

Article

Effects of Bacterial Inoculants and Ground Corn Grain on Fermentation Profile and In Situ Rumen Degradability of Tropical Grass Silage

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Abstract

The aim of this study was to evaluate different doses of bacterial inoculants and the inclusion of 8% ground corn grain (GCG) on fermentative characteristics, chemical composition, and in situ ruminal degradability of low-DM elephant grass (cv. BRS Capiacu) silage. The experiment followed a completely randomized design in a 6×3 factorial arrangement (six treatments $\times$ three fermentation periods). Treatments were a control without additive (CTR); 0.5 or 1 g/ton of *Lentilactobacillus buchneri* (LBU0.5 and LBU1); 1 or 2 g/ton of a *Lactiplantibacillus plantarum* + *Pediococcus acidilactici* inoculant (LPP1 and LPP2); and 8% GCG. After 60 d of fermentation, in situ ruminal degradability was evaluated using rumen-fistulated lactating cows with incubation times from 0 to 240 h. The GCG treatment increased DM, CP, and ether extract concentrations and reduced NDF, ADF, and lignin contents. Additionally, GCG silage exhibited lower pH, butyric acid, and ammonia nitrogen concentrations, along with higher lactic acid levels. No treatment effects were observed for water-soluble carbohydrates or total DM losses. The effective NDF degradability, degradation rate of the slowly degradable fraction, and undigested NDF after 240 h were not affected by treatments. In conclusion, the inclusion of GCG improved the fermentative profile of low-DM elephant grass silage, whereas bacterial inoculants did not significantly enhance the silage quality under the conditions evaluated.

Keywords: BRS Capiacu silage; fermentation profile; in situ ruminal degradability; inoculant



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1. Introduction

Approximately 23.5% of Brazilian dairy farmers use tropical grasses for silage production, with elephant grass (*Pennisetum purpureum* Schum.) being the most common [1]. This species is characterized by its high biomass yield, adaptability to diverse ecosystems, favorable nutrient composition under proper management, and high palatability for dairy cattle [2]. It also represents a viable silage alternative for tropical regions worldwide. In 2016, the Brazilian Agricultural Research Corporation (EMBRAPA) released a new cultivar of elephant grass, BRS Capiacu (BRSC), which is currently the highest-yielding cultivar of

the genus, producing up to 50 tons of dry matter (DM) per hectare per year [3]. BRS Capiacu can be used as chopped fresh forage and/or ensiled feed, with silage being preferred on dairy farms due to its lower daily labor requirements [1,4].

The cultivation of BRSC is especially recommended for dairy farms where corn silage production is unfeasible due to water scarcity or land constraints. However, producing high-quality grass silage remains challenging, primarily due to the high moisture content, low concentrations of water-soluble carbohydrates, and high buffering capacity at the time of ensiling [4]. These characteristics hinder rapid pH reduction, creating an environment conducive to undesirable microorganisms such as *Bacillus* sp., *Listeria* sp., and *Clostridium* sp. [5]. Therefore, the use of silage additives—particularly bacterial inoculants or moisture-absorbing agents—is critical to improving the fermentation quality of elephant grass silage [1,6].

Inoculants containing homofermentative lactic acid bacteria, such as *Lactiplantibacillus plantarum* and *Pediococcus acidilactici*, are especially important because they help minimize nutrient losses by inhibiting the production of carbon dioxide during undesirable fermentation. In contrast, inoculants containing heterofermentative species such as *Lentilactobacillus buchneri* promote the conversion of lactic acid and glucose into acetic acid, which inhibits the growth of yeasts and molds and enhances aerobic stability [7]. Additionally, incorporating absorbent additives into the silage can reduce fermentation losses. In this context, ground corn grain (GCG) may serve as an effective additive to minimize gas, effluent, and DM losses while improving the digestibility of fibrous fractions and enhancing overall nutritional value [8].

Based on this background, we hypothesized that varying doses of bacterial inoculants in BRSC silage with a low DM content would improve the fermentation profile and chemical composition of the silage. Furthermore, the inclusion of 8% GCG would reduce fermentative losses and enhance nutritional value, thereby altering the chemical composition and improving the in situ ruminal degradability of DM, organic matter (OM), and neutral detergent fiber (NDF) fractions. Therefore, the objective of this study was to evaluate the effects of different doses of bacterial inoculants and the inclusion of 8% GCG on the fermentative characteristics, chemical composition, and in situ ruminal degradability of DM, OM, and NDF of low-DM elephant grass (cv. BRS Capiacu) silage.

2. Materials and Methods

The experiment was conducted at the Dairy Research Facility of the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Animal Use Ethics Committee of the Department of Animal Science of the Universidade Federal de Viçosa, Minas Gerais, Brazil (protocol 060/2022).

2.1. Experimental Design

The experimental design was completely randomized, with treatments arranged in a 6×3 factorial scheme (six treatments $\times$ three fermentation periods) with three replicates. Each portion (mini-silo) was randomly assigned to one of the six additive treatments using a random-number sequence, and, simultaneously, each mini-silo was randomly pre-assigned to one of the three opening times (30, 60, or 90 d). Thus, the fermentation time was evaluated by destructive sampling, with independent silos opened only once at the scheduled time.

The treatments were (1) control without additive (CTR), (2) 0.5 g/ton of a commercial product containing *Lentilactobacillus buchneri* (LBU0.5), with a minimum guarantee level of 1.0×10^{11} CFU/g, resulting in a final inoculation rate of 5.0×10^4 CFU/g, (3) 1 g/ton of

the LBU product (LBU1), with a minimum guarantee level of 1.0×10^{11} CFU/g, resulting in a final inoculation rate of 1.0×10^5 CFU/g, (4) 1 g/ton of a commercial product containing *Lactiplantibacillus plantarum* (LPP) and *Pediococcus acidilactici* (LPP1), with minimum guarantee levels of 5.0×10^{10} CFU/g and 1.5×10^{10} CFU/g, respectively, reaching a final inoculation rate of 6.5×10^4 CFU/g, (5) 2 g/ton of the LPP product (LPP2), with the same minimum guarantee levels, resulting in a final inoculation rate of 1.3×10^5 CFU/g, and (6) 8% on a dry matter basis of GCG-type flint, finely ground to a diameter of less than 1250 μm . These products were applied to the forage mass, which was then ensiled in mini-silos and opened at 30, 60, and 90 d of fermentation. The chemical composition of the BRSC before ensiling is shown in Table 1.

Table 1. Chemical composition of elephant grass (BRS Capi  u) in natura with the application of bacterial inoculants or the inclusion of 8% ground corn grain before ensiling process.

	Additives					
	CTR ^a	LBU _{0.5} ^b	LBU ₁ ^c	LPP ₁ ^d	LPP ₂ ^e	GCG ^f
pH	5.9	5.8	5.8	5.9	5.9	5.8
Buffer capacity	15.0	14.6	14.5	14.7	14.3	11.4
Chemical composition, g/kg DM						
Dry matter	133.3	137.5	137.9	139.6	135.8	192.5
Neutral detergent fiber	704.5	689.4	678.9	682.9	681.0	524.9
Acid detergent fiber	452.8	480.4	438.8	467.9	472.4	317.1
Crude protein	65.0	68.5	67.6	66.9	67.3	71.4
Ether extract	15.5	13.5	16.6	15.0	15.7	19.0
Crude ash	112.4	110.8	101.9	106.4	109.6	77.0
Lignin (sa) ^g	95.2	74.5	74.4	78.9	89.8	66.0
Cellulose	358.0	406.0	364.4	389.0	382.6	251.1
Hemicellulose	251.7	208.9	240.1	215.1	208.6	207.8
Soluble carbohydrates	101.5	101.8	101.4	110.2	110.4	119.9
Non-fibrous carbohydrates	102.6	117.9	135.0	128.8	126.4	307.7
Insoluble protein in acid detergent fiber	5.0	5.7	5.4	5.3	5.3	4.4

^a Control. ^b 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^c 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^e 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f Inclusion of 8% of ground corn grain. ^g Lignin determined by solubilization of cellulose with sulfuric acid.

