SHORT COMMUNICATION

Addition of grape marc improves the silage of aerial parts of cassava plant

La adición de orujo mejora el ensilaje de las partes aéreas de la planta de yuca

A adição de bagaço de uva melhora a silagem da parte aérea da mandioca

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Abstract

Background: Although it is possible to preserve the aerial part of cassava in the form of silage, as demonstrated in previous studies, its low dry matter content can result in undesirable fermentation and increased effluent losses during silage, leading to a reduction in the final quality of the silage. A possible way to get around this problem would consist in mixing this silage with dehydrated by-products from the agroindustry. **Objective:** To evaluate the addition of dehydrated grape marc (DGM) to a silage of aerial parts of cassava (*Manihot esculenta*, Crantz) on the chemical composition, degradability and *in vitro* gas production of silage. **Methods:** A completely randomized experimental design was used, with two treatments: (I) silage of the aerial part of cassava without additive; (II) silage of the aerial part of cassava added with 10% dehydrated grape marc (DGM) in the natural matter, as an additive. After 30 days of fermentation, silages were evaluated to estimate the contents of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), total digestible nutrients (TDN), as well as pH, *in vitro* degradability, and cumulative gas production by semi-automatic *in vitro* gas production. **Results:** Addition of DGM did not affect CP, EE, NDF, nor TDN levels in silage. The DGM, however, promoted an increase in DM content (32.61 *vs* 30.31%) and a reduction in pH (4.00 *vs* 4.75) of the silage. The silage that received DGM presented higher degradability coefficients of soluble and potentially degradable fractions, which led to higher values of potential and effective degradability. Similarly, DGM promoted a reduction in particle colonization time (6.74 *vs* 10.01 h) and increased cumulative gas production (62.03 *vs* 57.82 mL/g DM). **Conclusion:** Addition of 10% dehydrated grape marc in the silage of aerial parts of cassava can be useful to reduce pH and increase dry matter contents of the silage.

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Keywords: *agroindustrial residue; cassava; chemical composition; conserved forage; degradability; grape marc; Manihot esculenta; silage; Vitis vinifera; yucca.*

Resumen

Antecedentes: Si bien es posible preservar la parte aérea de la yuca en forma de ensilaje, como se demostró en estudios anteriores, el bajo contenido de materia seca puede resultar en fermentaciones indeseables y mayores pérdidas de efluentes durante el ensilaje, levando a una reducción en la calidad final del ensilaje. Una de las formas de solucionar este problema sería el ensilaje mixto de la parte aérea de la yuca con subproductos deshidratados de la agroindustria. **Objetivo:** Evaluar el efecto de la adición de bagazo de uva deshidratado (BUD) a la parte aérea del ensilaje de yuca (*Manihot esculenta*, Crantz) sobre la composición química, degradabilidad y producción de gas *in vitro* del ensilado. **Métodos:** El diseño experimental utilizado fue completamente al azar y los tratamientos consistieron en: (I) ensilado de brotes de yuca sin aditivos; (II) ensilaje de la parte aérea de yuca con 10% de BUD en materia natural, como aditivo. Luego de 30 días de fermentación, los ensilajes fueron evaluados para materia seca (MS), proteína cruda (PC), extracto de éter (EE), fibra detergente neutra (FDN), nutrientes digestibles totales (NDT), pH, degradabilidad *in vitro* y producción acumulada de gas mediante la técnica de producción de gas *in vitro* semiautomática. **Resultados:** La adición de BUD no cambió los contenidos de PB, EE, FDN y NDT del ensilaje. Sin embargo, BUD promovió un aumento en el contenido de MS (32,61 *vs* 30,31%) y una reducción en el pH (4,00 *vs* 4,75) del ensilaje. El ensilado que recibió la BUD mostró mayores coeficientes de degradabilidad de las fracciones solubles y potencialmente degradables, lo que resultó en mayores valores de degradabilidad potencial y efectiva. De manera similar, BUD promovió una reducción en el tiempo de colonización de partículas (6,74 *vs* 10,01 h) y un aumento en la producción acumulada de gas (62,03 *vs* 57,82 mL/g MS). **Conclusiones:** La adición de 10% de bagazo de uva deshidratado al ensilaje de la parte aérea de yuca puede ser útil para reducir el pH y aumentar el contenido de materia seca del ensilaje.

