Ruminal kinetics and nutritive value of zuri grass silage harvested at different ages and added with powder molasses

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Abstract

The ideal regrowth age of forage plants and the use of fermentation stimulants additives may improve the fermentative characteristics and nutritive value of tropical grass silages. This study aimed to evaluate the effect of regrowth ages and the use of powdered molasses on nutritional characteristics of Zuri grass (*Megathyrsus maximus*) silages. A completely randomized design was used, in a 3x3 factorial scheme: three regrowth ages (65, 80, and 95 days) and three inclusion levels of powdered molasses of sugarcane (0, 20, and 40 g), with four replications. The chemical composition, fermentation parameters, *in vitro* digestibility, and *in vitro* degradation kinetics of the silages were evaluated. Greater DM contents were observed in silages with 95 days with 40 g of molasses. The 65 days of regrowth silages with 40 g of molasses had greater IVDMD compare to others treatments. However, lower pH values, N-NH₃, DM losses, gas losses, and density were observed in silages with 95 days of regrowth with 40 g of molasses. It is recommended to cut the Zuri grass in the range of 80 to 95 days of regrowth with the addition of 40g/kg of molasses in powders for the production of silages.

1 | Introduction

Tropical grasses have a high yield potential; however, they are not usually explored options for silage production in Brazil. The forage grass *Megathyrsus maximus* is known worldwide for its high productivity, quality, and adaptation to different edaphoclimatic conditions. They are grasses that produce a large amount of forage mass, with good nutritional value when it has done good management, becoming a relevant forage for animal production (Euclides et al., 2018).

BRS Zuri (*Megathyrsus maximus*) is a cultivar released in 2014, with productions ranging from 12 to 25 tons per year of DM.ha⁻¹ (Bender, 2017). However, to achieve this high production, several cuts are needed during the rainy season (Santos et al., 2014). Zuri grass has a seasonal production, as well as the tropical grasses majority, that drastically decreases or even stops its growth in periods of drought. Thus, silage of this grass can be one of the alternatives to guarantee the supply of forage throughout the year.

Plant age factors contribute to the productive and qualitative characteristics of forage, with a reduction in regrowth age resulting in low forage mass production and an increase in the plant's nutritional value (Maranhão et al., 2010). The plant late harvested, on the other hand, causes an increase in fiber content, a reduction in cell content, and, consequently, a reduction in the plant's nutritional value (Santos et al., 2014).

However, when the using purpose of this plant is for ensiling, other parameters interfere in the process result. Santos et al. (2014), studied four regrowth intervals (35, 45, 55, and 65 days) on the fermentation profile and dry matter recovery of mombaça grass silages, and observed lower pH and ammonia nitrogen values (N-NH₃) in silages with a longer regrowth interval (65 days) compared to a shorter interval (35 days).

On the other hand, if the regrowth age is greater to increase the DM content of the forage, there may be a reduction in the soluble carbohydrate content, which can cause difficulties in the material of silo compaction (Silva et al., 2015). The regrowth recommendation for ensilage grasses is cut at 60 to 70 days after regrowth, justified by the high nutritional values (Ávila et al., 2006); however, the low DM contents and excessive moisture at these ages may interfere with the quality of the silages (Pereira et al., 1999).

To decrease these impacts, in the present experiment, the regrowth age was increased from 65 to 95 days, to increase the forage mass and reduce the moisture content in the ensiled material.

In summer, weather conditions promote the fast growth of Zuri grass, and part of the forage produced can be used for silage production. Nevertheless, Zuri grass has some limitations because of its low DM content and soluble carbohydrates, and high buffering capacity at the ideal regrowth age (Silva et al., 2015). These factors make their conservation challenging or rough, leading these silages to great losses through effluents and the emergence of inopportune microorganisms such as clostridia and enterobacteria (Panditharatne et al., 1986; Nussio, 2005).

There are several ways to avoid this problem, including the fermentation stimulants use, such as sugarcane molasses, to raise fermentative bacteria, reduce pH and DM losses, and improve nutritional value and the silages preservation (Bolsen et al., 1996; Bernardes et al. 2013; Desta et al., 2016).

Powdered sugarcane molasses is a by-product of the sugar industry with about 83.98% DM, 3.3% CP, 1.36% EE, 3.43% FB, and 11.49 ash (Valadares Filho et al., 2010). It is rich in soluble carbohydrates, promotes adequate fermentation for lactic acid bacteria, and inhibits unwanted fermentations (Bolsen et al., 1996; Bernardes et al. 2013). The recommendations on the ideal levels of molasses to be used in grass ensilage are controversial. Thus, for this experiment, levels close to the minimum (20 g) and maximum (40 g) recommended by the manufacturers were used.

In this context, the objective was to evaluate the nutritional characteristics of Zuri grass silages harvested at 65, 80, and 95 days of regrowth and added at the time of ensiling with 0, 20, and 40 g of powdered molasses per kg of ensiled mass. We hypothesized that the forage harvest at a shorter regrowth age compromises the silage quality due to the high moisture content of the material and the use of additives that stimulate fermentation can improve the fermentative characteristics and nutritional value of tropical grass silages.

2 | Materials And Methods

The study was carried out at the Experimental Farm of the Federal University of Mato Grosso do Sul, Brazil (20°26'48.2"S 54°50'39.2"O and 530 m altitude), located in Terenos, Mato Grosso do Sul. Meteorological data were obtained from a Monitoring Center for Weather, Climate and Water Resources of Mato Grosso do Sul and at the experimental site (Figure 1). The pasture was established in 2016 by seeding of Zuri grass. The experimental area had a total of 108 m² divided into three plots of 36 m²,

which each plot was subdivided into three plots of 12 m², randomly allocating the three regrowth ages (65, 80, and 95 days).

