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Farm-Scale Effectiveness of Feed Additives Supplied through a Mineral Mix for Beef Cattle Grazing Tropical Pastures

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Abstract: The effectiveness of feed additives delivered through free-choice mineral mixtures (MMs) to grazing cattle remains unclear. Two farm-scale and one in vitro experiment (Exp.) were conducted to investigate the effects of salinomycin and virginiamycin, delivered through an MM, on growing bulls grazing tropical pastures. In Exp. 1, 316 zebu (*Bos indicus*) Nellore bulls (225 ± 26.7 kg liveweight (LW)) were randomly allocated to four treatments: (1) MM no additives (CON), (2) MM with salinomycin at 1950 mg/kg (SLI), (3) MM with salinomycin at 780 mg/kg (SHI), and (4) MM with virginiamycin at 1950 mg/kg (VGN). Over 123 days, these bulls grazed tropical grasses on pastures of guinea grass, palisade grass, or Bermuda grass. No significant treatment effects were observed for oocyst eggs or ruminal parameters. Bulls fed VGN had higher average daily gain (ADG) compared to CON ($p = 0.02$) and SLI ($p = 0.03$) but similar compared to SHI ($p = 0.07$). In Exp. 2, 308 zebu cross bulls (237 ± 23.0 kg LW) grazed Bermuda grass paddocks and were allocated into two treatments: (1) MM with no additives (CON) and (2) MM containing virginiamycin at 2522 mg/kg (VGN). Cattle fed VGN had a significantly higher ADG ($p = 0.007$). Exp. 3 tested salinomycin's effectiveness in vitro at different exposure times to MM, revealing no impact of exposure time on short-chain fatty acid production. In conclusion, virginiamycin delivered through free-choice MM can increase grazing beef bulls' ADG by 12% compared with CON, with no clear link to rumen fermentation or coccidiostat effects.

Keywords: antibiotic; cattle performance; ionophore; mineral mix

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1. Introduction

Livestock producers worldwide constantly seek ways to enhance the efficiency and profitability of their production systems [1,2]. Several authors have reported increased efficiency and/or performance of animals fed feed additives, such as ionophores and non-ionophore antibiotics, in both high-concentrate [3–6] and forage-based [7,8] diets. The latter studies, however, were carried out in housed facilities with animals fed in feed troughs. Thus, while they were able to show the potential benefits of a given feed additive for animals fed high-forage diets, mimicking pasture-based systems, the effectiveness of such technology in grazing systems remains unclear.

Grazing animals face spatial–temporal variability of pasture conditions throughout the year (e.g., seasonal variation of sward structure, forage availability) that directly affects their nutrient intake [9]. Further, the main obstacle to the widespread adoption of feed additives in pasture-based systems is the challenge of administering them effectively to ensure sufficient mineral consumption. The recommended delivery vehicles for ionophores

in grazing cattle are supplements containing grains at varying intake levels [1,10,11]. Nevertheless, the use of concentrate supplements is not a technology adopted widely and leads to increased production costs that may not translate into economic returns. In contrast, most cattle production systems utilize mineral mixture (MM) supplementation.

Validating a method for delivering feed additives via MMs would enable widespread adoption of this technology by producers. However, there are challenges associated with providing feed additives through MMs. Ionophores have a tendency to impact the consumption of MMs, potentially limiting mineral intake and making the intended ionophore dosage impractical, thereby impairing animal performance [12]. Additionally, MMs exhibit high variability in daily individual intake compared to grain supplements, which is often suggested as a cause for the lack of positive results [13]. Moreover, there is a possibility that additives lose their effectiveness when mixed with minerals and exposed to feed-trough conditions. Bagley et al. [12] observed a 47% reduction in salinomycin activity two weeks after it was mixed with mineral supplements. A lack of positive results with feed additives in MMs has been documented [14,15], but there are also greater average daily gain (ADG) responses caused by the administration of growth-promoting feed additives through mineral supplements [16,17]. The latter studies have reported ADG increases ranging from 0.05 to 0.10 kg/d. It is important to note that the absence or unexpected results in some studies can often be attributed to inadequate statistical design (e.g., low sample size and statistical power) [18]. Given that, we hypothesize that mineral supplement formulations with higher voluntary intake would reduce the variability in the daily intake of feed additives observed with MMs, ultimately enhancing the efficacy of the additives. Hence, the objective of this study was to evaluate the growth-promoting effects of both the ionophore salinomycin and the non-ionophore virginiamycin delivered through MM supplementation on zebu and zebu-cross bulls grazing tropical pastures.

