

# A two-location trial for selecting corn silage hybrids for the humid tropic: forage and grain yields and *in vitro* fermentation characteristics

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**ABSTRACT** - The goal of our study was to evaluate the nutritional potential of dented corn hybrids for silage production. We performed a two-location trial in which 19 dented corn hybrids and five corn controls grew in four randomized blocks within two experimental areas located in the Northern (Campos dos Goytacazes) and Northwestern (Itaocara) Rio de Janeiro State, Brazil. We recorded yields of fresh and dry forage matter and yields of fresh and dry grain matter, as well as chemical composition variables. We interpreted variables by assuming a Normal distribution for yield variables and a Beta distribution for chemical composition and ratios. The SAS GLIMMIX procedure fitted the linear model under those assumptions. Dual-pool models fitted the gas production profiles generated by *in vitro* anaerobic fermentations. We used the nlme of R software to fit the dual-pool models and the information-theoretic approach to evaluate their quality of fit. We did a cluster analysis (NbClust of R) to group corn hybrids based on fresh and DM yields and kinetic parameters of *in vitro* gas production. Three clusters of corn hybrids stood out, their basic differences relied on fresh and DM yields. Nonetheless, the least-squares means for gas production characteristics among groups did not present disjoint confidence intervals. Therefore, we can infer that dented corn hybrids rank by forage yield, but not by forage quality, and recommend the most productive ones that consistently stand out in both locations (hybrids UENF-2203, UENF-2192, UENF-2193, and UENF-506-11).

**Keywords:** corn silage, forage quality, gas production, nutritional value

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## 1. Introduction

A balance between amounts of nutrients available and intake, digestibility, and efficiency of nutrient utilization by animals characterizes an ideal forage. It is desirable that forage plants alike yield large amounts of forage mass per unit of area, with the lowest possible cost and with the objective of obtaining increments in livestock performance.

The act of ensiling the corn plant is a traditional forage preservation technique for livestock feeding around the world, for feeding herds either during dry periods or as the primary component of total-mixed rations in intensive production systems. Corn shows interesting characteristics for this purpose such as high yield of dry matter (DM), nutrient levels (e.g., starch), and palatability. The ensiling process of the corn plant preserves and sometimes increases its nutrient digestibility (Van Soest, 1994).

Companies have developed genetically improved corn hybrids based on grain yield, but it appears that the use of the same hybrids for silage production arose regardless of the main goal of grain production. Therefore, in the past, they did not focus their genetic improvement programs to release corn varieties and hybrids for silage production (Bunting, 1975; Hunter, 1978). In Brazil, there is a wide diversity of corn hybrids in the market, which are adapted to different environmental conditions and present divergent productive characteristics. Nonetheless, companies seldom have developed few corn hybrids by considering nutritional aspects other than chemical composition and grain and forage yields. Some aspects of ruminal fermentation or digestibility parameters of corn as a forage plant are missing.

Several studies demonstrate variations in starch digestibility for different corn hybrids, which can lead to different animal production responses (Philippeau and Michalet-Doreau, 1997; Philippeau et al., 1999; Correa et al., 2002; Taylor and Allen, 2005c). Therefore, an increased grain proportion does not necessarily imply a corn silage with high quality, because grain digestibility varies considerably among corn hybrids (Russell et al., 1992; Cox et al., 1994; Correa et al., 2002; Ferrareto and Shaver, 2015). In addition, environmental constraints affect the forage yield of corn hybrids, which demand specific recommendations to corn forage cropping by region to predict the effect of environmental factors (e.g., altitude and temperature) on silage quantity and quality (Buxton, 1996; Crevelari et al., 2017, 2018, 2019). Therefore, our goal was to evaluate the forage and nutritional potential of dented corn hybrids for silage production in two locations of low altitudes above sea level (a.s.l.) and under the influence of predominant humid tropical conditions.

## 2. Material and Methods

We conducted the evaluation of corn hybrids simultaneously in Campos dos Goytacazes (21°38'57,48" S and 41°20'34,65" W, and 15 m a.s.l. – Northern Rio de Janeiro State) and Itaocara (21°38'39,93" S e 42°3'7,18" W, and 90 m a.s.l. – Northwestern Rio de Janeiro State), in the 2013/2014 crop year. The climate in both regions is tropical with well-defined dry and wet seasons and classified as Aw (Kottek et al., 2006; Beck et al., 2018). The average annual rainfalls were 800 and 1221 mm·yr<sup>-1</sup> for the Northern and Northwestern regions, respectively. The experimental layout was a completely randomized block design with four replicates in both locations.

### 2.1. Cropping conditions

We used a conventional planting system to sow the hybrid corn seeds in November and December of 2013 at the experimental areas of Campos (Location 1) and Itaocara (Location 2). We fertilized the areas at sowing with 400 kg·ha<sup>-1</sup> of formulated NPK 8-28-16. Subsequently, we did two topdressing fertilizations: the first at 30 days after planting with 300 kg·ha<sup>-1</sup> of NPK 20-0-20, and the second on day 45 after planting with 200 kg·ha<sup>-1</sup> of urea.

We evaluated 24 corn hybrids, and considered five of them as control hybrids (Table 1). Each experimental plot consisted of a 5-m row (120 m per block), and rows (blocks) 1 m apart from each other. We sowed five seeds·m<sup>-1</sup>.

We harvested the hybrids when at least three of the four plots presented grains at silage harvesting point. We considered the harvesting point when the milk line reached 1/2 of the kernel profile. We harvested 15 plants per plot manually at 0.2 m above ground, and weighed the samples collected in the field with 50-kg pointer scales (0.1-kg increment).

**Table 1** - Corn hybrid topcrosses, testers, and controls sown in the two-location trial

Identification	Genotype <sup>a,b,c</sup>	Grain type
1	UENF-2194 <sup>a</sup>	Dent
2	UENF-2195 <sup>a</sup>	Dent
3	UENF-2199 <sup>a</sup>	Dent
4	UENF-2205 <sup>a</sup>	Dent
5	UENF-2198 <sup>a</sup>	Dent
6	UENF-2203 <sup>a</sup>	Dent
7	UENF-2192 <sup>a</sup>	Dent
8	UENF-2206 <sup>a</sup>	Dent
9	UENF-2207 <sup>a</sup>	Dent
10	UENF-2208 <sup>a</sup>	Dent
11	UENF-2209 <sup>a</sup>	Dent
12	UENF-2210 <sup>a</sup>	Dent
13	UENF- 2200 <sup>a</sup>	Dent
14	UENF-2202 <sup>a</sup>	Dent
15	UENF-2201 <sup>a</sup>	Dent
16	UENF-2204 <sup>a</sup>	Dent
17	UENF-2193 <sup>a</sup>	Dent
18	UENF-2191 <sup>a</sup>	Dent
19	<i>Piranão</i> 13 <sup>a</sup>	Dent
20	AG 1051 <sup>b</sup>	Dent
21	UENF-2197 <sup>b</sup>	Dent
22	UENF-2196 <sup>b</sup>	Dent
23	BR 106 <sup>b</sup>	Semi-dent
24	UENF-506-11 <sup>b</sup>	Semi-dent

<sup>a</sup> Topcross hybrids.<sup>b</sup> Control.<sup>c</sup> Genotypes 1-19, 21, and 22 were crossed with *Piranão* 12. *Piranão* 12 was a tester (Crevelari et al., 2017, 2019).

