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Metabolizable energy equivalence of guanidinoacetic acid in corn soybean meal-based broiler diets

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ABSTRACT - In this study, we evaluated how guanidinoacetic acid (GAA) addition in diets with various metabolizable energy (ME) contents affects the performance of broiler chickens. We also estimated the equivalence of GAA in ME. We distributed 1,280 one-day-old broilers in a completely randomized design with eight treatments, eight replicates, and twenty birds per experimental unit. Treatments were based on ME levels (2,775-2,875-2,975 kcal/kg; 2,850-2,950-3,050 kcal/kg; 2,925-3,025-3,125 kcal/kg; or 3,000-3,100-3,200 kcal/kg, from 1 to 7, 8 to 21, and 22 to 42 days of age) and the inclusion of GAA (0 or 600 mg/kg). Supplementation of GAA increased weight gain in broilers at an energy level of 2,908 kcal/kg and improved feed conversion ratio (FCR) at energy levels of 2,908 and 2,983 kcal/kg. There was a linear reduction in feed intake and an improvement in FCR of broilers with increasing levels of energy in diets, with and without GAA addition. Solving the equivalence equation, by applying each of the weighted average energy levels studied. indicates the GAA equivalence of 133, 103, 74, and 44 kcal/kg of diet. In conclusion, GAA supplementation improves broilers' efficiency of energy use; the average ME equivalence of 600 mg/kg of GAA is 88.5 kcal/kg.

Keywords: birds, creatine, performance

1. Introduction

Guanidinoacetic acid (GAA), the common name of N-(aminoimino-methyl)-glycine, is the precursor to creatine, which, together with phosphocreatine, is inextricably involved in cellular energy metabolism through adenosine triphosphate (ATP) regeneration (Portocarero and Braun, 2021). Guanidinoacetic acid is methylated to creatine by the action of enzyme S-adenosylL-methionine:N-guanidino acetate methyltransferase, which, in poultry, is also expressed in the kidneys as well as in the liver (Van Pilsum et al., 1972). The GAA supplementation to broilers has been found to promote growth performance, enhance breast meat yield, and improve feed conversion ratio (FCR; Oviedo-Rondón and Córdova-Noboa, 2020; Zarghi et al., 2020; de Souza et al., 2021). These effects may be partially related to significant increases in high-phosphate energy metabolites in muscle (DeGroot et al., 2018; Majdeddin et al., 2020). In addition, improvements in energy utilization by birds have been linked to better feed utilization (Khajali et al., 2020).

De novo synthesis of GAA requires amino acids glycine and arginine as precursors. Several studies on broilers have been performed to explore the potential of GAA as a "spare" of arginine (Ale Saheb Fosoul et al., 2019; DeGroot et al., 2019). Other studies have demonstrated that GAA supplementation can improve the energy use efficiency in broilers (Mousavi et al., 2013; Ale Saheb Fosoul et al., 2018). In poultry feed, energy is the most expensive component, accounting for 70% of feed cost (Pirgozliev and Rose, 1999; Noblet et al., 2022). Nevertheless, according to Khajali et al. (2020), the GAA equivalence in metabolizable energy (ME) in diets for broilers needs to be determined. The first studies with reduced energy in diets for broilers showed that GAA supplementation can contribute the equivalent of 47.8 kcal/kg ME (Çenesiz et al., 2020) and 50.0 kcal/kg ME (Ceylan et al. 2021).

In this study, we hypothesized that GAA supplementation may improve the energy use efficiency and, consequently, the performance of broilers. Therefore, we evaluated how GAA addition in diets with various energy contents affects the performance of broiler chickens; we also estimated an equivalence in ME of GAA.

2. Material and Methods

2.1. Ethical matters

The Institutional Animal Care and Use Committee approved all animal handling procedures (case number 34/2020), and the experiment was conducted according to the experimental protocol for the use of live birds from the Brazilian College of Animal Experimentation.

2.2. Birds, experimental design, and diets

The experiment was conducted in Viçosa, MG, Brazil (20°45'57.19" S, 42°51'35.42" W, and 682 m altitude). The male broiler chickens (Cobb 500) used in the experiment were obtained from a commercial hatchery (Rivelli Alimentos SA, Matheus Leme, MG, Brazil). The chicks were vaccinated against bursal disease and Marek's disease (Serotype 3, Live Marek's Disease Vector, Merial Inc., Athens, GA).

