Response of Phytogenic Additives on Enteric Methane Emissions and Animal Performance of Nellore Bulls Raised in Grassland

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Abstract: The objective of this study was to evaluate the intake and digestibility of nutrients, emission of enteric CH4, and productive performance of Nellore bulls grazing Urochloa brizantha cv. Marandu palisade grass pastures during the rainy season, receiving an energy supplement or mineral supplement, with or without the inclusion of phytogenic additives. Forty-eight Nellore bulls were treated with: (1) energy supplement without the inclusion of phytogenic additives; (2) energy supplement with the inclusion of phytogenic additives; (3) mineral supplement without the inclusion of phytogenic additives; and (4) mineral supplement with the inclusion of phytogenic additives. Consumption of total dry matter (DM), crude protein (CP), apNDF, and energy; digestibility of DM, CP, and energy; average daily gain; stocking rate; and gain per area were higher in animals consuming energy supplements than those consuming mineral supplements. Digestibility of DM, NDF, and energy levels were lower in animals that consumed phytogenic additives. Compared with mineral supplements, the supply of energy supplements provides higher nutrient intake, increases enteric CH4 emission, and improves nutrient digestibility, providing a greater productive performance. The inclusion of phytogenic additives negatively affected nutrient intake and digestibility, did not reduce enteric CH4 emission, and influenced productive performance.

Keywords: cattle; enteric methane; essential oils; supplementation; hydrolyzable tannins

1. Introduction

The intensification of tropical pasture production systems is strongly influenced by proper pasture management, which, under the criterion of height and light interception of 95%, provides an adjustment between the forage mass and stocking rate [1–3], enabling a higher proportion of leaves, and consequently, lower concentration of indigestible neutral detergent fiber (NDF), and structure and high quality of pasture [4].

With the management of tropical grass pastures, a strategic supply of supplements can provide an additional body weight gain of approximately 0.250 kg/animal/day during the rainy period [4–6]. Strategic supplementation reduces methane (CH4) emissions per kilogram of the produced product [7].

In the supplementation of animal diet, growth promoters, such as ionophore antibiotics, provide better feed efficiency and performance. However, they have been banned because of the possibility of increase in bacterial resistance and issues with human health [8]. Thus, phytogenic additives, such as essential oils and tannins, could be a better choice
because they have antimicrobial properties and can reduce the concentration of ruminal ammoniacal nitrogen, increasing the efficiency of N utilization, as with the use of essential oils [9,10]. Additionally, tannins have the potential to bind proteins in the diet, increasing the flow of protein to the small intestine, favoring both an increase in animal performance as well as a reduction in environmental impacts caused by ammonia volatilization, nitrous oxide (N₂O) emission, and nitrate leaching (NO₃⁻) due to an increase in the consumption of soluble protein, as seen in animals consuming pastures fertilized with nitrogen and maintaining a high proportion of leaves [11–13].

The average daily gain (ADG) of beef cattle in the feedlot with the inclusion of clover or cinnamon essential oils, at doses of 3.5 g/day or 7.0 g/day, respectively, was higher than that of the control animals because of higher consumption of dry matter (DM), possibly due to better acceptability of these diets, without causing adverse effects on food efficiency. In addition, the essential oils demonstrated an ability to manipulate the ruminal environment, as in the reduction in the molar proportion of acetate and acetate: propionate ratio [14,15]. Including hydrolyzable and condensed tannins can improve nitrogen utilization efficiency and reduce urea nitrogen in the plasma of beef cattle fed diets with a high proportion of forage [16,17]. The effect of hydrolyzable tannins on nitrogen utilization differs from that of condensed tannins. Hydrolyzable tannins may be rapidly hydrolyzed in the rumen and do not bind proteins. However, the effects of the combined use of hydrolyzable tannins and essential oils for grazing cattle have not been reported until now.

The hypotheses studied were: (i) the consumption of energy supplements increases the intake and digestibility of nutrients and (ii) reduces the emission of enteric CH₄ and consequently improves animal performance. Furthermore, the combined use of hydrolyzable tannins and essential oils improves nutrient utilization, mitigates the emission of enteric CH₄, and improves animal performance. The objective of this study was to evaluate the intake and digestibility of DM and nutrients, emission of enteric CH₄, and productive performance of Nellore bulls grazing Marandu palisade grass during the rainy period, receiving energy or mineral supplements, with or without the inclusion of phytogenic additives composed of carvacrol and cinnamaldehyde, and hydrolyzable tannins, supplied at a dose of 1.5 g/kg DM ingested.

2. Materials and Methods

2.1. Site and Experimental Area

The study was carried out in the Forage sector of the Faculty of Agricultural and Veterinary Sciences (FCAV) of the São Paulo State University “Júlio de Mesquita Filho” (UNESP), Jaboticabal campus, São Paulo, during the animal breeding phase in the 2018/2019 rainy season, between December and April, to evaluate the performance of animals and the production of enteric methane. The climate observed in the region is of subtropical type AW according to the Köppen classification. The soil in the experimental area was Yellow Red Latosol [18].

Meteorological data, temperature (°C), precipitation (mm), rainy days, and water balance (Table 1) were recorded during the experimental period by the Agroclimatological Station of Jaboticabal campus, located at latitude 21°14'05" south, and longitude 48°17'09" west, at an altitude of 615 m. At the end of the experiment, the data were extracted from the data collection of the Agroclimatological Station of the Department of Exact Sciences.
Table 1. Temperature (°C), precipitation (mm), rainy days, and water balance (mm) during the 2018/2019 rainy period.

| Month   | Temperature (°C) | Precipitation (mm) | Rainy Days | Water Balance (mm) |
|---------|------------------|--------------------|------------|--------------------|
|         | Max   | Min   | Average |                     | Def | Exc |
| October | 31.1  | 19.5  | 24.4    | 157.0                | 15  | 0   |
| November| 29.7  | 19.7  | 23.8    | 330.1                | 18  | 0   |
| December| 32.0  | 20.2  | 25.2    | 88.2                 | 16  | 163 |
| Year 2018 |       |        |         |                      |     |     |
| January | 32.7  | 20.9  | 26.1    | 148.1                | 11  | 0   |
| February| 30.9  | 20.4  | 24.4    | 282.6                | 17  | 0   |
| March   | 31.0  | 20.1  | 24.5    | 115.2                | 12  | 0   |
| April   | 30.6  | 19.0  | 23.9    | 97.6                 | 6   | 0   |

1 Temperature: Max, maximum; Min, minimum; 2 Water balance: Def, deficiency; Exc, excess water.

The animals were housed in an area formed by *Urochloa brizantha* cv. Marandu during the backgrounding phase, in 12 experimental paddocks, with a total area of 12 ha, divided into 0.7 and 1.3 ha paddocks. A three ha reserve paddock was used to adjust the stocking rate. All paddocks were provided with a trough with access from both sides (30 cm linear per animal), and a drinking fountain. The management center was equipped with a corral with a containment trunk, digital scale, and separator.