Palabras clave: *bagazo de uva; casava; composición química; degradabilidad; ensilaje; forraje conservado; mandioca; Manihot esculenta; orujo de uva; residuo agroindustrial; Vitis vinífera; yuca.*

Resumo

Antecedentes: Embora seja possível preservar a parte aérea da mandioca na forma de silagem, conforme demonstrado em estudos anteriores, o baixo teor de matéria seca pode resultar em fermentação indesejável e aumento das perdas por efluentes durante a ensilagem, levando à redução da qualidade final da silagem. Uma das formas de contornar esse problema seria a silagem mista da parte aérea da mandioca com subprodutos desidratados da agroindústria. **Objetivo:** Avaliar o efeito da adição do bagaço desidratado de uva (BDU) na ensilagem da parte aérea de mandioca (*Manihot esculenta*, Crantz) sobre a composição química, degradabilidade e produção de gases *in vitro* da silagem. **Métodos:** O delineamento experimental utilizado foi inteiramente casualizado e os tratamentos constituíram-se por: (I) silagem da parte aérea da mandioca sem aditivo; (II) silagem da parte aérea da mandioca com 10% de bagaço desidratado de uva (BDU) na matéria natural, como aditivo. Após 30 dias de fermentação, as silagens foram avaliadas quanto aos teores de matéria seca (MS), proteína bruta (PB), extrato etéreo (EE), fibra em detergente neutro (FDN), nutrientes digestíveis totais (NDT), pH, degradabilidade *in vitro* e produção cumulativa de gases por meio da técnica semiautomática de produção de gases *in vitro*. **Resultados:** A adição do BDU não alterou os teores de PB, EE, FDN e NDT da silagem. No entanto, o BDU promoveu aumento no teor de MS (32,61 *vs* 30,31%) e redução no pH (4,00 *vs* 4,75) da silagem. A silagem que recebeu o BDU apresentou maiores coeficientes de degradabilidade das frações solúvel e potencialmente degradável, o que acarretou em maiores valores de degradabilidade potencial e efetiva. Do mesmo modo, o BDU promoveu uma redução no tempo de colonização da partícula (6,74 *vs* 10,01 h) e um aumento na produção cumulativa de gás (62,03 *vs* 57,82 mL/g MS). **Conclusões:** A adição de 10% do bagaço desidratado de uva na silagem da parte aérea de mandioca pode ser útil para reduzir o pH e aumentar os teores de matéria seca da silagem.

Palavras-chave: *composição química; bagaço de uva; degradabilidade; forragem conservada; mandioca; macaxeira; Manihot esculenta; resíduo agroindustrial; silagem; Vitis vinífera.*

Introduction

Preserving forage as silage is a common strategy used by farmers to feed animals during periods of low forage availability (Brito *et al*., 2020; Haselmann *et al*., 2020). Preservation of the aerial parts of cassava (*Manihot esculenta*, Crantz) in the form of silage could maximize the use of this resource (Fluck *et al*., 2017), considering that this material is usually discarded on the field at the time of root harvesting (Nunes irmão *et al*., 2008).

The aerial part of cassava presents, on average, 14.0 to 19.0% crude protein, 61.1% to 63.6% neutral detergent fiber, 6.7 to 8.3% ethereal extract, and 50.0 to 60.0% total digestible nutrients (Souza *et al*., 2011). These characteristics make the aerial part of cassava a low-cost forage alternative, which can be included in ruminant nutrition in its *in natura* hay or silage form (Souza *et al*., 2018; Nascimento *et al*., 2021).

Although it is possible to preserve the aerial part of cassava in the form of silage, as demonstrated in previous studies (Fluck *et al*., 2017; Oliveira *et al*., 2018; Nascimento *et al*., 2021), its low dry matter content (Modesto *et al*., 2008) may result in undesirable fermentation and increased effluent losses during silage, leading to a reduction in final silage quality. A possible way to circumvent this problem consists in mixing the silage of aerial part of cassava with dehydrated byproducts of the agro-industry.

On the other hand, viticulture is one of the most extended agro-economic activities worldwide, with over 60 million tons produced globally each year. An important part of the production of grapes is destined to the production of wine, which is a relevant traditional activity in several countries (Teixeira *et al*., 2014). Production of wines and juices involves the generation of large amounts of by-products, mainly organic waste, wastewater, greenhouse gas emissions, and inorganic waste (Musee *et al*., 2007). Grape pomace is a residue obtained from the grape pressing process, constituting on average 20% of the total used for the production of wine and juice (Teixeira *et al*., 2014; Flores *et al*., 2020). Inclusion of this residue in animal diets can reduce the environmental impact of this agricultural activity.

