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Influence of dietary supplementation of guanidinoacetic acid on growth performance and blood chemistry profile of growing steers

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ABSTRACT

Guanidinoacetic acid (GAA, Cas no. 352-97-6) is a feed additive that positively influences the energy and protein metabolism of animals, so it has the potential to improve the productivity of animals without affecting their health. The objective of the present study was to evaluate the influence of dietary supplementation with GAA on growth performance and blood chemistry profile of growing steers for 60 d. Forty growing crossbred male steers (*Bos taurus* × *Bos indicus*; 146.0 ± 0.5 kg body weight (BW)) were randomly divided into two experimental groups (n = 20): the first, consisted of a total mixed ration (TMR) without; and the second, substituted the 0.1 % of the TMR with GAA. Dietary supplementation of GAA decreased ($P < 0.0001$) dry matter intake (DMI) by 12.8 % compared to the steers that were fed the TMR without GAA. Although these changes did not influence ($P = 0.4180$) BW and average daily gain (ADG), they improved ($P < 0.0490$) feed conversion efficiency (FCE) by decreasing DMI per kilogram of ADG by 10.4 %. Furthermore, the inclusion of GAA in the TMR reduced ($P < 0.0050$) triglycerides by 23.2 % and increased the urea nitrogen (BUN) in the steers by 22.4 % ($P < 0.0002$). However, sampling time influenced ($P \leq 0.0042$) all blood chemistry parameters except calcium, and the interaction between GAA supplementation and time did not significantly influence ($P \geq 0.0750$) any parameter. In conclusion, dietary supplementation of 1 g GAA kg^{-1} of TMR, decreased the DMI and improved FCE without negative effects on blood chemistry profiles.

1. Introduction

The world population is growing at an accelerated rate, which implies an increase in the demand for food of both animal and plant origin and consequently, there is intense pressure on natural resources due to the competition generated by the use of land for crops and livestock [1–3]. Additionally, climate change negatively impacts food security due to the variability of the precipitation distribution and the atmospheric temperature that causes instability of agricultural production and the availability of forage for livestock [4,5]. In this scenario, there is a global concern about how to sustainably satisfy the growing and changing demand for food, mainly foods of animal origin [6]. Ruminant livestock plays an important role due to their ability to transform fibrous feeds into foods of high nutritional value for humans [7]. However, it is necessary to improve the productivity and efficiency in the production of this type of livestock to guarantee its sustainability, and therefore food security [6].

In Mexico, livestock farming is mainly based on direct grazing, also known as the extensive system, under this system the animals have low productivity, generate greater environmental impact, and consume more natural resources [8,9], which leads to inefficient use of these resources and increases greenhouse gas (GHG) emissions. Consequently, studies that are being carried out are focused on creating strategies to reduce the ruminant's GHG emissions and increase the sustainable use

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Table 1
Ingredients and chemical composition of the total mixed rations (TMR) used in each period evaluation.

Items	Total mixed rations[a]	
	Period 1	Period 2
Ingredients, g kg^{-1} TMR		
Ground sorghum grain	407	560
Buffel grass hay	190	50
Sugarcane molasses	105	90
Sorghum straw	140	180
Dehydrated brewer's yeast	60	40
Cottonseed meal[b]	70	50
Urea	3.5	5.0
Farmix BC 250 FX4 C/Lev[c]	25.0	25.0
Nutrient composition, g kg^{-1} DM[d]		
Crude protein	154.3	148.0
Net energy maintenance (Mcal kg^{-1})	1.58	1.69
Net energy gain (Mcal kg^{-1})	0.95	1.06
Crude fiber	145.1	104.4
Crude fat	18.9	22.8
Water	122.6	121.5
Calcium	9.4	8.9
Phosphorus	3.5	3.4

[a] Period 1: from day 1–30; Period 2: from day 31–60.
[b] Cottonseed meal is a by-product derived from the milling (oil extraction) of cotton seeds, which is used as a source of protein.
[c] Farmix BC 250 FX4 C/Lev (Trouw Nutrition México, S.A. de C.V.) is a premix of vitamins, minerals and additives for intensive fattening livestock medicated with monensin sodium, which contains calcium carbonate ($CaCO_3$) 22 %, crude protein 60 %, urea, cane molasses, sodium chloride 4.80 %, vitamin A acetate, vitamin E acetate, manganese, zinc, copper, EDD (iodine source), selenium, cobalt, live yeast (*Saccharomyces cerevisiae*) and monensin sodium (1200 g t^{-1}).
[d] Based on analysis except for calcium, phosphorus and metabolizable energy estimated from the NRC value tables (NRC, 2000).

of natural resources [10]. In this sense, fattening livestock in stalls, also known as an intensive system, improves the use of resources and the productivity of livestock until they reach slaughter weight, but it involves more economic costs associated with the infrastructure and human resources and, mostly with the livestock feeding [11]. Then, the use of feed additives in livestock farming has gained a lot of popularity because they can improve the feed efficiency and health of livestock, as well as reduce feed costs [12].