Based on their body weight, we assigned a total of 1,280 one-day-old broilers to a completely randomized design with eight treatments, eight repetitions, and twenty birds per experimental unit. The birds were housed in 64 floor pens (2 m^2), each equipped with four nipple drinkers and a feed dispenser.

Corn-soybean meal-based diets were formulated to meet the nutritional recommendations given by Rostagno et al. (2017) according to phase, except for ME levels (Table 1). Basal diets contained 2,775, 2,875, and 2,975 kcal/kg in the phases of 1 to 7, 8 to 21, and 22 to 42 days, respectively. Treatments were based on four ME levels per phase (Table 2) and the inclusion of 0 or 600 mg/kg GAA (CreAMINO®, minimum 96% GAA, AlzChem, Trostberg, Germany). The increases in the ME levels of the basal diets were 75, 150, and 225 kcal/kg based on the experimental treatments. These energy density increases were carried out exclusively with the addition of soybean oil instead of the inert. The addition of GAA to the experimental diets was also in place of the inert. The diets were prepared in mashed form. Birds had free access to water and feed throughout the experimental period (1 to 42 days of age), and were exposed to 24 h of light from ages 1–14 days old, after which an 18 h light:6 h dark cycle was implemented until the end of the experiment.

Table 1 - Ingredients and nutrient composition of basal diets (as fed basis)

Ingredient (g/kg)	Age (days)		
	1 to 7	8 to 21	22 to 42
Corn	459.46	493.24	596.75
Soybean meal	447.01	410.72	317.33
Soybean oil	21.81	29.92	25.72
Dicalcium phosphate	19.97	16.83	13.24
Limestone	9.56	8.39	6.92
Salt	5.38	5.16	4.80
DL-Methionine, 999 g/kg	3.54	3.25	2.68
L-Lysine HCl, 780 g/kg	1.81	1.53	2.02
Vitamin premix[1]	1.50	1.30	1.20
Trace mineral premix[2]	1.40	1.20	1.00
Choline chloride, 600 g/kg	1.00	1.00	0.80
L-Threonine, 985 g/kg	0.66	0.58	0.53
L-Valine, 990 g/kg	0.15	0.13	0.26
Coccidiostatic[3]	0.55	0.55	0.55
Inert	26.20	26.20	26.20
Calculated composition (g/kg, unless shown)			
Metabolizable energy (kcal/kg)	2,775	2,875	2,975
Crude protein	243.4	229.1	195.0
Calcium	10.11	8.78	7.05
Available phosphorus	4.82	4.19	3.41
Sodium	2.27	2.18	2.30
Digestible glycine + serine	19.65	18.46	15.49
Digestible lysine	13.64	12.56	10.77
Digestible methionine + cysteine	9.89	9.29	7.97
Digestible valine	10.29	9.67	8.29
Digestible threonine	8.82	8.29	7.11
Digestible tryptophan	2.82	2.64	2.17

[1] Vitamin premix per kilogram contained: vitamin A, 9,637,000 IU; vitamin D3, 2,409,000 IU; vitamin E, 36,100 IU; vitamin K3, 1,930 mg; vitamin B1, 2,590 mg; vitamin B12, 15.9 mg; vitamin B6, 3,610 mg; vitamin B5, 12.95 g; vitamin B3, 39.2 g; vitamin B9, 903 mg; biotin, 89.8 mg.
[2] Trace mineral premix per kilogram contained: Mn, 58.36 g; Zn, 54.21 g; Fe, 41.68 g; Cu, 8.31 g; I, 843 mg; Se, 250 mg.
[3] Salinomycin 12%.