Dehydrated grape marc (*Vitis vinifera*) in silage form can be used as a feedstuff for ruminants (Flores *et al*., 2020). It has, approximately, 17.0% crude protein, 40.0% non-fibrous carbohydrates and 42.0 to 62.0% neutral detergent fiber (Basalan *et al*., 2011; Santos *et al*., 2014; Guerra-Rivas *et al*., 2016; Flores *et al*., 2020). Furthermore, depending on the dehydration process, the byproduct can have dry matter levels of 60 to 80%. Thus, using this by-product as an additive can elevate silage dry matter content to a desirable concentration during the fermentative process.

It should be noted that the proper amount of grape marc to be added to silage should be evaluated due to its high fiber values, which can negatively affect nutrient digestibility (Basalan *et al*., 2011; Guerra-Rivas *et al*., 2016). Therefore, our hypothesis was that adding 10% grape marc dehydrated to a silage from aerial parts of cassava would improve the chemical composition, degradability, and production of *in vitro* gases from the silage.

Materials and Methods

Location of the experiment

The study was conducted at the Agricultural Sciences campus of Universidade Federal do Vale do São Francisco, municipality of Petrolina – Pernambuco, Brazil. Geographic coordinates: Latitude 9° 09' South and longitude: 40º 22' West. According to the Köppen classification, the climate in the region corresponds to a Bswh type (hot and dry region).

Experimental design and conduct of the test

The experimental design used was completely randomized and the treatments consisted on silage of the aerial part of cassava without additive (CAP), and with 10% dehydrated

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grape marc (DGM) in the natural matter, as an additive (CAP+DGM), with 14 repetitions per treatment. The upper third of the aerial part of cassava was used at approximately 12 months of age. The material was minced to approximately 5 mm in a stationary forage chopper. The DGM was the result of the extraction of natural grape juice (Cooperativa Agrícola Nova Aliança). This residue was disposed over an open area to the sun for five days and rotated daily until reaching approximately 80% DM. After this period, the residue was ground in a stationary forage chopper with 3-mm sieve.

The samples *in natura* from the aerial part of cassava and DGM were collected to determine dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF), as described in AOAC (2000). For the estimation of total digestible nutrients (TDN) we used the following equation proposed by Cappelle *et al*. (2001): TDN (%) = 99.39-0.7641*NDF (Table 1).

Table 1. Chemical composition of *in natura* ingredients used for silage production.

	DM (%)	CP	EE (%)	NDF (%)	ADF (%)	TDN (%)
Cassava aerial part	29.61	13.93	5.90	56.08	31.11	56.54
Dehydrated grape marc	82.79	12.52	7.48	62.85	35.98	51.37

DM: Dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients.

Twenty-eight cylindrical PVC (Tigre®, Rio Claro, SP, Brazil) silos with 10-cm diameter and 50-cm length were used, with PVC caps equipped with a Bunsen-type valve to allow escape of fermentation gases. Compaction of the material was performed with wooden sockets and sealing with PVC covers (sealed with adhesive tape). The material was compacted to a specific mass of 650 kg/m^3. Determination of chemical composition and *in vitro* evaluation of silages were carried out in triplicates.

Chemical composition and pH of silages

For silage evaluation, the silos were opened after 30 days of fermentation. The contents of the upper and lower three centimeters of each silo was disregarded and the rest of the contents were homogenized (initial, intermediate and final). After this procedure, silage samples were collected, packaged in plastic bags, and sent to the laboratory to determine DM, CP, EE, NDF and TDN in an analogous way to the evaluations performed to *in natura* material.

The pH of the silage was determined after dilution of 9 g of fresh silage into 60 mL distilled water; after 30 minutes of rest, an electrode was introduced in the solution waiting 15 seconds for stabilization of each sample (Jobim *et al*., 2007).

In vitro degradability

Evaluation of *in vitro* degradability followed methodology proposed by Tilley and Terry (1963), modified and adapted to Ankom Daisy System (Ankom Technology Corp., Macedon, NY, USA), described by Holden (1999). The procedure was performed by incubating samples of 0.500 g of silages in polypropylene tissue bags (5x5 cm, 100 μm). The samples were incubated in jugs with Bunsen valve using 1.600 mL of buffer solution composed of solutions A and B, and 400 mL of ruminal inoculum from sheep.