## 2.2. Sampling and processing of corn plants

We threshed and recorded the mass of grains from corn ears of five plants and chopped their husks and cobs along with vegetative parts (stem and leaves). We sampled grains separately and quantitatively from the husks, cobs, and remaining vegetative part (fibrous part or stover). The chopped husks, cobs, stems, and leaves formed one single sample of ca. 0.6 kg per plot. We stored grain and fibrous samples for further processing and analysis under freezing conditions.

We dried grains and chopped fibrous parts at 55 °C for 72 h in a forced-air oven, subsampled the partially dried grains and fibrous parts, and ground them quantitatively in a Wiley-type mill fitted with a 1-mm-sieve. We carried out the analyses of total DM (method 967.03; AOAC, 2019) on grains and fibrous parts. In sequence, we combined the milled parts of grains and fibrous parts quantitatively in appropriate DM portions to resemble the composition of the whole plant (Kruse et al., 2008). That procedure was necessary for saving samples for chemical analysis and *in vitro* incubations. We also determined crude fat (CF, method 2003.06; Thiex et al., 2003), ash (method 942.05; AOAC, 2019), and crude protein (CP, method 984.13 and method 2001.11; AOAC, 2019; Thiex et al., 2002). We determined the neutral detergent fiber (NDF) fraction with a standardized heat-stable amylase solution, 0.5 g of Na<sub>2</sub>SO<sub>3</sub>, and expressed results exclusive of residual ash (aNDFom as method 2002.04; Mertens, 2002). The lignin fraction or Lignin(sa) is the organic matter (OM) determined on the acid detergent residue by solubilization of cellulose with standardized sulfuric acid solution (Möller, 2009). The aNDFom and Lignin(sa) analyses were made with Berzelius beakers with spouts. We estimated the amount of non-fibrous carbohydrates as follows: NFC = DM – ASH – CP – CF – aNDFom.

We determined the ASH contents in porcelain crucibles (method 942.05; AOAC, 2019), fitted with porcelain covers to avoid spillages during incineration and manipulation of crucibles. However,

we used two types of muffle furnaces: one with the temperature electronically programmable (model Q318M24; Quimis Aparelhos Científicos, Diadema, SP, Brazil), and the other was analogic (model Q318D24; Quimis Aparelhos Científicos, Diadema, SP, Brazil). The temperatures in the analogic furnace were manually increased (approximate 50 °C increase) to gradually reach 525±15 °C.

The Kjeldahl CP method (method 2001.11) was used for digesting samples in aluminum blocks using copper catalyst, K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, and 1 g of feed sample into 250 mL borosilicate digestion tubes. Nonetheless, we used 100 mL micro-assay borosilicate tubes for digesting samples in two 40-probe aluminum blocks (model TE-040/25; Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil); the 100 mL tubes also fit to a specific steam distillation unit (TE-0363; Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil). We proportionally adjusted the amounts of catalyst-salt mixture for 0.25 g samples and sulfuric acid (5 mL per tube). The concentration of the NaOH solution used for neutralization in the distillation step was 40% (w/w). We determined duplicate blanks per 40-probes run, and used certified purity NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and Lysine-HCl to check for N-recovery of the distillation step and the entire method, respectively (Thiex et al., 2002).

### 2.3. Statistical analysis

We analyzed yields (kg·ha<sup>-1</sup>) of fresh (Yha, as is) and dry (YDMha, DM) forage mass, yields of fresh (Ygha, as is) and dry (YgDMha, DM) grain matter, and yields (kg·ha<sup>-1</sup>) of the chemical components CP (CPYha), aNDFom (aNDFomYha), Lignin(sa) (Lignin(sa)Yha), and NFC (NFCYha), which were estimated using the following statistical models:

$$Y_{ijk} \sim \text{Normal}(\mu_{ijk}, \sigma^2) \text{ and} \quad (1)$$

$$Y_{ijk} = \mu + \alpha_i + \beta_k + b_{j(k)} + \alpha\beta_{ik} + e_{ijk} \quad (2)$$

in which  $Y_{ijk}$  corresponds to the observed production of the  $i$ -th corn hybrid harvested in the plot corresponding to the  $j$ -th block within the  $k$ -th location. Parameter  $\mu$  is a constant,  $\alpha_i$  is the hybrid effect ( $i = 1, 2, 3, \dots, 24$ ),  $b_{j(k)} \sim \text{Normal}(0, \sigma_{b(\beta)}^2)$  corresponds to block effect ( $j = 1, 2, 3, 4$ ) within each location,  $\beta_k$  is the location effect ( $k = 1, 2$ ),  $\alpha\beta_{ik}$  is the hybrid × location interaction, and  $e_{ijk} \sim \text{Normal}(0, \sigma^2)$  represents the random error term. Hybrid, location, and their interaction were fixed effects in the model, whereas blocks within locations were random effects.

The GLIMMIX procedure of SAS (Statistical Analysis System, University Edition) fitted equations 1 and 2 to the data. First, we fitted the model using the estimation method of maximum likelihood to choose the best variance-covariance structure to verify the need to account for dependence and heterogeneity for variables normally distributed. Subsequently, the model was chosen based on the lowest value of the Akaike information criterion (Akaike, 1974), as recommended by Wolfinger (1993) and Littell et al. (2006). We adopted  $P = 0.001$  as a strong evidence of an effect, and  $P$ -values between 0.01 and 0.001 as evidences of effects. We did this to minimize the risk of finding false positives (Johnson, 2013). The Tukey-Cramer adjustment compared the least-squares means of treatments to avoid the inflation of the type-I error rate (Littell et al., 2006).

We presented the chemical composition on a DM basis. Because chemical components are continuous proportions, the most suitable probability density function for such variables is the Beta distribution (Mood et al., 1974; Ferrari and Cribari-Neto, 2004; Carlton and Devore, 2014; Stroup, 2015). Therefore, we estimated the chemical components DM, CF, ash, CP, NFC, aNDFom, Lignin(sa), and Lignin(sa):aNDFom ratio, as well as the grain proportions in fresh and dry forage mass (Ygha/Yha or YgDMha/YDMha) using the following stochastic models:

$$p_{ijk} \sim \text{Beta}(\pi_{ik}, \phi), \quad (3)$$

$$\eta_{ijk} = \log(p_{ijk}/(1 - p_{ijk})) = \eta + \alpha_i + b_{j(k)} + \beta_k + \alpha\beta_{ik} \text{ or} \quad (4)$$

$$\eta_{ijk} = -\log[-\log(p_{ijk})] = \eta + \alpha_i + b_{j(k)} + \beta_k + \alpha\beta_{ik} \quad (5)$$

The general constant  $\eta$  is associated with the transformed mean  $\eta_{ijk}$  which is linked by logarithmic functions to a linear predictor (Vonesh, 2012; Stroup, 2015). The expected value of a given chemical component, i.e.,  $\hat{\pi}_{ik} \in (0, 1)$ , is the inverse link function, as follows:

$$E[p_{ijk}] = \hat{\pi}_{ik} = 1/(1 + \exp(-\hat{\eta}_{ik})), \quad (6)$$

in which  $\hat{\eta}_{ik}$  found in equations 4 and 5 is the expected linear predictor  $\hat{\eta}_{ik} = E[\eta_{ijk}] = \hat{\eta} + \hat{\alpha}_i + \hat{\beta}_k + \hat{\alpha}\hat{\beta}_{ik}$ , and  $\hat{\pi}_{ik}$  is the expected mass proportion of the chemical component. The observed  $p_{ijk} \in (0, 1)$  is the proportion corresponding to the measured chemical constituent (e.g., DM/1000, CP/1000, etc.) of the  $i$ -th hybrid ( $\alpha_i$ ) planted in the  $j$ -th block ( $b_{j(k)}$ ) within the  $k$ -th location ( $\beta_k$ ). Parameter  $\phi$  is a positive scale parameter that completes the variance of the Beta distribution, as follows (Ferrari and Cribari-Neto, 2004):

$$V[p_{ijk}] = \hat{\pi}_{ik} (1 - \hat{\pi}_{ik}) / (1 + \hat{\phi}). \quad (7)$$