2.2. Production of BRSC Silage

One day prior to harvest, 100 plants of BRSC were randomly sampled from a 0.7-hectare area and transported to the laboratory for evaluation. Harvesting was performed using a mechanical forage harvester (Model PREMIUM FLEX 2008, Menta Mit, S  o Paulo, Brazil), adjusted to chop the forage to a theoretical length of 11 mm.

Following harvesting, 15 kg of unwilted fresh forage was thoroughly homogenized and divided into 54 portions (15 kg each), corresponding to the 6 treatments $\times$ 3 fermentation times $\times$ 3 replicates. Bacterial inoculants were applied to the forage mass according to the experimental treatments: LBU0.5, LBU1, LPP1, and LPP2. The inoculants were diluted in distilled water and sprayed onto the forage mass using a 500 mL hand sprayer, with each silo treated individually, to ensure proper homogeneity. For the CTR treatment, the same volume of distilled water was applied to the forage using the same procedure. For the treatment involving 8% GCG, 15 kg of fresh forage was mixed with the additive and distributed among the corresponding replicates.

The forage was ensiled in experimental silos made from plastic buckets with a capacity of 10 L (29.5 cm in height, 24 cm in upper diameter, and 22 cm in base diameter). Each silo contained 3 kg of sand at the bottom, covered with a cloth to separate the forage from the sand and to retain effluent. A total of 54 silos were prepared, comprising three replicates for each treatment and fermentation period. The forage was compacted into the silos using a wooden stake, resulting in an average final density of 767 kg/m³. After packing, buckets were sealed with bucket lids and adhesive tape to ensure airtight conditions and stored in a covered area protected from sunlight and rainfall. The silos were opened after 30, 60, and 90 d of fermentation for analysis.

2.3. Fermentation Profile of BRSC Silage

Upon opening the mini-silos, the upper 10 cm of silage was discarded to eliminate any material potentially exposed to air. The remaining silage was thoroughly homogenized, and subsamples were collected for chemical composition and fermentation profile analyses. For fermentation profile evaluation, an aqueous extract was prepared by blending 25 g of silage with 225 mL of distilled water for 1 min [9]. The extract was used to determine pH using a digital potentiometer (Tecnal, model Tec-3MP, São Paulo, Brazil). Portions of the extract were acidified in a 1:1 ratio with sulfuric acid and diluted with distilled water for the determination of ammoniacal nitrogen as a percentage of total nitrogen, using the phenol-hypochlorite colorimetric method described by Okuda [10]. Water-soluble carbohydrates were analyzed according to Nelson [11], and organic acids—including lactic, acetic, propionic, and butyric acids—were quantified following the methodology of Siegfried et al. [12].

For the determination of organic acids, samples were treated with calcium hydroxide and cupric sulfate and analyzed by high-performance liquid chromatography (SPD-10 AVP, Shimadzu, Columbia, MD, USA). The high-performance liquid chromatography system was equipped with a refractive index detector and an Aminex HPX-87H column (BIO-RAD, Hercules, CA, USA), following the procedures outlined by Siegfried et al. [12].

Buffer capacity was measured following the method of Playne and McDonald [13]. Approximately 15 g of fresh forage was macerated and diluted in 250 mL of distilled water. The solution was then titrated to pH 3.0 using hydrochloric acid (0.1 N), followed by back-titration to pH 6.0 using sodium hydroxide (0.1 N).

2.4. Chemical Composition

Samples of the ensiled material were dried at 55 °C in a forced-air oven for 72 h, then ground to pass through a 1 mm sieve using a Wiley mill (Fortinox, model STAR FT 50/6, São Paulo, Brazil). The ground samples were subsequently analyzed for DM (method 934.01), crude protein (CP; method 984.13), crude ash (method 942.05), and ether extract (EE; method 920.39) following the procedures outlined by AOAC [14].

Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were determined sequentially according to the procedures described by Van Soest et al. [15], using an Ankom 2000 fiber analyzer (Ankom Technology, Fairport, NY, USA). Results were expressed on a DM basis and included residual ash. For aNDF determination, a thermostable α -amylase (FAA, Ankom Technology, Macedon, NY, USA) was added as recommended by the manufacturer.

Acid detergent insoluble protein was determined following the methodology of Licitra et al. [16]. Cellulose was solubilized using 72% sulfuric acid, and the lignin content was determined by the resulting weight loss, as described by Goering and Van Soest [17]. Hemicellulose was calculated by the difference between aNDF and ADF. Non-fibrous

carbohydrates were calculated using the equation: non-fibrous carbohydrates = $100 - (\text{NDF} + \text{CP} + \text{EE} + \text{ash})$ [18].

2.5. Total Dry Matter, Gas, and Effluent Loss

Total DM, gas, and effluent losses were determined by weighing the empty and full laboratory silos before and after the ensiling period, along with the corresponding DM contents, following the methodology described by Jobim et al. [19]. Total DM losses were calculated as the difference between the initial and final gross DM weight in each silo, relative to the total amount of forage ensiled, as described by Schmidt [20]. Gas losses were estimated by comparing the weight of the silos at sealing and at opening, relative to the initial weight of the stored forage mass.

Effluent losses were estimated using dry sand placed at the bottom of each silo, which served to collect and retain the liquid. Effluent production was calculated by determining the difference in the weight of the silo (including sand, cloth, and cover) after the forage was removed (at opening) and before ensiling, in relation to the amount of fresh forage initially ensiled [20].

2.6. In Situ Ruminal Degradability Measurement

Three ruminally fistulated lactating Holstein cows (5 ± 1.3 years old; 650 ± 50.7 kg body weight) producing 30 ± 5.2 kg of milk per day were used for in situ ruminal degradability evaluations. The cows were arranged in a randomized block design, with each animal representing a block. The experimental design included six treatments and one fermentation time (60 d). For each round, all six treatments were incubated in each cow; therefore, each treatment had three replicates.

The cows were housed in a free-stall barn with a concrete floor and sand bedding and had ad libitum access to water and feed (Table 2). Prior to the degradability trial, the animals underwent a 14 d adaptation period [21] during which they were fed a corn silage- and concentrate-based diet, maintaining the diet and the forage-to-concentrate ratio of 55:45 on a DM basis throughout the entire experimental period. Ruminal degradability assessments were carried out over 10 consecutive days.

Table 2. Proportion of ingredients and chemical composition of the experimental diet fed to 3 ruminally fistulated cows used for determination of in situ degradability of nutrients.

Ingredient	Level of Inclusion (g/kg DM)
Corn silage	560.1
Ground corn grain	158.4
Soybean meal	136.8
Cottonseed whole	93.3
Sodium bicarbonate	15.5
Urea	10.1
Mineral and vitamin premix a	10.3
Magnesium oxide	7.72
Dicalcium phosphate	5.08
Limestone	3.06
Chemical Composition	
Dry matter	440.7
Organic matter, DM basis	922.5
Neutral detergent fiber, DM basis	256.2
Crude protein, DM basis	166.7
Ether extract, DM basis	43.1
Non-fibrous carbohydrates, DM basis	456.5

Silage samples were ground to a 2 mm particle size using a Wiley mill (Fortinox, model STAR FT 50/6, São Paulo, Brazil). Approximately 4 g of each sample was weighed into individual nylon bags (Sefar Nitex, Heiden, Switzerland; 50 µm pore size, 10 cm × 20 cm). The sample-to-surface area ratio was maintained at 20 mg DM/cm², as recommended by Nocek [22]. The nylon bags were incubated in the rumen for the following time intervals: 0, 3, 6, 12, 24, 30, 48, 72, 120, and 240 h, as recommended by Detmann et al. [23]. The number of bags per sample varied by the incubation time to ensure sufficient material remained for post-incubation analysis.