Soil analysis of the paddocks was performed and presented the following chemical characteristics: pH of 5.27 in CaCl₂; 35 g/dm³ of organic matter (OM); 3.07 mmolc/dm³ phosphorus resin; 0.7 mg/dm³ potassium; 0.14 mmolc/dm³ calcium; 0.04 mmolc/dm³ magnesium; 29.89 mmolc/dm³ hydrogen+aluminum; and 54.44% base saturation.

Maintenance of fertilization in the experimental area was performed according to soil analysis. A total of 100 kg/ha and P₂O₅, 200 kg/ha K₂O were applied in two applications, and 180 kg/ha of N was applied in three applications. The nitrogen source used was ammonium nitrate, and the applications were conducted on 16 December 2018; 24 January 2019; and 2 March 2019 coinciding with the favorable climatic conditions of the application.

2.2. Animals and Experimental Treatments

The animals were allocated to the experimental area 15 d before the beginning of the experiment to adapt to the experimental conditions. The performance evaluation period of grazing animals was 102 days (15 days of adaptation and 87 days of evaluation), beginning on 18 December 2018, and ending on 30 March 2019. Enteric CH₄ production was measured during the performance evaluation period.

Forty-eight Nellore bulls were used, with an average initial body weight of 211 ± 30 kg, distributed in a completely randomized design in a 2 × 2 factorial arrangement (A: energy supplement and mineral supplement; B: without and with phytogenic additives), with four treatments and three replicates (paddocks), totaling four testers per paddock and 12 animals per treatment. The animals were wormed and identified with numbered earrings to monitor their individual performance.

Pastures were continuously stocked with variable stocking rates and a canopy target of 25 cm high, using the “put and take” technique [19]. Additional animals (regulators) were used to adjust and maintain the height target of pasture at 25 cm, in addition to the animal testers.

The treatments were: (1) energy supplement without the inclusion of phytogenic additives (ES); (2) energy supplement with the inclusion of phytogenic additives (ESPA); (3) mineral supplement without the inclusion of phytogenic additives (MS); and (4) mineral supplement with the inclusion of phytogenic additives (MSPA) (Table 2). The blend and phytogenic additives contained carvacrol, cinnamaldehyde, and hydrolyzable tannins, supplied in treatments at a dose of 1.5 g/kg of ingested DM. The phytogenic additives
were composed of 10% carvacrol and cinnamaldehyde oil, and 90% hydrolyzable tannins extracted from berries and grapes.

Table 2. Chemical composition of the supplements used in the rearing phase during the rainy season.

| Item              | Supplements 1 |
|-------------------|---------------|
|                   | ES | ESPA | MS 3 | MSPA 4 |
| Chemical composition 2 |    |      |      |        |
| MM (%)            | 34.02 | 33.12 | -    | -      |
| OM (%)            | 65.98 | 66.88 | -    | -      |
| apNDF (%)         | 30.32 | 30.31 | -    | -      |
| iNDF (%)          | 15.75 | 15.61 | -    | -      |
| Lignin (%)        | 1.70  | 1.16  | -    | -      |
| EE (%)            | 1.81  | 2.11  | -    | -      |
| GE (MJ/kg DM)     | 12.58 | 13.33 | -    | -      |
| CP (%)            | 14.87 | 15.02 | -    | -      |
| NFC (%)           | 5.07  | 6.40  | -    | -      |
| Fraction (%CP) 5  |      |      |      |        |
| A                 | 29.01 | 36.98 | -    | -      |
| B1                | 6.33  | 6.48  | -    | -      |
| B2                | 50.92 | 45.01 | -    | -      |
| B3                | 10.89 | 8.76  | -    | -      |
| C                 | 2.85  | 2.77  | -    | -      |

1 ES: Energy supplement without the inclusion of phytogenic additives; ESPA: energy supplement with the inclusion of phytogenic additives; MS: mineral supplement without the inclusion of phytogenic additives; MSPA: mineral supplement with the inclusion of phytogenic additives; 2 Chemical composition—MM: mineral matter; OM: organic matter; NFC: non-fibrous carbohydrates; apNDF: insoluble fiber in neutral detergent corrected for ash and protein; GE: gross energy; CP: crude protein. 3 MS: mineral supplement warranty levels: calcium—123.0 g kg⁻¹; phosphorus: 90 g kg⁻¹; copper—1040.00 mg kg⁻¹; manganese: 500 mg kg⁻¹; Zinc—2000.0 mg kg⁻¹; Cobalt—15.0 mg kg⁻¹; Iode—67.0 mg kg⁻¹; selenium—14.0 mg kg⁻¹. 4 MSPA: Guarantee levels similar to DM include phytogenic additives composed of carvacrol, cinnamaldehyde (10% of DM), and hydrolyzable tannins from berries and grapes. 5 Fraction A = non-protein nitrogen, fraction B1 = easily degradable protein, fraction B2 = moderately degradable protein, fraction B3 = slowly degradable protein, and fraction C = non-degradable and unavailable to the animal.

Supplements were provided daily at 09:00 a.m. through a feeder. The energy supplements were administered in the proportion of 0.3% of body weight (CP), in which weekly adjustments were made in the amount offered. Mineral supplements (mineral supplements) were provided ad libitum. The energy supplements used were commercial, and suppliers were not authorized to disclose the percentage composition of the ingredients. These supplements did not contain urea.