The soils in the experimental area are classified as Dystrophic Red Latosols, with clayey texture and low natural fertility. The soil chemical analysis was carried out before the trial establishment, at a depth of 0-20 cm, were: pH (CaCl₂): 5.31; pH (H₂O): 5.91; P: 4.52 mg dm⁻³; organic matter: 35.34 mgdm⁻³; K: 0.20 cmol dm⁻³; Ca: 7.35 cmol dm⁻³; Mg: 1.20 cmol dm⁻³; Ca + Mg: 8.55 cmol dm⁻³; Al: 0.00 cmol dm⁻³; H + Al: 5.18 cmol dm⁻³; cation exchange capacity: 13.93 cmol dm⁻³ and base saturation: 62.81%.

Soil correction was performed using 1.2 ton/ha⁻¹ of dolomitic limestone (Relative power of total neutralization = 80%). Subsequently, fertilizer applications were applied using 100 kg/ha⁻¹ of P₂O₅, 100 kg/ha⁻¹ N (urea or nitrocalcium), and 60 kg/ha⁻¹ of K₂O. The following year, maintenance fertilization was carried out using 100 kg/ha⁻¹ of N (urea or nitrocalcium).

Our study was conducted using a 3x3 factorial experiment based on a completely randomized design, assessing three regrowth ages (65, 80, and 95 days) and three levels of molasses (0, 20, and 40 g), using four replications per treatment.

2.1 | Forage production and morphological composition

To evaluate the forage mass, Zuri grass was cut at 20 cm from the ground, with a frame of 1.0 m², and weighed. Samples were separated into the stem (stem + sheath), leaf (leaf blade), and dead material. Subsequently, samples were oven-dried at 55°C for 72 h to a constant weight.

Total fresh forage mass (TFFM; kg/ha), total dry forage mass (TDFM; kg/ha) and leaf, stem and dead material proportion was estimated (Table 1). Samples from forage mass were utilized for chemical composition before ensiling (Table 2). The chemical composition of molasses used was checked on the packaging label, according to the manufacturer's data where: crude protein (CP): 45 g.kg⁻¹; humidity: 50 g.kg⁻¹; total sugar: 550 g.kg⁻¹; ash: 200 g.kg⁻¹; crude fiber (CF): 800 mg.kg⁻¹; ether extract (EE): 4000 mg.kg⁻¹; metabolizable energy (ME): 3.10 kcal.kg⁻¹; Ca: 70 g.kg⁻¹; P: 1 g.kg⁻¹.

Table 1
Productive and morphological characteristics of Zuri grass before ensiling at three regrowth ages (65, 80, and 95 days).

Itens	Regrowth ages		
	65 days	80 days	95 days
Fresh forage mass (ton.ha ⁻¹)	28.5	31.2	37.6
Forage mass (ton.ha ⁻¹ DM)	6.30	7.30	10.9
Leaf (g.kg ⁻¹)	690	611	528
Stem (g.kg ⁻¹)	305	365	416
Dead material (g.kg ⁻¹)	5.02	24.2	55.6
Leaf:stem ratio	2.26	1.26	1.27

Table 2
Chemical composition of Zuri grass before ensiling at three regrowth ages (65, 80, and 95 days).

Itens	Regrowth ages		
	65 days	80 days	95 days
DM (g kg ⁻¹)	177	202	235
CP (g.kg ⁻¹ DM)	72.2	70.3	53.8
OM (g.kg ⁻¹ DM)	898	893	905
NDF (g.kg ⁻¹ DM)	777	803	811
ADF (g.kg ⁻¹ DM)	587	593	594
Lignin (g.kg ⁻¹ DM)	78.4	81.1	84.8
IVDMD (g.kg ⁻¹ DM)	569	559	534
IVNDFD (g.kg ⁻¹ DM)	548	532	491
IVADFD (g.kg ⁻¹ DM)	472	466	463
DM: dry matter; CP: crude protein; OM: organic matter; NDF: neutral detergent fibre; FDA: acid detergent fibre; IVDMD: <i>in vitro</i> dry matter digestibility; IVNDFD: <i>in vitro</i> neutral detergent fibre digestibility; IVADFD: <i>in vitro</i> acid detergent fibre digestibility.			

2.2 | Silage making

Whole plant of Zuri grass was chopped in a stationary shredder into particles of 2 to 3.0 cm, approximately, homogenized with molasses according to the amount proposed for each treatment (0, 20, and 40 g), compacted with wooden sockets in experimental PVC microsilos (50 cm high and 10 cm in diameter). At the bottom of each microsilos, 750 g of dry sand wrapped in a non-woven fabric was placed, and the upper part between the ensiled mass and the PVC caps equipped with Bunsen valve was placed a thin plastic mesh.

The silo components (PVC + lids + sand + mesh) were weighed before ensiling, and after filling in order to determine gas losses (GL), dry matter recovery (DMR), and effluent production (EP), according to gravimetric differences. After closing and sealing, the microsilos were stored at room temperature for a period of 50 days.

2.3 | Evaluation of fermentation parameters and losses

After the fermentation period, the microsilos were weighed to determine the total loss of dry matter, by gas and effluents, according to Jobim et al. (2007).

The top of each microsilos was discarded, and the center's material was homogenized and separated into two samples. The first sample was used for pH determination using a digital pH meter (MA522 model), and ammonia nitrogen (N-NH_3) according to the methodology described by Bolsen et al. (1992). The second sample was packed in paper bags, weighed and placed in a forced air circulation oven at 55°C for 72 hours, then ground in a Willey knife mill and analyzed for chemical compositions.

