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

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Abstract
This study aimed to evaluate the effect of regrowth ages and the use of powdered molasses on nutritional characteristics of Zuri grass (Mogathyrsus maximus) silages. A completely randomized design was used, in a 3×3 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 dry matter (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 in vitro digestibility of dry matter compared to other 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 40 g/kg of molasses in powders for the production of silages.

Keywords Additive · Grass · Regrowth age · Fermentation · Quality

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 dry matter (DM) per hectare (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 (Avila 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 stimulant 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% crude protein (CP), 1.36% ether extract (EE), 3.43% crude fiber (CF), 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 kilogram 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.

### 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 (Fig. 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, in 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 analyses carried out before the trial establishment, at a depth of 0–20 cm, were as follows: 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 3 × 3 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.

![Fig. 1](image-url)  
**Fig. 1** Medium, minimum, and maximum temperature and monthly precipitation during the experimental period in the 2017 year
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 (kg/ha), total dry forage mass (kg/ha), and leaf, stem, and dead material proportion were estimated (Table 1). Samples from forage mass were utilized for chemical composition before ensiling (Table 2).

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

| Items                     | 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)

| Items                     | Regrowth ages |
|---------------------------|---------------|
|                           | 65 days | 80 days | 95 days |
| DM (g k⁻¹)                | 177     | 202     | 235     |
| CP (g k⁻¹ DM)             | 72.2    | 70.3    | 53.8    |
| OM (g k⁻¹ DM)             | 898     | 893     | 905     |
| NDF (g k⁻¹ DM)            | 777     | 803     | 811     |
| ADF (g k⁻¹ DM)            | 587     | 593     | 594     |
| Lignin (g k⁻¹ DM)         | 78.4    | 81.1    | 84.8    |
| IVDM (g k⁻¹ DM)           | 569     | 559     | 534     |
| IVNDFD (g k⁻¹ DM)         | 548     | 532     | 491     |
| IVADFD (g k⁻¹ DM)         | 472     | 466     | 463     |

DM dry matter, CP crude protein, OM organic matter, NDF neutral detergent fiber, FDA acid detergent fiber, IVDM in vitro dry matter digestibility, IVNDFD in vitro neutral detergent fiber digestibility, IVADFD in vitro acid detergent fiber digestibility

Silage making

The 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 microsilo, 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, dry matter recovery, and effluent production, according to gravimetric differences. After closing and sealing, the microsilos were stored at room temperature for a period of 50 days.

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 microsilo 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–NH₃) 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 h, then ground in a Willey knife mill and analyzed for chemical compositions.

Chemical analysis and in vitro digestibility

In order to analyze chemical analysis and in vitro digestibility, samples were collected before and after ensiling. These samples were dried in a forced-air circulation oven at 55 °C for 72 h, weighed, and ground in a Willey knife mill. Subsequently, DM contents were quantified (method 934.01: AOAC, 2000), and organic matter (MM; 942.05), mineral matter (MM; 942.05), and CP were obtained through concentration of N (total N×6.25) using 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 (IVADFD) was 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 h (Holden, 1999).

**In vitro degradation kinetics**

In order to quantify the cumulative production of gases in vitro, three repetitions were utilized per treatment, using ruminal fluid collected manually from fistulated sheep, using the wireless Ankom Rf Gas Production System (Ankom Technology, NY, USA) consisting of 24 bottles equipped with pressure sensors (pressure range $-69$; +3447 kPa; resolution ±0.27; accuracy ± 0.1%).

Samples of diets were weighed (0.5 g) and placed in synthetic polypropylene filter bags measuring 25 cm², which were then heat-sealed and incubated in bottles. The inoculums were homogenized and placed in a thermal container previously heated to 39 °C, and after filtering the contents using a plastic sieve, 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 ruminal fluid was taken from three fistulated previously fed for 7 days with a diet similar to that used in this study.

Buffer solution A (g/L), i.e., 10.0 g KH₂PO₄, 0.5 g MgSO₄7H₂O, 0.5 g NaCl, 0.1 g CaCl₂2H₂O, and 0.5 g urea, and buffer solution B (g/100 mL), i.e., 15.0 g Na₂CO₃ and 1.0 g Na₂S9H₂O in a ratio of 5:1 (A:B), pH 6.8, bottles were purged with CO₂ for 3 s before being closed. The bottles were stirred continuously at a constant temperature of 39 °C.

The system was coupled with nine vials in digital pressure sensors connected wirelessly to a computer. After 48 h, the cumulative gas production was quantified in milliliters/100 mg of incubated DM, according to the bicompart mental logistic model proposed by Schofield et al. (1993).