2. Materials and Methods

2.1. Experiment 1 (Exp. 1)

2.1.1. Sites and Treatments

Exp. 1 was carried out on two different sites. Site 1 was located at Areão Farm at the Department of Animal Science of the "Luiz de Queiroz" College of Agriculture, University of Sao Paulo (ESALQ/USP) in Piracicaba, SP, Brazil (22°42′ S, 47°38′ W and 546 a.s.l.) and site 2 at Campanario Ranch in Carapó, MS, Brazil (22°47′ S, 55°04′ W and 425 a.s.l.). At site 1, the experimental period lasted 108 days from January to May 2009, whereas at site 2, it was carried out from May to October 2009 (137 days). According to the Köppen classification, the local climate of site 1 is Cwa, humid sub-tropical with hot summers and dry winters, and site 2 is Cfa, humid sub-tropical with hot summers and without dry winters [19]. At site 1, the average temperature and accumulated rainfall recorded during Exp. 1 were 452 mm and 24.2 °C, respectively. The historical average temperature and accumulated rainfall from May to October recorded at site 2 were 18.8 °C and 288 mm, respectively.

The four treatments were (1) control as MM containing 5% soybean meal without additive (CON), (2) MM containing 5% soybean meal + salinomycin at 1950 mg/kg (salinomycin low intake (SLI)), (3) MM containing 15% soybean meal + salinomycin at 780 mg/kg (salinomycin high intake (SHI)), and (4) MM containing 5% soybean meal + virginiamycin at 1950 mg/kg (VGN). The concentration of additives in the MMs was defined based on an expected intake of 30 mg/100 kg liveweight (LW) for salinomycin and virginiamycin [1,12,20]. The expected daily voluntary intake of animals fed MM, SLI, and VGN was 30, 30, and 100 g, respectively. The treatment SHI was included based on the hypothesis that a greater inclusion of soybean meal would increase voluntary intake and decrease the individual intake variation, leading to a more constant rate of intake [13]. The mineral composition of all treatments was 100 g/kg of Ca, 133 g/kg of Na, 57 g/kg of P, 52 g/kg of S, 7 g/kg of Mg, 4009 mg/kg of Zn, 1069 mg/kg of Cu, 1336 mg/kg of Mn, 67 mg/kg of I, 40 mg/kg of Co, and 19 mg/kg of Se, whereas for SHI, the concentration of each mineral in the MM represented 40% of the previous MM. Mineral mixtures were offered daily in the morning

in a covered feed trough. Supplement refusals were collected and oven dried at 65 °C for 72 h to estimate the average voluntary intake on a dry matter basis.

2.1.2. Animals, Paddocks' Grass Species, and Management

One hundred and twenty zebu (*Bos indicus*) Nellore bulls averaging 243 ± 41 kg (mean ± SD) LW and 14 months of age (site 1) and a hundred and ninety-six zebu-cross bulls (1/2 Bonsmara × 1/2 Nellore and 3/4 Senepol × 1/4 Nellore) averaging 214 ± 18 kg LW and 10 months age (site 2) were stratified and grouped according to LW and breed, and then randomly assigned to one of four treatments. The number of animals in each paddock varied according to the size of the paddock and the forage mass available. On average, there were 7 to 8 animals per paddock in Site 1 and 24 to 25 in Site 2. The stocking rate of each paddock was monitored in order to maintain the sward heights accordantly to the recommended grazing height for each pasture species. In both sites, the experimental units (paddocks) were randomly assigned to one of four treatments. At site 1, animals continuously grazed an area of 16.5 ha consisting of 16 paddocks divided into 4 blocks. Every block was composed of a different grass species as follows: Mombaça guinea grassguinea grass (*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Mombaça), Colonião guinea grass (*M. maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Colonião), Tanzânia guinea grass (*M. maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Tanzânia), and Xaraés palisade grass (*Urochloa brizantha* (A. Rich.) Stapf cv. Xaraes). Paddocks of the guinea grass species were continuously stocked, aiming to keep the canopy height between 40 and 50 cm [21], whereas for palisade grass, the target was 20 cm [22]. Paddocks were fertilized with 50 kg N/ha every 30 days, totalling 200 kg N/ha during the experimental period. At site 2, bulls continuously grazed an area of 108 ha formed by Coastcross Bermuda grass (*Cynodon dactylon* (L.) Pers) distributed in a set of eight paddocks divided into 2 blocks. Grazing management aimed to keep the canopy height between 15 and 20 cm [23], and the paddocks were not fertilized. In both sites, the treatment groups were rotated between paddocks weekly to minimize the effect of canopy structure on animal responses among treatments within the blocks [12].