The guanidinoacetic acid (GAA; also called glycocyamine, Cas No. 352-97-6), a product from the amino acids arginine and glycine, is the direct metabolic precursor of creatine biosynthesis [13] and participates in energy metabolism and protein synthesis in cattle muscle [14,15], so it is considered to have the potential to improve the growth performance of livestock without compromising their health [16,17]. Including GGA as a feed additive in ruminants' diets had improved the body weight (BW), the average daily gain (ADG), and the feed conversion efficiency (FCE) [18,19], avoiding excessive fat deposits in the subcutaneous and visceral adipose tissue and therefore, improving the yield and quality of the carcass [20,21]. Additionally, GGA might increase the abundance of total bacteria and fungi and decrease the abundance of total protozoa in the rumen, which improves nutrient digestion and utilization and reduces the GHG production from both fibrous and non-fibrous feeds [22,23]. However, most of the studies carried out on beef cattle included bulls or steers older than 12 months old and body weight greater than 200 kg, and there is a lack of information about the effect of GGA inclusion in diets for younger and lower BW steers.

It has been hypothesized that dietary supplementation with GAA could also favorably influence the productivity of growing steers without negative implications on the health of the animals, therefore, the objective of the present study was to evaluate the influence of dietary supplementation with GAA on the growth performance and blood chemistry profile of growing steers.

2. Materials and methods

2.1. Experimental site

The work was performed at the facilities of the company Pa'Lante México S.A of C.V., which is dedicated to the purchase of livestock for export nationwide in Mexico, and in the state of Tamaulipas it is located at kilometer 14 from the Cd. Victoria - Soto la Marina highway, at the height of the town of San Juan and El Ranchito, municipality of Victoria (geographic coordinates: 23° 43′ 11″ N and 99° 00′10″ W, 240 m above the sea level).

2.2. Animal bioethics regulations

During the entire experimental period, the management of the steers was carried out with strict adherence to the Official Mexican Norms NOM-051-ZOO-1995, NOM-062-ZOO-1999 and NOM-024-ZOO-1995. These official norms establish the actions for the humane treatment of animals, specify techniques for the production, care and use of laboratory animals and indicate the zoosanitary measures for the transport of animals, their products and by-products, chemical, pharmaceutical, biological and feed products for use in animals or consumption by them.

2.3. Treatments and experimental procedures

Forty growing crossbred male steers (crossbreeding of the *Bos taurus* × *Bos indicus* breeds) of six months old, and 146.0 ± 0.5 kg of BW were used (those steers were weaned at five months old). Before the evaluation, the steers were treated against internal and external parasites (200 μg kg^{-1} BW; Dectiver®, Lapisa®, La Piedad, Michoacan, Mexico) and vaccinated intramuscularly (5 mL $animal^{-1}$; Bacterina Biobac 11 Vías®, Biozoo®, Zapopan, Jalisco, Mexico). During the evaluation animals were housed as a group in pens of 88.65 m^2 (16.16 × 5.34 m) equipped with shade, feeders, and waterers, and all steers had freshwater *ad libitum* at all times.

The steers were randomly assigned into two experimental groups (each with 20 animals), and each group to treatment: the first, consisted of a total mixed ration (TMR) without (GAA−); and the second, substituted the 0.1 % of the TMR with (GAA+) (GuanAMINO® for ruminants, at 96 % of purity), which is equivalent to 1 g GAA kg^{-1} TMR, according to the results of Li et al. [24] study. The TMR was formulated to satisfy the nutritional requirements of the steers and to obtain an ADG of 1200 g d^{-1}, as recommended by the NRC (Table 1) [25], and was supplied at 07:00 and 14:00 h, in proportions of 60 and 40 % of the total daily consumption, respectively. The evaluation period was composed of two periods of 30 d each (the experiment lasted 60 d), after both groups were being adapted to their respective treatment. During the evaluation period, samples of TMR were collected once a week and stored until chemical analysis.