For incubation, the nylon bags were attached to a stainless-steel chain (90 cm × 2 cm) with a 300 g weight at the end to ensure immersion in the rumen contents [24]. Bags were inserted in reverse chronological order, so all incubation times concluded simultaneously. After removal, all bags were washed under running cold water until the rinse water was clear [25]. The 0 h samples were treated similarly by being briefly immersed in the rumen, then rinsed and processed in the same manner as the incubated bags, following the protocol of Detmann et al. [23]. All bags were then dried in a forced-air oven at 55 °C for 72 h, followed by a final drying at 105 °C for 2 h to determine DM disappearance [24].

Residues from each bag were ground to pass through a 1 mm sieve (Wiley mill, Fortinox, model STAR FT 50/6, São Paulo, Brazil) and stored in labeled plastic bags according to the animal, treatment, and incubation time. Residual samples were analyzed for DM (method 934.01) and OM (method 942.05) using AOAC procedures [14]. Neutral detergent fiber was determined using the method described by Van Soest et al. [15] with an Ankom 2000 fiber analyzer (Ankom Technology, Fairport, NY, USA). A thermostable α-amylase (FAA, Ankom Technology, Macedon, NY, USA) was added during NDF analysis, following the manufacturer's recommendations. These data were used to estimate ruminal degradation parameters for DM, OM, and NDF.

2.7. Degradation Models

The in situ degradability of DM and OM was estimated by finding the weight difference in each component before and after ruminal incubation, expressed in g/kg DM. The coefficients “a,” “b,” and “c” for DM and OM were determined according to Ørskov and McDonald [26] using the following equation:

$$\text{Deg}(t) = a + b(1 - e^{(-ct)}) \quad (1)$$

where “Deg(t)” represents the degradability or disappearance of the constituent (DM, OM) of the feed, as expressed in g/kg of DM; “a” is the water-soluble fraction of the feed at time zero; “b” is the fraction that is insoluble in water but is potentially degradable in the rumen at a given time; “c” is the degradation rate of the potentially degradable fraction in the rumen (h); and “t” is the incubation time (h).

The in situ degradability of NDF was also estimated by finding the weight difference between the weights performed before and after ruminal incubation. It was expressed in g/kg of DM. To obtain the NDF “D”, “C”, and uNDF240 coefficients, the model proposed by Waldo et al. [27] was used:

$$R(t) = D(e^{(-ct)}) + I \quad (2)$$

where “R(t)” is the incubation residue at time t (h); “D” is the fraction of potentially degradable NDF in the rumen; “c” is the rate of degradation of fraction D; and “I” is the non-degradable fraction of NDF.

The effective degradability of DM and OM in the rumen was calculated using the following model:

$$\text{Effective degradability} = A + (B \times c/c + K) \quad (3)$$

where “K” corresponds to the estimated rate of passage by particles in the rumen, which was used at 2, 5, and 8 h^{−1}, simulating low, medium, and high passage rates, respectively [28].

To calculate the effective degradability of NDF, the following model was used:

$$\text{Effective degradability} = \text{ad} \times c / (c + K) \quad (4)$$

where “ad” is the standardized potentially degradable fraction (%).

2.8. Statistical Analyses

Prior to statistical analyses, assumptions of normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene’s tests, respectively.

The data from the fermentation trial were analyzed in a completely randomized design in a 6 × 3 factorial arrangement. The model includes the fixed effect of treatments, the fermentation period, and the interaction between these factors according to the following statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}, \quad (5)$$

where Y_{ijk} = dependent variable, μ = mean, α_i = fixed effect of the i -th treatment, β_j = fixed effect of days of fermentation, $(\alpha\beta)_{ij}$ = interaction between treatments and days of fermentation, and ϵ_{ijk} = random error.

The data of the in situ trial was analyzed according to the following statistical model:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}, \quad (6)$$

where Y_{ij} = dependent variable, μ = mean, α_i = fixed effect of the i -th treatment, and ϵ_{ij} = random error.

The nonlinear models for each treatment and time of incubation were adjusted by the cow level using the nls function in R (R Development Core Team) for each animal and treatment. Subsequently, the extracted function parameters were organized in a dataset format, including the treatments’ effects. R Core Team was used for the analysis of variance and comparison of means.

Means were separated by Tukey’s test, and differences were declared at $p < 0.05$.

3. Results

3.1. Buffering Capacity and Chemical Composition of Silages

The buffering capacity of BRSC for different treatments is presented in Table 1. No significant interaction ($p > 0.05$) was observed between treatments and fermentation periods for any of the chemical composition variables. However, DM content varied significantly ($p < 0.001$) across fermentation periods, with lower values recorded at 60 and 90 d compared to 30 d (Table 3). Crude protein content was also affected by fermentation time, with silage fermented for 30 d showing higher ($p < 0.001$) CP concentrations than silage fermented for 60 and 90 d.

The contents of aNDF and ADF were significantly influenced ($p < 0.001$) by the fermentation period. aNDF values were similar at 30 and 60 d but increased significantly ($p < 0.001$) at 90 d. ADF content also increased progressively with the length of fermentation, although to varying degrees depending on the treatment.

Table 3. Effect of fermentation time and bacterial inoculant levels or ground corn grain inclusion on chemical composition (g/kg DM) of elephant grass silage (BRS Capiçu).

(g/kg DM)	Fermentation Time (d)			SEM ^a	Additives						SEM	p-Value		
	30	60	90		CTR ^b	LBU _{0.5} ^c	LBU ₁ ^d	LPP ₁ ^e	LPP ₂ ^f	GCG ^g		Additives	Days	A × D
Dry matter	158.1 ^A	145.0 ^B	144.2 ^B	1.28	136.1 ^b	142.3 ^b	138.2 ^b	143.0 ^b	140.0 ^b	193.1 ^a	1.81	<0.001	<0.001	0.2141
Crude protein	59.6 ^A	48.3 ^B	38.4 ^C	1.80	42.6 ^b	46.7 ^b	43.6 ^b	46.6 ^b	48.0 ^b	65.3 ^a	2.55	<0.001	<0.001	0.7802
aNDF ^h	644.0 ^B	654.4 ^B	673.3 ^A	4.80	700 ^a	680.1 ^a	692.1 ^a	696.3 ^a	681.0 ^a	494.0 ^b	6.79	<0.001	<0.001	0.9312
ADF ⁱ	432.2 ^C	442.3 ^B	474.1 ^A	2.89	480 ^a	467.0 ^a	477.2 ^a	476.2 ^a	468.4 ^a	327.5 ^b	4.09	<0.001	<0.001	0.3314
ADIP ^j	3.8 ^B	4.1 ^{AB}	4.5 ^A	0.12	4.32 ^a	4.3 ^a	4.6 ^a	4.2 ^a	4.3 ^a	2.8 ^b	0.18	<0.001	0.001	0.2422
Lignin(sa) ^k	79.3 ^B	87.1 ^A	78.9 ^B	1.56	86.7 ^a	82.4 ^a	90.1 ^a	84.4 ^a	87.0 ^a	60.0 ^b	2.20	<0.001	<0.001	0.5632
Hemicellulose	213.0	212.3	199.0	4.37	220 ^a	213.5 ^a	215.2 ^a	219.3 ^a	212.2 ^a	167.2 ^b	6.18	<0.001	0.056	0.9512
NFC ^l	171.0 ^A	166.2 ^A	154.4 ^B	3.28	121 ^b	137.2 ^b	134.3 ^b	126.4 ^b	136.7 ^b	329.2 ^a	4.64	<0.001	0.002	0.1500
Crude ash	99.2 ^B	109.8 ^A	113.4 ^A	1.21	115 ^a	112.3 ^a	112.4 ^a	109.2 ^a	114.0 ^a	82.0 ^b	1.71	<0.001	<0.001	0.0654
Ether extract	25.7 ^A	22.2 ^B	20.9 ^B	0.76	21.4 ^{bc}	23.6 ^b	18.9 ^c	22.2 ^{bc}	21.2 ^{bc}	30.2 ^a	1.07	<0.001	<0.001	0.8112

Note: Capital letters represent pairwise comparisons among times of fermentation. And lowercase letters represent pairwise comparisons among additives by the Tukey test ($p < 0.05$).