2.4 | Chemical analysis and *in vitro* digestibility

Samples were dried in a forced-air circulation oven at 55°C for 72 hours, weighed, ground in a Willey knife mill. Subsequently, dry matter (DM) contents were quantified (method 934.01: AOAC, 2000), organic matter (OM; 942.05), mineral matter (MM; 942.05), and crude protein (CP) was obtained through concentration of N (total N x 6.25) Kjeldahl procedure (method 920.87; AOAC, 2000).

Fiber fractions were determined by filtration in porous crucibles in neutral detergent (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991), and lignin was determined by the ADF analysis sequence, according to the 973.18D method (AOAC, 2000).

The *in vitro* digestibility of dry matter (IVDMD), crude protein (IVCPD), neutral detergent fiber (IVNDFD), and acid detergent fiber (IVADF) were determined according to the DAISYII method (Ankom Technology Corp., Fairport, NY; Holden, 1999) using the rumen fluid collected from three fistulated Texel sheep, and fed a mixed diet (brachiaria pasture and Zuri grass silage of the three regrowth ages). Subsequently, samples were submitted to pepsin incubation for 24 hours (Holden, 1999)

2.5 | *In vitro* degradation kinetics

In order to quantify the cumulative production of gases *in vitro*, were utilized three repetitions per treatment, using ruminal fluid collected manually from the three-fistulated sheep. The inoculums were

homogenized and placed in a thermal container previously heated to 39°C, and after filtering the contents, 25 mL of ruminal fluid and 100 mL of buffer solution pre-warmed to 39°C were injected into three glass vials per sample with a graduated syringe.

The vials containing samples were purged with CO₂ and then coupled to the pressure sensors under constant agitation for 48 hours at 39°C, in an electronic equipment Ankom Technology RF (GasProduction System). The system was coupled with nine vials in digital pressure sensors connected wirelessly to a computer. After 48 hours, the cumulative gas production was quantified in mL/100 mg of incubated DM, according to the bicompartamental logistic model proposed by Schofield et al. (1993).

$$Y = A / \{1 + \exp[2 + 4 * B * (LAG - t)]\} + D / \{1 + \exp[2 + 4 * E * (LAG - t)]\}$$

,

Where:

Y = total gas volume at “t” time (extent of degradation);

A = gas volume of rapidly degradation (mL);

D = gas volume of slowly degradation (mL);

B = degradation rate of rapidly fraction (/hour);

E = slow fraction degradation rate (/hour);

LAG = colonization time of bacteria (hour).

2.6 | Statistical analysis

The results obtained were subjected to analysis of variance using the statistical program PROC MIXED of the SAS software (SAS Institute, 2000). The means were compared by the "Tukey" test at 5% of significance level, using the following statistical model:

$$Y_{ijk} = \mu + I_i + M_j + Y_{ij} + e$$

Where:

Y_{ijk} – value observed in the ith regrowth ages of the jth molasses levels

μ – overall average;

I_i – fixed effect associated with the ith regrowth ages, i = 1, 2, 3;

M_j – fixed effect associated with the jth molasses levels;

Y_{ij} – interaction associated with the i th regrowth ages and the j th molasses levels;

e – Error associated in all observation (ij)

3 | Results

The chemical composition of Zuri grass silages is described in Table 3. There was an interaction between regrowth ages and molasses levels on DM, NDF, ADF, and lignin contents ($P < 0.05$). The contents of NDF and ADF were greater at 65, 80, and 95 days without molasses inclusion ($P = 0.001$ and $P = 0.006$, respectively). On the other hand, the lignin content was greater at 95 days without molasses inclusion ($P < 0.0001$).

Table 3
Chemical composition of Zuri grass silages at three regrowth ages (65, 80, and 95 days) and three levels powdered molasses (0, 20, and 40 g).

Regrowth ages (days)						<i>p</i> -value ¹			
Items	Molasses	65	80	95	Mean	SEM	Age	Molasses	A*M
DM g.kg ⁻¹	0	195 Bc	196 Bc	237 Ac	209				
	20	212 Cb	228 Bb	270 Ab	237	2.533	< 0.0001	< 0.0001	0.0002
	40	223 Ca	252 Ba	283 Aa	253				
	Mean	210	225	263					
OM g.kg ⁻¹	0	900	888	892	893 ^a				
	20	895	889	895	893 ^a	1.146	0.0016	0.0126	0.0991
	40	890	888	888	889 ^b				
	Mean	895 ^A	888 ^B	892 ^{AB}					
Ash g.kg ⁻¹	0	99.7	111.8	108.1	106.5 b				
	20	104.6	110.8	105.1	106.9 b	1.985	0.0016	0.0126	0.0985
	40	110.0	111.9	111.8	111.2 a				
	Mean	104.8 B	111.5 A	108.4 AB					
CP g.kg ⁻¹	0	57.0	45.7	56.2	53.0 b				
	20	59.4	52.4	56.1	56.0 b	1.685	< 0.0001	< 0.0001	0.1783
	40	68.1	58.5	61.7	62.8 a				

¹Probability of significant effect due to interaction between the molasses and regrowth age ($p < .05$) and regrowth age and molasses ($p < .05$).

Regrowth ages (days)					<i>p</i> -value ¹				
	Mean	61.5 A	52.2 C	58.0 ^B					
NDF g.kg ⁻¹	0	775 Aa	749 Ba	782 Aa	769				
	20	725 Ab	711 Ab	709 Ab	715	6.318	< 0.0001	< 0.0001	0.0006
	40	685 Ac	616 Ac	664 ^{Bc}	655				
	Mean	729	692	718					
ADF g.kg ⁻¹	0	608 Aa	579 Ba	606 Aa	598				
	20	523 Ab	507 Ab	518 Ab	516	5.739	< 0.0001	< 0.0001	0.0065
	40	499 Ac	438 Bc	487 Ac	475				
	Mean	543	508	537					
Lignin g.kg ⁻¹	0	62.5 Ca	72.7 Ba	93.3 Aa	76,2				
	20	52.4 Cb	68.2 Ab	63.0 Bb	61.2	1.228	< 0.0001	< 0.0001	< 0.0001
	40	49.5 Cb	57.3 Bc	61.7 Ab	56.2				
	Mean	54.8	66.1	72.7					
¹ Probability of significant effect due to interaction between the molasses and regrowth age (<i>p</i> < .05) and regrowth age and molasses (<i>p</i> < .05).									