Equations 3 and 4 are applicable to values within the open interval  $0.1 < p_{ijk} < 0.9$ . However, outside this range, but still within the interval  $(0, 1)$ , we also tested the model defined by equations 3 and 5, as recommended by Stroup (2013, 2015). It is important to emphasize that the quality of both fits was compared using the concordance correlation coefficient (Lin, 1989), according to the procedures recommended by Vonesh et al. (1996), and implemented as a macro in the SAS program (Vonesh, 2012). We used the restricted pseudo-likelihood method (RSPL) of GLIMMIX to fit this model to the observed chemical component concentrations and ratios. One of the requested outputs (given by the "ilink" function) of the GLIMMIX procedure is given by equation 6, and variances of the estimates given by equation 7. The final chemical concentrations resulted from the product  $1000 \cdot \hat{\pi}_{ik}$  and the respective standard deviation of the estimate obtained from the product  $1000 \cdot (\hat{V}[p_{ijk}])^{0.5}$ .

#### 2.4. *In vitro* incubations and related quantitative interpretation

We obtained cumulative gas production profiles through *in vitro* anaerobic incubations (Hall and Mertens, 2008, 2012). The gas pressures generated from the onset of fermentation were recorded by manometric readings (0-7 psi manometer, 0.05-psi increments), as well as the volumes displaced within a graduated pipette (0-25 mL, 0.1-mL increments) by the pressurized gas diverted into the pipette using a three-way valve. Pressure and volume readings were taken at initial equilibrium (zero time) and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, 48, 72, and 96 h after rumen inoculum addition. We collected the inoculum from three mature sheep (wethers with approximately 60 kg live weight) fitted with permanent rumen cannulas (Protocol 207, revised as protocol 308 by the Institutional Committee on Ethics of Animal Use). The inoculum donor animals were fed a TMR diet of corn silage and concentrate (2:1, DM basis) at 2X the maintenance energy needs computed from NRC (2007). We obtained the cumulative volume readings of fermentation gases by summing actual volume readings throughout measurement times. The gas volume readings were standardized as mL·dg<sup>-1</sup> of DM from the incubated sample (Malafaia et al., 1999; Abreu et al., 2014).

The general structure attributed to the nonlinear model used to quantitatively explain the cumulative gas production profiles were  $V_t = \mu_{y_t} + e_t$ , with  $V_t \sim \text{Normal}(\mu_{y_t}, \sigma_{y_t}^2)$ . The mean function  $\mu_{y_t}$  is described as follows:

$$\mu_{y_t} = V_{f1}(1 - \exp(-\kappa_1 t)) + V_{f2}(1 - (\delta^N \exp(-\kappa t) + \exp(-\lambda t) \sum_{i=1}^{N-1} (1 - \delta^{N-i}) (\lambda t)^i / i!)), \text{ and} \quad (8)$$

$$\mu_{y_t} = V_{f1}(1 - \exp(-\kappa_1 t)) + V_{f2}(1 - \exp(-\gamma t) \sum_{i=0}^N (\gamma t)^i / i!) \quad (9)$$

Equations 8 and 9 are dual-pool models formed by the combination of the monomolecular and GNG1 models (M-GNG1), and M-GN as a combination of the monomolecular and GN models (Matis et al., 1989; Ellis et al., 2005; Vieira et al., 2008a). In equation 8, parameters  $V_{f1}$  and  $V_{f2}$  are the asymptotic gas volumes (mL·dg<sup>-1</sup>) produced by rapidly and slowly degradable fractions;  $\kappa_1$  is the fractional rate (h<sup>-1</sup>) of the rapidly fermentable fraction, and  $\kappa$  is the rate (h<sup>-1</sup>) of slowly fermentable fraction of the sample DM. Parameter  $N$  is a positive integer that represents the order of time dependency,  $\lambda$  is the

asymptotic rate ( $\text{h}^{-1}$ ) of preparation for digestion of the insoluble and slowly degradable substrate, and constant  $\delta = \lambda(\lambda - \kappa)^{-1}$  (Vieira et al., 2008a, 2012). In equation 9,  $V_{f1}$ ,  $V_{f2}$ , and  $\kappa_1$  have the same biological meanings as in equation 8, excepting parameter  $\gamma$  ( $\text{h}^{-1}$ ), which represents an asymptotic rate of substrate fermentation. We challenged the conventional homoscedasticity assumption for fitting both nonlinear models using the nlme function of the R statistical package and modeled variances as follows (Pinheiro and Bates, 2000; Pinheiro et al., 2017):

$$\sigma_{y_t}^2 = \sigma^2, \text{ or} \quad (10)$$

$$\sigma_{y_t}^2 = \sigma^2 |\mu_{y_t}|^{2\psi}. \quad (11)$$

Parameter  $\sigma^2$  is the homogeneous and true residual variance scaled or not by the expected  $\mu_{y_t}$  value to the power  $\psi$ . We also had to challenge the assumption about independence among residuals, because gas readings are repeated measures over time for each flask. Therefore, we fitted equation 8 or 9 with equations 10 or 11, and with a function (corCAR1) that accounted for a first-order autoregressive correlation ( $\varphi$ ) structure of nlme. The required condition to run the analysis was grouping data (groupedData object) using the following formula:

$$V \sim \text{Time|gbl}. \quad (12)$$

Term V corresponds to the accumulated gas production records, Time corresponds to time points when volume readings were taken for each flask, and gbl corresponds to the random effect associated to the experimental unit, that is, the plot within each block  $\times$  location intersection where corn hybrids grew. In all cases, we used the Akaike information criterion (Akaike, 1974) to evaluate the quality of fit of the models.

We determined the matrices of standardized mean Euclidean distances (ED) among hybrids based on canonical analysis considering variables Yha (as is), YDMha (DM),  $V_{f1}$  and  $V_{f2}$  ( $\text{mL} \cdot \text{d} \cdot \text{g}^{-1}$ ),  $\kappa_1$ ,  $\lambda$ , and  $\kappa$  ( $\text{h}^{-1}$ ). Those relative scale distances were dissimilarity measures for cluster analysis of hybrids according to the Ward method, and we used the NbClust function of R to determine the best number of clusters (Charrad et al., 2014).

### 3. Results

The Normal (Gaussian) distribution was valuable to interpret fresh forage, DM, and grain yields of corn hybrids in the two locations (Table 2). There was no hybrid  $\times$  location effect for those variables, as well as no location effects, but hybrids differed significantly. Fresh forage (Yha, as is) and grain (Ygha, as is) yields presented considerable block variations within location and heteroscedastic residual variations. Block variation was also important for YDMha and YgDMha, but homogeneous variances were more likely to describe forage and grain DM yields. In addition, Yha, YDMha, Ygha, and YgDMha differed significantly among hybrids, but location and hybrid  $\times$  location interaction did not affect those yields (Table 2).