^a Standard error of mean. ^b Control. ^c 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^e 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^g Inclusion of 8% of ground corn grain. ^h Analyzed with a thermostable amylase and expressed including residual ash. ⁱ Expressed including residual ash. ^j Acid detergent insoluble protein. ^k Lignin determined by solubilization of cellulose with sulfuric acid. ^l Non-fibrous carbohydrates.

Among treatments, as expected, silage containing GCG had the highest ($p < 0.001$) DM and CP concentrations compared to LBU0.5, LBU1, LPP1, LPP2, and CTR treatments. Treatment effects were also significant ($p < 0.001$) for aNDF and ADF, with GCG silage showing the lowest fiber contents. Specifically, GCG silage presented average aNDF and ADF values of 494 and 327 g/kg DM, respectively, whereas silages treated with bacterial inoculants and CTR had aNDF values ranging from 687 to 700 g/kg DM and ADF values from 472 to 480 g/kg DM.

In addition, lignin and hemicellulose contents were considerably lower in GCG-treated silage, with average values of 60.0 and 167 g/kg DM, respectively, differing significantly ($p < 0.01$) from those observed in silages treated with bacterial inoculants.

3.2. Fermentation Profile of Silages

No significant interaction ($p > 0.05$) was observed between treatments and fermentation days for pH values, soluble carbohydrates, lactic acid/ acetic acid ratio, production of effluents, and total DM losses (Table 4). However, pH, water-soluble carbohydrates, and total DM losses differed significantly ($p < 0.001$) across fermentation periods, with higher values observed at 60 and 90 d. Total DM losses at 60 and 90 d were approximately 275% greater than those at 30 d. The lactic-to-acetic acid ratio also decreased over time. At 30 d of fermentation, the lactic-to-acetic acid ratio was 2.64, while at 60 and 90 d, the ratio declined and stabilized at approximately 0.51. Fermentation length did not affect effluent production ($p > 0.05$).

Treatment effects ($p < 0.05$) were observed for pH, effluent production, and the lactic-to-acetic acid ratio. Silage containing GCG exhibited the lowest pH, differing significantly ($p < 0.05$) from both the CTR and the bacterial inoculant treatments. The highest effluent production value was observed in the LPP2 treatment, although it did not differ significantly ($p > 0.05$) from LBU0.5, LBU1, or LPP1. Regarding the lactic-to-acetic acid ratio, GCG silage showed the highest value (3.375), significantly greater ($p < 0.01$) than the values observed for CTR (0.832) and inoculated silages (0.776).

A significant interaction ($p < 0.001$) between treatments and fermentation days was observed for ammoniacal nitrogen expressed as a proportion of total nitrogen and for gas losses (Table 5). The lowest ammoniacal nitrogen expressed as a proportion of total nitrogen values at 60 d was found in silages treated with GCG and LBU0.5 ($p < 0.001$). At 90 d, GCG silage maintained the lowest ammoniacal nitrogen expressed as a proportion of total nitrogen concentration, significantly differing from all inoculated treatments and CTR. At 30 d, no significant differences ($p > 0.05$) were observed among treatments.

Across fermentation times, ammoniacal nitrogen expressed as a proportion of total nitrogen concentration did not change for the GCG silage. However, in silages treated with LBU0.5 and LBU1, ammoniacal nitrogen expressed as a proportion of total nitrogen concentration increased with longer fermentation periods. For LPP1, LPP2, and CTR, lower ammoniacal nitrogen expressed as a proportion of total nitrogen concentration was observed for 30 d, compared to 60 and 90 d, and no difference was observed between 60 and 90 d of fermentation time.

For gas losses, silages treated with GCG exhibited the lowest values at 30 and 60 d, although not significantly different ($p > 0.05$) from the inoculated or control silages. At 90 d, CTR silages showed the highest gas losses ($p < 0.001$), differing from all other treatments, which did not differ among themselves. Gas losses in GCG silage remained consistent across the 30, 60, and 90 d fermentation periods. In contrast, CTR silages showed increased gas losses with longer fermentation times. Silages treated with LBU0.5 and LBU1 exhibited increasing gas losses from 30 to 60 d, stabilizing thereafter. Similarly, LPP1 and LPP2 silages showed increased gas losses until 60 d, with values remaining constant beyond that point.

Table 4. Effect of levels of bacterial inoculants or inclusion of ground corn grain and different fermentation days on pH values, soluble carbohydrates, lactic acid/acetic acid ratio, production of effluents, and total DM losses.

Variable	Fermentation Time (d)			SEM ^a	Additives						SEM	p-Value		
	30	60	90		CTR ^b	LBU _{0.5} ^c	LBU ₁ ^d	LPP ₁ ^e	LPP ₂ ^f	GCG ^g		Additives	Days	A × D
pH	4.1 ^B	4.9 ^A	4.9 ^A	0.10	4.8 ^a	4.6 ^a	4.8 ^a	4.8 ^a	4.7 ^a	4.0 ^b	0.14	<0.001	<0.001	0.1410
WSC ^h , g/kg DM	4.8 ^B	5.6 ^A	5.5 ^A	0.14	5.5	5.2	5.3	5.0	5.2	5.6	0.20	0.3241	<0.001	0.2033
L/A ratio ⁱ	2.6 ^A	0.5 ^B	0.5 ^B	0.19	0.8 ^b	0.8 ^b	0.6 ^b	1.0 ^b	0.7 ^b	3.4 ^a	0.16	<0.001	<0.001	0.5241
EP ^j , kg/ton of fresh matter	89.4	88.4	87.6	2.18	85.3 ^b	88.1 ^{ab}	85.5 ^{ab}	89.2 ^{ab}	98.3 ^a	85.0 ^b	3.09	0.0301	0.8312	0.8103
Total DM losses, % of DM	3.09 ^B	10.8 ^A	11.6 ^A	0.66	8.4	6.8	9.8	7.9	8.9	9.2	0.93	0.2602	<0.001	0.1021

Note: Capital letters represent pairwise comparisons among times of fermentation, and lowercase letters represent pairwise comparisons among additives by the Tukey test ($p < 0.05$).

^a Standard error of mean. ^b Control. ^c 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^e 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g.

^g Inclusion of 8% of ground corn grain. ^h Water-soluble carbohydrates. ⁱ Lactic/acetic acid ratio. ^j Effluent production.

Table 5. Effect of levels of bacterial inoculants or inclusion of ground corn grain on different fermentation days on ammoniacal nitrogen, gas loss, and organic acid production of elephant grass silage (BRS Capiacu).