2.1.3. Sward Monitoring and Animal Sampling

At site 1, canopy height was monitored from ground level to the top leafy horizon by 40 systematic readings per paddock, using a stick graduated in centimetres [24]. Herbage mass was sampled on day 5 and day 70 using four square frames of 1 m^2 per paddock randomly allocated in areas that represented the average canopy condition [25]. The determination of canopy height and herbage mass in site 2 followed the same methodology applied in site 1, with the difference that these assessments were only conducted on day 60 (Table 1).

At site 1, the individual intake of MM was monitored using lithium sulphate as a marker [26] on days 75 (Intake 1) and 90 (Intake 2). During these dates, each treatment was enriched with 13 g of lithium/kg MM and offered at 6:00 h, and residues were collected at 16:00 h. Eight animals were selected randomly per treatment to be removed from the paddocks and remained in curfew until the following day when blood samples were taken via jugular venipuncture using K_2EDTA vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood samples were centrifuged (3000× *g* at 4 °C for 20 min) and plasma was analysed by atomic absorption spectrometry for the determination of lithium concentration in the blood and to estimate the individual MM intake of each animal [27]. The percentage of non-eaters was calculated based on the number of animals that presented lithium blood concentrations lower than 50 µg/L divided by the total number of animals in the group multiplied by 100 [27]. Faecal samples were collected from the rectum of all animals on days 90 and 98 for sites 1 and 2, respectively. The faecal samples were then processed and analysed to quantify *Eimeria* spp. and *Strongylida* spp. oocysts per gram of faeces (OpG) [28]. Ruminal samples were taken using a flexible orogastric tube from two random animals of each treatment on days 100, 103, and 106 of the Exp. 1 for site 1 and

one unique collection was conducted in site 2 on day 137. The samples were thawed at room temperature, centrifuged (15,000× *g* at 4 °C for 30 min), and analysed for short-chain fatty acid (SCFA) concentrations using the gas chromatography procedure [29], and for ammonia N (NH_3-N) concentration using the phenol–hypochlorite technique [30]. Animals were individually weighed after 14 h of feed and water curfew at the beginning and every 30 days throughout the Exp. 1 to estimate the ADG.

Table 1. Average canopy characteristics during the experimental periods at sites 1 and 2 (Exp. 1).

Site	Block	Pasture Species	Canopy Characteristics		Chemical Composition [6]	
			Height (cm)	Mass (kg DM/ha)	CP [7] (%)	NDF [8] (%)
1	1	Colonião guinea grass [1]	45.8	8626	14.3	73.7
	2	Mombaça guinea grass [2]	52.2	7205	13.3	65.3
	3	Tanzânia guinea grass [3]	35.6	8515	12.8	67.3
	4	Xaraés palisade grass [4]	24.3	6746	12.4	67.3
2	5	Coastcross Bermuda grass [5]	20.8	2811	15.1	64.9
	6	Coastcross Bermuda grass [5]	17.2	1491	15.1	64.9

[1] *M. maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Colonião; [2] *M. maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Mombaça; [3] *M. maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs cv. Tanzânia; [4] *U. brizantha* (A. Rich.) Stapf cv. Xaraés; [5] *C. dactylon* (L.) Pers., [6] Values estimated from Valadares et al. [31], [7] Crude protein, [8] Neutral-detergent fibre.

2.2. Experiment 2 (Exp. 2)

Exp. 2 was conducted at the same set of paddocks of site 2 in Exp. 1, but this second time using the 8 paddocks distributed into 4 blocks. It lasted 133 days from January to May 2010, and treatments were (1) MM with no feed additive (CON) and (2) MM containing virginiamycin at 2522 mg/kg (VGN). This study aimed to assess the repeatability of the virginiamycin results from the previous experiments; however, in a condition of excessive rainfall with MMs being provided in uncovered feed troughs during the rainy season, a typical scenario where beef cattle are raised in tropical conditions. The mineral composition of both treatments was 110 g/kg of Ca, 155 g/kg of Na, 73 g/kg of P, 30 g/kg of S, 20 g/kg of Mg, 4799 mg/kg of Zn, 1346 mg/kg of Cu, 1512 mg/kg of Mn, 115 mg/kg of I, 101 mg/kg of Co, and 21 mg/kg of Se.