The hybrid effect was significant over the yields of chemical components (DM basis), namely aNDFomYha, CPYha, NFCYha, and Lignin(sa)Yha, whereas location and hybrid  $\times$  location interaction did not affect chemical component yields. Block and homoscedastic residual variations were more likely for aNDFom, CP, and Lignin(sa) yields; however, the NFC yield presented block and heteroscedastic variances for the location effect (Table 2).

Beta distribution was most suited to describe the YgDMha:YDMha ratio, and the fit of equations 1-5 revealed that block variation was considerable (Table 2). Similarly, Beta distribution was the most suitable for the quantitative description of the chemical components. The model fitting allowed us to identify the significant effect of hybrid  $\times$  location interaction on DM content, and it was necessary to add parameter  $\hat{\sigma}_{j(k)}$ . Fixed effects (hybrid and location) did not significantly influence CP and aNDFom levels, but these chemical components showed heteroscedastic residual variances for each location ( $\hat{\sigma}_{k=1}$  and  $\hat{\sigma}_{k=2}$ ). Block variation was important for the quantitative description of the other chemical composition variables, including Lignin(sa):aNDFom ratio. According to the adopted statistical criteria,

the fixed effects (hybrid and location) did not influence chemical components. The exception was the significant effect of hybrid over the ASH content (Table 2).

The best model for describing *in vitro* gas production profiles resulted from the monomolecular phase with the sigmoid phase G6G1 combination. A random effect was ascribed to the diagonal matrix (D) with parameters  $V_{f1}, k_1, V_{f2}, \lambda$ , and  $\kappa$ , a correlation between repeated measures over time ( $\varphi$ ), and the variance power of the mean ( $\psi$ ). As a result, 248 parameters were estimated. Due to the high probability for this solution, we can consider that it was the best model solution, given the data (Table 3). In addition, this model contained the hybrid  $\times$  location interaction effects, which yielded feasible gas production parameter estimates (Table 4). In fact, despite the absence of the interaction effect over the chemical composition, there was a detectable interaction effect over the fermentation parameters (Table 4).

The cluster analysis allowed gathering information about fresh and DM yields, as well as on biological fermentation parameters of gas production from *in vitro* incubations (Table 5). We identified three main clusters, and each cluster grouped together hybrids that grew at different locations (Figure 1 and Table 4), even when no significant effects for fresh and DM yields were detected for individual variables (Table 2).

The cluster analysis identified similar treatment (hybrids and locations) groups, and we computed least-squares means for each group (Table 5). One can note that the main dissimilar variables are those related to fresh and DM yield, which characterizes each group based on disjoint confidence

**Table 2** - P-values regarding fixed effects of corn hybrids ( $\alpha_i$ ), locations ( $\beta_k$ ), their interaction ( $\alpha\beta_{ik}$ ), and estimates for variance components ( $\hat{\sigma}_{j(k)}$  as SD for blocks within locations, residual SD for each location  $\hat{\sigma}_{k=1}$  or  $\hat{\sigma}_{k=2}$ ,  $\hat{\phi}$  as a scale parameter for the Beta distribution, and homoscedastic residual SD as  $\hat{\sigma}$ ) for yields and chemical composition

Variable <sup>a,b</sup>	Distribution	P-value for fixed effects			Variance component
		$\alpha_i$	$\beta_k$	$\alpha\beta_{ik}$	
Yha (as is)	Normal	<0.001	0.057	0.057	$\hat{\sigma}_{j(k)} = 576.1, \hat{\sigma}_{k=1} = 6680.4, \hat{\sigma}_{k=2} = 5468.6$
YDMha (DM)	Normal	0.005	0.458	0.865	$\hat{\sigma}_{j(k)} = 435.7, \hat{\sigma} = 2252.5$
Ygha (as is)	Normal	0.016	0.041	0.772	$\hat{\sigma}_{j(k)} = 263.9, \hat{\sigma}_{k=1} = 1551.4, \hat{\sigma}_{k=2} = 1157.8$
YgDMha (DM)	Normal	0.012	0.069	0.884	$\hat{\sigma}_{j(k)} = 189.9, \hat{\sigma} = 787.4$
aNDFomYha (DM)	Normal	0.003	0.370	0.697	$\hat{\sigma}_{j(k)} = 257.9, \hat{\sigma} = 1148.3$
CPYha (DM)	Normal	0.001	0.821	0.673	$\hat{\sigma}_{j(k)} = 25.7, \hat{\sigma} = 157.3$
NFCYha (DM)	Normal	0.001	0.619	0.862	$\hat{\sigma}_{j(k)} = 34, \hat{\sigma}_{k=1} = 115.4, \hat{\sigma}_{k=2} = 93.2$
Lignin(sa)Yha (DM)	Normal	0.011	0.394	0.639	$\hat{\sigma}_{j(k)} = 27.0, \hat{\sigma} = 79.2$
Ygha/Yha (as is)	Beta	<0.001	0.076	<0.001	$\hat{\sigma}_{j(k)} = 0.0214, \hat{\phi} = 361.04$
YgDMha/YDMha (DM)	Beta	<0.001	0.007	0.007	$\hat{\sigma}_{j(k)} = 0.0320, \hat{\phi} = 224.63$
DM	Beta	<0.001	0.009	<0.001	$\hat{\sigma}_{j(k)} = 0.0037, \hat{\phi} = 472.91$
CP (DM)	Beta	0.297	0.445	0.961	$\hat{\sigma}_{k=1} = 0.012, \hat{\sigma}_{k=2} = 0.006, \hat{\phi} = 1590.54$
aNDFom (DM)	Beta	0.066	0.577	0.036	$\hat{\sigma}_{k=1} = 0.054, \hat{\sigma}_{k=2} = 0.018, \hat{\phi} = 312.12$
Lignin(sa) (DM)	Beta	0.016	0.209	0.021	$\hat{\sigma}_{j(k)} = 0.010, \hat{\phi} = 2721.11$
Lignin(sa)/aNDFom (DM)	Beta	0.016	0.172	0.376	$\hat{\sigma}_{j(k)} = 0.010, \hat{\phi} = 138.49$
CF (DM)	Beta	0.031	0.364	0.130	$\hat{\sigma}_{j(k)} = 0.008, \hat{\phi} = 3473.51$
Ash (DM)	Beta	0.001	0.268	0.440	$\hat{\sigma}_{j(k)} = 0.011, \hat{\phi} = 2624.7$
NFC (DM)	Beta	0.290	0.753	0.164	$\hat{\sigma}_{j(k)} = 0.052, \hat{\phi} = 253.36$

<sup>a</sup> Yields ( $\text{kg}\cdot\text{ha}^{-1}$ ) of forage matter (Yha), grain mass (Ygha), insoluble fiber (aNDFomYha); crude protein (CPYha), nonfibrous carbohydrates (NFCYha), and lignin (Lignin(sa)Yha).

<sup>b</sup> Ratios and chemical component fractions presented as  $\text{kg}\cdot\text{t}^{-1}$  or  $\text{g}\cdot\text{kg}^{-1}$  but statistically analyzed as dimensionless proportions: grain to forage ratio (Ygha:Yha), dry matter (DM), insoluble fiber (aNDFom), lignin (Lignin(sa)), lignin to insoluble fiber ratio (Lignin(sa):aNDFom), crude fat (CF), ash, and nonfibrous carbohydrates (NFC).