	Fermentation Time						SEM ^g	<i>p</i> -Value		
	30 d							Additives	Days	A × D
	CTR ^a	LBU _{0.5} ^b	LBU ₁ ^c	LPP ₁ ^d	LPP ₂ ^e	GCG ^f				
NH ₃ -N, % total N ^h	17.0 ^{aB}	13.4 ^{aC}	17.3 ^{aC}	14.7 ^{aB}	13.6 ^{aB}	8.6 ^{aA}	3.91	<0.001	<0.001	<0.001
GL, % of DM ⁱ	4.7 ^{aC}	4.4 ^{aB}	5.7 ^{aB}	4.6 ^{aB}	4.6 ^{aB}	3.6 ^{aA}	1.10	<0.001	<0.001	0.001
Lactic acid, g/kg DM	14.9 ^{bcA}	16.7 ^{abA}	13.0 ^{cA}	17.5 ^{abA}	15.7 ^{bcA}	19.5 ^{aA}	0.89	<0.001	<0.001	<0.001
Acetic acid, g/kg DM	6.2 ^{abB}	7.52 ^{abC}	8.5 ^{aB}	6.4 ^{abC}	7.7 ^{abC}	4.4 ^{bA}	0.83	<0.001	<0.001	<0.001
Propionic acid, g/kg DM	1.03 ^{aC}	0.9 ^{aC}	1.2 ^{aB}	0.5 ^{aB}	1.0 ^{aB}	0.6 ^{aA}	0.17	<0.001	<0.001	<0.001
Butyric acid, g/kg DM	26.9 ^{aB}	15.0 ^{bcC}	29.9 ^{aB}	18.1 ^{bB}	11.2 ^{cB}	12.1 ^{cC}	1.17	<0.001	<0.001	<0.001

Table 5. Cont.

	Fermentation Time						SEM ^g	<i>p</i> -Value		
	30 d							Additives	Days	A × D
	CTR ^a	LBU _{0.5} ^b	LBU ₁ ^c	LPP ₁ ^d	LPP ₂ ^e	GCG ^f				
60 d										
NH ₃ -N, % total N	54.9 ^{aA}	28.0 ^{bcB}	41.0 ^{abB}	38.5 ^{abA}	38.4 ^{abA}	19.4 ^{cA}	3.91	<0.001	<0.001	<0.001
GL, % of DM	10.3 ^{aB}	10.0 ^{aA}	10.3 ^{aA}	10.0 ^{aA}	7.5 ^{aAB}	5.7 ^{aA}	1.10	<0.001	<0.001	0.001
Lactic acid, g/kg DM	0.6 ^{bB}	0.9 ^{bB}	0.5 ^{bB}	0.6 ^{bB}	3.2 ^{bB}	14.6 ^{aB}	0.89	<0.001	<0.001	<0.001
Acetic acid, g/kg DM	17.0 ^{bcA}	19.9 ^{bA}	15.6 ^{cA}	19.4 ^{bA}	25.6 ^{aA}	5.3 ^{dA}	0.83	<0.001	<0.001	<0.001
Propionic acid, g/kg DM	2.2 ^{abB}	1.7 ^{bB}	2.8 ^{aA}	2.1 ^{bA}	1.8 ^{bA}	10.0 ^{cA}	0.17	<0.001	<0.001	<0.001
Butyric acid, g/kg DM	33.7 ^{bA}	22.5 ^{deB}	42.1 ^{aA}	19.1 ^{eB}	27.8 ^{cA}	26.3 ^{cdA}	1.17	<0.001	<0.001	<0.001
90 d										
NH ₃ -N, % total N	65.4 ^{aA}	57.1 ^{abA}	58.9 ^{abA}	48.1 ^{bA}	45.0 ^{bA}	13.7 ^{cA}	3.91	<0.001	<0.001	<0.001
GL, % of DM	18.0 ^{aA}	9.9 ^{bA}	9.9 ^{bA}	8.3 ^{bAB}	8.4 ^{bA}	6.3 ^{bA}	1.10	<0.001	<0.001	0.001
Lactic acid, g/kg DM	0.5 ^{bB}	0.5 ^{bB}	0.6 ^{bB}	2.7 ^{bB}	0.8 ^{bB}	12.7 ^{aB}	0.89	<0.001	<0.001	<0.001
Acetic acid, g/kg DM	17.5 ^{aA}	13.8 ^{bB}	13.5 ^{bA}	15.3 ^{abB}	15.3 ^{abB}	4.7 ^{cA}	0.83	<0.001	<0.001	<0.001
Propionic acid, g/kg DM	5.1 ^{aA}	5.1 ^{aA}	3.0 ^{bA}	2.1 ^{cA}	2.1 ^{cA}	1.0 ^{dA}	0.17	<0.001	<0.001	<0.001
Butyric acid, g/kg DM	33.5 ^{aA}	27.2 ^{bA}	29.6 ^{abB}	34.0 ^{aA}	29.5 ^{abA}	21.2 ^{cB}	1.17	<0.001	<0.001	<0.001

Note: Means followed by the same lower case, in the line, and capital letter, in the column, do not differ by the Tukey test ($p > 0.05$). ^a Control. ^b 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^c 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^e 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f Inclusion of 8% of ground corn grain. ^g Standard error of mean. ^h Ammoniacal nitrogen content in relation to total nitrogen. ⁱ Gas losses.

3.3. Profile of Organic Acids in BRSC Silages

A significant interaction ($p < 0.001$) was observed between treatments and fermentation days for all organic acids evaluated (Table 5). At 30 d of fermentation, higher ($p < 0.001$) lactic acid concentrations were recorded in silages treated with LBU0.5, LPP1, and GCG. At 60 and 90 d, GCG-treated silage exhibited the highest lactic acid concentrations ($p < 0.001$), differing significantly from all bacterial inoculant treatments and the CTR treatment. Across all treatments, lactic acid levels were highest at 30 d and declined thereafter, with values at 60 and 90 d being significantly lower.

For acetic acid, the GCG treatment showed the lowest concentration ($p < 0.001$) at 30 d, differing from LBU1, although not significantly different ($p > 0.05$) from CTR, LBU0.5, LPP1, and LPP2. At 60 d, GCG silage again showed the lowest ($p < 0.001$) acetic acid concentration (5.26 g/kg DM), followed by LBU1 (15.64 g/kg DM). At 90 d, GCG silage continued to show the lowest acetic acid concentration ($p < 0.001$), differing from all other treatments, which also varied among themselves, with LBU0.5 and LBU1 showing the highest values. Acetic acid concentrations in LBU0.5, LBU1, LPP1, and LPP2 treatments increased from 30 to 60 d, then decreased slightly at 90 d, except for LBU1, where values remained stable between 60 and 90 d. For CTR, acetic acid levels increased and stabilized at 60 d. In contrast, GCG-treated silage showed no significant variation ($p > 0.05$) in acetic acid concentration across fermentation days, maintaining an average of 4.77 g/kg DM.

Significant differences ($p < 0.001$) in propionic acid concentrations were observed among fermentation periods for CTR and LBU0.5 silages. However, in GCG-treated silage, propionic acid levels remained consistent across all fermentation periods. In LBU1, LPP1, and LPP2 silages, propionic acid concentrations were significantly higher ($p < 0.001$) at 60 and 90 d compared to 30 d.

Except for LPP1, all treatments showed an increase ($p < 0.001$) in butyric acid concentration from 30 to 60 d of fermentation. For CTR and LPP2 silages, butyric acid levels did not change significantly ($p > 0.05$) between 60 and 90 d. However, silages treated with LBU0.5 and LPP1 had higher butyric acid concentrations at 90 than at 60 d. In contrast, butyric acid concentrations decreased in LBU1 and GCG silages between 60 and 90 d. At 90 d, GCG-treated silage had the lowest butyric acid concentration (21.2 g/kg DM), differing significantly from all other treatments. A similar trend was observed at 30 d, where GCG silage also showed a lower butyric acid content, although it did not differ significantly ($p > 0.05$) from LBU0.5 and LPP2.