Three hundred and eight bulls (Nellore, Black Angus and 1/2 Red Angus × 1/2 Charolais) averaging 237 ± 23 kg LW and 14 months of age were stratified and grouped in pairs according to breed and LW and randomly assigned to one of two treatments throughout 4 blocks. Feed troughs were monitored every three days and additional MM was provided if necessary. The MM refusals were collected only at the end of the Exp. to estimate the average intake. The treatment groups were rotated between paddocks every fortnight to minimize the effect of canopy structure on animal responses among treatments within the blocks [12]. The animals were individually weighed after 14 h of feed and water curfew on days 30, 100, and 133 to estimate ADG. Canopy height was measured on day 30 as previously described for Exp. 1 and the average canopy heights were 19.6, 20.6, 17.0, and 16.5 cm for blocks 1 to 4, respectively.

2.3. Experiment 3 (Exp. 3)

Exp. 3 was carried out at the Laboratory of Animal Nutrition of the Center of Nuclear Energy in Agriculture (CENA), USP, in Piracicaba, SP, Brazil. This study aimed to verify the effectiveness of salinomycin after different exposure times to the MM [12]. The treatments were (1) MM without salinomycin on day 1 (T1–C), (2) MM + salinomycin mixed on day 1 (T1), (3) MM + salinomycin mixed after 40 days (T40), and (4) MM + salinomycin mixed after 300 days (T300). The composition of MMs used in this in vitro assay was the same as SLI from Exp. 1. The objective of Exp. 3 was to assess if the exposure time of salinomycin to the MM could affect in vitro gas production and fermentation profile.

The diet used as a fermentation substrate had a 20:80 forage-to-concentrate ratio with the ingredients as follows (DM basis): chopped Tifton-85 hay (*Cynodon* spp.) (20.0%), ground corn (62.7%), soybean meal (15.0%), mineral premix (1.3%), and limestone (1.0%). The chemical composition was dry matter (91.4%), crude protein (15.7%), neutral-detergent fibre (20.3%), acid-detergent fibre (8.8%), ether extract (3.3%), and ash (5.4%). A representative substrate sample was oven-dried and ground in a Wiley mill to pass through a 1 mm screen for use in the incubation assay.

To minimize the effects of atypical ruminal conditions, five adult rumen-cannulated Santa Inês sheep (50 ± 5.6 kg LW) were used as rumen-content donors. Animals had free access to fresh water, continuously grazed a tropical grass pasture, and were supplemented daily (150 g of ground corn + 65 g of soybean meal + 4.5 g of molasses per animal). Prior to morning feeding, the liquid phase from ruminal content was collected using a tube attached to a 60 mL syringe. The solid phase was obtained through the cannula from the dorsal rumen sac using a stainless-steel probe (2.5 mm screen) attached to a large-capacity syringe. Both liquid and solid phases were mixed at 1:1 (v/v) for 10 s and strained through three layers of cheesecloth before combining with buffer solution, and then kept under CO_2 in a water bath (39 °C) until used [32].

The in vitro gas production technique used was according to Theodorou et al. [33] adapted with a semi-automatic system [34]. A completely randomized block design with ten blocks and four treatments was used. The in vitro assay was conducted in three different gas production runs using 160 mL serum bottles. Half a gram of substrate was weighed, put into the 160 mL serum bottles, and stored overnight at 39 °C. Each bottle was then injected with 50 mL of incubation medium (Theodorou's buffered medium; [35]) and 25 mL of rumen inoculum. Then, the bottles were closed with rubber stoppers, manually shaken, and placed in a forced air incubator at 39 °C for 16 h. The internal gas headspace (85 mL) pressure of each bottle was recorded at 3, 8, 12, and 16 h of incubation using a pressure transducer and a data logger (Druck DPI 800, GE, Boston, MA, USA) calibrated and connected in a laminar airflow chamber located beside the incubator. The gas production volume (mL) was calculated as 7365 × measured pressure (psi) (n = 500; r^2 = 0.99). The net cumulative gas production at 16 h of incubation was the sum of partial gas production at each time interval. The individual SCFA concentrations were determined according to El-Zaiat et al. [36] using a gas chromatograph (HP 7890A, Automatic Injector HP 7683B; Agilent Technologies, Palo Alto, CA, USA).