Incubation periods were 2, 6, 12, 24, 48, 72 and 96 h. The bags were positioned in different times and removed at the same time. After removal, bags were washed with cold water until the water ran clear. Later, the bags were dried in a forced-air circulation oven at 55 °C for 72 h, and then weighed. The DM content in the waste was determined according to AOAC (2000).

In vitro degradation kinetics was calculated using the nonlinear model proposed by Orskov and Mcdonald (1979), defined as:

$$P = A + B*(1-Exp(-C*t))$$

Where: P is the percentage of degraded substrate up to time t; A represents the rapidly

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soluble fraction; B is the fraction potentially degraded; C is the degradation rate of the B fraction, and t is the incubation time. With the estimation of the model parameters, it was possible to calculate the potential degradability (A + B), the non-degradable fraction (100 - (A + B)), and the effective degradability (ED), which was obtained from the following expression:

$$ED = A + B*C/(C+K)$$

Where, A, B and C are the same parameters as the model proposed by Orskov and Mcdonald (1979), and K is the rate of passage of solids in the rumen: 2, 5 and 8% per hour (AFRC, 1992).

Cumulative in vitro gas production

The *in vitro* semiautomatic gas production technique proposed by Maurício *et al*. (2003) and modified by Menezes *et al*. (2015) was applied to perform cumulative gas production evaluations. For each silage, three samples were incubated using ruminal fluid from sheep (Theodorou *et al*., 1994). The incubation solution was composed of 160 mL solution, 80% (128 mL) buffer and minerals, and 20% (32 mL) ruminal inoculum manually introduced using a graduated syringe in glass vials, previously washed and dried in an oven with forced ventilation and one gram of sample added. The ruminal inoculum, used in the *in vitro* degradation and gas production evaluations, was collected from ewes that were fed minced forage and concentrate when the ruminal fluid was collected, in a 70:30 proportion of roughage/concentrate.

In addition to the vials containing silage samples, two other vials contained only the inoculum and buffer solution for the purpose of measuring possible pressures that were not linked to the diets. The incubation solution was prepared as described by Theodorou *et al*. (1994) using cysteine-HCl as a reducing agent (Mould *et al*., 2005). After introducing the incubation solution and samples, CO_2 was manually injected for seven seconds into each flask, later closed, and introduced into the forced-air ventilation oven at 39 °C during 96 hours for incubation.

To obtain the parameters of gas production kinetics, the bicompartmental logistic model proposed by Schofield *et al*. (1994) was used:

$$Y = A/(1+exp(2-4*B*(Lag-t))) + D/(1+exp(2-4*E*(Lag-t)))$$

Where: Y is the cumulative production of gas at time t (mL g/DM); A and D represent the volume of gas produced by the degradation of non-fibrous carbohydrates and soluble compounds, and fibrous carbohydrates, respectively; B and E are the respective degradation rates of non-fibrous carbohydrates and soluble compounds and fibrous carbohydrates; and Lag is the time (hours) of particle colonization by bacteria (lag time).

Statistical analysis

The parameters of the Orskov and Mcdonald models (1979), and bicompartmental logistics, were estimated using the modified Gauss-Newton method using the SAS NLIN procedure (SAS University Edition -Version 12-, SAS Institute Inc. Cary, CA, USA). The maximum number of iterations used was 100.

All data were subjected to analysis of variance using a completely randomized design by the PROC GLM command, and the averages were compared by the Fischer test, analyzed by the SAS statistical package (SAS University Edition -Version 12-, SAS Institute Inc. Cary, CA, USA). A significance level of 5% was adopted in all statistical analyses.

Results

Chemical composition and pH of silages

Addition of DGM did not change CP, EE, NDF, nor the TDN levels in the silage (Table 2). However, DGM promoted an increase in DM contents of the silage. Likewise, the pH of silage plus DGM was 15.79% lower than the value observed in silage without DGM (Table 2).

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In vitro degradability

The rate of DM degradation did not change with the addition of DGM. However, the silage that received DGM presented higher degradability coefficients of soluble and potentially degradable fractions, which led to higher values of potential and effective degradability of this silage (Table 3). On the other hand, the non-degradable fraction was higher in the silage composed only by aerial parts of cassava.

Cumulative in vitro gas production

Inclusion of DGM had no effect on the volume of gas (mL) produced from degradation of non-fibrous (A) and fibrous (D) carbohydrates (Table 4). There was also no difference in the values of non-fibrous (B) and fibrous (E) carbohydrate degradation rates. However, the inclusion of DGM promoted a reduction in particle colonization time (Lag time) and increased cumulative gas (Y) production.