2.3. Evaluation of Forage: Forage Mass, Morphological Composition, and Forage Supply

The mean height of the forage canopy was measured weekly during the experimental period from 80 random points per hectare, with the aid of a graduated ruler, to maintain a pasture height of 25 cm by adjusting the stocking rates. Forage mass and morphological components were evaluated every 28 days. Three points representing the average height of the forage canopy of each paddock were identified, and the forage cutting area close to the ground was delimited using a circular frame of 0.25 m².

Samples were collected, weighed, and subsequently obtained as two sub-samples. The first sub-sample was manually separated to determine the morphological composition of the dead material (dry leaf blade and dry stem + sheath), green leaf blade, and green stem + sheath. An estimate of the allowance of total herbage mass (HM, kg DM/ha) of forage for each paddock was obtained in the second sub-sample. The sub-samples were weighed and dried in an oven with air circulation at 55 °C for 72 h, and then weighed again.

The animal capacity in each paddock was evaluated and used along with forage mass to obtain the forage supply estimate (kg DM/kg BW). Similarly, the forage mass of leaves (kg of the green leaf blade) and animal weight were used to obtain the supply of forage leaves (kg DM of leaves/kg of BW).
Potentially digestible DM (pdDM) was calculated according to Paulino [20]:

\[ \text{pdDM} = 0.98 \times (100 - \text{NDF}) + (\text{NDF} - \text{iNDF}) \]

In which: 0.98 = true digestibility coefficient of cell content, NDF = neutral detergent fiber, and iNDF = indigestible neutral detergent fiber.

2.4. Chemical Analysis and Nutritive Value Estimation

The chemical bromatological composition of the forage was evaluated in forage samples collected by manual grazing simulation (hand-plugged samples) [21]. The samples were collected every 28 d, and the post-collection was dried in a greenhouse with forced air circulation at 55 °C for 72 h, processed in a Wiley mill with a 2 mm mesh sieve for samples intended for NDF analysis (iNDF), according to Valente et al. [22], and with a 1 mm mesh sieve for other analyses. Samples of the energy supplements were collected and subjected to the same processing procedure.

The analyses of DM, OM, mineral matter (MM), ether extract (EE), and crude protein (CP) to obtain the nutritional value were performed according to the AOAC procedures [23]. The Dumas method was used to determine the CP content by dry combustion using Leco® equipment, model FP-528 (Leco Corporation, St. Joseph, MI, USA). Neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (ADF) were determined using Ankom® 2000 equipment (Ankom Technologies, Fairport, NY, USA). A thermostable α was used to determine the fiber content of the concentrates [24].

ADF and lignin (H2SO4 72%) were determined according to Robertson and Van Soest [25], and neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined according to the recommendations of Licitra et al. [26]. The in situ incubation procedure for 288 h by Valente et al. [22] was used to obtain iNDF estimates for forage, concentrates, and feces. An adiabatic calorimetric pump was used to determine the gross energy content (GE) of the food and feces (PARR Instrument Company 6300, Moline, IL, USA).

The digestible energy (DE) was calculated from the apparent digestibility of GE. The metabolizable energy was estimated from the multiplication of DE by the corrected factor found after the loss of gross energy by CH4 and urine, calculated by the equation of Street:

\[ \text{GE urine (kcal/g)} = 0.026 + 0.117 \times \% \text{ urine nitrogen}, \text{ for each treatment: SE = 0.8457; SEAF = 0.8509; MS = 0.8246 and SMAF = 0.8058. Analysis of the fractionation of nitrogen compounds in supplements supplied to animals during the rainy period [26].} \]

2.5. Animal Performance

The testers were weighed at the beginning (18 December 2018), and end of the experimental period (30 March 2019), at 5:30 a.m., after a previous 14 h feed and water fasting. The ADG was calculated as the ratio of the difference between the final and initial weights of the animals and the period of performance evaluation (101 days). Follow-up weighing was performed every 28 days without fasting to adjust the stocking rate and supplement supply.

The stocking rate and gain per area were calculated by considering the days of occupation of the regulatory animals in each paddock. The average individual gains and the number of testers and regulator animals in each paddock per day during the evaluation period were considered to calculate the gain per area. The stocking rate in animal units per hectare (450 kg BW/ha) was obtained from the total body weight (BW) of the testers and regulators in each paddock per day. Thus, the average stocking rate during the evaluation period was calculated.

2.6. Intake and Digestibility of Dry Matter and Nutrients

Nutrient intake and digestibility were evaluated in six tester animals per treatment (two animals/paddock; total 24 animals) after the performance evaluation, simultaneously with the assessment of enteric CH4.
Fecal production was estimated from the use of the external marker chromic oxide (Cr$_2$O$_3$), packed in paper cartridges, and supplied at a dose of 10 g for 10 days, at 6:00 a.m., via an esophageal tube, and provided for 7 days for adaptation and the final 3 days of feces collection. The feces were collected on the 8th day at 5:00 p.m., 9th day at 11:00 a.m., and 10th day at 6:00 a.m. Later, they were dried at 55 °C for 72 h, processed, a sample was composed of the 3 days of collection per animal, and stored for subsequent chemical analysis. Fecal excretion was determined using the following equation:

Fecal excretion (g/day) = Cr$_2$O$_3$ supplied (g/day)/Concentration Cr$_2$O$_3$ (g/kg DM)

Herbage intake and digestibility of DM were estimated based on fecal production data using iNDF as an internal marker. To quantify the NDF concentration, hand-plucked fecal samples were adequately conditioned in ANKOM F-57 filter bags and arranged in the rumen of fistulated animals for in situ incubation for 288 h, according to the methodology of Valente et al. [22]. After removing the rumen, the bags were washed until thoroughly bleached and dried in an oven with forced circulation at 55 °C for 72 h. The average supplement intake at the base of the DM was calculated from the average amount of supplement provided (% BW) per paddock.

2.7. Enteric Methane Emission

Methane emissions were measured after the performance evaluation period in the same animals to evaluate intake and digestibility. Thus, using the sulfur hexafluoride tracer technique, six tester animals were used per treatment (two animals/paddock; totaling 24 animals), with an average body weight of 326 ± 38 kg SF$_6$ [27,28].