2.4. Statistical Analysis

All statistical analyses were conducted using the open-source software R (version 4.2.3) [37], data were assessed prior to analysis for normality and homoscedasticity, and data transformation was based on the box–cox procedure [38]. Exp. 1 and 2 were analysed as completely randomised block designs with paddocks considered to be experimental units [39]. Average daily gain, MM intake, ruminal SCFA, and oocyst counts of Exp. 1 as well as ADG and MM intake of Exp. 2 were analysed using the linear mixed models procedure of the package "nlme" [40]. In Exp. 1, MM intake, ammonia concentration, and acetate:propionate were transformed prior to statistical analysis using the logarithmic transformation, while MM intake was transformed using the inverse square root and prevalence of oocysts in faeces utilizing the cube root [38]. No transformation was necessary for data from Exp. 2. In Exp. 3, the concentration of isovalerate and total short-chain fatty acid was transformed using reciprocal transformation, while propionate concentration was transformed using the reciprocal square root transformation [38].

In Exp. 1, the models included treatment as a fixed factor and block within the location as a random factor. The analysis of the effect of treatment on the percentage of non-eaters was conducted using a generalized linear model for quasi-binomial distribution and a Chi-square test. Pearson correlation analysis was conducted on data from the individual intake of Exp. 1 between the two collection dates (i.e., Intake 1 and 2) and the experimental unit was considered to be each bull and not the paddock. In Exp. 2, the statistical models

included treatment as a fixed factor and block as a random factor. In Exp. 3, the effect of time of exposure to the MM of salinomycin on rumen parameters was evaluated using a generalized linear model. Differences were declared significant at $p \leq 0.05$, and trends were declared at $p \leq 0.10$.

3. Results

3.1. Exp. 1

Across all treatments, the proportion of cattle that did not consume the MM supplement (i.e., non-eaters) varied from 23.5 to 51.2% of the group without differences between treatments ($p = 0.234$; Table 2). Intake of MM was greater ($p < 0.001$) for SHI treatment than all other treatments (Table 2). Cattle offered the CON had a similar ($p = 0.12$) intake to animals consuming VGN treatment but greater ($p = 0.008$) than the SLI group. The consumption of active ingredients for animals offered VGN was similar ($p = 0.63$) when compared to SLI and higher than ($p = 0.007$) from animals fed SHI. However, both SLI and SHI treatments did not present any differences in active ingredient intake ($p = 0.17$). The average daily gain of cattle fed MM that included salinomycin (i.e., SLI and SHI) was not different from the CON ($p = 0.99$ and $p = 0.97$, respectively); however, the group fed VGN presented higher ADG than animals fed SLI ($p = 0.03$) and CON ($p = 0.02$). The analysis of ruminal SCFA did not show any difference between the treatment groups in Exp. 1 ($p > 0.05$; Table 2). In addition, the number of *Strongylida* spp. and *Eimeria* spp. also did not present any difference ($p > 0.05$) between treatments. Correlation analysis between Intake 1 (d75) and 2 (d90) (Exp. 1—site 1) showed no significant ($p = 0.42$) relationship between the individual intake of animals in the two different periods (Figure 1).

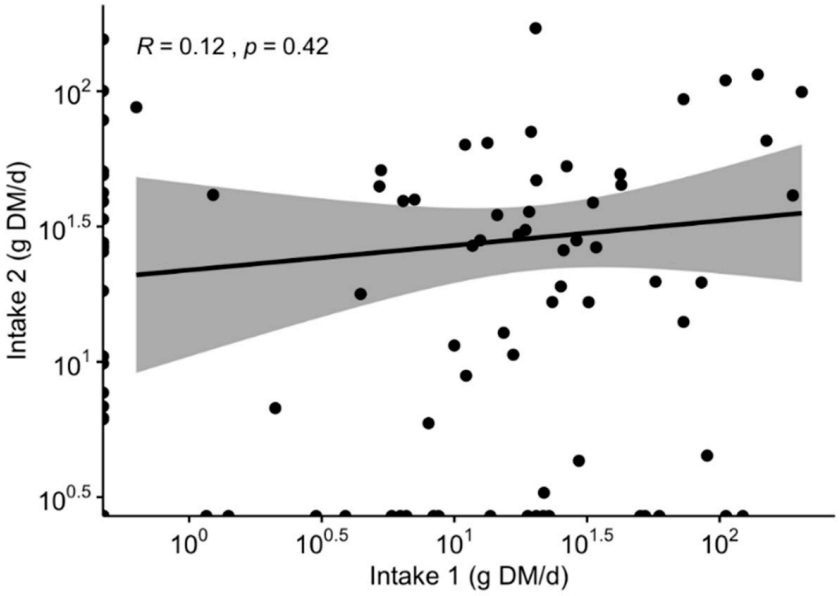

Figure 1. Pearson correlation analysis between Intake 1 and Intake 2. Data (n = 240) present individual intake of cattle (black circles) across all treatments during days 75 and 90 of Exp. 1, the results were log transformed on base 10. Shaded grey area represents 95% confidence interval.