**Table 3** - Estimate of the negative logarithm of the likelihood function ( $-\ell_r$ ) and derived information criteria<sup>a</sup> for the best fitted one-compartment (GN), and double-compartment (GNG1) nonlinear mixed effects models

Effect	Model <sup>b</sup>	Random <sup>b</sup>	Variance <sup>c</sup>	$-\ell_r$	$AICc_r^a$	$\Delta_r^a$	$w_r^a$	$ER_r^a$	$\theta_r^a$
General	M-G1G1	No effect	Homog	5871.0	11754.1	10623.1	$\rightarrow 0$	$\rightarrow \infty$	6
	M-G6G1	No effect	Homog	5852.9	11717.8	10586.8	$\rightarrow 0$	$\rightarrow \infty$	6
	M-G6G1	No effect	VP	4085.4	8184.8	7053.8	$\rightarrow 0$	$\rightarrow \infty$	7
	M-G6G1	No effect	CORR	2874.3	5762.6	4631.6	$\rightarrow 0$	$\rightarrow \infty$	7
	M-G6G1	No effect	VP, CORR	1437.2	2890.4	1759.4	$\rightarrow 0$	$\rightarrow \infty$	8
$\beta_k$	M-G6G1	No effect	VP, CORR	1185.5	2397.2	1266.2	$1.1 \cdot 10^{-275}$	$9.0 \cdot 10^{274}$	9
$\alpha_i$	M-G6G1	$\kappa$	VP, CORR	918.5	2095.2	964.2	$4.2 \cdot 10^{-210}$	$2.4 \cdot 10^{209}$	124
	M-G6G1	$D(V_{\beta_1}, k_1, V_{\beta_2}, \lambda, \kappa)$	VP, CORR	575.7	1418.1	287.1	$4.5 \cdot 10^{-63}$	$2.2 \cdot 10^{62}$	128
$\alpha\beta_{ik}$	M-G6G1	$D(V_{\beta_1}, k_1, V_{\beta_2}, \lambda, \kappa)$	VP, CORR	296.5	1131	0.0	$\cong 1$	$\cong 1$	248
	M-G1	$D(V_{\beta_1}, k_1, V_{\beta_2}, \lambda, \kappa)$	VP, CORR	604.6	1634	503	$6.0 \cdot 10^{-110}$	$1.7 \cdot 10^{109}$	199
	M-G2	$D(V_{\beta_1}, k_1, V_{\beta_2}, \lambda, \kappa)$	VP, CORR	567.2	1559	428	$1.2 \cdot 10^{-93}$	$8.7 \cdot 10^{92}$	199
	M-G3	$D(V_{\beta_1}, k_1, V_{\beta_2}, \lambda, \kappa)$	VP, CORR	675.8	1776.2	645.2	$7.9 \cdot 10^{-141}$	$1.3 \cdot 10^{140}$	199

<sup>a</sup>  $AICc_r$  - Akaike information criterion corrected for small samples;  $\Delta_r$  - Akaike difference;  $w_r$  - model probability;  $ER_r$  - evidence ratio; and number of parameters ( $\theta_r$ ) of the  $r$ -th model combination.

<sup>b</sup> Model combinations described by the fixed part of the model, i.e., monomolecular combined with GN or GNG1 equations, followed by the parameter(s) to which random effects were ascribed with a diagonal ( $D$ ) variance-covariance structure if applied to two or more parameters.

<sup>c</sup> The homoscedastic variance (Homog) and residual independence correspond to the traditional assumptions for fitting a given model. Variance functions: VarPower (VP), and correlation (CORR).

intervals. Regarding the fermentative potential, it is not possible to discriminate parameters related to gas production with the same criteria. Therefore, qualitatively, the evaluated hybrids showed similar characteristics in terms of the potential nutritive value, but differed in total forage yield.

The group with greater fresh and DM forage yields (group 1, Table 5) contains hybrids recommendable for both locations, for example, hybrids 6, 7, 17, and 24. Hybrid 20 and the other hybrids of group 1 did not show consistent performances between the two locations. The other two groups contain hybrids with lower fresh and DM forage yields (Figure 1 and Table 5).

#### 4. Discussion

Corn genetic improvement can enhance its agronomic performance and nutritional qualities for silage production (Roth et al., 1970; Deinum and Bakker, 1981; Crevelari et al., 2019; Pereira et al., 2020). Some results indicate that environmental factors, such as agronomic factors that change grain:stover ratio, do not greatly influence the final digestibility of the corn silage, nor improve ensiling characteristics, but the factors affecting yields and economic returns may be worth investigating (Russell et al., 1992). Nonetheless, the development of corn hybrids with higher rumen OM degradability or fermentability becomes essential for producing high-quality forage for feeding ruminants (Philippeau and Michalet-Doreau, 1997; Taylor et al., 2005a,b,c), particularly if we consider the importance of fiber quality and starch concentration and digestibility in the forage mass produced under humid tropical conditions. Low-altitude areas represent an additional challenge because of their warmer nights, when metabolism and respiration of the forage plant do not slow down and reduce starch and other NFC accumulation (Van Soest, 1994). Environmental conditions generated by year-to-year variations exert greater influence over the fibrous (aNDFom) content of the whole-corn crop than genotypes do (Kruse et al., 2008). This is important because both corn silage concentration and aNDFom quality affect intake (Oba and Allen, 1999; Tjardes et al., 2002; Huhtanen et al., 2008a; Vieira et al., 2008b, 2020). Because productive animals generally have an increased demand for nutrients, including fiber for rumen health (Van Soest, 1994), the selection of corn hybrids with higher nutritional values can provide more energy that is useful to the animal (digestible and metabolizable energies), especially when the aNDFom content constrains intake (Van Soest, 1994; Mertens, 2010; Akins and Shaver, 2014;

**Table 4 - Estimates of gas production parameters<sup>a</sup> (with standard errors within parentheses) for each genotype (Gen) × location interaction**