At the beginning of the 15-day adaptation period, a permeation tube containing SF$_6$ with a known release rate, calibrated by gravimetric difference until reaching a constant weight, was inserted into the animal to balance the tracer gas in the rumen [28]. Seven days before the beginning of the collection of gas samples (the 8th day of adaptation), the animals were equipped with halters and adapted to the canisters.

At the beginning of the evaluation period, the adaptation halter and canisters were replaced by a halter equipped with a capillary tube adjusted on the head of the animal and connected to polyvinyl chloride chamber (canister) fitted with a valve and register. The canisters were then vacuumed and measured at the time of use. The collection events were also placed in strategic locations in the paddocks where the animals were to correct for the amount of gas present in the environment, which could influence the samples collected by the animal.

After 24 h, the canisters were exchanged and pressurized with pure N$_2$, and the evaluation was performed for five consecutive days. The concentrations of CH$_4$ and SF$_6$ were determined via gas chromatography using a Shimadzu 2014 AF gas chromatograph under the following conditions to measure SF$_6$: injector at 250 °C, column at 80 °C, using N$_2$ as the carrier gas (30 mL/min), and the electric capture detector at 325 °C; and CH$_4$, using H$_2$ as the carrier gas (30 mL/min) and flame intake detector at 280 °C.

Methane production was calculated based on the concentrations of CH$_4$ and SF$_6$ present in the animal samples, subtracted from the concentrations present in the environment, and the release rate of SF$_6$ from the capsules, using the following equation described by Berndt et al. [28]:

Methane (g/day) = [release rate SF$_6$ × (CH$_4$ animal sample − CH$_4$ background air)]/[SF$_6$ animal sample − SF$_6$ background air] × (molecular weight CH$_4$)/(molecular weight SF$_6$) × 1000.

2.8. Statistical Analyses

The variables studied during the performance and emission experiment of CH$_4$ were analyzed in a completely randomized design, with a factorial arrangement of 2 × 2 (A × B; Factor A: 2 supplements—energy supplement and mineral supplement and factor B: additives phytogenic—with or without phytogenic additives. The effects of factors A and B and
the interaction between the factors, which are considered fixed effects, were tested. The experimental unit was a paddock \((n = 12)\).

Initially, the data were analyzed for normality of residuals and homoscedasticity of variance. Repeated measurements were included in the time (months of collection) for the production and composition of forage variables. For the analysis, the covariance matrix structure of the errors that fit the data was chosen according to the corrected Akaike criterion (AICc). The structure with the best fit was ‘unstructured.’ Data were analyzed using the PROC MIXED procedure in SAS (version 9.2). Effects were declared significant when \(p \leq 0.05\), and trends were considered when the Tukey test compared \(p > 0.05\), and \(<0.10\), and the means.

3. Results

3.1. Herbage Mass and Chemical Composition

The possible effects of the treatments on the production and composition of the forage, which were minimized by adjusting the stocking rates, were not significant \((p > 0.05)\). Thus, the effects on morphological and chemical production characteristics were considered to be due to climatic conditions during the evaluation period (Table 3). The fractionation of nitrogen compounds in the forage was as follows: fraction A (% CP), 26.64%; fraction B1 (% CP), 7.40%; fraction B2 (% CP), 45.70%; fraction B3 (% CP), 14.20%; fraction C (% CP), 6.06%.

Table 3. Effect of the evaluation months on forage mass, morphological composition, and chemical-bromatological composition of forage \((Urochloa brizantha\ cv. Marandu)\ during the rainy season.

| Item \(^1\) | Months | EPM \(^2\) | p-Value |
| --- | --- | --- | --- |
| | January | February | March | April |
| HM (t/DM/ha) | 5.58 b | 8.16 a | 6.31 b | 6.06 b | 0.21 | <0.0001 |
| Herbage allowance | 3.84 b | 4.70 a | 3.62 b | 3.29 b | 0.19 | 0.001 |
| Herbage allowance (pdDM) | 3.18 ab | 3.78 a | 2.95 b | 2.62 b | 0.40 | 0.002 |
| Green leaves allowance | 1.18 ab | 1.40 a | 1.08 b | 1.01 b | 0.06 | 0.009 |
| Morphological composition (%) | | | | | | |
| Green leaves | 30.98 | 30.54 | 30.90 | 31.20 | 0.68 | 0.990 |
| Green stems | 30.02 a | 27.77 ab | 19.71 c | 25.09 b | 0.82 | <0.0001 |
| Dead material | 39.00 b | 41.69 b | 49.39 a | 43.71 b | 1.13 | 0.005 |
| Leaves: Stem | 1.06 b | 1.12 b | 1.58 a | 1.27 ab | 0.04 | <0.0001 |
| Chemical composition \(^3\) (%) | | | | | | |
| OM | 90.88 c | 91.65 a | 90.58 d | 91.30 b | 0.07 | <0.0001 |
| apNDF | 53.10 c | 57.89 a | 52.14 c | 55.55 b | 0.38 | <0.001 |
| iNDF | 46.61 c | 41.74 a | 38.32 c | 41.91 a | 0.06 | 0.002 |
| Lignin | 2.66 a | 2.28 c | 2.61 ab | 2.40 bc | 0.05 | 0.001 |
| EE | 17.85 bc | 18.17 a | 17.79 c | 18.10 ab | 0.04 | 0.002 |
| GE (MJ/kg DM) | 14.17 c | 14.69 bc | 17.09 a | 15.37 b | 0.22 | <0.0001 |
| pdDM | 82.70 a | 80.46 b | 81.52 ab | 79.69 b | 0.24 | <0.0001 |

\(^1\) HM: Total herbage mass; herbage allowance (kg DM/kg BW); green leaf allowance (kg DM/kg BW); \(^2\) SEM: Standard error of mean; \(^3\) Chemical composition of samples obtained by simulated grazing technique. 1 MJ = 239 Kcal. OM, organic matter; apNDF, neutral detergent fiber corrected for ash and protein; iNDF, insoluble fiber indigestible neutral detergent; EE, ether extract; GE, gross energy; CP, crude protein; pdDM, potentially digestible dry matter. In the line, numbers followed by the same letter did not differ according to the Tukey test at 5% of probability.