Table 2. Performance, ruminal, and prevalence of oocyst eggs in faeces of young beef bulls continuously grazing tropical pastures supplemented or not (CON, n = 6) with salinomycin low-intake (SLI, n = 6), salinomycin high-intake (SHI, n = 6), or virginiamycin (VGN, n = 6) (Exp. 1).

Item	S [1]	CON [2]	SLI [2]	SHI [2]	VGN [2]	SEM	*p*-Value [3]
Non-eaters, %	8	23.5	49.3	31.3	51.2	10.2	0.234
Mineral mixture intake, g DM/d	-	36.1 b	26.4 c	56.8 a	28.9 bc	4.39	<0.001
Active ingredient intake, mg DM/d	-	0 c	53.2 ab	45.0 b	58.1 a	2.67	<0.001
Average daily gain, kg/d	79	0.547 b	0.551 b	0.557 ab	0.616 a	0.0814	0.037

Table 2. *Cont.*

Item	S [1]	CON [2]	SLI [2]	SHI [2]	VGN [2]	SEM	*p*-Value [3]
Total short-chain fatty acids, mmol/L	3	63.0	59.6	66.5	58.1	12.3	0.422
Acetate	3	44.6	42.3	47.1	41.7	10.5	0.481
Propionate	3	11.3	10.7	11.8	10.2	0.98	0.407
Butyrate	3	7.01	6.61	7.49	6.23	0.96	0.358
Acetate:propionate	3	3.95	3.95	3.90	3.97	0.68	0.997
Ammonia, mg/100 mL	3	19.2	19.4	20.0	17.7	11.5	0.745
Strongylida spp., OpG	79	122	126	128	106	69.9	0.258
Eimeria spp., OpG	79	458	344	325	387	113.7	0.773

[1] Number of animals sampled as sub-sample to compose the sample considering that the experimental unit was the paddock. [2] CON, mineral mixture (MM) with no feed additives; SLI, MM + salinomycin at 1950 ppm; SHI, MM + salinomycin at 780 ppm; VGN, MM + virginiamycin at 1950 ppm. [3] Treatment means within a row with different letters are significantly different ($p < 0.05$).

3.2. Exp. 2

There was a trend of greater MM intake for cattle receiving CON treatment when compared to the group receiving the MM containing VGN ($p = 0.062$; Table 3). The average daily gain of cattle fed VGN was greater than the CON group ($p = 0.031$).

Table 3. Performance of young beef bulls continuously grazing tropical pastures supplemented or not (CON, n = 4) with virginiamycin (VGN, n = 4) (Exp. 2).

Item	s [1]	CON [2]	VGN [2]	SEM	*p*-Value
Mineral mixture intake, g DM/d	-	43.0	39.0	2.44	0.062
Active ingredient intake, mg DM/d	-	0	98.4	-	-
Average daily gain, kg/d	154	0.636	0.703	0.066	0.031

[1] Number of animals sampled as sub-sample for each treatment to compose the average considering that the experimental unit was the paddock. [2] CON, mineral mixture (MM) with no feed additives; VGN, MM + virginiamycin at 2522 ppm.

3.3. Exp. 3

In Exp. 3, treatments that included salinomycin (i.e., T1, T40, and T300) increased the concentration of propionate and decreased butyrate and isobutyrate concentration as well as the acetate:propionate ratio when compared to treatment T1–C ($p < 0.05$; Table 4). Different time exposure of salinomycin to MM did not affect the concentration of any SCFA nor the total production of SCFA ($p > 0.05$)

Table 4. In vitro short-chain fatty acid (SCFA) gas production of mineral mixture with salinomycin at different timings after mixing (n = 5) (Exp. 3).

	Treatments				SEM	*p*-Value [1]
	T1–C [2]	T1 [2]	T40 [2]	T300 [2]		
Total SCFA, mmol/L	73.90	72.43	78.68	72.14	3.249	0.278
Acetate Propionate	47.4	46.3	50.4	46.1	2.081	0.212
Propionate	13.1 a	16.2 b	17.7 b	16.1 b	0.707	<0.001
Butyrate	10.37 a	7.26 b	7.70 b	7.21 b	0.422	<0.001
Isobutyrate	0.74 a	0.59 b	0.63 ab	0.59 b	0.045	<0.005
Isovalerate	1.27	1.17	1.27	1.19	0.099	0.715
Valerate	0.86	0.83	0.87	0.84	0.043	0.799
Acetate:propionate	3.61 a	2.85 b	2.84 b	2.85 b	0.041	<0.001

[1] Treatment means within a row with different letters are significantly different ($p < 0.05$); [2] T1–C, MM without salinomycin at day 1; T1, MM + salinomycin mixed at day 1; T40, MM + salinomycin mixed after 40 days; and T300, MM + salinomycin mixed after 300 days.