Table 2 - Experimental treatments

Guanidinoacetic acid (mg/kg)	Age (days)			Weighted average 1 to 42
	1 to 7	8 to 21	22 to 42	
0	2,775 kcal	2,875 kcal	2,975 kcal	2,908 kcal
0	2,850 kcal	2,950 kcal	3,050 kcal	2,983 kcal
0	2,925 kcal	3,025 kcal	3,125 kcal	3,058 kcal
0	3,000 kcal	3,100 kcal	3,200 kcal	3,133 kcal
600	2,775 kcal	2,875 kcal	2,975 kcal	2,908 kcal
600	2,850 kcal	2,950 kcal	3,050 kcal	2,983 kcal
600	2,925 kcal	3,025 kcal	3,125 kcal	3,058 kcal
600	3,000 kcal	3,100 kcal	3,200 kcal	3,133 kcal

2.3. Performance and carcass characteristics

Birds and feed leftovers, were weighed at 42 days of age to calculate feed intake (FI), weight gain (WG), and FCR. Mortalities were recorded throughout the experimental period, and the necessary corrections of performance data were calculated.

At 42 days old, two birds with weights closest to the average weight of their respective experimental unit were selected. After 8 h of fasting, these broilers were euthanized and slaughtered to measure the yield of carcass, breast, and thigh with drumstick, as well as the relative weight of abdominal fat. Carcass yield was calculated in relation to living weight before slaughter (carcass weight × 100/live weight) and breast and thigh with drumstick yield as a function of carcass weight (part weight × 100/carcass weight). The relative weight of abdominal fat was calculated in relation to the birds' live weight before slaughter.

2.4. Statistical analysis and ME equivalence calculations

For each variable, the analysis of variance was performed according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \tag{1}$$

in which Y_{ij} is the measured dependent variable, μ is the overall mean, α_i is the effect of treatments, and ε_{ij} is the random error.

Analyses were carried out using the PROC GLM of SAS (Statistical Analysis System, version 9.4). The significance of the effects was tested at the 5% probability level. To assess the effect of including GAA at each energy level, contrast analyses were performed. Linear equations for energy levels with or without GAA supplementation were also estimated using the PROC REG of SAS. Significance for each of the regression model parameters was tested at the 5% probability level using Student's t test.

The ME equivalence of the GAA was estimated with a methodology adapted from Jendza et al. (2006) and Stefanello et al. (2017). Linear effects of increasing ME in diets with or without GAA addition were tested. Regression equations of ME levels were generated for FCR, and an equivalence equation was obtained by equating the two linear equations estimated as follows:

$$Y = a + bX_1 \text{ (FCR response according to ME levels in diets with GAA)} \tag{2}$$

$$Y = a + bX_2 \text{ (FCR response according to ME levels in diets without GAA)} \tag{3}$$

Equivalence equation:

$$a + bX_2 = a + bX_1 \tag{4}$$

in which Y is the FCR response; X_1 is the ME level in diets with GAA; X_2 is the ME level in diets without GAA; a is the intercept in each respective equation; and b is the slope in each respective equation.

The equivalence equation was solved by substituting the weighted average energy levels studied in X_1 and obtaining X_2. The equivalence in ME of the GAA was estimated at each energy level studied by subtracting X_1 from X_2, and the average of the estimates was calculated.

3. Results

There was no effect of GAA supplementation on the FI of broilers at any energy level studied (P>0.05; Table 3). However, GAA supplementation increased the WG of broilers at the energy level of 2,908 kcal/kg (P = 0.036) and improved the FCR at the energy levels of 2,908 kcal/kg (P = 0.004) and 2,983 kcal/kg (P = 0.049). Regarding the energy levels, there was a linear reduction in the FI of broilers with increasing levels of energy in the diets without (P = 0.015) and with (P = 0.018) GAA addition (Table 4). The FCR improved linearly with increasing levels of energy in the diets without (P<0.001) and with (P = 0.008) GAA. Solving the equivalence equation (by applying the weighted average energy levels studied) indicates that the ME equivalence of GAA were 133, 103, 74, and 44 kcal/kg of diet, with an average equivalence of 88.5 kcal/kg (Table 5).

The carcass, breast and thighs with drumstick yield, and abdominal fat of the birds were not influenced by GAA supplementation at any energy level studied (P>0.05; Table 6); these parameters were also unaffected by the energy levels in the diets (P>0.05).