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

where

- \(Y\) is the total gas volume at “t” time (extent of degradation)
- \(A\) is the gas volume of rapidly degradation (mL)
- \(D\) is the gas volume of slowly degradation (mL)
- \(B\) is the degradation rate of rapidly fraction (hour)
- \(E\) is the slow fraction degradation rate (hour)
- \(\text{LAG}\) is the colonization time of bacteria (hour).

**Statistical analysis**

The results obtained were subjected to analysis of variance using the statistical program PROC MIXED of the SAS software (SAS 2001). The means were compared by the “Tukey” test at 5% of significance level, using the following statistical model:

\[
Y_{jk} = \mu + I_i + M_j + Y_{ij} + e
\]

where

- \(Y_{jk}\) is the value observed in the ith regrowth ages of the jth molasses levels;
- \(\mu\) is the overall average;
- \(I_i\) is the fixed effect associated with the ith regrowth ages, \(i = 1, 2, 3;\)
- \(M_j\) is the fixed effect associated with the jth molasses levels;
- \(Y_{ij}\) is the interaction associated with the ith regrowth ages and the jth molasses levels;
- \(e\) is the error associated in all observation (ij).

**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 (\(P < 0.0001\)).

No effects on regrowth ages and molasses level interaction were observed on CP, OM, and ash contents. Nevertheless, CP content 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 40-g 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 40 g of molasses was added \((P < 0.001)\).

Fermentation parameters are described in Table 5. Interaction effect between regrowth ages and molasses levels on pH and N–NH\(_3\) was 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–NH\(_3\) 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 level 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 90 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 40 g of molasses was added 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/100 g DM) and with 95 days of regrowth with 40 g of molasses (4.33 mL/100 g 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).

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

### 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; Gruber et al., 2018).

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; Luo et al., 2021). This effect may have increased the DM content of the silage and thus stimulated the growth of lactic acid bacteria, improving fermentation parameters. 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, reflecting the increase in gas production by in vitro degradation (Fig. 2A).

The DM contents of Zuri grass with 95 days of regrowth age showed little variation from 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; Luo et al., 2021). This effect may have increased the DM content of the silage and thus stimulated the growth of lactic acid bacteria, improving fermentation parameters. 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, reflecting the increase in gas production by in vitro degradation (Fig. 2A).

The DM contents 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) | Molasses | 65  | 80  | 95  | Mean | SEM | p-value1 | Age | Molasses | A*M |
|----------------------|----------|-----|-----|-----|------|-----|----------|-----|----------|-----|
| IVDMD (g kg⁻¹ DM)    | 0        | 517 | 492 | 494 | 501  |     |          |     |          |     |
|                      | 20       | 581 | 557 | 559 | 566  |     | 3.424    |     |          |     |
|                      | 40       | 638 | 645 | 638 | 640  |     | 0.0099   |     | <0.0001  | 0.0654 |
|                      | Mean     | 578 | 565 | 564 | 564  |     | <0.0001  |     |          |     |
| IVNDFD (g kg⁻¹ DM)   | 0        | 435 | 424 | 434 | 431  |     | 4.884    |     | <0.0001  | 0.0948 |
|                      | 20       | 486 | 447 | 439 | 457  |     | 4.884    |     | <0.0001  | 0.0948 |
|                      | 40       | 505 | 476 | 492 | 491  |     | 4.884    |     | <0.0001  | 0.0948 |
|                      | Mean     | 475 | 449 | 455 | 455  |     | <0.0001  |     |          |     |
| IVADFD (g kg⁻¹ DM)   | 0        | 407 | 404 | 411 | 407  |     |          |     |          |     |
|                      | 20       | 463 | 313 | 388 | 388  |     | 11.463   |     | <0.0001  | <0.0001 |
|                      | 40       | 482 | 343 | 437 | 421  |     | 11.463   |     | <0.0001  | <0.0001 |
|                      | Mean     | 451 | 353 | 412 | 412  |     |          |     |          |     |
| IVCPD (g kg⁻¹ DM)    | 0        | 552 | 460 | 556 | 556  |     |          |     |          |     |
|                      | 20       | 724 | 645 | 724 | 724  |     | 16.646   |     | <0.0001  | <0.0001 |
|                      | 40       | 825 | 797 | 800 | 807  |     | 16.646   |     | <0.0001  | <0.0001 |
|                      | Mean     | 701 | 634 | 727 | 727  |     |          |     |          |     |

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

Different capital or small letters denote statistical differences by regrowth ages or molasses levels, respectively, by the Tukey test

**IVDMD** in vitro dry matter digestibility, **IVNDFD** in vitro neutral detergent fiber digestibility, **IVADFD** in vitro acid detergent fiber digestibility, **IVCPD** in vitro crude protein digestibility
an increase in cell wall constituents and a reduction in cell content (Van Soet et al., 1991; Oliveira et al., 2014).