No effects on regrowth ages and molasses levels interaction were observed on CP, OM, and ash contents. Nevertheless, CP contents was greater at 65 days and with 40 g of molasses inclusion (61.54 g.kg⁻¹ and 62.75 g.kg⁻¹, respectively; *P* < 0.0001).

The *in vitro* digestibility coefficients are shown in Table 4. The IVDMD and IVNDFD were greater at 65 days (578.52 g.kg⁻¹ and 475.17 A g.kg⁻¹, respectively) and with a 40g molasses inclusion (640.54 g.kg⁻¹ and 490.93 g.kg⁻¹, respectively) (*P* < 0.05). There were effects on the interaction between regrowth ages and molasses levels for IVADFD and IVCPD (*P* < 0.001). The IVCPD was greater at 65, 80, and 90 days when was added 40 g of molasses (*P* < 0.001).

Table 4

In vitro digestibility of Zuri grass silages at three regrowth ages (65, 80, and 95 days) and three levels of inclusion of powdered molasses (0, 20, and 40 g).

Itens	Regrowth ages (days)					<i>p-value</i> ¹			
	Molasses	65	80	95	Mean	SEM	Age	Molasses	A*M
IVDMD (g kg ¹ DM)	0	517	492	494	501 ^c				
	20	581	557	559	566 ^b	3.424	0.0099	< 0.0001	0.0654
	40	638	645	638	640 ^a				
	Mean	578 _A	565 _B	564 _C					
IVNDFD (g kg ¹ DM)	0	435	424	434	431 ^c				
	20	486	447	439	457 ^b	4.884	0.0024	< 0.0001	0.0948
	40	505	476	492	491 ^a				
	Mean	475 _A	449 _B	455 _B					
IVADFD (g kg ¹ DM)	0	407 _{Ab}	404 _{Aa}	411 _{Aab}	407				
	20	463 _{Aa}	313 _{Cb}	388 _{Bb}	388	11.463	< 0.0001	0.0061	< 0.0001
	40	482 _{Aa}	343 _{Cb}	437 _{Ba}	421				
	Mean	451	353	412					
IVCPD (g kg ¹ DM)	0	552 _{Bc}	460 _{Cc}	655 _{Ac}	556				
	20	724 _{Ab}	645 _{Bb}	724 _{Ab}	698	16.646	< 0.0001	< 0.0001	< 0.0001
	40	825 _{Aa}	797 _{Aa}	800 _{Aa}	807				
	Mean	701	634	727					

¹Probability of significant effect due to interaction between the molasses and regrowth age ($p < .05$) and regrowth age and molasses ($p < .05$).

TABLE 5 Fermentative parameters of Zuri grass silages at three regrowth ages (65, 80, and 95 days) and three levels of inclusion of powdered molasses (0, 20, and 40 g)

Regrowth ages (days)						<i>p-value</i> ¹			
Items	Molasses	65	80	95	Mean	SEM	Age	Molasses	A*M
pH	0	5.17 Aa	5.05 Aa	4.61 Ba	4.94				
	20	5.02 Aa	4.90 Aa	4.33 Bb	4.75	0.067	< 0.0001	< 0.0001	0.0023
	40	4.22 Ab	3.91 Bb	3.93 Bc	4.02				
	<i>Mean</i>	4.80	4.62	4.29					
N-NH ₃ (% DM)	0	10.6 Aa	6.04 Ba	3.25 Ca	6.64				
	20	4.58 Ab	4.73 Ab	1.93 Ba	3.74	0.298	< 0.0001	< 0.0001	< 0.0001
	40	3.12 Ac	2.75 Ac	2.13 Ab	2.67				
	<i>Mean</i>	6.10	4.51	2.44					
DM losses g.kg ⁻¹	0	142	68.5	69.5	93.3				
	20	138.0	59.5	48.0	81.8	5.843	< 0.0001	0.356	0.755
	40	152.2	66.5	52.7	90.5				
	<i>Mean</i>	144.1 A	64.8 B	56.7 B					
Gas losses g.kg ⁻¹	0	98.4	16.5	9.14	44.7 A				
	20	91.3	7.40	5.57	34.8 AB	2.200	< 0.0001	0.0122	0.8205
	40	95.3	11.0	7.23	37.8 B				
	<i>Mean</i>	95.0 A	11.6 B	10.6 B					
Effluent losses g.kg ⁻¹	0	42.6	52.5	59.9	48.3				
	20	48.7	49.5	41.7	46.6	2.903	0.0167	0.6532	0.1698
	40	42.1	51.9	44.9	46.3				

	Mean	44.4 B	51.3 A	45.5 A					
DM recovery g.kg ⁻¹	0	858	932	931	907				
	20	862	940	952	918	5.843	< 0.0001	0.3654	0.7556
	40	848	933	947	909				
	Mean	855 ^B	935 ^B	943 ^A					
Density kg/cm ³	0	759	668	607	678 ^C				
	20	760	759	663	728 ^B	11.333	< 0.0001	< 0.0001	0.1872
	40	804	788	716	767 ^A				
	Mean	775 ^A	738 ^A	662 ^B					

¹Probability of significant effect due to interaction between the molasses and regrowth age ($p < .05$) and regrowth age and molasses ($p < .05$).