The total herbage mass \((p < 0.0001)\) and herbage allowance \((kg DM/kg BW)\) \((p = 0.001)\) were higher in February (Table 3), and the proportion of dead material was higher \((p = 0.005)\) during March. In addition, in March, the crude protein content \((p < 0.0001)\), and leaf-to-stem ratio \((p < 0.0001)\) were higher.
3.2. Animal Performance

There was no interaction between the supplements and phytogenic additives in the productive performance variables evaluated \((p > 0.05)\) (Table 4). The final body weight (kg) of the animals was not influenced by the supplements provided or the inclusion of phytogenic additives \((p > 0.05)\) (Table 4). However, the ADG (kg/day) \((p = 0.013)\), stocking rate (AU/ha) \((p = 0.024)\), and gain per area (kg/ha/day) \((p = 0.005)\) were higher in the animals that received energy supplements compared to those that received mineral supplements, regardless of the inclusion of phytogenic additives (Table 4).

Table 4. Effects of the supply of energy supplements or mineral supplements, with or without phytogenic additives, on the productive performance of grazing cattle during the rainy season in the rearing phase.

| Item 1 | Treatments 2 | SEM 3 | p-Value |
|-------|--------------|-------|---------|
| Item 2 | ES | ESPA | MS | MSPA | Supl. | Adit. | Supl. × Adit. |
| iBW (kg) | 212 | 213 | 209 | 211 | 4.41 | 0.802 | 0.831 | 0.952 |
| fBW (kg) | 349 | 352 | 338 | 338 | 6.15 | 0.314 | 0.893 | 0.898 |
| ADG (kg/day) | 0.840 | 0.896 | 0.767 | 0.774 | 0.02 | 0.013 | 0.422 | 0.535 |
| SR (AU/ha) | 5.32 | 5.21 | 4.60 | 4.72 | 0.13 | 0.024 | 0.977 | 0.609 |
| Gain per area (kg/ha/day) | 6.66 | 7.45 | 5.94 | 6.09 | 0.22 | 0.005 | 0.108 | 0.250 |

1 iBW, initial body weight; fBW, final body weight; ADG, average daily gain (kg/day); SR, stocking rate (AU/ha)
2 ES: energy supplement without the inclusion of phytogenic additives; ESPA: Energy supplement with the inclusion of phytogenic additives; MS: mineral supplement without the inclusion of phytogenic additives; MSPA: mineral supplement with the inclusion of phytogenic additives; SEM: standard error of the mean.

3.3. Intake of Dry Matter and Nutrients

There was no interaction between the supplements and phytogenic additives in the DM and nutrient intake variables \((p > 0.05)\) (Table 5). DM forage intake (kg/day and % BW) was not affected \((p > 0.05)\) by using phytogenic additives, but showed an increasing trend (forage DMi kg/day, \(p = 0.073\); DMi forage % BW, \(p = 0.052\)) with energy supplement consumption (Table 5).

The intake of non-fibrous carbohydrate (NFC, % BW) was not affected by supplements or phytogenic additives. The consumption of total DM \((p = 0.015)\), OM \((p = 0.023)\), CP \((p = 0.012)\), and apNDF (kg/day, \(p = 0.044)\), kg/day, total DM consumption, % BW \((p = 0.005)\), gross energy consumption (GE MJ/day: \(p = 0.026)\), digestible energy (DE MJ/day: \(p = 0.012)\), and metabolizable energy (MJ/day: \(p = 0.008)\) were higher in animals that received energy supplements compared with animals that received mineral supplements, and were not affected by the inclusion of phytogenic additives \((p > 0.05)\) (Table 4). The average consumption of mineral supplementation was recorded, and the mean DM was 106 g/animal/day and 108 g/animal/day, respectively.

The relationship between protein content and the amount of digestible organic matter (DOM; g CP/kg) tended to increase \((p = 0.077)\) in animals that consumed mineral supplements (energy supplement: 258 g CP/kg DOM; mineral supplement: 272 g CP/kg DOM) compared with animals that consumed energy supplements. In addition, animals that consumed phytogenic additives showed lower efficiency \((p = 0.0001)\) in the relationship between protein content and amount of organic matter than animals that did not consume phytogenic additives (without phytogenic additives: 247 g CP/kg DOM; with phytogenic additives: 283 g CP/kg DOM).
Table 5. Effects of the supply of energy supplement or mineral supplement, including or not phytogenic additives, on the voluntary consumption of nutrients in grazing cattle during the rainy season, in the rearing phase.

| Item | Treatments | SEM | p-Value |
|------|------------|-----|---------|
|      | ES | ESPA | MS | MSPA |
| **Intake (kg/day)** | | | | |
| Total DM | 7.87 | 7.92 | 5.17 | 5.03 | 0.57 | 0.015 | 0.964 | 0.931 |
| DM Pasture | 6.99 | 7.02 | 5.17 | 5.03 | 0.50 | 0.073 | 0.958 | 0.934 |
| DM Supplement | 0.88 | 0.89 | - | - | 0.05 | - | 0.896 | - |
| OM | 6.97 | 7.01 | 4.71 | 4.59 | 0.50 | 0.023 | 0.964 | 0.932 |
| CP | 1.18 | 1.21 | 0.77 | 0.78 | 0.08 | 0.012 | 0.893 | 0.954 |
| apNDF | 4.11 | 4.12 | 2.88 | 2.87 | 0.29 | 0.044 | 0.999 | 0.982 |
| g CP/kg DOM | 244 | 272 | 249 | 294 | 7.04 | 0.077 | 0.0001 | 0.254 |
| **Intake (% BW)** | | | | |
| DM Total | 2.40 | 2.33 | 1.63 | 1.55 | 0.14 | 0.005 | 0.751 | 0.987 |
| DM forage | 2.13 | 2.06 | 1.63 | 1.55 | 0.12 | 0.052 | 0.747 | 0.990 |
| apNDF | 1.12 | 1.31 | 0.98 | 1.00 | 1.10 | 0.151 | 0.486 | 0.570 |
| **MJ/day** | | | | |
| EB | 136 | 137 | 93.9 | 91.2 | 9.66 | 0.026 | 0.959 | 0.921 |
| ED | 85.2 | 80.6 | 57.1 | 47.9 | 5.97 | 0.012 | 0.527 | 0.830 |
| EM | 72.0 | 68.6 | 47.1 | 38.6 | 8.97 | 0.008 | 0.516 | 0.782 |

1 Chemical composition of total diet. DM: dry matter; OM: organic Matter; CP: crude Protein; apNDF: neutral detergent insoluble fiber corrected for ash and protein; EB: gross energy; ED: digestible energy; EM: metabolizable energy; 2 ES: energy supplement without the inclusion of phytogenic additives; ESPA: energy supplement with the inclusion of phytogenic additives; MS: mineral supplement without the inclusion of phytogenic additives; MSPA: mineral supplement with the inclusion of phytogenic additives; 3 SEM: standard error of the mean.