3.4. In Situ Ruminal Degradability Parameters of BRSC Silage

A significant treatment effect ($p < 0.001$) was observed for the soluble fraction “a” of DM and OM, with notably higher values in the silage containing GCG, which differed from silages treated with bacterial inoculants and CTR treatments. The GCG silage presented values of 419 and 400 g/kg DM for fractions “a” of DM and OM, respectively (Table 6). Treatment effects ($p < 0.001$) were also observed for the potentially degradable fraction “b” of DM and OM. In this case, silages supplemented with GCG showed reduced values for fraction “b” compared to those treated with bacterial inoculants and CTR. Among the bacterial inoculant treatments and CTR, no substantial variation ($p > 0.05$) was observed in this parameter.

Despite treatment effects on fractions “a” and “b” of DM and OM, no significant differences ($p > 0.05$) were observed for the degradation rate constant (fraction “c”) of either variable. The average degradation rates for DM and OM were 0.0202 and 0.0205%/h, respectively. Regarding NDF, treatments significantly affected ($p < 0.05$) the potentially degradable fraction “D”. GCG-treated silages had the highest “D” value (578 g/kg DM), which did not differ statistically ($p > 0.05$) from CTR, LPP1, or LPP2 silages. However, the silages treated with bacterial inoculants and CTR did not differ ($p > 0.05$). There were no significant treatment effects ($p > 0.05$) on the undegradable NDF fraction (uNDF240) or on the NDF degradation rate constant (“C”), which averaged 376.5 g/kg DM and 0.019%/h, respectively.

Table 6. Effect of levels of bacterial inoculants or inclusion of ground corn grain on in situ degradation parameters and potential degradability of dry matter, organic matter, and neutral detergent fiber of elephant grass silage (BRS Capiaçu).

Parameters	Additives						SEM ^g	p-Value
	CTR ^a	LBU0.5 ^b	LBU1 ^c	LPP1 ^d	LPP2 ^e	GCG ^f		
Dry matter								
a, g/kg ^h	224.1 ^c	248.2 ^b	222.3 ^c	243.4 ^{bc}	232.0 ^{bc}	419.2 ^a	4.87	<0.001
b, g/kg ⁱ	451.0 ^a	436.4 ^a	451.3 ^a	440.4 ^a	457.6 ^a	361.2 ^b	6.37	<0.001
c, %/per h ^{-1, j}	0.019	0.020	0.021	0.018	0.019	0.024	0.0019	0.3221
Organic matter								
a, g/kg	179.0 ^{bc}	197.3 ^b	166.4 ^c	188.1 ^{bc}	175.2 ^{bc}	400.2 ^a	5.42	<0.001
b, g/kg	474.2 ^a	464.1 ^a	481.2 ^a	470.4 ^a	491.6 ^a	370.3 ^b	7.51	<0.001
c, %/per h ⁻¹	0.019	0.020	0.021	0.019	0.020	0.025	0.0020	0.3711
Neutral detergent fiber								
D, g/kg ^k	540.1 ^{ab}	528.4 ^b	530.4 ^b	535.2 ^{ab}	559.0 ^{ab}	578.1 ^a	10.2	0.029
C, %/per h ^{-1, l}	0.018	0.019	0.020	0.018	0.018	0.022	0.0020	0.7942
I, g/kg, ^m	385.0	384.1	387.3	377.3	369.2	357.0	6.56	0.050
Potential degradability								
Dry matter, g/kg	675.1 ^b	684.2 ^b	673.1 ^b	682.6 ^b	689.4 ^b	780.0 ^a	4.77	<0.001
Organic matter, g/kg	653.0 ^b	661.4 ^b	647.6 ^b	658.8 ^b	666.9 ^b	770.1 ^a	4.79	<0.001
Neutral detergent fiber, g/kg	584.1 ^{ab}	579.2 ^{ab}	578.4 ^b	587.4 ^{ab}	603.8 ^{ab}	618.8 ^a	8.45	0.0340

Note: Lowercase letters represent pairwise comparisons among additives by the Tukey test ($p < 0.05$). ^a Control. ^b 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^c 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^e 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f Inclusion of 8% of ground corn grain. ^g Standard error of mean. ^h Water-soluble fraction of the feed at time zero (g/kg DM). ⁱ b: Potentially degradable fraction in the rumen (g/kg DM). ^j Degradation rate of the potentially degradable fraction in the rumen (b; %/per h ⁻¹). ^k NDF fraction potentially degradable in the rumen (g/kg DM). ^l Degradation rate of the fraction D (%/per h ⁻¹). ^m Non-degradable fraction of the NDF (g/kg DM).

Treatments also significantly affected ($p < 0.001$) the potential degradability (measured degradability at 240 h) of DM and OM (Table 6). The highest values were observed in GCG-treated silage, with 780 and 770 g/kg DM for DM and OM, respectively. The lowest values were found in CTR and bacterial inoculant silages, which did not differ from one another and had average potential degradability values. The potential degradability of NDF was also higher ($p < 0.05$) in GCG-treated silages; however, these values did not differ significantly ($p > 0.05$) from CTR, LBU0.5, LPP1, and LPP2. The lowest potential NDF degradability was observed for LBU1-treated silage, which did not differ ($p > 0.05$) from CTR or other inoculant treatments.

A similar pattern was observed for effective degradability of DM and OM (Table 7). The GCG-treated silages exhibited the highest effective degradability values ($p < 0.001$)

compared to CTR and bacterial inoculant silages. For all passage rates (2, 5, and 8%/h), GCG-treated silages showed effective degradability values above 500 g/kg DM for DM and OM, except for OM at the 8%/h rate, which averaged 487 g/kg DM. No significant treatment effects ($p > 0.05$) were found for effective NDF degradability across the three passage rates, with mean values of 287, 162, and 113 g/kg DM for 2, 5, and 8%/h, respectively.

Table 7. Effect of levels of bacterial inoculants or inclusion of ground corn grain on effective degradability of dry matter, organic matter, and neutral detergent fiber of elephant grass silages (BRS Capi  u).

Effective Degradability (g/kg)	Additives						SEM ^g	<i>p</i> -Value
	CTR ^a	LBU _{0.5} ^b	LBU ₁ ^c	LPP ₁ ^d	LPP ₂ ^e	GCG ^f		
Dry matter								
Passage rate (%/h ⁻¹)								
2	443.2 ^b	463.4 ^b	450.8 ^b	451.0 ^b	457.3 ^b	616.2 ^a	10.21	<0.001
5	348.2 ^b	371.0 ^b	354.3 ^b	360.4 ^b	360.5 ^b	537.6 ^a	8.73	<0.001
8	310.3 ^b	334.1 ^b	315.5 ^b	324.4 ^b	321.1 ^b	503.6 ^a	7.41	<0.001
Organic matter								
Passage rate (%/h ⁻¹)								
2	411.2 ^b	427.3 ^b	411.2 ^b	413.4 ^b	418.2 ^b	603.2 ^a	10.60	<0.001
5	311.0 ^b	328.0 ^b	308.4 ^b	315.6 ^b	313.3 ^b	522.3 ^a	9.33	<0.001
8	271.1 ^b	289.2 ^b	266.5 ^b	277.2 ^b	272.4 ^b	487.4 ^a	7.93	<0.001
Neutral detergent fiber								
Passage rate (%/h ⁻¹)								
2	278.2	279.4	284.6	277.8	287.2	320.2	13.40	0.2622
5	156.1	157.7	162.2	155.6	161.1	186.4	11.10	0.4003
8	108.2	110.5	113.6	108.2	112.7	131.8	8.72	0.4402

Note: Lowercase letters represent pairwise comparisons among additives by the Tukey test ($p < 0.05$). ^a Control. ^b 0.5 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^c 1 g/ton of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guaranteed levels of 1.0×10^{11} CFU/g. ^d 1 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^e 2 g/ton of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guaranteed levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guaranteed levels of 1.5×10^{10} CFU/g. ^f Inclusion of 8% of ground corn grain. ^g Standard error of mean.