Gen	Location 1 (Campos dos Goytacazes)					Location 2 (Itaocara)				
	$V_{H1}$	$\kappa_1$	$V_{F2}$	$\lambda$	$\kappa$	$V_{H1}$	$\kappa_1$	$V_{F2}$	$\lambda$	$\kappa$
1	15.2(2.03)	0.137(0.0338)	17.0(1.85)	0.740(0.1604)	0.026(0.0051)	14.7(2.03)	0.148(0.0339)	19.4(1.92)	0.833(0.2701)	0.012(0.0050)
2	15.7(2.03)	0.134(0.0337)	17.4(1.84)	0.712(0.1538)	0.023(0.0050)	14.7(2.00)	0.110(0.0334)	21.4(2.10)	3.117(2.0605)	0.006(0.0049)
3	14.6(2.00)	0.139(0.0338)	17.2(1.77)	0.756(0.1550)	0.027(0.0051)	9.5(2.00)	0.229(0.0371)	17.5(1.74)	1.035(0.2077)	0.030(0.0054)
4	15.7(2.01)	0.136(0.0337)	16.4(1.78)	0.726(0.1537)	0.027(0.0051)	15.7(2.03)	0.112(0.0335)	15.2(1.89)	1.097(0.4000)	0.013(0.0050)
5	14.7(1.97)	0.148(0.0337)	16.3(1.74)	0.621(0.1482)	0.027(0.0051)	12.6(2.04)	0.144(0.0345)	18.0(1.87)	0.892(0.2471)	0.018(0.0052)
6	16.4(1.98)	0.126(0.0336)	17.0(1.76)	0.567(0.1495)	0.024(0.0050)	13.0(2.03)	0.139(0.0339)	14.8(1.79)	0.927(0.2209)	0.024(0.0055)
7	15.2(2.01)	0.131(0.0337)	17.7(1.78)	0.750(0.1554)	0.025(0.0050)	12.4(2.03)	0.112(0.0336)	15.2(1.81)	2.432(0.7646)	0.023(0.0054)
8	12.6(1.98)	0.149(0.0339)	18.1(1.75)	0.889(0.1650)	0.028(0.0051)	10.2(2.01)	0.194(0.0363)	17.5(1.78)	0.811(0.1954)	0.023(0.0055)
9	16.0(2.02)	0.137(0.0338)	16.2(1.79)	0.753(0.1569)	0.027(0.0051)	12.0(2.03)	0.184(0.0350)	17.7(1.79)	0.892(0.2042)	0.025(0.0054)
10	16.8(2.06)	0.130(0.0341)	15.4(1.85)	0.732(0.1886)	0.025(0.0051)	14.0(2.04)	0.148(0.0343)	16.2(1.87)	0.839(0.2543)	0.019(0.0053)
11	17.4(2.02)	0.129(0.0337)	15.5(1.80)	0.683(0.1516)	0.024(0.0052)	14.4(2.03)	0.118(0.0336)	13.7(1.83)	0.987(0.2725)	0.020(0.0054)
12	16.2(2.04)	0.135(0.0338)	16.5(1.84)	0.731(0.1561)	0.020(0.0054)	9.6(1.99)	0.168(0.0347)	17.7(1.76)	1.079(0.2271)	0.022(0.0052)
13	15.2(2.00)	0.137(0.0337)	17.2(1.77)	0.735(0.1547)	0.024(0.0050)	11.2(2.33)	0.179(0.0377)	16.0(2.07)	0.849(0.2281)	0.027(0.0060)
14	15.7(2.01)	0.128(0.0338)	16.8(1.80)	0.672(0.1505)	0.027(0.0060)	11.4(2.02)	0.192(0.0353)	17.2(1.77)	1.008(0.2279)	0.024(0.0054)
15	14.8(2.00)	0.141(0.0338)	17.9(1.77)	0.756(0.1549)	0.026(0.0050)	7.3(1.98)	0.283(0.0412)	20.0(1.74)	1.262(0.2141)	0.034(0.0054)
16	12.4(2.02)	0.150(0.0339)	16.8(1.79)	1.561(0.13263)	0.031(0.0054)	12.5(2.02)	0.154(0.0342)	17.6(1.83)	0.814(0.2095)	0.019(0.0052)
17	14.9(2.00)	0.140(0.0338)	18.1(1.77)	0.753(0.1551)	0.025(0.0050)	13.7(2.04)	0.167(0.0345)	16.2(1.83)	0.705(0.1932)	0.021(0.0054)
18	14.6(2.00)	0.142(0.0338)	16.5(1.76)	0.731(0.1529)	0.029(0.0052)	13.0(2.03)	0.171(0.0346)	17.0(1.81)	0.816(0.2065)	0.022(0.0053)
19	15.6(2.03)	0.137(0.0338)	17.8(1.99)	0.777(0.1675)	0.021(0.0050)	12.4(2.04)	0.149(0.0342)	16.0(1.85)	0.954(0.2505)	0.020(0.0057)
20	14.6(2.00)	0.143(0.0338)	17.1(1.76)	0.741(0.1532)	0.028(0.0051)	12.6(2.07)	0.119(0.0337)	17.2(1.86)	4.310(1.3763)	0.024(0.0056)
21	12.4(1.97)	0.167(0.0341)	17.3(1.73)	0.789(0.1551)	0.031(0.0053)	9.1(2.28)	0.190(0.0395)	17.2(1.99)	1.106(0.2621)	0.027(0.0059)
22	15.9(2.02)	0.129(0.0337)	16.7(1.79)	0.719(0.1529)	0.026(0.0051)	2.2(2.02)	0.166(0.0345)	17.7(1.79)	0.943(0.2093)	0.024(0.0053)
23	15.5(2.01)	0.135(0.0337)	16.5(1.78)	0.727(0.1545)	0.025(0.0050)	15.1(2.03)	0.128(0.0336)	17.6(1.91)	0.839(0.2706)	0.013(0.0050)
24	14.8(2.00)	0.134(0.0337)	18.4(1.80)	0.753(0.2706)	0.025(0.0050)	11.5(2.02)	0.174(0.0348)	17.1(1.77)	0.878(0.1882)	0.028(0.0055)

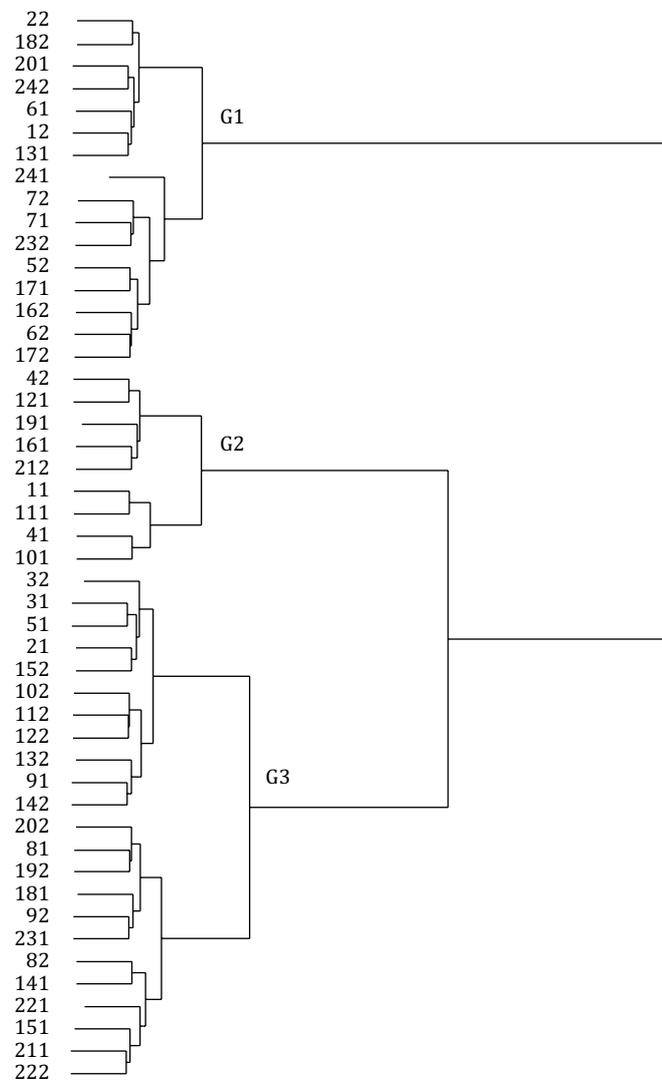
<sup>a</sup> Standard deviations of random effects (0.95 confidence intervals within brackets) of experimental units associated to each parameter of the monomolecular-G6G1 model:  $\sigma_{V_{H1}} = 3.6$  (2.2, 6.0);  $\sigma_{\kappa_1} = 0.063$  (0.038, 0.105);  $\sigma_{V_{F2}} = 3.1$  (1.8, 5.1);  $\sigma_{\lambda} = 0.271$  (0.158, 0.467);  $\sigma_{\kappa} = 0.009$  (0.006, 0.015); and the correlation  $\rho = 0.868$  (0.848, 0.886), power exponent  $\psi = 0.510$  (0.485, 0.534), and residual standard error  $\sigma = 0.20$  (0.18, 0.22).