3.4. Digestibility of Dry Matter and Nutrients

There was no interaction between the supplements and phytogenic additives in the digestibility variables of DM and nutrients evaluated (p > 0.05) (Table 6). DM digestibility (p = 0.001), CP (p = 0.044), and energy (p = 0.050) were higher in animals that received energy supplements than in animals that received mineral supplements. The animals that also received phytogenic additives had lower digestibility of DM (p < 0.0001; without phytogenic additives: 64.7%; with phytogenic additives: 55.5%), NDF (p = 0.025; without phytogenic additives: 70.0%; with phytogenic additives: 65.4%), and energy (p = 0.023; without phytogenic additives: 61.6%; with phytogenic additives: 56.3%). The animals that received phytogenic additives also showed a tendency for lower digestibility of OM (without phytogenic additives: 65.61%; with phytogenic additives: 61.26%) (Table 5).

Table 6. Effects of the supply of energy supplements or mineral supplements, with or without phytogenic additives on nutrient digestibility in grazing cattle during the rainy season, in the rearing phase.

| Item | Treatments | SEM | p-Value |
|------|------------|-----|---------|
|      | ES | ESPA | MS | MSPA |
| **DMd (%)** | | | | |
| DMd | 67.95 | 57.08 | 61.45 | 54.01 | 1.20 | 0.001 | <0.0001 | 0.138 |
| OMd | 66.08 | 64.63 | 65.13 | 57.89 | 1.19 | 0.081 | 0.052 | 0.180 |
| CPd | 62.71 | 60.04 | 58.86 | 52.88 | 1.39 | 0.044 | 0.104 | 0.519 |
| apNDFd | 70.36 | 67.74 | 69.82 | 63.01 | 1.09 | 0.185 | 0.025 | 0.287 |
| GEd | 62.26 | 60.03 | 60.90 | 52.53 | 1.28 | 0.050 | 0.023 | 0.163 |

1 DMd: dry matter digestibility; OMd: organic matter digestibility; CPd: crude protein digestibility; apNDFd: neutral detergent fiber corrected for ash and protein; GEd: gross energy digestibility; 2 ES: energy supplement without the inclusion of phytogenic additives; ESPA: energy supplement with the inclusion of phytogenic additives; MS: mineral supplement without the inclusion of phytogenic additives; MSPA: mineral supplement with the inclusion of phytogenic additives; 3 SEM: standard error of the mean.
3.5. Enteric Methane Emission

There was no interaction between the supplements and phytogenic additives in the enteric CH$_4$ emission variables evaluated ($p > 0.05$). The daily production of enteric CH$_4$ showed a tendency ($p = 0.065$) to increase with the supply of energy supplements. The production of CH$_4$ by DM intake, amount of DOM, average daily gain, consumption of crude energy and metabolizable energy, and conversion rate of crude energy in CH$_4$ Ym were not influenced by supplements or phytogenic additives ($p > 0.05$) (Table 7).

Table 7. Effects of the supply of energy supplements or mineral supplements, with or without phytogenic additives, on the production of CH$_4$ of cattle grazing during the rainy season, in the rearing phase.

| Item                      | Treatments $^1$ | SEM $^2$ | $p$-Value |
|---------------------------|----------------|----------|-----------|
| CH$_4$ (g/day)            | ES 172        | ESPA 183 | MS 127    | MSPA 160 | 9.28 | 0.065 | 0.208 | 0.504 |
| CH$_4$ (g/kg DMi)         | 23.5          | 21.6     | 23.1     | 28.8     | 1.59 | 0.310 | 0.568 | 0.262 |
| CH$_4$ (g/kg DOM)         | 40.5          | 39.0     | 38.8     | 52.9     | 3.20 | 0.365 | 0.345 | 0.249 |
| CH$_4$ (kg/kg ADG)        | 0.21          | 0.18     | 0.18     | 0.19     | 0.01 | 0.619 | 0.459 | 0.329 |
| CH$_4$ (g GE MJ/day)      | 1.37          | 1.24     | 1.28     | 1.60     | 0.09 | 0.483 | 0.610 | 0.241 |
| Ym (%) $^3$               | 7.58          | 6.86     | 7.06     | 8.84     | 0.48 | 0.484 | 0.610 | 0.241 |

$^1$ ES: energy supplement without the inclusion of phytogenic additives; ESPA: Energy supplement with the inclusion of phytogenic additives; MS: mineral supplement without the inclusion of phytogenic additives; MSPA: mineral supplement with the inclusion of phytogenic additives; $^2$ SEM: standard error of the mean; $^3$ conversion rate of gross energy consumed by the animal that is transformed into CH$_4$.

4. Discussion

Pasture management in forage-based production systems is crucial for planning an optimized design. In grazing systems, bit size is strongly influenced by canopy height [29], as the height shapes the structure of the pasture. Therefore, the management criterion of light interception of 95% allows the alteration of pasture structure, improving the composition of forage, implying forage with low levels of iNDF due to the high proportion of green leaves, which results in high levels of CP with a higher proportion of fractions with higher solubility [30].

The management of pastures at a height of 25 cm by adjusting the stocking rate allows the concentration of NDF to be minimized and a higher proportion of soluble protein to be available so that it does not limit pasture intake, providing potentially digestible dry matter (pdDM), which is based on the concepts of quality and supply of fodder available to the animal at the time of grazing. A 4–5% supply of CP in pdDM is recommended to promote production by an animal; by area and grazing efficiency of 70% [31]. The mean pdDM verified during the study period was 81%, that is, the supply of potentially digestible forage was approximately 3.13 kg pdDM/kg CP.

