



Effect of row spacing and maturity at harvest on the fermentative profile, aerobic stability, and nutritional characteristics of biomass sorghum (BRS 716) silage in the semiarid region of Brazil

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ABSTRACT - The objective was to evaluate the effects of maturity at harvest and row spacing on fermentative profile, aerobic stability, and nutritional value of biomass sorghum (BRS 716) silage. The experiment was conducted using a split-plot completely randomized block design with three row spacings (45, 70, and 90 cm) and four maturities at harvest (70, 100, 130, and 160 days) and eight replications. Polyvinyl chloride silos of known weight measuring 50 cm length and 10 cm diameter were used for silage production. Dry matter and total carbohydrate contents of the silage increased linearly, whereas crude protein and ash decreased linearly with maturity at harvest. Row spacing did not influence pH, ammoniacal nitrogen (N-NH₃), gas and effluent losses, and dry matter recovery of silage. The concentrations of malic, succinic, and acetic acids and ethanol responded quadratically to maturity at harvest. The levels of neutral detergent fiber, lignin, and indigestible neutral detergent fiber increased linearly with maturity at harvest. Ruminal degradation kinetics of dry matter of biomass sorghum silage was not influenced by row spacing. BRS 716 biomass sorghum should be planted at 70-cm row spacing and harvested at 160 days for silage production based on fermentative profile, dry matter losses, and nutritional characteristics.

Keywords: dry matter, effluents, organic acids, pH, ruminal kinetics, *Sorghum bicolor*

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) has been grown in several regions of the world for silage due to its high mass production per unit area, good nutritional value, tolerance to water deficit, pests, and diseases, and appropriate fermentation characteristics when harvested at the correct maturity. However, productivity of different cultivars and sorghum hybrids varies extensively and is a topic of research interest. Researchers aim for sorghum materials with high productivity to reduce animal feed costs, especially in areas with water deficit, such as the semiarid region (Borges et al., 2019; Monção et al., 2019, 2020).

Ensiling forage plants is a conservation technique used worldwide to correct nutrient deficiencies of ruminants in different production systems (Bernardes et al., 2018). Different forage species can be

ensiled if the factors associated with fermentative capacity (dry matter [DM] content, water-soluble carbohydrates, and buffering capacity) are adequate (Borreani et al., 2018).

Biomass sorghum cv. BRS 716, released in 2014 by EMBRAPA Maize and Sorghum, is an important alternative crop for cogeneration of energy by direct biomass burning in thermoelectric and sugarcane ethanol industries. Moreover, this sorghum cultivar has potential for ensiling and use in ruminant diets due to its high mass productivity, reaching up to 50 t DM/ha. However, according to Bernardes et al. (2018) and Monção et al. (2019, 2020), plant maturity can modify the processes involved with fermentation of the ensiled mass and nutritional value of the silage produced. Borreani et al. (2018) and Kung Jr. et al. (2018) stated that forage harvested at different maturities at harvest might reduce fiber digestibility and lose DM in the form of gases and effluents during fermentation and nutrients through proteolysis. Moreover, row spacing modifies the light-gathering capability of the plant by changing pasture production, structure, and nutritional characteristics (May et al., 2016), which can interfere with the fermentation process (i.e., DM content, non-fibrous carbohydrates) as well as the diet balancing for ruminants (i.e., fiber content and nitrogen fractions). There is no information in the literature on the ideal row spacing and maturity at harvest of sorghum BRS 716 for silage production. According to Monção et al. (2019, 2020), the intense solar radiation in the semiarid region throughout the year associated with high temperature can change plant growth pattern. Therefore, the best management practices on forage cut for silage production should be investigated. We hypothesize that there is a better maturity at harvest of biomass sorghum cv. BRS 716 with equilibrium between mass yield and nutritional value of silage.

Therefore, the objective of this study was to evaluate the fermentation profile, aerobic stability, and nutritional value of silage of biomass sorghum BRS 716 harvested at different maturities at harvest and planted in three row spacings in the semiarid region.