**Table 5** - Least-squares means of yields and fermentation parameters related to the groups arranged after cluster analysis (similar predicted performances for hybrid × location were grouped by Euclidean distances)

Variable <sup>a,b</sup>	Group		
	G1	G2	G3
Yha (as is)	37.9±0.62 (36.7, 39.2)	26.3±0.47 (25.4, 27.2)	31.6±0.39 (30.8, 32.4)
YDMha (DM)	13.3±0.25 (12.7, 13.8)	10.1±0.19 (9.7, 10.5)	11.7±0.16 (11.3, 12.0)
$V_{f1}$	15±0.9 (13, 17)	14±0.3 (13, 15)	13±0.5 (12, 14)
$\kappa_1$	0.140±0.0071 (0.125, 0.154)	0.141±0.0047 (0.132, 0.151)	0.160±0.008 (0.144, 0.176)
$V_{f2}$	16±0.4 (16, 17)	17±0.3 (17, 18)	17±0.3 (16, 18)
$\lambda$	0.9±0.10 (0.7, 1.1)	1.0±0.17 (0.7, 1.4)	1.0±0.15 (0.7, 1.3)
$\kappa$	0.024±0.0017 (0.021, 0.028)	0.021±0.0015 (0.018, 0.024)	0.026±0.0007 (0.024, 0.027)

<sup>a</sup> Variables regarding fresh forage mass (as is) and dry matter (DM) yields ( $t \cdot ha^{-1}$ ).

<sup>b</sup> Kinetic variables of cumulative gas production: gas volume produced from the rapidly fermented fraction ( $V_{f1}$ ,  $mL \cdot dg^{-1}$  DM) and its fractional fermentation rate ( $\kappa_1$ ,  $h^{-1}$ ), gas volume produced from the slowly fermented fraction ( $V_{f2}$ ,  $mL \cdot dg^{-1}$  DM), rate of preparation of the slowly fermentable fraction for digestion and fermentation ( $\lambda$ ,  $h^{-1}$ ), and effective fermentation rate of the slowly fermentable fraction ( $\kappa$ ,  $h^{-1}$ ).



The corresponding yields of the groups are listed on Table 5.  
Location 1 represents Campos dos Goytacazes and location 2 represents Itaocara.

**Figure 1** - Hierarchical clustering of hybrids (1 to 24, numbers on the left) × location (1 to 2, numbers on the right) combinations forming the three groups (G1, G2, and G3).

Allen, 2014). Oba and Allen (1999) demonstrated that an increase in one unit of fiber digestibility results in an increase of  $0.17 \text{ g} \cdot \text{d}^{-1}$  in DM intake and an increase of  $0.23 \text{ kg} \cdot \text{d}^{-1}$  in milk yield.

We observed a significant genetic variation about the potential DM digestibility of the studied corn hybrids, as revealed by the *in vitro* gas production technique and the need for a nonlinear model that accounted for the hybrid  $\times$  location interaction effect for a better description of gas production profiles. This result may be attributable to the corn variability studied by our research group (Crevelari et al., 2017, 2018, 2019). The variable digestibility relates to the quality of the fibrous fraction because of somewhat different environmental conditions, as mentioned before (Van Soest, 1994). The year-to-year variation interacts with the genotype, and the interaction affects the fibrous content of the final whole-crop corn; temperature and radiation might be major determinant factors that explain a greater proportion of variability about fiber and lignin contents at harvest for corn silage production, with soil water playing a minor influence over those response variables (Kruse et al., 2008). Different growing conditions from place to place favor genetic variations among corn hybrids used for silage production, mainly because of temperature, radiation, and water supply, but the *in vitro* digestibility amplitude for corn silages under temperate and snow environments ranging from Mediterranean to Scandinavian conditions is about 647 to  $814 \text{ g} \cdot \text{kg}^{-1}$  DM, regardless of the hybrid cultivated (Deinum, 1988). We did not detect effects of hybrids and locations over the Lignin(sa) and aNDFom levels; differences in rainfall were remarkable (data not shown), but average temperature differences between locations from Nov/2013 to Mar/2014 were not ([https://www.agritempo.gov.br/agritempo/jsp/PesquisaClima/index.jsp?siglaUF=RJ&lang=pt\\_br](https://www.agritempo.gov.br/agritempo/jsp/PesquisaClima/index.jsp?siglaUF=RJ&lang=pt_br), accessed on 11/13/2020).

Differences regarding feed efficiency for milk production from cows fed silage made with nutrient-dense, brown midrib, or dual-purpose corn hybrids are difficult to detect for some corn types cultivated in America; nonetheless, the phenotypic variation about fiber digestibility correlates to fiber quality, e.g., low lignin contents in brown midrib corn hybrids (Akins and Shaver, 2014). These are further evidences that the relationship between dietary aNDFom content and its digestibility is not constant (Van Soest, 1967; Huhtanen et al., 2006), and measures of fiber digestibility are necessary for predicting animal performance (Van Soest et al., 1992; Oba and Allen, 1999; Huhtanen et al., 2008b; Vieira et al., 2008b, 2020). However, we warn the readers that we biased our fiber results to some extent, because at the time we performed the fiber analyses, there were no Berzelius beakers without spouts in our laboratory (Silva et al., 2018). We presently corrected this fault by asking our lab suppliers to produce Berzelius beakers without spouts as requested by reference methods for measuring insoluble fiber and lignin in feeds and fibrous foods (Goering and Van Soest, 1970; Mertens, 2002; Möller, 2009).

The variations about corn DM and aNDFom digestibilities among different genetic materials concur for different animal performances (Roth et al., 1970; Deinum and Bakker, 1981; Dhillon et al., 1990). Digestibility of silages made with different corn hybrids also vary according to cropping conditions observed in different environments (Deinum, 1988). Voluntary intake and digestibility are related to the kinetic parameters of gas production (Menke et al., 1979; Huhtanen et al., 2008a,b); therefore, fermentation characteristics may be useful attributes to discriminate potential nutritive traits for clustering corn hybrids into distinct groups, as observed for sugarcane varieties (Freitas et al., 2006). However, we did not detect important differences among fermentation parameters, despite the necessary use of a 248-parameter-model as the best solution to account for the genotype  $\times$  environment interaction.

Unlike neutral detergent solubles, which behave uniformly (Van Soest, 1967; Van Soest et al., 1992; Huhtanen et al., 2006), fiber is a heterogeneous physicochemical feed fraction (Van Soest, 1967; Robinson et al., 1986; Schofield et al., 1994; Huhtanen et al., 2006; Vieira et al., 2008a,b; 2012; Regadas Filho et al., 2014). Nonetheless, only one parameter ( $\kappa$ ) represented the gas production rate of the insoluble, but potentially digestible fraction in our study. Huhtanen et al. (2008a) associated this rate to the fibrous fraction (aNDFom). This parameter and the asymptotic volume ( $V_{p2}$ ) may be of use as measures to select hybrids with an improved quality of the fibrous fraction (greater potential and faster digestibilities). Therefore, the goal for silage corn selection may be to achieve an increased OM

digestibility of the corn forage mass through changes in the vegetative part (Oba and Allen, 1999; Akins and Shaver, 2014), or by improving grain characteristics that favor its fermentation in the ruminoreticulum (Ferraretto and Shaver, 2015).

Another important feature for choosing corn hybrids for silage production is the quantity and proportion of grains; a higher grain proportion in the ensiled mass means higher metabolizable and net energy contents for maintenance and productive functions (Woody et al., 1983; Akins and Shaver, 2014). Our research group predicted the genetic potential of corn hybrids for the Northern and Northwestern Rio de Janeiro State and found hybrids with high heritabilities for forage mass ( $h^2 = 0.69$ ) and grain ( $h^2 = 0.51$ ) yields in the whole crop mass at the harvesting point for silage production (Crevelari et al., 2019).