Silage supplemented with GCG consistently showed higher DM and OM degradability compared to CTR and bacterial inoculant treatments across all incubation times (Figures 1 and 2). Maximum degradability of DM and OM was observed after 240 h of rumen incubation. Notably, at 30 h of incubation, GCG silage had already achieved high DM and OM degradation levels, exceeding 650 g/kg DM. No plateau in degradability was observed for any treatment, indicating that silages continued to degrade beyond 240 h. However, a noticeable reduction in the degradation rate occurred after 120 h of incubation.

The NDF degradation curves revealed treatment differences in fiber degradation dynamics (Figure 3). Up to 24 h of ruminal incubation, NDF degradation values across treatments averaged approximately 289 g/kg DM. The GCG silage reached a maximum of 653 g/kg DM after 240 h. After 24 h, the NDF degradability of GCG silage was higher than that of CTR and bacterial inoculant treatments. At 120 h, the highest NDF degradation values (>500 g/kg DM) were observed. The NDF degradability did not reach a plateau within the 240 h incubation period, although a marked deceleration was evident after 120 h.

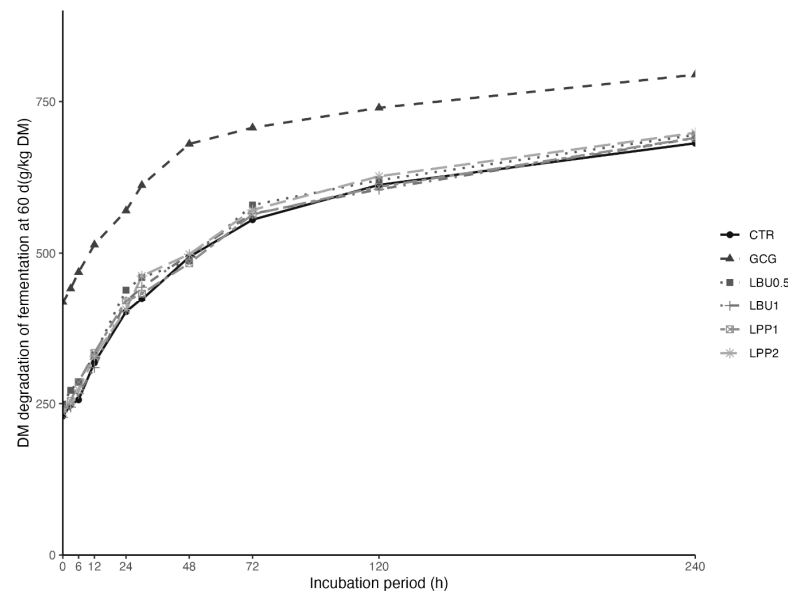


Figure 1. Total dry matter (DM) degradability of elephant grass silages treated with bacterial inoculants or with the inclusion of ground corn grain. Estimated by the equation $\text{Deg}(t) = a + b(1 - e^{-ct})$. a = water-soluble fraction of the feed at time zero; b = fraction insoluble in water, but potentially degradable in the rumen at a given time; c = degradation rate of the potentially degradable fraction in the rumen (b); t = incubation time (h). CTR: control; LBU_{0.5}—0.5 g/t of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guarantee levels of 1.0×10^{11} CFU/g; LPP₂—2 g/t of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guarantee levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guarantee levels of 1.5×10^{10} CFU/g; GCG—inclusion of 8% of ground corn grain.

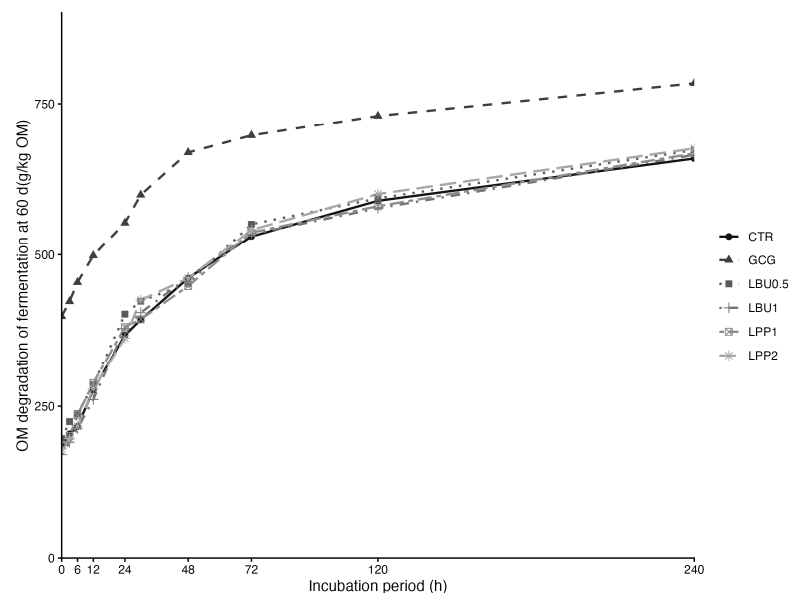


Figure 2. Total degradability of organic matter (OM) in elephant grass silages with doses of bacterial inoculants or ground corn grain. Estimated by the equation $\text{Deg}(t) = a + b(1 - e^{-ct})$. a = water-soluble fraction of the feed at time zero; b = fraction insoluble in water, but potentially degradable in the rumen at a given time; c = degradation rate of the potentially degradable fraction in the rumen (b); t = incubation time (h). CTR, control; LBU_{0.5}—0.5 g/t of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guarantee levels of 1.0×10^{11} CFU/g; LPP₂—2 g/t of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guarantee levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guarantee levels of 1.5×10^{10} CFU/g; GCG—inclusion of 8% of ground corn grain.

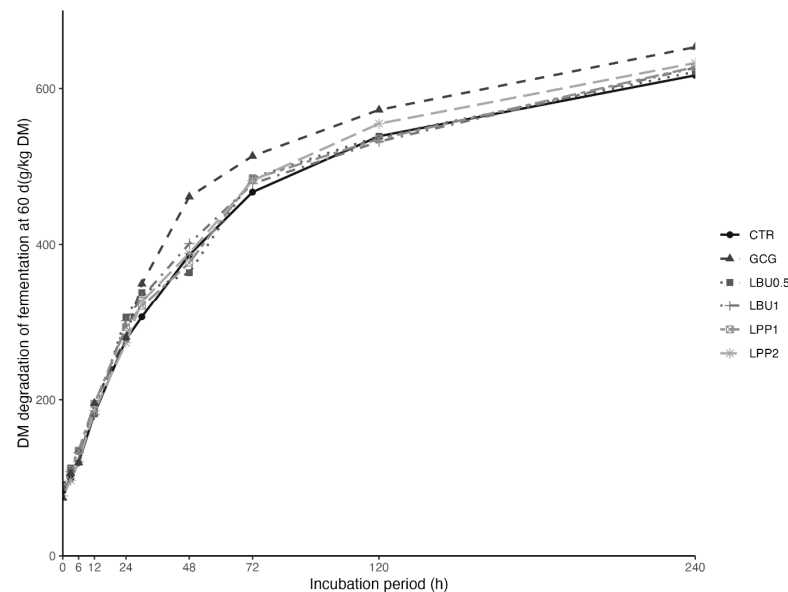


Figure 3. Total degradability of neutral detergent fiber (NDF) of elephant grass silages with doses of bacterial inoculants or ground corn grain. Estimated by the equation $R(t) = D(e - ct) + I$. a = water-soluble fraction of the feed at time zero; b = fraction insoluble in water, but potentially degradable in the rumen at a given time; c = degradation rate of the potentially degradable fraction in the rumen (b); t = incubation time (h). CTR, control; LBU0.5—0.5 g/t of a commercial product composed of *Lentilactobacillus buchneri*, with minimum guarantee levels of 1.0×10^{11} CFU/g; LPP2—2 g/t of a commercial product composed of *Lactiplantibacillus plantarum*, with minimum guarantee levels of 5.0×10^{10} CFU/g, and *Pediococcus acidilactici*, with minimum guarantee levels of 1.5×10^{10} CFU/g; GCG—inclusion of 8% of ground corn grain.