Such changes may reflect in our silage density results, 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 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 in older silages may be related to higher NDF and ADF contents, since those fibrous components are directly related to digestibility and their increase results in lower feed digestibility (Godlewksa and Ciepiela 2020). 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 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 have 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) and added with 40 g of molasses is according to the

### 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) | Items | Molasses | 65     | 80     | 95     | Mean | SEM | Age | Molasses | A*M | p-value<sup>1</sup> |
|----------------------|-------|----------|--------|--------|--------|------|-----|-----|----------|-----|-------------------|
|                      | pH    |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 0        | 5.17<sup>A</sup>a | 5.05<sup>A</sup>a | 4.61<sup>B</sup>a | 4.94 | 0.067 | <0.0001 <0.0001 | 0.0023 |
|                      |       | 20       | 5.02<sup>A</sup>a | 4.90<sup>A</sup>a | 4.33<sup>B</sup>b | 4.75 | 0.067 | <0.0001 <0.0001 | 0.0023 |
|                      |       | 40       | 4.22<sup>Ab</sup> | 3.91<sup>Bb</sup> | 3.93<sup>Bc</sup> | 4.02 |     |     |          |     |                   |
|                      | Mean  |          | 4.80   | 4.62   | 4.29   |      |     |     |          |     |                   |
|                      | N-NH<sub>3</sub> (% DM) |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 0        | 10.6<sup>A</sup>a | 6.04<sup>Aa</sup> | 3.25<sup>Ca</sup> | 6.64 | 0.298 | <0.0001 <0.0001 | 0.0001 |
|                      |       | 20       | 4.58<sup>Ab</sup> | 4.73<sup>Ab</sup> | 1.93<sup>Ba</sup> | 3.74 | 0.298 | <0.0001 <0.0001 | 0.0001 |
|                      |       | 40       | 3.12<sup>Ac</sup> | 2.75<sup>Bc</sup> | 2.13<sup>Ab</sup> | 2.67 |     |     |          |     |                   |
|                      | Mean  |          | 6.10   | 4.51   | 2.44   |      |     |     |          |     |                   |
|                      | DM losses g.kg<sup>-1</sup> |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 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 |     |     |          |     |                   |
|                      | Mean  |          | 144.1<sup>A</sup>a | 64.8<sup>B</sup> | 56.7<sup>B</sup> |     |     |     |          |     |                   |
|                      | Gas losses g.kg<sup>-1</sup> |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 0        | 98.4   | 16.5   | 9.14   | 44.7<sup>A</sup> |     |     |          |     |                   |
|                      |       | 20       | 91.3   | 7.40   | 5.57   | 34.8<sup>AB</sup> | 2.200 | <0.0001 0.0122 0.8205 |               |
|                      |       | 40       | 95.3   | 11.0   | 7.23   | 37.8<sup>B</sup> |     |     |          |     |                   |
|                      | Mean  |          | 95.0<sup>A</sup>a | 11.6<sup>B</sup> | 10.6<sup>B</sup> |     |     |     |          |     |                   |
|                      | Effluent losses g.kg<sup>-1</sup> |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 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<sup>B</sup> | 51.3<sup>A</sup> | 45.5<sup>A</sup> |     |     |     |          |     |                   |
|                      | DM recovery g.kg<sup>-1</sup> |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 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<sup>B</sup> | 935<sup>B</sup> | 943<sup>A</sup> |     |     |     |          |     |                   |
|                      | Density kg/cm<sup>3</sup> |          |        |        |        |      |     |     |          |     |                   |
|                      |       | 0        | 759    | 668    | 607    | 678<sup>C</sup> |     |     |          |     |                   |
|                      |       | 20       | 760    | 759    | 663    | 728<sup>B</sup> | 11.333 | <0.0001 <0.0001 0.1872 |               |
|                      |       | 40       | 804    | 788    | 716    | 767<sup>A</sup> |     |     |          |     |                   |
|                      | Mean  |          | 775<sup>A</sup> | 738<sup>A</sup> | 662<sup>B</sup> |     |     |     |          |     |                   |

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

Different capital or small letters denote statistical differences (p < .10) by regrowth ages or molasses levels, respectively, by the Tukey test.
reduction of N–NH₃ silage with greater 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 (Zhao 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 (Santana et al., 2020).