4. Discussion

The primary objective of the present study was to assess the potential growth-promoting effects of both salinomycin and virginiamycin delivered through MM supplementation for

beef cattle grazing tropical pastures at the farm scale. Our hypothesis was based on the literature reporting a greater performance of ruminants fed feed additives compared with their non-fed cohorts [2]. Nonetheless, most research has focused on ionophores, such as monensin and lasalocid, fed with high-concentrate diets for beef cattle under controlled trials in housed conditions [41]. Furthermore, the scarce information regarding the impacts of feed additives fed with low-concentrate diets is inconsistent [1]. The latter authors were not able to assess the potential of both salinomycin and virginiamycin individually in their meta-analysis due to the limited number of studies. Additionally, they reported that the main reason for inconsistent results of feed additives as a growth promoter for grazing beef cattle is finding a suitable carrier that ensures a constant intake of the recommended dosage. In this way, energy or protein concentrate supplements would fit perfectly; however, their cost associated with limited and variable use throughout the year in tropical grazing systems would prevent its wide adoption.

Conversely, MM supplements are widely adopted in beef cattle production systems, and validating them as a carrier for feed additives would enable widespread adoption of this technology by producers. However, there are challenges associated with delivering additives through MMs. First, because the individual daily intake of MMs is highly variable [13]. Results from our Exp. 1 corroborate it, showing that, on average (no differences in proportion of non-eater animals between treatments), 39% did not consume MMs regardless of feed additive inclusion. The methodology used in this study was derived from Dixon et al. [26] and considered only the 10 h period prior to the sampling to define the proportion of non-eater animals. A wider window of animal exposure to the marker might have resulted in a lower proportion of non-eaters. Further, ionophores such as salinomycin tend to decrease the MM intake [12]. Both can jeopardize the animals' achievement of the recommended daily intake of the feed additive active ingredient. Our results from Exp. 1 corroborate that the ionophore salinomycin (SLI) can decrease the MM intake by 27% compared to CON. In this way, the strategy of increasing soybean meal concentration from 5 to 15% for the SHI group was effective in increasing the MM intake; however, because the salinomycin concentration was reduced by 60% in that MM, the active ingredient intake was the same for both SLI and SHI treatments.

In the present study, salinomycin supplements did not change either ruminal parameters or ADG compared to the CON group, which aligns with previous forage-based diet studies carried out either in housed [7] or grazing conditions [12]. In one of their experiments, Bagley et al. [12] reported linear increases in ruminal propionate and ADG of beef steers grazing Bermuda grass fed from 0 to 150 mg of salinomycin/d. The ionophore, however, was delivered through 0.9 kg of ground corn daily (0.4% LW, DM basis), which probably resulted in a more uniform intake of active ingredients and a lower proportion of non-eater animals. One possible cause commonly mentioned for the lack of positive results from a given feed additive is the daily dose consumed [2]. The average salinomycin intake for both SLI and SHI groups from Exp. 1 in the current study was 19 mg/100 kg LW. The literature has reported daily intakes of salinomycin ranging from 20 to 36 mg/100 kg LW in forage-based diets but with no positive results on ADG [7,12]. Bagley et al. [12] also suggested that the lack of positive results of salinomycin supplementation could be explained by the loss of activity of the additive after mixing with MM. Results from our Exp. 3 did not support this hypothesis. Neither the total SCFA nor individual SCFA were influenced either 40 or 300 days after salinomycin was mixed with MM. In addition, the results showed an increased production of propionate with the inclusion of salinomycin in the MM, which corroborates the results of Bagley et al. [12].