The highest total herbage mass and herbage allowance during February and the higher proportion of dead material during March were influenced by higher rainfall during February and were reflected in the following month. The herbage mass (kg/ha) promoted by the management proposed in the study was similar, except for the higher value verified in March due to climatic conditions, which was found in studies such as that by Delevatti et al. [1], who used the management criterion of 25 cm height in continuous grazing with variable stocking rate.

The higher grazing intensity provides management of the canopy height at 25 cm and allows a higher stocking rate, with positive effects on the nutritive value of the forage, because the range of visits of the animals to the same tiller is smaller, with a reduction in the average maturity of regrowth and a higher proportion of leaves in the forage mass available [32,33].

Regarding pasture structure, the greater leaf:stem ratio verified in March was due to a lower proportion of green stems during the same period, as there was no change in the
proportion of green leaves. Moreover, despite the higher supply of forage during February, the highest levels of apNDF and the indigestible fraction, NDF, may have interfered with CP content.

The CP concentration suggested that the availability of ruminal ammoniacal nitrogen is necessary to prevent microbial growth. Consequently, the use of fibrous carbohydrates from forage results in approximately 100 g/kg DM [34]. After there is an increase in voluntary forage consumption, N availability should be higher than that necessary for ruminal fiber degradation, increasing the concentration of CP to approximately 145 g/kg DM [35]. Throughout the evaluation period of the study, CP content was close to or higher (15.33% CP) than that recommended to avoid limiting forage intake.

Owing to the high quality of the forage present in the study, there was only a tendency to stimulate forage consumption caused by the supply of the energy supplement. The increase in the total consumption of the animals was at the same level as the SE, indicating an additive effect on consumption [2,34].

The high CP content and high digestibility of the forage were responses to pasture management. It was also observed that the forage contained fraction A of N at approximately 26.64%. Thus, to improve the efficiency of protein use, it is necessary to synchronize ruminal degradation between energy and pasture protein, justifying the use of energy supplements.

Poppi and McLennan [36] stated that when the relationship between CP content and forage energy exceeded 210 g CP/kg of DOM, losses and incomplete transfer of ingested protein into the intestine occurred. The highest efficiency occurred when these values were less than 160 g CP/kg MOD. In all treatments, these values exceeded the reference of 210 g CP/kg of MOD (general values on average 265 g CP/kg MOD), suggesting the need to provide supplements with higher energy content in situations such as that in our study.

Moreover, the lower efficiency of the animals that consumed supplements with phyto-organic additives than those that consumed supplements without phyto-organic additives is justified by the tendency of lower digestibility of OM observed in the rumen of these animals. The total DM intake of animals that received only mineral supplementation was lower (5.17 kg/day) than the predicted value (6.05 kg/day) by the nutritional requirements established by BR-Corte 3. Zero in animals with an average body weight of 270 kg and an average daily gain of 0.800 kg/day. The lower consumption of total DM also resulted in lower consumption of digestible energy and metabolizable energy than that recommended by BR-Corte 3.0 [37].

Consequently, lower consumption of total DM in animals supplemented only with mineral supplements influenced the ADG. The ADG of animals that received only mineral supplement was 11.5% lower than that of animals that received an energy supplement. Considering these nutritional requirements, the total DM consumption of the animals that received energy supplement (7.87 kg/day) was above the maximum recommended by BR-Corte 3.0 (6.65 kg/day) with a higher digestible and metabolizable energy intake. Because of the higher consumption of DM from the SE, the CP intake of these animals was 55.23% higher than the recommended BR-Corte 3.0 of 760.13 g/day for gain of 800 g/day [37].

According to the chemical–bromatological composition of the energy supplements, the mineral matter content was high (approximately 33.5%), indicating a high inert material content in its design. In addition, the energy supplement was composed of corn gluten meal, providing a supplement with lower crude energy (MJ/kg DM) when compared to a supplement consisting of corn and citrus pulp as an energy source, as seen in the study by Barbero et al. [4]. The supply of forage (kg DM/kg PC) and composition were similar in both studies, justifying the lower weight gain provided by the energy supplement.

The model proposed by Mott [38] describes the optimal point of gain per animal (kg/animal/day) and gain per area (kg/ha). When there is a higher gain per animal, there are sub grazing conditions because the stocking rate (AU/ha) is reduced, resulting in higher grazing height and high forage supply, but lower gain per area. The highest individual gain occurs because there is less feed competition for the animals, allowing for greater
forage selectivity. An increase in the gain per area can occur with an increase in stocking rate. However, maximum gain per animal is no longer obtained.

The average daily gain responds to the increase in the supply of forage (kg DM/kg BW), reaching higher weight gain per animal (kg/head/day); however, to reconcile with a higher gain per area (kg/ha), it is necessary to provide the animals with complementary nutrients through supplementation [39]. Therefore, the inclusion of the energy supplement increased grazing intensity, resulting in a higher stocking rate (AU/ha), and consequently, a higher gain per area (kg/ha), in addition to the higher ADG.

Additionally, the composition of the energy supplement may have been one of the factors that influenced the lack of effect of phytogenic additives in ADG because other nutrients, such as energy, may have been limiting, as the EB in the supplement used by Barbero et al. [4] was 20.1 MJ/kg DM. In contrast, in our study it was 12.96 MJ/kg DM. In addition, the dose of phytogenic additives provided in the present study was 0.15% of the ingested DM. In comparison, Min et al. [40] found ADG 20% higher in heifers supplemented with chestnut tannins, a source of hydrolyzable tannins, with a supplied dose of 1.5% DM intake.

Tannin concentrations above 2% of DM in the diet can reduce DM intake and, consequently, animal performance [10,41]. Consequently, the results obtained in the present study were consistent because the inclusion of a low concentration of hydrolyzable tannins did not affect the DMi and ADG. Similarly, Beauchemin et al. [42] found no effects of 0% condensed tannins and 1% and 2% DM of the diet on these parameters in steers (223 kg initial body weight) fed a diet containing 70% forage DM. The phytogenic additives included in the diet did not alter the emission of enteric CH₄, which tended to be higher in animals that received an energy supplement regardless of the inclusion of additives.

