_2	T_3		
Glucose (mg/dl)	232.71^{b}±5.09	210.46^{c}±8.31	255.80^{a}±6.76	6.85	0.004
Creatinine (mg/dl)	0.27±0.03	0.30±0.02	0.24±0.02	0.02	0.191
SGPT (U/L)	13.32±0.96	12.64±3.16	11.98±0.88	1.97	0.892
SGOT (U/L)	317.11±16.90	253.81±21.80	286.80±36.39	26.36	0.286
Total protein (g/dl)	3.07±0.27	3.01±0.19	3.08±0.09	0.19	0.966

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

3.4 Effect on breast muscle creatine

Breast muscle creatine concentration of broilers fed MBM-based diets differed significantly (p<0.05) among treatments shown in Table 6. Birds receiving the Corn-Soya MBM diet supplemented with GAA at normal energy (T_2) recorded the highest muscle creatine level (2810.09±83.94 mg/kg), followed by reduced energy + GAA diet (T_3: 2716.78±53.28 mg/kg), while the lowest creatine content was observed in the control MBM group without GAA (T_1: 2226.03±138.49 mg/kg). These results clearly demonstrate that creatine deposition in breast muscle is improved by GAA supplementation in MBM-based diets.

The significant enhancement in muscle creatine content in GAA-supplemented diets (T_2 and T_3) reflects the direct role of dietary GAA as a precursor for creatine synthesis in muscle tissue (Lemme *et al.*, 2007; Michiels *et al.*, 2012) [15, 17]. GAA is methylated to creatine in the liver and subsequently transported to skeletal muscle, where it is used for ATP buffering via the creatine-phosphocreatine system. Increased creatine deposition enhances cellular energy availability, boosting muscle growth and meat yield (Wyss and Kaddurah-Daouk, 2000) [25]. The results of present study are in agreement with Majdeddin *et al.* (2023) [16] reported that total creatine content in breast muscle was significantly increased at both 0.6 g/kg and 1.2 g/kg GAA supplementation. However, contradictory results were reported by Khalil *et al.* (2021) [13] and He *et al.* (2019) [12] reported that GAA supplementation at 600-1200 mg/kg of feed had no significant influence on breast muscle creatine concentration.

Table 6: Breast muscle creatine concentration of experimental broilers at the end of experiment

Treatments	T_1	T_2	T_3	SEM	P value
Breast muscle creatine (mg/kg)	2226.03^{b}±138.49	2810.09^{a}±83.94	2716.78^{a}±53.28	98.42	0.005

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

4. Conclusions

Supplementation of guanidinoacetic acid (600 g/tonne) in meat and bone meal-based broiler diets suggest that dietary GAA effectively elevates muscle creatine concentration and dressing percentage without any adverse effects on nutrient utilization or physiological health, indicating its suitability as a functional feed additive in MBM-based commercial broiler production.

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