2.4. Data collection

The BW was measured before the morning feeding on d 15, 30, 45 and 60 of the evaluation periods, and was reduced by 4 % to adjust for gastrointestinal filling [26]. The feed offered and refused was recorded daily, and in the case of the feed offered, it was daily adjusted according to the daily feed intake of the previous day. From these data, DMI, BW (at different evaluation times), and ADG were estimated, as well as the final BW (FBW) and the FCE for the entire evaluation period. Asides this, blood samples were taken from each steer from the coccygeal vein before morning feeding on d 1, 15, 30, 45 and 60 of the evaluation periods, and samples were collected in tubes (BD Vacutainer® brand, model 368175, Franklin Lakes, NJ, USA) for serum analysis with coagulation activator. These samples were centrifuged at 4 °C and 3400 g for 20 min to separate serum and plasma, and then stored at −20 °C until analysis.

Table 2
Means (± standard error) of dry matter intake, average daily gain and feed conversion efficiency of growing steers fed a total mixed ration without (GAA−) and with (GAA+) the inclusion of guanidinoacetic acid.

Items	Treatment[a]		*P*-values
	GAA−	GAA+	
Dry matter intake (kg d^{-1})	8.1 ± 0.1[a]	7.0 ± 0.1[b]	<0.0001
Body weight (kg)			
0 d	149.6 ± 1.5[a]	144.2 ± 1.4[b]	0.0230
15 d	163.3 ± 2.0	165.6 ± 1.9	0.4180
30 d	182.9 ± 2.9	183.2 ± 2.7	0.9400
45 d	200.1 ± 3.1	203.5 ± 3.0	0.7600
60 d	217.5 ± 3.2	219.5 ± 3.2	0.6800
Average daily gain (kg d^{-1})			
1–15 d	1.0 ± 0.1	1.3 ± 0.1	0.3630
16–30 d	1.3 ± 0.1	1.2 ± 0.1	0.5480
31–45 d	1.2 ± 0.1	1.2 ± 0.1	0.9750
46–60 d	1.0 ± 0.1	1.1 ± 0.1	0.7120
1–60 d	1.2 ± 0.1	1.2 ± 0.1	0.9140
Feed conversion efficiency[b]	6.8 ± 0.2[a]	6.1 ± 0.2[b]	0.0490

Means in the same row with different superscript letters indicate significant differences (Tukey, $P \leq 0.05$).
[a] GAA−: total mixed ration without GAA; GAA+: total mixed ration with 1 g GAA kg^{-1}.
[b] Feed conversion efficiency was estimated by dividing dry matter intake by average daily gain.

2.5. Analysis of feed and blood samples

The samples of the TMR, the feed offered, and rejected were dehydrated in a forced air oven at 60 °C for 48 h and, subsequently, the dry matter (DM) content was determined according to the method 930.15 of the Association of Official Analytical Chemists (AOAC) [27] to obtain the estimate of the DMI by the difference in weights. The blood serum samples were analyzed for the concentration of cholesterol, glucose, triglycerides, urea nitrogen (BUN), calcium (Ca), phosphorus (P), creatinine, and bilirubin using a semi-automatic clinical chemistry analyzer (Minray brand, model BA-88A, Guangzhou, China).

2.6. Statistical analysis

The growth performance variables were analyzed under a completely randomized design with 20 repetitions per treatment; the blood chemistry analysis was repeated over time with 10 repetitions per treatment, and in both cases, the statistical analysis program SAS version 9.2 (SAS, Cary, NC, USA) was used. The analysis of variance (ANOVA) of growth performance was carried out with the GLM procedure, including the treatments as fixed effects and initial BW as a covariate. In all blood chemistry parameters, except glucose, the GLIMMIX procedure was used, including treatments, time, and the interaction of both as fixed effects, while for glucose the MIXED procedure was used, considering treatments, time, and the interaction of both as fixed effects. The multiple comparison of means was performed using the Tukey test, and the means were considered significantly different when they presented a *P*-value ≤0.05 and a trend of $P \leq 0.10$.

3. Results

3.1. Growth performance

The growing crossbred steers supplemented with GAA+ reduced their DMI by 12.8 % ($P < 0.0001$) compared with the group of steers that did not receive supplementation, however, BW and ADG were not affected ($P \geq 0.4180$). Although at the beginning of the evaluation (0 d), the steers supplemented with GAA weighed 3.4 % less than the steers without supplementation with GAA ($P < 0.0230$), at the end of the evaluation (60 d) both groups of steers had similar BW. Steers supplemented with GAA had better FCE than those not supplemented (6.12 *vs.* 6.83), and this difference corresponded to 10.4 % less DMI per kilogram of ADG (Table 2).

3.2. Blood chemistry profile

The blood of growing crossbred steers supplemented with GAA showed a 23.2 % less triglyceride concentration ($P < 0.0050$) and 22.4 % more BUN ($P < 0.0002$) than steers without that supplementation (Table 3). However, the concentration of cholesterol, glucose, Ca, P, creatinine, and bilirubin were not affected by the GAA inclusion ($P \geq 0.135$).