The differences observed in DM degradability parameters (Table 3) and *in vitro* gas production kinetics (Table 4) promoted changes in the respective curves projected from the parameters estimated by the Orskov models (Figure 1A) and bicompartmental logistic (Figure 1B).

Table 2. Chemical composition of cassava aerial part silage (CAP) with or without dehydrated grape marc (DGM).

Variables	Silage		SEM	p-Value
	CAP	CAP + DGM		
DM (%)	30.31b	32.61a	0.360	<0.001
CP (%)	14.75a	14.75a	0.130	0.980
EE (%)	5.62a	5.38a	0.160	0.290
NDF (%)	53.98a	55.34a	0.440	0.060
TDN (%)	57.10a	58.14a	0.370	0.060
pH	4.75a	4.00b	0.110	<0.001

DM: Dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; TDN: Total Digestible Nutrients; SEM: standard error of the mean. Different letters within the same line differ at 5% probability by the Fischer test ($p<0.05$).

Table 3. *In vitro* degradability parameters of dry matter of cassava aerial part silage (CAP) with or without dehydrated grape marc (DGM).

Degradability coefficients	Silage		SEM	p-Value
	CAP	CAP + DGM		
Soluble fraction (%)	13.84b	15.06a	0.334	<0.001
Potentially degradable fraction (%)	31.46b	37.13a	1.245	<0.001
Degradation rate (%/h)	0.045	0.044	0.004	0.0218
Non-degradable fraction (%)	54.71a	47.82b	1.452	<0.001
Potential degradability (%)	45.29b	52.18a	1.362	0.0341
Effective degradability (%)	24.23b	27.80a	0.631	0.0172

Different letters within the same line differ at 5% probability by the Fisher test ($p<0.05$). SEM: standard error of the mean.

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Table 4. *In vitro* gas production kinetics parameters of cassava aerial part silage (CAP) with and without dehydrated grape marc (DGM).

Parameters	Silage		SEM	p-Value
	CAP	CAP + DGM		
Y (mL/g DM)	57.82b	62.03a	0.750	0.001
A (mL g/DM)	31.29	30.18	1.280	0.740
B (h)	0.066	0.061	0.002	0.310
Lag time (h)	10.01a	6.74b	0.581	<0.001
D (mL g/DM)	26.52	31.86	1.621	0.180
E (h)	0.021	0.021	0.001	0.640

Y = cumulative gas production at time t (extent of degradation); A = gas volume (mL g/DM) produced by degradation of non-fibrous carbohydrates and soluble compounds; B = degradation rate of non-fibrous carbohydrates and soluble compounds (h); Lag = time taken by the bacteria to colonize particle (h); D = gas volume (mL g/DM) produced by degradation of fibrous carbohydrates; E = fibrous carbohydrate degradation rate (h); SEM: standard error of the mean. Distinct letters on the same line differ at 5% probability by the Fischer test ($p<0.05$).

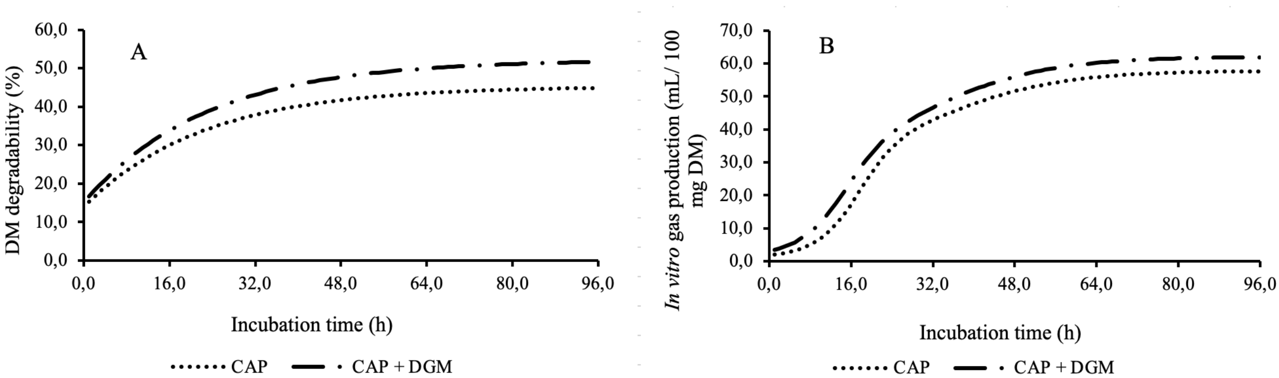

Figure 1. Material degradability curves (A) and cumulative gas production (B) of cassava aerial part silage (CAP) with and without dehydrated grape marc (DGM) projected from parameters estimated by Orskov models (A) and bicompartmental logistics (B).