Table 3 - Growth performance of broiler chickens from 1 to 42 days of age

	GAA (mg/kg)	Energy level (kcal/kg)[1]				Linear P-value
		2,908	2,983	3,058	3,133	
FI (kg/bird)	0	5.246	5.208	5.134	5.122	0.015
	600	5.234	5.169	5.140	5.100	0.018
	SEM	0.025	0.035	0.030	0.021	
	P-value	0.805	0.585	0.921	0.603	
WG (kg/bird)	0	3.026b	3.062	3.084	3.106	0.069
	600	3.115a	3.117	3.116	3.139	0.605
	SEM	0.019	0.031	0.020	0.016	
	P-value	0.036	0.391	0.423	0.314	
FCR	0	1.734a	1.701a	1.665	1.649	<0.001
	600	1.680b	1.658b	1.650	1.625	0.008
	SEM	0.008	0.010	0.014	0.008	
	P-value	0.004	0.049	0.567	0.135	

GAA - guanidinoacetic acid; FI - feed intake; WG - body weight gain; FCR - feed conversion ratio; SEM - standard error of the mean (n = 8 for treatment).
[1] Metabolizable energy weighted average calculated from the following values: 2,775-2,875-2,975 kcal/kg; 2,850-2,950-3,050 kcal/kg; 2,925-3,025-3,125 kcal/kg; 3,000-3,100-3,200 kcal/kg, from 1 to 7, 8 to 21, and 22 to 42 days of age, respectively.
Means within each column followed by different letters differ by Tukey's test (P<0.05).

Table 4 - Linear regression equations estimated for each variable on the response of energy levels with or without guanidinoacetic acid (GAA) supplementation

	GAA (mg/kg)	Regression equation	SE intercept	P-value intercept	SE slope	P-value slope	r^2
FI	600	$Y = 6.89883 - 0.0005755X_1$	0.69392	<0.001	0.00022965	0.018	0.17
	0	$Y = 6.97577 - 0.00059533X_2$	0.69719	<0.001	0.00023073	0.015	0.18
FCR	600	$Y = 2.36209 - 0.0002345X_1$	0.24917	<0.001	0.00008246	0.008	0.21
	0	$Y = 2.85391 - 0.000386X_2$	0.25028	<0.001	0.00008283	<0.001	0.42

FI - feed intake; FCR - feed conversion ratio; SE - standard error.

Table 5 - Equivalence equation for feed conversion ratio (FCR) and to estimate the energy equivalence of guanidinoacetic acid (GAA)

Equivalence equation for FCR[1]	Energy level (kcal/kg)			
	X_1			
$2.85391 - 0.000386X_2 = 2.36209 - 0.0002345X_1$	2,908	2,983	3,058	3,133
	X_2			
	3,041	3,086	3,132	3,177
Energy equivalence of GAA	$X_2 - X_1$			
	133	103	74	44

[1] The equivalence equation was obtained by equating the two linear equations estimated to the FCR. The equivalence equation was solved by substituting the weighted average energy levels studied in X_1 and obtaining X_2. The ME equivalence of the GAA was estimated at each level studied by subtracting X_1 from X_2.

4. Discussion

In this study, we hypothesized that GAA supplementation may improve broilers' energy use efficiency. This was confirmed by the improvement in WG and FCR of broilers fed diets with the two lower energy levels studied. According to the recommendations of the National Research Council (NRC, 1994), along with more recent standards adopted by the Brazilian Poultry Sector (Rostagno et al., 2017), these ME levels are a performance-limiting factor. The improvements in WG and FCR may be explained by higher creatine and phosphocreatine levels and higher ATP:ADP and phosphocreatine:ATP ratios in the

Table 6 - Carcass yield, abdominal fat (% of live weight), breast, and thighs with drumstick (% of carcass) of broiler chickens at 42 days of age

	GAA (mg/kg)	Energy level (kcal/kg)[1]				Linear P-value
		2,908	2,983	3,058	3,133	
Carcass (%)	0	81.18	80.60	81.15	80.17	0.313
	600	81.83	81.00	80.79	80.92	0.260
	SEM	0.39	0.39	0.49	0.32	
	P-value	0.419	0.617	0.718	0.262	
Breast (%)	0	37.11	37.32	35.67	36.04	0.373
	600	37.34	37.17	36.95	36.69	0.071
	SEM	0.34	0.42	0.52	0.31	
	P-value	0.731	0.861	0.234	0.311	
Thighs with drumstick (%)	0	25.71	25.85	24.33	25.92	0.738
	600	25.53	26.03	25.60	25.94	0.550
	SEM	0.18	0.22	0.55	0.19	
	P-value	0.617	0.692	0.263	0.962	
Abdominal fat (%)	0	0.76	0.80	0.89	0.83	0.312
	600	0.81	0.79	0.75	0.81	0.968
	SEM	0.06	0.04	0.05	0.04	
	P-value	0.705	0.896	0.158	0.877	