Animal scientists always face the challenge to improve the use of corn by animals, because genetic differences between corn hybrids concur for different starch digestibilities in the ruminoreticulum. The most important differences rely on the endosperm vitreousness (Philippeau et al., 1999). Differences regarding vitreous and floury endosperms in grains are crucial to ruminal starch digestibility. Those differences depend on the encapsulating degree of starch granules by a protein matrix (Kotarski et al., 1992), which determines the grain vitreousness (Lopes et al., 2009). The amount of the amino acid proline in the protein matrix of the corn grain (Hamaker et al., 1995) is another potential inhibitor of protein degradation by microbial enzymes (Yang and Russell, 1992), which might explain the reduced starch digestibility for some corn hybrids. Vitreousness is negatively associated with starch degradation in the rumen (Correa et al., 2002; Taylor and Allen, 2005a,b; Corona et al., 2006; Nkonyamo-Majee et al., 2008; Lopes et al., 2009). The presence of a protein matrix encompassing starch granules makes them densely packed and less susceptible to microbial digestion (Kotarski et al., 1992). Pereira et al. (2004) found 44% of vitreous endosperms in hybrids sold in the Brazilian market classified as dented corn. Correa et al. (2002) and Philippeau and Michalet-Doreau (1997) found 43 and 48% of vitreous endosperms in dented corn hybrids cultivated in tropical and temperate environments, respectively. Nonetheless, we used only hybrids classified as dented or semi-dented corn hybrids (Crevelari et al., 2017, 2019). Our research group is currently making further comparisons among dented and flint corn hybrids (Crevelari et al., 2018), with special reference to fermentation characteristics.

In this study, we did not determine the relationship between vitreous and floury endosperms in the grains harvested from the cultivated corn hybrids. However, some hybrids showed higher yields of a gas-producing fraction with faster fermentation characteristics ( $V_{f1}$  and  $\kappa_1$ ; Table 4), which indicate a larger contribution from NFC to the fermentable OM (Beuvink and Spoelstra, 1992; Schofield et al., 1994; Schofield and Pell, 1995a,b). Whether this fermentable fraction can be associated to a higher endosperm starch availability or not is an open question, because we were not able to detect qualitative differences in this regard. In addition, it is worth remembering that we incubated the harvested and partially dried forage *in vitro*. Therefore, not only starch but also the most soluble carbohydrates were mostly preserved and contributed substantially to the measured gas production, because the NFC fraction in our study was not fermented, as partially occurs with ensiled forages (Van Soest, 1994). Nonetheless, because we applied the same procedure to all corn hybrids, we can speculate that some hybrids naturally were richer in floury endosperm, had a finer and incomplete protein matrix with empty spaces surrounding the starch granules, or had a reduced starch density (Huntington, 1997).

The microbial accessibility to the corn endosperm is essential for the use of starch granules (McAllister and Cheng, 1996). Thus, the selection of corn hybrids considering the fermentative potential and grain texture might result in a better utilization of starch by the animals. Corn hybrids present a median heritability ( $h^2 = 0.46$ ) for rumen degradability of grains and high heritabilities ( $h^2 = 0.74$ ) for stalk fiber degradability (stem, leaves, and leaf sheaths) and OM digestibility ( $h^2 = 0.80$ ), which enhance forage quality (Dolstra et al., 1992; Davide et al., 2011). Nonetheless, we remind the reader that *in vitro* fermentation records rely on finely grounded samples (Goering and Van Soest, 1970; Hall and Mertens, 2008, 2012). Therefore, limitations observed in the silage bunk (e.g., kernel processing and theoretical length of cut) are not overcome (Ferraretto et al., 2018), and inferences taken insofar may be at risk.

The cluster analysis allowed the grouping of genotypes using a combination of variables to highlight corn hybrids with the best performance according to genotypes and environmental conditions. Freitas et al. (2006) performed a similar task when they selected sugarcane varieties. In our study, forage yield was determinant for clustering, and this trait is of economic importance (Kruse et al., 2008). Therefore, we can infer that hybrids that presented higher production levels in both locations have potential for silage production, because they presented good forage yield without compromising nutritional quality, as revealed by comparable *in vitro* fermentation traits (parameters) regardless of group (Table 5). In addition, the fresh mass and DM yields indicated an increase in nutrient availability per hectare, which might be another trait of economic importance. Crevelari et al. (2017) found an expected genetic gain of 6.13% for fresh mass yield at the ensiling phase, which highlights the forage potential of the selected corn hybrids for the Northern and Northwestern regions of the Rio de Janeiro State. This study and the studies of Crevelari et al. (2017, 2019) allowed the selection (mostly based on agronomic characteristics, e.g., stay green trait) of two main hybrids that were registered at the Brazilian Ministry of Agriculture, Livestock and Supply (UENF MS2208 – no. 41705 and UENF MSV2210 – no. 41721). The regions we are referring to are typically of low-altitude areas a.s.l. with a characteristic humid tropical savanna climate (Kotteck et al., 2006; Beck et al., 2018). Nonetheless, we have to challenge those corn genotypes (Table 1) and other potential genotypes (floury and flint starch) in long-term, multi-location trials to check the consistency of results, as well as other genetic materials with divergent nutritional potential for forage and silage production.

The mean DM yield of the group that outstood the others was within 12.8 to 18.7 t·ha<sup>-1</sup>·harvest<sup>-1</sup>, as shown in some literature reports (Almeida Filho et al., 1999; Paziani et al., 2009). The sowing density may have contributed to the observed yields, because studies with a higher forage yield used between 55,000 and 58,000 plants·ha<sup>-1</sup>. In our study, the sowing density was about 50,000 plants·ha<sup>-1</sup>, which indicates the good performance of the selected corn hybrids for these two locations. Some reported experiments in America contain stands that yield DM forage mass from 19 to 28 t·ha<sup>-1</sup>·harvest<sup>-1</sup>, with a sowing density of 84,000 seeds·ha<sup>-1</sup> or stands with 66,700 plants·ha<sup>-1</sup> (Tjardes et al., 2002; Akins and Shaver, 2014).

## 5. Conclusions

The corn silage hybrids UENF-2203, UENF-2192, UENF-2193, and UENF-506-11 stand out as potential genotypes for the production of silage in the North and Northwest regions of the Rio de Janeiro State for consistently presenting, in both locations, the highest yields of fresh and dry forage matter, without compromising their *in vitro* fermentation characteristics.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: R.A.M. Vieira. Data curation: L.C.R. Bendia, F.H.V. Azevedo, D.F.D. Sant'Anna and J.A. Crevelari. Formal analysis: R.A.M. Vieira. Funding acquisition: M.G. Pereira. Investigation: L.C.R. Bendia, F.H.V. Azevedo, J.A. Crevelari and R.A.M. Vieira. Methodology: F.H.V. Azevedo, J.A. Crevelari, M.G. Pereira and R.A.M. Vieira. Project administration: M.G. Pereira and R.A.M. Vieira. Supervision: M.G. Pereira. Validation: L.C.R. Bendia and D.F.D. Sant'Anna. Writing-original draft: L.C.R. Bendia, J.G. Oliveira, M.A.R. Nogueira, L.V. Silva, E.S. Aniceto and R.A.M. Vieira. Writing-review & editing: R.A.M. Vieira.

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