Discussion

Addition of 10% DGM was not sufficient to increase or reduce CP, EE, NDF and TDN levels of silage, which can be attributed to the fact that the DGM did not present a discrepant difference in the concentration of these nutrients in relation to the aerial part of cassava (Table 1). Moreover, when silage processing is performed according to established basic procedures, no significant change in the concentration of these nutrients in silage is detected (Zardin *et al*., 2017; Santana *et al*., 2019).

However, inclusion of DGM promoted an increase in DM of silage, as this residue went through the drying process with the objective of raising DM to approximately 80%. The aerial part of cassava has a high moisture content (Modesto *et al*., 2008; Fluck *et al*., 2017), which may potentiate clostridia activity and cause undesirable fermentation, significantly increasing losses in nutritional quality due to proteolysis (Liu *et al*., 2011; Xie *et al*., 2012; Wilkinson & Muck, 2019). Therefore, the use of absorbent additives, such as agro-industrial waste, can be an alternative to improve the fermentation process of wet materials (Gurgel *et al*., 2019), such as the aerial part of cassava.

The higher DM content observed in CAP+DGM silage reflected in better compaction and, consequently, in lower silage pH (Table 2).

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In addition, the lower the DM content of ensiled forage, the greater the resistance that the forage mass has to the pH decrease (Jobim *et al*., 2007). The pH value of silage is one of the most important indicators of quality (Wilkinson & Davies, 2013), in this sense, the pH values found in DGM additive silage are satisfactory for the conservation of forage, since pH between 3.6 and 4.2 are considered ideal (Mcdonald *et al*., 1991).

The higher degradability coefficients of soluble, potentially degradable, potential and effective fractions observed in CAP + DGM silage resulted in lower non-degradable fraction of this silage, suggesting that DGM may have provided more substrates to micro-organisms and elevated the degradation of the incubated material. It should be noted that although DGM has high fiber contents (Flores *et al*., 2020), which could hinder access by microorganisms, inclusion of 10% of this additive was not sufficient to change the fibrous fraction of silage (Table 2).

Agroindustry residues are rich in starch, simple sugars, and pectin, the latter being a structural carbohydrate of rapid ruminal degradation (Ítavo *et al*., 2020). Pectin is a non-starch carbohydrate considered as soluble fiber readily available, despite being part of the cell wall. Considering its better-quality fiber, agroindustry residues such as DGM are alternative additives that can improve ruminal degradability of silages.

High cumulative production of gas in CAP + DGM silage was observed. Gas production potential of total carbohydrates was prolonged due to possible increase of fermentable substrates by DGM addition. The shorter particle colonization time (lag time) shows, in parts, that DGM addition increased substrate supply to microorganisms. Lag time is related to the presence of readily fermentable substrates and physical and chemical properties of food, capable of facilitating microbial fermentation (Schofield *et al*. 1994). In addition, there is a direct relationship between food degradability and cumulative gas production (Leal *et al*., 2020, Santana *et al*., 2020).

Addition of DGM to silage of aerial parts of cassava proved to be a promising alternative. The DGM promoted an increase in DM levels and reduction of silage pH, improving fermentative parameters. Therefore, the hypothesis that adding 10% DGM to silage from aerial parts of cassava would improve the chemical composition, degradability and production of *in vitro* gases from silage was confirmed by the results observed.

In conclusion, addition of 10% dehydrated grape marc in the silage of aerial parts of cassava is recommended to reduce pH, increase dry matter contents, and improve parameters of silage fermentation kinetics.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Jean Neuton de Oliveira, Fabio Nunes Lista, João Virgínio Emerenciano Neto, Antonio

Leandro Chaves Gurgel, Gelson dos Santos Difante, Rodrigo da Silva Santos and William Gama de Oliveira worked on the aspects involved in the methodology and statistical analysis. Antonio Leandro Chaves Gurgel wrote and prepared the manuscript. Luís Carlos Vinhas Ítavo, Patrick Bezerra Fernandes and Carolina Marques Costa contributed to the review and approval of the final version of the manuscript. All authors read and agreed with the published version of the manuscript.

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