Note: Different capital or small letters denote statistical differences ($p < .10$) by regrowth ages or molasses levels, respectively, by the Tukey test.

Fermentation parameters are described in Table 9. Interaction effect between regrowth ages and molasses levels on pH and N-NH₃ were observed ($P = 0.002$ and $P < 0.001$, respectively). The pH was smaller at 95 days with 40 g of molasses inclusion (3.93; $P = 0.02$). In addition, N-NH₃ was 66% greater at 65 days without molasses inclusion in relation to other treatments (10.61; $P < 0.001$).

No effects on regrowth ages and molasses levels interaction were observed on DM and gas losses, effluents, DM recovery, and density ($P > 0.05$). There was an effect on molasses levels in gas losses, which was observed value 23% greater without molasses inclusion ($P = 0.012$). The DM and gas losses were greater at 65 days of regrowth age ($P < 0.01$). Conversely, 65 days showed smaller effluents losses in relation to 80 and 95 days ($P = 0.016$). In addition, DM recovery was greater at 95 days of regrowth age in relation to 65 and 80 days ($P < 0.01$). The density was greater at 65 and 80 days of regrowth age and when was added 40 g of molasses on silage ($P < 0.001$).

In vitro degradation kinetics are described in Table 6. There were observed effects on interactions between regrowth age and molasses levels for all items *In vitro* degradation kinetics ($P < 0.05$). The fast fraction (A) showed the highest gas volume in the silages with 65 days of regrowth without additive (3.53 mL/100g DM) and with 95 days of regrowth with 40 g of molasses (4.33 mL/100g DM). The lowest rate of rapid degradation was found in silages with 65 and 80 days of regrowth without additives. Greater

volume of gas from the fraction D was observed in silages with 95 days added with 40 g of molasses (29.97 mL/100 g DM) with a lower degradation rate (0.01%/hour) and colonization time (0.01%/hours).

Table 6

In vitro degradation kinetics of Zuri grass silages with three regrowth ages (65, 80, and 95 days) and three levels of inclusion of powdered molasses (0, 20, and 40 g)

Regrowth ages (days)						<i>p-value</i> ¹			
Items	Molasses	65	80	95	Mean	SEM	Age	Molasses	A*M
A	0	3.53 Aa	2.78 ^{Bb}	2.18 ^{Cc}	2.83				
	20	2.71 Ac	2.31 ^{Bc}	2.55 ^{Ab}	2.52	0.099	0.3831	< 0.0001	< 0.0001
	40	3.09 Cb	3.91 ^{Ba}	4.33 ^{Aa}	3.78				
	Mean	3.11	3.00	3.02					
B	0	0.08 Ba	0.08 ^{Bc}	0.11 ^{Aa}	0.09				
	20	0.10 Ca	0.15 ^{Aa}	0.13 ^{Ba}	0.13	0.006	< 0.0001	< 0.0001	0.0043
	40	0.10 Aa	0.12 ^{Ab}	0.12 ^{Aa}	0.11				
	Mean	0.09	0.12	0.12					
LAG	0	5.27 Aa	5.99 ^{Aa}	6.66 ^{Aa}	5.97				
	20	5.63 Aa	5.35 ^{Aab}	3.38 ^{Bb}	5.63	0.530	0.0082	< 0.0001	< 0.0049
	40	4.20 Aa	3.79 ^{Ab}	0.01 ^{Bc}	3.00				
	Mean	5.03	5.04	3.68					
D	0	8.28 Bb	10.37 ^{Aa}	7.32 ^{Cb}	8.66				
	20	9.07 Aa	9.55 ^{Ab}	7.64 ^{Bb}	8.75	0.219	< 0.0001	< 0.0001	< 0.0001
	40	8.09 Bb	8.48 ^{Bc}	9.97 ^{Aa}	15.51				
	Mean	8.48	9.47	14.98					

¹Probability of significant effect due to interaction between the molasses and regrowth age ($p < .05$) and regrowth age and molasses ($p < .05$).

Regrowth ages (days)					<i>p-value</i> ¹				
E	0	0.02 ABa	0.01 ^{Bb}	0.03 ^{Aa}	0.02				
	20	0.02 Ba	0.03 ^{Aa}	0.03 Aba	0.03	0.002	0.7394	0.0002	< 0.0001
	40	0.02 ABa	0.02 ^{Ab}	0.01 ^{Bb}	0.02				
	Mean	0.02	0.02	0.02					
¹ Probability of significant effect due to interaction between the molasses and regrowth age ($p < .05$) and regrowth age and molasses ($p < .05$).									

Data of gas production and colonization time are detailed in Figure 2 (A and B). There was an effect observed between regrowth age and molasses levels in relation to gas production, which 95 days of regrowth age added with 40 g of molasses showed greater gas volume (Figure 2A).

4 | Discussion

The increase in DM of older silages (95 days) and the inclusion of molasses may be related to the advancement of growth of Zuri grass and the positive effect of the inclusion of molasses. The DM and fiber content increase when forages reach the maturation stage, reducing the number of leaves and cell content and, consequently, their nutritional value (Santos et al., 2014; Garcez et al., 2016).

Despite the DM contents (Table 1) at the three ages being below those recommended by Jobim et al. (2007), the powdered sugarcane molasses, as it is rich in DM and soluble carbohydrate, may have contributed to the improvement in nutritional value and preservation of silages (Bernardes et al., 2013; Desta et al., 2016). This effect may have increased the DM content of the silage (Table 3) and thus stimulated the growth of lactic acid bacteria, improving fermentation parameters (Table 5). This may have contributed to the improvement of the nutritional quality of the silage produced with grass harvested at 95 days and added with 40 g/kg of molasses, since the *in vitro* digestibility was greater (Table 4), reflecting the increase in gas production by *in vitro* degradation (Figure 2A).