Although the time affected almost all blood chemistry variables ($P \leq 0.0042$), except for Ca ($P > 0.755$) 0.0042), there was no interaction between the GAA supplementation and the time ($P \geq 0.0750$).

4. Discussion

4.1. Growth performance

In adult steers and bulls, GAA has been shown to promote greater growth development and improvement in FCE without compromising animal health. In the present study, dietary supplementation with GAA decreased the DMI of growing crossbred steers, which is in contrast to the findings of Yi et al. [23], who reported that the inclusion of GAA at doses of 0.8 and 1.6 g kg^{-1} of DM in the diet of 16-month-old Angus steers did not significantly influence DMI, and with what was reported by Li et al. [24], who by supplementing 11.8-month-old Angus bulls with 0.3–0.9 g GAA kg^{-1} (on DM basis) observed an increase in DMI. These discrepancies are probably due not only to the doses evaluated in each study, but also to the age of the steers and bulls and the TMR used,

Table 3
Means (± standard error) of blood chemistry profile of growing steers fed a total mixed ration without (GAA−) and with (GAA+) the inclusion of guanidinoacetic acid.

Item	Treatment[a]		*P*-value[b]		
	GAA−	GAA+	Treatment	Time	Interaction
Cholesterol (mg dL^{-1})	103.54 ± 2.41	99.57 ± 2.94	0.3020	0.0420	0.3360
Glucose (mg dL^{-1})	87.78 ± 3.09	87.90 ± 3.04	0.9780	0.0330	0.2350
Triglycerides (mg dL^{-1})	35.99 ± 1.56[a]	29.21 ± 1.63[b]	0.0050	<0.0001	0.0750
BUN (mg dL^{-1})[c]	16.81 ± 0.54[b]	21.67 ± 1.19[a]	0.0002	<0.0001	0.2560
Calcium (mg dL^{-1})	9.23 ± 0.15	9.44 ± 0.09	0.2330	0.7550	0.3010
Phosphorus (mg dL^{-a})	7.25 ± 0.23	6.82 ± 0.18	0.1350	<0.0001	0.7060
Creatinine (mg dL^{-1})	1.25 ± 0.03	1.34 ± 0.06	0.2200	0.0010	0.5520
Bilirubin (mg dL^{-1})	0.43 ± 0.02	0.39 ± 0.02	0.2400	<0.0001	0.6210

Means in the same row with different superscript letters indicate significant differences (Tukey, $P \leq 0.05$).
[a] GAA−: total mixed ration without GAA; GAA+: total mixed ration with 1 g GAA kg^{-1}.
[b] Effect of the treatment with the inclusion of guanidinoacetic acid in the total mixed ration, sampling time and the interaction of both factors.
[c] BUN: blood urea nitrogen.

and in the case of the current study, the effect of GAA on DMI may be associated with the participation of GAA in energy metabolism, which possibly led to an increase in the energy concentration of the TMR. If so, it is possible that steers supplemented with GAA decreased DMI as a mechanism to regulate their energy consumption [28].

However, it was observed a positive response in FCE when GAA was included in the diets of the growing crossbred steers, despite of the fact that they had a lower DMI. In previous studies including 0.6–1.6 g GAA kg^{-1} of diet (on DM basis) increased the FCE of Angus steers and bulls from 9.4 to 16.1 % [19,23,24,29], while in bulls Jinjiang Li et al. [18] reported that supplementation with 2 g GAA kg^{-1} of diet (on DM basis) improved FCE by 35.0 %.

Including GAA in ruminants' diets could increase activity of fibrolytic enzymes, α-amylase, and proteases inside the rumen, as well as the total fungal and bacteria counts, and the number of specific microorganisms such as *Ruminococcus albus*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Ruminobacter amylophilus* and *Prevotella ruminicola* [18,19,30]. Ruminal microbes can use GAA as a source of energy and nitrogen for their biological activity and synthesize their proteins, which could have favored the degradation of nutrients, the growth of microbes and the increase in microbial protein production in the rumen of steers supplemented with GAA, as has been reported in other studies [31,32]. Additionally, GAA can decrease the total population of ruminal protozoa and methanogens, and at high levels (≥0.8 g GAA kg^{-1} diet DM) has the capacity to increase the total concentration of short-chain fatty acids (SCFA) and the molar proportion of propionate, which together with acetate and butyrate represent an important source of energy for ruminants [30,33], this also results in lower methane production and higher energy conversion efficiency [33].