Animals from VGN did not decrease their MM intake compared to the CON group in Exp. 1, but there was a trend in Exp. 2. There is no evidence in the literature reporting decreased MM intake due to virginiamycin inclusion [20,42]. This trend observed in Exp. 2 may be explained by the higher concentration of the active ingredient in the MM containing virginiamycin in Exp. 2 compared to Exp. 1 (2522 vs. 1950 mg/kg). The VGN groups had an average daily intake of the active ingredient of 58.1 and 98.4 mg,

which represented 23 and 35 mg/100 kg LW for Exp. 1 and Exp. 2, respectively, values closer to the 33.5 mg/100 kg LW reported by Maciel et al. [42]. Results from both Exp. 1 and Exp. 2 showed increases of 12.6 and 10.5% (0.069 and 0.067 kg/d), respectively, on ADG of the VGN animals compared to CON. Potential benefits on growth performance of supplementing ruminants with virginiamycin can include (1) inhibition of growth of ruminal microorganisms associated with liver abscess formation, (2) reduction in ruminal lactate concentration and potential increase in propionate, and (3) decrease in deamination activity of proteins in the rumen [43]. Liver abscesses are more often reported in high-concentrate feedlot diets, which is not the case in the present study. Shifts in ruminal fermentation due to virginiamycin supplementation towards more propionate and less lactate are reported for animals fed high-concentrate diets [43] but not for forage-based diets [8,38,44]. Results regarding rumen parameters from the present study corroborate the latter authors. However, it might also be partially explained by the less accurate sampling method used in the current study (i.e., tube) and/or the restricted number of subsamples (i.e., number of animals) composing our sample (i.e., paddock). Furthermore, the high proportion of non-eaters made it more difficult to capture any potential change in the ruminal fermentation profile among treatments. Based on that, we cannot exclude the possibility from the literature that an increased ADG of animals fed virginiamycin might be explained by changes in ruminal microorganisms and fermentation profile. However, for any additive, it is important to consider that despite the possible positive impact on cattle performance, it is essential to evaluate the cost of the strategy, emphasizing the need for a balanced evaluation that takes both effectiveness and economic factors into account.

The variability in daily individual intake of MMs poses a challenge in effectively delivering ionophores and non-ionophore antibiotics to grazing animals. Any strategy that promotes the inefficient use of minerals can be detrimental to animals and the economic viability of the system. Phosphorus deficiency, for example, impairs animal productivity and this mineral is one of the most expensive nutrients used in ruminant nutrition [45]. Despite the existence of means to diagnose mineral deficiencies [46,47], having effective management practices is essential to prevent setbacks in production. Most feed additives do not drastically affect the intake of minerals if added in combination with supplementation strategies to promote a high intake of energy or protein [48–50]. However, when only MM is in place, erratic consumption can be quite detrimental to production due to mineral deficiencies. Our results showed high variability in individual intake of MM using lithium sulphate as a marker [26]. The coefficients of variation averaged 134% and, even with large numerical differences between treatments, no statistical difference was detected and the average proportion of animals that did not consume the supplement was 39% across all groups. The hypothesis that a potentially higher intake of SHI would reduce its daily variability [13,26] was not confirmed. Higher levels of soybean meal or other ingredients (e.g., molasses) can be tested as bait to attract more animals and/or stimulate the frequency of visits to consume the supplement containing the feed additive.

Behavioural patterns in young bulls grazing on pasture may exhibit distinctions compared to other animal categories in the same environment and the current findings may or may not be applicable in the broader context. However, the relevance of the animal category studied to commercial operations is notorious, mainly in tropical regions. The current study used 624 animals to evaluate the farm-scale effectiveness of feed additives supplied through an MM for beef cattle grazing tropical pastures. Probably the most comprehensive study in terms of the number of animals where the supplement was fed in both covered and uncovered feed troughs during the rainy season. Our results indicate that virginiamycin supplied through an MM increased the ADG of grazing beef bulls by 12%, whereas salinomycin was not effective. The growth-promoting effect of virginiamycin was not explained either by changes in the rumen fermentation profile or by a potential coccidiostat effect, which may be associated with the number of subsamples taken for these parameters. Further research should be undertaken to investigate strategies to decrease the

individual variability of MM intake and maximize the proportion of the herd accessing the feeder for consuming the supplement.

5. Conclusions

Production systems would benefit from a more stable and widespread mineral mix intake throughout the herd. Our findings demonstrated that the inclusion of virginiamycin via the mineral supplement can result in a 12% increase in the average daily gain of grazing beef bulls, while salinomycin did not yield similar benefits. Interestingly, this growth-promoting effect of virginiamycin was not attributable to changes in rumen fermentation or coccidiostat effects and could potentially be due to the number of animals sampled. In conclusion, the study highlights the need for further research on feed additives targeting grazing cattle offered free-choice mineral mix. It should enhance herd-wide access to the supplement, paving the way for more effective and efficient cattle management practices.

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