The DM contents of Zuri grass with 95 days of regrowth age, described in Table 1, showed little variation from those recommended by Jobim et al. (2007). The DM contents to be ensiled must vary between 250 to 350 g.kg⁻¹, values below this range can result in the action of undesirable microorganisms, compromising the silage quality. In addition, high DM contents make it difficult to compact the ensiled material.

The increase in NDF, ADF, and lignin as a function of increasing age at a cut was expected (Table 1). The younger the plant, the smaller the cell wall and the greater the cell content. The opposite occurs when is a

plant with older age; there is an increase in cell wall constituents and a reduction in cell content (Van Soest et al., 1991; Oliveira et al., 2014).

Such changes may reflect in our silage density results (Table 5), where greater values were observed for silages with a lower regrowth age (65 days). Furthermore, the reduction in NDF and ADF contents on silages with additive in may be attributed to the molasses chemical composition (non-fibrous source), which can dilute and reduce the fibrous contents of the silages (Muck & Kung, 1997).

With the sugarcane molasses inclusion, there was an increase in CP contents in all silages and showed little variation as recommended by Van Soest (1994), CP contents of 60 g/kg are ideal for microbial activities in accelerating ruminal fermentation and fiber degradation.

The *in vitro* digestibility reduction (Table 4) in older silages may be related to higher NDF and ADF contents (Table 1), since those fibrous components are directly related to digestibility and their increase results in lower feed digestibility (Cavalcanti et al., 2016; Rosa et al., 2004). Moreover, molasses provided fiber degradation, influencing the digestibility of silages added with molasses in relation to the non-added.

The pH reduction in silage harvested at 95 days and added with molasses compared to 65 and 80 days without additive (Table 5) may be caused by the increase in dry matter content and molasses efficiency in stimulating fermentation. High values in silages at younger ages and without additives, may be resulted from the low concentration of soluble carbohydrates and the low dry matter content of tropical grasses (Silva et al., 2015), promoting undesirable fermentations and lower pH drop.

The pH value at 95 days of regrowth age silage (4.29; Table 5) and added with 40 g of molasses is according recommended by McDonald et al. (1991). Therefore, low pH values indicate high lactic acid production, reduced production of other undesirable acids, and reduced proteolysis (Van Soest, 1994). This finding is probably due to the inclusion of fermentable carbohydrates from the addition of 40 g/kg of molasses in the material at the time of ensiling.

The reduction of N-NH₃ silage with greater regrowth ages with molasses added may be due to the low Clostridium activity, stability in the fermentation of the silages, and lower humidity in the ensiled mass. According to McDonald et al. (1991), the low stability during the fermentation of the silages, results in the extensive degradation of amino acids into ammonia, CO₂, and amines, providing an increase in the contents of ammoniacal nitrogen.

The greater losses of DM and by gas in silages with 65 days of regrowth age may be attributed to the lower contents of DM, which can cause undesirable fermentations, affecting the quality of the silages (McDonald et al., 1991).

On the other hand, the increase in effluent production in silages with greater regrowth ages can be attributed to the lower compaction density of the material, due to the high fiber and lignin contents.

Greater silo density improves the conservation of soluble carbohydrates of the silage, reduces the O₂ concentration, reducing effluent losses and dry matter (Sucu et al., 2016; Neumann et al., 2009).

In Table 6 and Figure 2A, a positive relationship was observed between the soluble and fibrous fractions and IVDMD, with a greater volume of gas produced in the soluble fraction of silages with lower regrowth ages, which may be associated with the greater cellular content of the Zuri grass at this age (Reis et al., 2012). The greater volume of gas from the fibrous fraction in the silages may be due to the increase in DM and the efficiency of the molasses on fibrous carbohydrates degradation in the silages (Zao et al., 2019).

Much of the volume of gases produced comes from slowly degrading compounds (Silva et al., 2014), and an increase in gas volumes on silages with higher digestibility in fibrous fractions, explaining the greater fiber degradability. Therefore, fermentable or digestible forages showed high gas production rates, resulting in greater fermentation of the material in a shorter incubation time (Guimarães Jr. et al., 2008).

Digestibility depends on the time that the diet remains in the digestive tract (Galyean & Owens, 1991). Thus, it was observed in Fig. 2B shorter colonization time (0.01 hours) in silages harvested at 95 days added with 40 g of molasses, which may be an effect of molasses on bacterial growth colonization (Rodrigues et al., 2012). Therefore, the reduction in colonization time (LAG) is influenced by the type of substrate used in fermentation and by the physical and chemical characteristics of the food cell wall (Tomich et al., 2011).

The degradation rates of the soluble fraction showed a slight variation from 0.09%/h in a younger age to 0.12%/h in older age, and 40 g of molasses showed an average value of 0.11%/h, being higher rates in relation to fibrous fractions that remained constant (0.02%/h) at the three ages. This effect was due to the powdered molasses use, which is rich in soluble carbohydrate, thus, increasing the soluble fraction of the material (Table 6).

According to Vans Soest (1994), non-fibrous carbohydrates are available for microbial degradation and have a rapid rate of fermentation, while fibrous carbohydrates need to be colonized by microorganisms to be degraded, making fermentation slow. Silva et al. (2014), and Rodrigues et al. (2012) reported the behavior similar to the present experiment, where higher rates of degradation of non-fibrous fractions were observed in relation to fibrous ones.

5 | Conclusion

Given the better fermentative and nutritional characteristics of the silages, it is recommended to cut the Zuri grass at 80 and 95 days of regrowth for the production of silage, adding 40 g of molasses powder at the time of ensilage.