GAA - guanidinoacetic acid; SEM - standard error of the mean (n = 8 for treatment).
[1] Metabolizable energy weighted average calculated from the following values: 2,775-2,875-2,975 kcal/kg; 2,850-2,950-3,050 kcal/kg; 2,925-3,025-3,125 kcal/kg; 3,000-3,100-3,200 kcal/kg, from 1 to 7, 8 to 21, and 22 to 42 days of age, respectively.

muscles of broilers fed diets with GAA (Yazdi et al., 2017; DeGroot et al., 2018; Majdeddin et al., 2020); these improved parameters indicate more efficient energy metabolism. The phosphocreatine:ATP ratios in the breast muscles of broilers were reported to be 28.4 and 20.3 for those that received GAA at 600 mg/kg and for the control group, respectively (Yazdi et al., 2017). Ale Saheb Fosoul et al. (2018) reported that the enhancement in the buffering capacity for ATP in the muscles exerted by supplemental GAA affects the metabolism of energy in broiler chickens fed diets with energy reduction, resulting in an improved FCR. In addition to functioning directly in muscle accretion as the precursor to creatine, dietary GAA can also effectively “spare” arginine from being used for GAA synthesis, so that the arginine may be used for muscle accretion and other physiological functions (Portocarero and Braun, 2021). As in the present study, Mousavi et al. (2013) reported that GAA supplementation can potentially improve the FCR and energy efficiency of broilers.

Previous study showed that the use of GAA improved the breast meat yield, but without effect on carcass and other cuts (Córdova-Noboa et al., 2018). In the present study, no effects of GAA were observed on carcass, breast, and thighs with drumstick yield. Similar results were observed by Mousavi et al. (2013), who also evaluated the effect of GAA addition to diets containing different levels of ME.

With increasing levels of ME in diets without and with GAA addition, the birds reduced their FI. This result was expected, based on the literature, because broilers may adjust their FI in response to their energy needs (Leeson et al. 1996; Hu et al., 2021). This is linked to metabolic signalization. Hu et al. (2019) reported that the central adenosine monophosphate-activated protein kinase signaling pathway and appetite are modulated in accordance with the energy level in the diet to regulate nutritional status and maintain energy homeostasis in broilers.

With the reduction in FI and no effect on WG, the FCR of broilers improved with increasing levels of energy in the diets with and without GAA addition, in accordance with several reports (Leeson et al., 1996; Ale Saheb Fosoul et al., 2018; Hu et al., 2021). The FCR responses observed in this study suggest an average ME equivalence of 88.5 kcal/kg, different from the values of 47.8 kcal/kg ME (Çenesiz et al., 2020) and 50.0 kcal/kg ME (Ceylan et al., 2021) observed in studies with reduced energy in diets and the same GAA supplementation. However, further research is needed to validate this dietary ME in practical diets.

5. Conclusions

Guanidinoacetic acid supplementation improves the energy use efficiency in broilers, and the average metabolizable energy equivalence of 600 mg/kg of guanidinoacetic acid is 88.5 kcal/kg. This is a prominent novel finding from our study.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: G.C. Rocha, M. Schmidt and A.A. Calderano. Data curation: A.A. Calderano. Formal analysis: A.A. Calderano. Funding acquisition: A.A. Calderano. Investigation: H.R. Salgado, R.A. Nunes, S.O. Borges and A.A. Calderano. Methodology: H.R. Salgado, G.C. Rocha, T.G. Petrolli, M. Schmidt and A.A. Calderano. Project administration: A.A. Calderano. Resources: A.A. Calderano. Supervision: M. Schmidt and A.A. Calderano. Validation: M. Schmidt, J.A. Rivera and A.A. Calderano. Visualization: G.C. Rocha, J.A. Rivera and A.A. Calderano. Writing – original draft: H.R. Salgado, R.A. Nunes and S.O. Borges. Writing – review & editing: T.G. Petrolli, J.A. Rivera and A.A. Calderano.

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