4. Discussion

The pre-ensiling DM content ranged from 133.2 to 192.4 g/kg, below the 300 g/kg threshold recommended for optimal fermentation and reduced effluent production [26], which may partially explain the observed butyric fermentation, and other parameters evaluated.

The GCG inclusion increased DM and CP contents compared to CTR and inoculant treatments, likely due to its high DM (88%) and CP (9%) contents and its function as a moisture absorbent. Paula et al. [8] reported similar improvements in DM and reductions in NDF and ADF with increasing GCG inclusion, although CP and EE did not significantly differ from control treatments in their study. In addition, silage treated with GCG exhibited a greater reduction in pH compared to bacterial inoculants and CTR treatment, likely due to its lower buffering capacity and higher DM [5,29]. Moreover, the 8% inclusion of GCG provided an additional source of carbohydrate, which, although not classified asWSC, functions as a slowly released substrate. This carbohydrate (starch) undergoes partial hydrolysis, releasing fermentable sugars that support lactic acid production and enhance the fermentative stability of the silage by limiting undesirable fermentation pathways [6]. Lactic acid concentrations in GCG silage ranged from 12.7 to 19.5 g/kg DM, slightly lower than values reported by Amaral et al. [5] and Jesus et al. [30], but still sufficient for adequate preservation.

Bacterial inoculants were associated with higher pH values and greater production of acetic and butyric acids, indicating undesirable fermentation [31]. The lower butyric acid concentration observed in GCG silage at 90 d is attributed to its higher DM content, which inhibits *Clostridium* growth [32]. Although a higher DM content could reduce clostridial activity, the silage was produced at this DM level because it corresponds to the stage at which elephant grass presents its highest nutritional quality, with lower indigestible neutral

detergent fiber (iNDF). Harvesting the forage at a higher dry matter concentration would likely increase the iNDF content and could negatively affect intake, thereby representing a trade-off between fermentation quality and nutritional value.

The elevated butyric acid levels in the CTR treatment and in some inoculant-treated silages at 30 and 60 d further support the occurrence of clostridial fermentation. In addition, the high moisture content of elephant grass likely favored butyric acid formation, which could potentially reduce silage palatability and intake by cattle. Although butyric acid concentrations declined over time (from 60 to 90 d) in GCG silage, absolute values remained higher than those reported in studies using forages with a greater DM content or more fermentable carbohydrate availability. This suggests that, despite improvements in fermentation stability at later stages, the characteristics of elephant grass, particularly its high moisture content and buffering capacity, still predisposed the silage to clostridial activity, even under improved fermentation conditions.

At 90 d, CTR silage showed the highest gas losses and ammonia nitrogen, while GCG-treated silage showed reduced levels of both parameters. Lower pH in GCG silage likely inhibited proteolytic bacteria, reducing protein degradation and ammonia formation [33].

While treatments did not significantly affect total DM losses, the fermentation time did, with losses increasing by 275% from 30 to 90 d. Effluent production is closely linked to forage moisture [34], and due to the low DM content of the forage used in this study, no reduction in effluent production was observed as expected, for instance, for the GCG treatment, which is likely attributable to its final DM.

Regarding ruminal degradability, GCG-treated silage had the highest values for fraction “a” of DM and OM, likely due to the high fermentability of corn [32]. Fraction “a” represents the readily available portion for microbial degradation [35]. Previous studies with corn and rice bran [32,36] support these findings, showing linear increases in fraction “a” with increasing energy-dense by-product inclusion.

Conversely, fraction “b” of DM and OM was lower in GCG silage, likely due to its lower acid detergent insoluble nitrogen content. Pereira et al. [37] observed similar reductions in fraction “b” with increased inclusion of corn and soybean residues.

Bacterial inoculants had limited impact on fractions “a” and “b” due to their negligible influence on chemical composition [38]. Fraction “c” (degradation rate) remained constant across treatments, consistent with previous studies [32].

In terms of NDF degradability, GCG silage showed the highest values for fraction “D,” although differences were not significant compared to CTR, LPP1, and LPP2. The higher starch content in GCG likely favored amylolytic over cellulolytic bacterial activity [32]. The “C” fraction and uNDF240 were unaffected by treatments, aligning with the ideal 0.02–0.06%/h range for high-quality silages [39].

Consistent with Chesson et al. [40], no changes in uNDF240 were observed, suggesting that microbial selectivity was not altered by GCG inclusion. Similar conclusions were drawn by Salawu et al. [41] and Filya [42], who reported no impact of LBU and LPP on degradability in low-DM silages. Although effective NDF degradability was numerically higher in GCG silage, differences were not significant, except when compared to LBU1. These results indicate that while GCG may enhance microbial activity through increased energy availability [40], this effect was insufficient to overcome the intrinsic structural limitations of the fiber matrix, such as lignin concentration, lignin–carbohydrate complexes, cellulose crystallinity, and the physical accessibility of fiber to ruminal microorganisms, explaining the lack of significant changes in degradation kinetics.

In summary, the inclusion of GCG improved the fermentation profile, nutrient composition, and ruminal degradability of BRSC silage, and no additional benefits were observed for the treatments with inoculants. The lack of significant effects of bacterial inoculants may

be associated with reduced inoculant viability under high-moisture conditions, which can limit their ability to establish dominance during fermentation. Furthermore, the low DM content of elephant grass and competition with native rumen microorganisms may have restricted inoculant performance, resulting in minimal effects on fermentation characteristics and degradability. A limitation of this study is that aerobic stability was not evaluated after silo opening. This parameter is particularly important when using inoculants containing acetic acid-producing bacteria, as their beneficial effects typically manifest during the aerobic exposure phase rather than during fermentation. Assessing aerobic stability would have provided a more comprehensive understanding of the impact of these additives on silage preservation and spoilage control. Future studies should evaluate aerobic stability when acetic acid-producing bacteria are used and should also investigate higher levels of GCG inclusion to increase the dry matter content of the forage prior to ensiling.

5. Conclusions

The inclusion of 8% ground corn grain in BRS Capiacu elephant grass silage improved the fermentative profile and nutritional value compared to varying doses of bacterial inoculants under low-DM conditions. While GCG supplementation enhanced lactic acid production and reduced silage pH, it was not fully effective in suppressing butyric fermentation at 60 and 90 d of ensiling. Bacterial inoculants, on the other hand, did not improve the fermentative profile and nutritional value in low-DM conditions. Silages supplemented with GCG and opened after 60 d exhibited greater in situ ruminal degradability of DM and OM, with favorable alterations in degradation fractions. However, no improvements were observed in the degradability of NDF or its associated fractions. It is recommended to add 8% ground corn grain rather than rely on conventional inoculants in low-dry-matter elephant grass silage.

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