Declarations

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Author contribution AMD, GSD and LCVI conceived and designed the experiment; OA, LJ, and ENOG conducted the experiment; AMD, GSD and LCVI supervised the experiment; LJ, ENOG, JOB, ARC, ROL, APS and JCS sample analysis; AMD, FKG, LCVI and EMC prepared the manuscript. All Authors approved of the manuscript.

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Consent to participate All authors agree to participate in the current work.

Consent for publication All authors agree to publish the findings of current research.

Conflict of interest We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

References

1. AOAC., 1990. AOAC Official Methods of Analysis. *Association of Official Agricultural Chemists*. Vol. 2, 15th Ed., Arlington, VA, USA: Association of Oficial Analytical Chemists, Inc.
2. Ávila, C.L.S., Pinto, J.C., Tavares, V.B., Santos, Í.P.A., 2006. Avaliação dos conteúdos de carboidratos solúveis do capim-tanzânia ensilado com aditivos. *Revista Brasileira de Zootecnia*, 35, 648–654. <https://doi.org/10.1590/S1516-35982006000300004>
3. Bender, S.E., 2017. Vitrine de forrageiras. In: Zanela, M.B., Dereti, R.M. (Ed). Boas práticas agropecuárias na produção de leite: da pesquisa para o produtor. Embrapa Clima Temperado, 15-38.
4. Bernardes, T.F., Souza, N.S.D.S. De, Silva, J.S.L.P. Da, Santos, I.A.P., Faturi, C., Domingues, F., 2013. Uso de inoculante bacteriano e melaço na ensilagem de capim-elefante. *Revista de Ciências Agrárias/Amazonian Journal of Agricultural and Environmental Sciences*, 56, 173–178. <http://doi.org/10.4322/rca.2013.026>
5. Bolsen, K.K., Lin, C., Brent, B.E., Gadeken, D., 1992. Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. *Journal of Dairy Science*, 75, 3066–3083. [https://doi.org/10.3168/jds.S0022-0302\(92\)78070-9](https://doi.org/10.3168/jds.S0022-0302(92)78070-9)
6. Bolsen, K.K., Ashbell, G., Weinberg, Z.G., 1996. Silage fermentation and silage additives-Review. *Asian-Australasian journal of animal sciences*, 9, 483–493. <https://doi.org/10.5713/ajas.1996.483>

7. Cavalcanti, A.C., Saliba, E.O.S., Gonçalves, L.C., Rodriguez, N.M., Borges, I., Borges, A.L.C.C., 2016. Consumo e digestibilidade aparente do feno de andropogon gayanus colhido em três idades diferentes. *Ciência Animal Brasileira*, 17, 482–490. <https://doi.org/10.1590/1089-6891v17i416026>
8. Desta, S.T., Yuan, X., Li, J., Shao, T., 2016. Ensiling characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility of Napier grass ensiled with additives. *Bioresource Technology*, 221, 447–454. <https://doi.org/10.1016/j.biortech.2016.09.068>
9. Euclides, V.P.B., Carpejani, G.C., Montagner, D.B., Nascimento Junior, D., Barbosa, R. A., Difante, G.S., 2018. Maintaining post-grazing sward height of *Panicum maximum* (cv. Mombaça) at 50 cm led to higher animal performance compared with post-grazing height of 30 cm. *Grass and Forage Science*, 73, 174–182. <https://doi.org/10.1111/gfs.12292>
10. Galyean, M.L., Owens, F. N., 1991. Effects of diet composition and level of feed intake on site and extent of digestion in ruminants. In *Physiological aspects of digestion and metabolism in ruminants*, 483–514. Academic Press. <https://doi.org/10.1016/B978-0-12-702290-1.50028-X>
11. Garcez, B.S., Alves, A.A., Araújo, D.L.C., Lacerda, M.S.B., Sousa, L.G.C., Carvalho, L. F., 2016. Degradabilidade ruminal do capim colonião (*Panicum maximum* Jacq. cv. colonião) em três idades pós-rebrota. *Acta Veterinária Brasileira*, 10, 130–134. <https://doi.org/10.21708/avb.2016.10.2.5513>
12. Guimarães Junior, R., Gonçalves, L.C., Maurício, R.M., Pereira, L.G.R., Tomich, T.R., Pires, D.A.A.; Jayme, D.G., Sousa, L.F., 2008. Cinética de fermentação ruminal de silagens de milho. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 60, 1174–1180. <https://doi.org/10.1590/S0102-09352008000500020>
13. Holden, L.A., 1999. Comparison of methods of in vitro dry matter digestibility for feeds. *Journal of Dairy Science*, 82, 1791–1794.
14. Jobim, C., Nussio, L., Reis, A.R., Schmidt, P., 2007. Avanços metodológicos na avaliação da qualidade da forragem conservada. *Revista Brasileira de Zootecnia*, 36, 101–119.
15. Maranhão, C.M.A., Bonom, P., Pires, A.J.V., Costa, A.C.P.R, Martins, G.C.F., Cardoso, E.O., 2010. Características produtivas do capim-braquiária submetido a intervalos de cortes e adubação nitrogenada durante três estações. *Acta Scientiarum. Animal Sciences*, 32, 375–384. <https://doi.org/10.4025/actascianimsci.v32i4.8574>
16. McDonald, P., Henderson, A.R., Heron, S., 1991. The biochemistry of silage. 2ed. Marlow: Chalcombe Publications, 340.
17. Muck, R. E., Kung, L., 1997. Effects of silage additives on ensiling In: SILAGE: FIELD TO FEEDBUNK, 1., Pennsylvania. In: *Proceedings*. p. 187–199.
18. Neumann, M., Restle, J., Mühlbach, P.R.F., Nörnberg, J., Romano, M.A., Lustosa, S., 2009. Comportamento ingestivo e de atividades de novilhos confinados com silagens de milho de diferentes tamanhos de partícula e alturas de colheita. *Ciência Animal Brasileira*, 10, 462–473.
19. Nussio, L. G., 2005. Silage production from tropical forages. *Silage Production and Utilisation*, 97–107.