The improvement of ruminal fermentation promoted by GAA inclusion might lead to greater energy availability for growth development, therefore GAA induced changes in the energy availability of the TMR, which is related to the decrease of the DMI. However, it is also not ruled out that the null influence of dietary supplementation with GAA on the body weight and average daily gain of the steers is related to the deficiency of methyl groups, since, although cattle can produce it through catabolism of the feed, the GAA is an important consumer of methyl and probably the steers did not produce what was necessary to cover the demand and synthesize enough creatine [23,34].

4.2. Blood chemistry profile

The parameters of the blood chemical profile of the growing crossbred steers supplemented with GAA did not present alterations, except triglycerides and BUN, however, all variables remained within the range reported for cattle by Roa-Vega et al. [35] and in the reference values reported for glucose, P, and Ca by Latimer et al. [36] who also concluded that GAA does not produce negative effects on the health of animals, when they reported that the inclusion of 0.3–0.9 g GAA kg^{-1} of DM did not influence the BUN and serum glucose of Angus bulls [24], while in sheep the supplementation with GAA it decreased glucose and BUN, increased creatinine and did not influence cholesterol and triglycerides [37]. In the current study, glucose concentration was not influenced by GAA supplementation, which can be attributed to the concentration of creatinine in the blood because it exerts a stimulating effect on the accumulation of glycogen in the muscles, which is the main form of glucose storage in the body [38]. The BUN is an index of nitrogen balance and protein utilization, so it is negatively correlated with the utilization rate of amino acids for protein synthesis [39], and in the present study, BUN was higher in steers supplemented with GAA. These findings agree with those reported by Speer et al. [40], who observed that BUN increased with the abomasal infusion of 7.5 and 15 g GAA d^{-1} in Angus steers and can be attributed to a greater deamination of the proteins and amino acids of the TMR, as well as an increase in protein digestibility [23]. In sheep, GAA supplementation was reported to increase creatinine and reduce BUN, glucose, and triglycerides [41], demonstrating that GAA reduces muscle fat deposition. Similarly, in the current study, GAA decreased triglycerides in the supplemented steers, but the values were within a reasonable range [42,43]. In addition to this, Aziza et al. [44] reported that increasing the dose of GAA from 0.6 to 1.8 g kg^{-1} diet (on DM basis) linearly increased cholesterol and triglycerides.

Other studies indicated that creatinine may increase with the inclusion of GAA [24], but this effect was not present in the current study and was opposite to that reported by Li et al. [18], who observed that creatinine increased compared to the control group with a dose of 0.5–4 g GAA kg^{-1} diet (on DM basis). In addition to this, increasing creatinine levels can promote the digestibility of nutrients at the ruminal level and increase the concentration of SCFA and the proportion of propionate, which favors energy metabolism [45]. Furthermore, the increase in creatinine is also an indicator of the conversion of GAA to creatine [38], since creatine is synthesized from GAA and the co-substrate S-adenosylmethionine that provides the methyl group [46], indicating that the dose evaluated in the present study was probably suboptimal. In total bilirubin, the values obtained were within the reference range (0.1−0.5 mg dL^{-1}), in accordance with what was reported by Kaneko et al. [42]. Regarding the fluctuations due to sampling time, it is likely that they were caused by the environment because blood is overly sensitive to environmental changes, as reported by Bhan et al. [47]. These same authors reported that quantitative and morphological changes in blood cells are also related to the physiological or pathological state of the animal.

5. Conclusion

Adding guanidinoacetic acid into the total mixed ration of steers at a dose of 1.0 g kg^{-1} reduced dry matter intake and improved feed conversion efficiency while the body weight and average daily gain were unaffected. Furthermore, it did not negatively alter the blood chemistry profile, so it can be stated that this dose does not compromise the health status of the steers. However, it is recommended to evaluate different doses of guanidinoacetic acid inclusion, and diets high and low in fibrous carbohydrates to know if the potential of this feed additive depends on the proportion of these carbohydrates.

CRediT authorship contribution statement

Jaime Sánchez-Villasana: Investigation, Writing – original draft. **Daniel López-Aguirre:** Conceptualization, Methodology, Writing – review & editing. **Luz Yosahandy Peña-Avelino:** Methodology, Resources, Data curation, Formal analysis, Writing – original draft. **Cecilia Carmela Zapata-Campos:** Methodology, Resources, Data curation, Formal analysis, Writing – original draft. **Edwin Rafael Alvarado-Ramírez:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Project administration, Data curation, Writing – review & editing. **Deli Nazmín Tirado González:** Writing – review & editing. **Abdelfattah Zeidan Mohamed Salem:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Project administration, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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