### 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) | Molasses | 65 | 80 | 95 | Mean | SEM | Age | Molasses | A*M |
|----------------------|----------|----|----|----|------|-----|-----|---------|------|
| A                    | 0        | 3.53<sup>Ab</sup> | 2.78<sup>Bb</sup> | 2.18<sup>Cc</sup> | 2.83 | 0.099 | 0.383 | <0.0001 | <0.0001 |
| 20                   | 2.71<sup>Bc</sup> | 2.31<sup>Ac</sup> | 2.55<sup>Ab</sup> | 2.52 | 0.099 | 0.383 | <0.0001 | <0.0001 |
| 40                   | 3.09<sup>Aa</sup> | 3.91<sup>Bb</sup> | 4.33<sup>Ab</sup> | 3.78 | 0.099 | 0.383 | <0.0001 | <0.0001 |
| Mean                 | 3.11      | 3.00 | 3.02 | 0.09 | 0.099 | 0.383 | <0.0001 | <0.0001 |
| B                    | 0        | 0.08<sup>Ba</sup> | 0.08<sup>Bc</sup> | 0.11<sup>Cc</sup> | 0.09 | 0.099 | 0.383 | <0.0001 | 0.0043 |
| 20                   | 0.10<sup>Cb</sup> | 0.15<sup>Aa</sup> | 0.13<sup>Bb</sup> | 0.13 | 0.099 | 0.383 | <0.0001 | 0.0043 |
| 40                   | 0.10<sup>Aa</sup> | 0.12<sup>Ab</sup> | 0.12<sup>Aa</sup> | 0.11 | 0.099 | 0.383 | <0.0001 | 0.0043 |
| Mean                 | 0.09      | 0.12 | 0.12 | 0.09 | 0.099 | 0.383 | <0.0001 | 0.0043 |
| LAG                  | 0        | 5.27<sup>Ab</sup> | 5.99<sup>Aa</sup> | 6.66<sup>Aa</sup> | 5.97 | 0.082 | 0.0082 | <0.0001 | <0.0049 |
| 20                   | 5.63<sup>Bb</sup> | 5.35<sup>Aab</sup> | 3.38<sup>Bb</sup> | 5.63 | 0.530 | 0.0082 | <0.0001 | <0.0001 |
| 40                   | 4.20<sup>Aa</sup> | 3.79<sup>Ab</sup> | 0.01<sup>Bc</sup> | 3.00 | 0.082 | 0.0082 | <0.0001 | <0.0001 |
| Mean                 | 5.03      | 5.04 | 3.68 | 0.08 | 0.082 | 0.0082 | <0.0001 | <0.0001 |
| D                    | 0        | 8.28<sup>Bb</sup> | 10.37<sup>Aa</sup> | 7.32<sup>Cb</sup> | 8.66 | 0.219 | <0.0001 | <0.0001 |
| 20                   | 9.07<sup>Aa</sup> | 9.55<sup>Aa</sup> | 7.64<sup>Bb</sup> | 8.75 | 0.219 | <0.0001 | <0.0001 |
| 40                   | 8.09<sup>Bb</sup> | 8.48<sup>Bc</sup> | 9.97<sup>Aa</sup> | 15.51 | 0.219 | <0.0001 | <0.0001 |
| Mean                 | 8.48      | 9.47 | 14.98 | 0.21 | 0.219 | <0.0001 | <0.0001 |
| E                    | 0        | 0.02<sup>Ab</sup> | 0.01<sup>Bb</sup> | 0.03<sup>Aa</sup> | 0.02 | 0.002 | 0.7394 | <0.0001 |
| 20                   | 0.02<sup>Aa</sup> | 0.03<sup>Ab</sup> | 0.03<sup>Aa</sup> | 0.03 | 0.002 | 0.7394 | <0.0001 |
| 40                   | 0.02<sup>Aa</sup> | 0.02<sup>Ab</sup> | 0.01<sup>Bb</sup> | 0.02 | 0.002 | 0.7394 | <0.0001 |
| Mean                 | 0.02      | 0.02 | 0.02 | 0.02 | 0.002 | 0.7394 | <0.0001 |

<sup>1</sup>Probability of significant effect due to interaction between the molasses and regrowth age (<p> <0.05) and regrowth age and molasses (<p> <0.05)

Different capital or small letters denote statistical differences (<p> <0.10) by regrowth ages or molasses levels, respectively, by the Tukey test (<p> <0.05)

A: Volume of gas produced from the fast and/or soluble fraction (mL/100 mg MS incubated); B: fast and/or soluble degradation rate (%/hour); LAG: colonization time (hours); D: volume of gas produced from the slow and/or fibrous fraction (mL/100 mg MS incubated); E: slow and/or fibrous fraction degradation rate (%/hour)
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 h) 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 carbohydrates, thus increasing the soluble fraction of the material. These results agree with those reported by Musco et al. (2016) which found a significant correlation between some chemical parameters and in vitro fermentation data for tropical forages.

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.

**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.
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Author contribution AMD, GSD, and LCV1 conceived and designed the experiment; OA, LJ, and ENOG conducted the experiment; AMD, GSD, and LCV1 supervised the experiment; LJ, ENOG, JOB, ARC, ROL, APS, and JCS conducted the sample analysis; AMD, FKG, LCV1, and EMC prepared the manuscript. All authors approved of the manuscript.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

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

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

Conflict of interest The authors declare no competing interests.

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