In an analysis of an intercontinental database (data from Europe, North America, Brazil, Australia, and South Korea), the equations indicated a positive relationship between CH₄ production and DMi, because the amount of substrate available for microbial fermentation and, consequently, for methanogenesis is higher [43]. Cows fed hay containing 4.5% hydrolyzable tannins were also positively correlated with CH₄ [44].

The positive association between additive-free energy supplementation and ruminal abundance of Archaea from the phylum Euryarchaeota and the genus Methanobrevibacter spp. was verified in a previous study using the same supplement [45]. This association may be related to the effect of organic matter degradation [16].

Feed energy losses, calculated by the conversion rate of gross energy consumed by the animal that is transformed into CH₄ (Ym %), are approximately 2–12% [46], with higher losses in animals fed forage [47]. The supply of concentrated feed can influence the conversion rate of crude energy into CH₄ because the digestibility of the diet affects this conversion. According to the IPCC [47], diets with digestibility between 63% and 70% can be characterized as good-quality diets and present emissions of CH₄ with a Ym of 6.3%. In the present study, the digestibility of the diets was lower than 63% (except in animals supplemented with an energy supplement without an additive).

Concerning the effect of the supplied tannin dose, the emission of CH₄ enteric from beef cattle fed a high-forage diet, in an amount of 1.5% of DM of the diet of condensed tannins, hydrolyzable, or the combination trend in reducing CH₄ emissions [48]. The authors also pointed out that low concentrations of tannins (0.25% of DM in the diet) are associated with diet components, such as fibers and proteins, and are insufficient to cause an inhibitory effect on methanogens.

Methanogenic microorganisms are susceptible to toxicity caused by the degradation products of hydrolyzable tannins, such as conical acids, galloping acids, and pyrogallol acids [49,50]. However, condensed tannins are more strongly linked to nutrients than hydrolyzable tannins, do not degrade in the ruminal environment, and may even reduce the digestibility of nutrients [30–52].

In in vitro studies, CH₄ production was reduced by more than 40% with supplementation with extracts of chestnut tannin at the dose of 37.8 g/kg DM and 35.6 g/kg DM, both
extracts of hydrolyzable tannins [53]. Conversely, the dose of 11.9 g/kg DM of chestnut tannin extract was insufficient to reduce the production of CH\(_4\) [54]. Thus, in addition to the source of tannin used, the composition and level of supplementation interfered with the results obtained with hydrolyzable tannin extracts.

In addition to hydrolyzable tannins, the blend of phytogenic additives used in this study was composed of essential oils (carvacrol and cinnamaldehyde). The CH\(_4\) emissions were not affected by the inclusion of phytogenic additives. This can occur because essential oils can be rapidly metabolized into less active forms in the rumen, causing a lack of effect, and their effectiveness is dose-dependent [55]. High doses of essential oils can negatively affect nutrient intake and digestibility, and low doses can stimulate these variables. Consequently, the animal performance can be improved [45,56].

Higher doses are suggested to verify the possible effect of these phytogenic additives on the emission of CH\(_4\) owing to low concentrations that can be rapidly metabolized by rumen microorganisms and are insufficient to cause an impact on rumen microorganisms. The complexity of the ruminal environment and the interaction of the compounds present in the essential oils with the fermentation of the diet makes it difficult to predict the effects of essential oils. Animals fed high-forage diets and a blend of essential oils composed of timol, guaiacol, and other compounds showed reduced NDF digestibility [42]. In contrast, Oh et al. [57] did not observe an effect of a blend composed of carvacrol, eugenol, and timol, supplied at a dose of 35 g/cab/day, on NDF digestibility.

Hydrolyzable tannins, present in the blend used in our study, generate degradation products that are toxic to fibrinolytic microorganisms, such as *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and anaerobic fungal populations, reducing fiber degradation in the rumen [58]. A trend of lowering The abundance of a genus of bacteria of the family *Ruminococcaceae* (Ruminococcaceae.UGC-010), *Roseburia* spp., and *Morella* spp. Teobaldo et al. [45] evaluated a blend of phytogenic additives composed of hydrolyzable tannins and essential oils (carvacrol and cinnamaldehyde) used in our study.

The inclusion of 3% condensed tannins in the diet of cows reduced the digestibility of DM, NDF, and ADF. To a lesser extent, hydrolyzable tannins at the same dose negatively affect the digestibility of these nutrients [16]. Additionally, intraluminal infusion of condensed tannins at doses of 4% and 6% ingested DM resulted in a reduction in NDF digestibility [51,59]. This study observed a similar effect in animals that received phytogenic additives in terms of DM, fiber, and energy digestibility.

In addition, tannins can form complexes with digestive enzymes secreted by bacteria, thereby affecting the digestibility of nutrients in the rumen [60]. In addition, ruminal fermentation of carbohydrates can be reduced by the unavailability of carbohydrates for digestion due to the complex formation of tannins with carbohydrates in the cell wall, which can prevent microbial fixation [61,62].

Despite reducing the digestibility of these nutrients, the performance of the animals that received phytogenic additives was not compromised. Additionally, a reduction in the digestibility of DM and fiber was observed in studies with higher doses of tannins or essential oils. Because of the low concentration of phytogenic additives used in this study, these results were unexpected.

Regardless of the inclusion of phytogenic additives in the supplements, the supply of energy supplements increased the digestibility of DM, CP, and energy. In high-quality fodder, the chemical profile of CP contains high proportions of non-protein nitrogen compounds (NPN) and nitrogen compounds associated with insoluble fiber [63]. Thus, the speed of forage energy availability is not compatible with the high degradation of forage NPN, with a metabolic imbalance of metabolizable protein/metabolizable energy, which can be improved by supplying energy supplementation [34].
5. Conclusions

The higher consumption of nutrients by energy supplements did not influence the emission of enteric methane. The inclusion of an energy supplement improved the digestibility of nutrients and, consequently, the productive performance of Nellore bulls grazing *Urochloa brizantha* cv. Marandu during the rainy season.

Including phytogenic additives composed of essential oils (carvacrol, cinnamaldehyde, and hydrolyzable tannins) ingested at a dose of 1.5 g/kg DM negatively affected nutrient digestibility, resulting in no potential to reduce the emission of enteric CH\textsubscript{4}.

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