20. Oliveira, E.R., Monção, F.P., Gabriel, A.M.A, Góes, R.H.T.B., Lemp, B., Moura, L.V., 2014. Ruminant degradability of neutral detergent fiber of *Cynodon* spp. grasses at four regrowth ages. *Acta Scientiarum. Animal Sciences*, 36, 201–208. <https://doi.org/10.4025/actascianimsci.v36i2.22469>
21. Panditharatne, S., Allen, V.G., Fontenot, J.P., Jayasuriya, M.C.N. (1986). Ensiling characteristics of tropical grasses as influenced by stage of growth, additives and chopping length. *Journal of Animal Science*, 63, 197–207. <https://doi.org/10.2527/jas1986.631197x>
22. Pereira, R.C., Banys, V.L., Silva, A.C., Pereira, R.G.A., 1999. Adição de polpa cítrica peletizada na ensilagem de capim-elefante (*Pennisetum purpureum* schum) cv. cameroon. *Revista da Universidade de Alfenas*, 5, 147–152.
23. Reis, R.A., Ruggieri, A.C., Oliveira, A.A., Azenha, M.V., Casagrande, D.R., 2012. Suplementação como estratégia de produção de carne de qualidade em pastagens tropicais. *Revista Brasileira de Saúde e Produção Animal*, 13, 642–655. <http://dx.doi.org/10.1590/s1519-99402012000300005>.
24. Rodrigues, R.T.D.S., Menezes, D.R., Pereira, L.G.R., Araújo, G.G.L., Dantas, F.R., Silva, T.M., 2012. Produção de gases e digestibilidade *in vitro* de silagens de maniçoba aditivadas com coproduto vitivinícola. *Revista Brasileira de Ciências Agrárias*, 7, 684–690.
25. Rosa, J.R.P., Silva, J.H.S., Restle, J., Pascoal, L.L., Brondani, I.L., Alves filho, D.C., Freitas, A.K., 2004. Avaliação do comportamento agrônomo da planta e valor nutritivo da silagem de diferentes híbridos de milho (*Zea mays*, L.). *Revista Brasileira de Zootecnia*, 33, 302–312.
26. Santos, E.M., Pereira, O.G., Garcia, R., Ferreira, C.L.L.F., Oliveira, J.S., Silva, T.C., 2014. Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages. *Journal of Dairy Science*, 97, 1–10. <http://dx.doi.org/10.3168/jds.2013-7634>.
27. SAS, 2001. SAS/STAT Users guide, version 8.1, vol. 1, 943 pp. Cary, NC: SAS Institute.
28. Schofield, P., Pitt, R.E., Pell, A., 1993. Kinetics of fiber digestion from *in vitro* gas production. *Journal Animal Science*. 72, 2980–2991. <https://doi.org/10.2527/1994.72112980x>.
29. Silva, T.C., Santos, E.M., Macedo, C.H.O., Lima, M.A., Azevedo, J.A.G., Pinho, R.M.A., Perazzo, A. F., Oliveira, J. S., 2014. Cinética de fermentação ruminal *in vitro* de silagens de híbridos de sorgo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 66, 1865–1873. <http://dx.doi.org/10.1590/1678-6221>
30. Silva, M.S.J., Jobim, C.C., Poppi, E.C., Tres, T.T., Osmari, M.P., 2015. Production technology and quality of corn silage for feeding dairy cattle in Southern Brazil. *Revista Brasileira de Zootecnia*, 44, 303–313. <http://dx.doi.org/10.1590/S1806-92902015000900001>.
31. Sucu, E., Kalkan, H., Canbolat, O., Filya, I., 2016. Effects of ensiling density on nutritive value of maize and sorghum silages. *Revista Brasileira de Zootecnia*, 45, 596–603. <http://dx.doi.org/10.1590/S1806-92902016001000003>
32. Tomich, T.R., Gonçalves, L.C., Maurício, R.M., Pereira, L.G.R., Rodrigues, J.A.S., 2011. Composição bromatológica e cinética de fermentação ruminal de híbridos de sorgo com capim-sudão. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 55, 747–755. <https://doi.org/10.1590/S0102-09352003000600013>

33. Valadares filho, S.C., Machado, P.A.S., Chizzotti, M.L., 2010. CQBAL 3.0. Tabelas Brasileiras de Composição de Alimentos para Bovinos. 3.ed. Viçosa: Universidade Federal de Viçosa. 502.
34. Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation animal nutrition. *Journal Dairy Science*, 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
35. Van Soest, P. J., 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press. Ithaca, 476.
36. Zhao, J., Dong, Z., Li, J., Chen, L., Bai, Y., Jia, Y., Shao, T., 2019. Effects of sugar sources and doses on fermentation dynamics, carbohydrates changes, *in vitro* digestibility and gas production of rice straw silage. *Italian Journal of Animal Science*, 18(1), 1345–1355. <https://doi.org/10.1080/1828051X.2019.1659106>

Figures

Figure 1

Medium, minimum and maximum temperature and monthly precipitation during the experimental period in the 2017 year.

Figure 2

Cumulative gas production (A) and colonization time (B) in vitro of Zuri grass silages at three regrowth ages (65, 80, and 95 days) and three levels of inclusion of molasses powder (0, 20, and 40 g).