2. Material and Methods

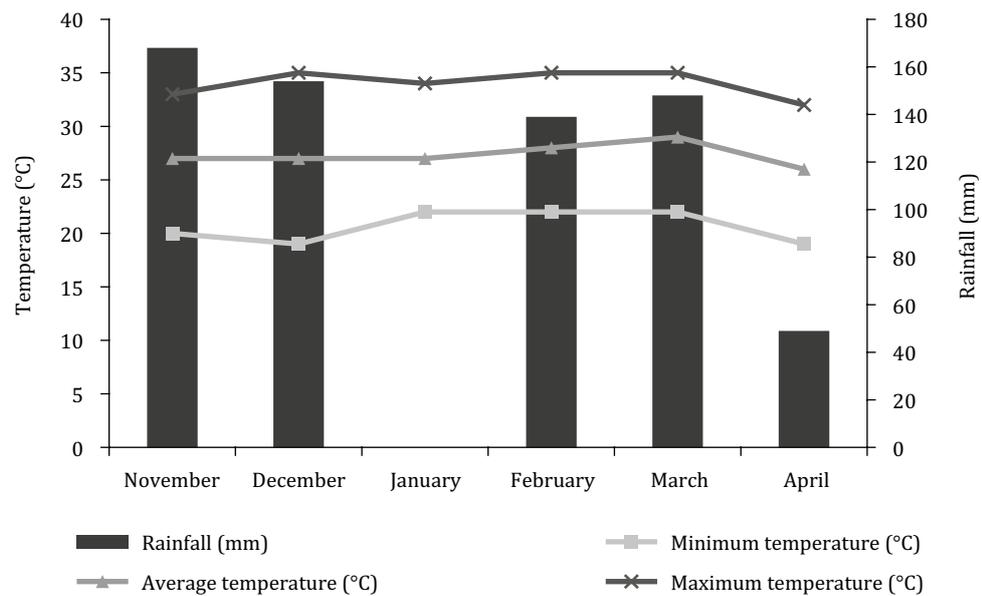
The procedures for care and handling of animals used in the experiment were in accordance with guidelines of the Brazilian College of Animal Experimentation (COBEA) and were approved by the institutional Ethics, Bioethics and Animal Welfare Committee (CEBEA) (case no. 173/2018).

The experiment was carried out in the municipality of Janaúba (15°52'38" S, 43°20'05" W), in the state of Minas Gerais, from November 13, 2018 to April 27, 2019. According to Koeppen's classification (Koeppen, 1948), the region has an Aw-type climate, with rainy summers and well-defined drought periods in winter. The mean annual rainfall is 876 mm, with an average annual temperature of 24 °C. The region has a mesothermal to megathermal tropical climate due to the altitude, and sub-humid and semiarid conditions are characterized by irregular rainfall, resulting in prolonged drought periods. Climate data during the experimental period are shown in Figure 1.

The experiment was carried out in a flat area (25 × 100 m) with biomass sorghum (*Sorghum bicolor* (L.) Moench) planted in a clayey dystrophic red-yellow latosol with the following chemical characteristics: pH in CaCl₂, 6.3; P (Mehlich), 21.2 mg dm⁻³; K (Mehlich), 110 mg dm⁻³; Na (Mehlich), 0.3 cmolc dm⁻³; Ca²⁺, 3.9 cmolc dm⁻³; Mg²⁺, 1.1 cmolc dm⁻³; Al³⁺, 0.0 cmolc dm⁻³; H + Al (0.5 mol L⁻¹ calcium acetate), 1.2 cmolc dm⁻³; sum of bases of 5.5 cmolc dm⁻³; cation exchange capacity of 6.7 cmolc dm⁻³; base saturation (V) of 82%. Soil samples were collected for analysis 70 days before planting.

The experiment was conducted using a split-plot completely randomized block design with three row spacings (45, 70, and 90 cm) and four maturities at harvest (70, 100, 130, and 160 days) and eight blocks, resulting in a total of 96 plots with 5.0 × 25.0 m each or a useful area of 3 × 15 m. Maturities at harvest were chosen due to the high growth of BRS 716 biomass sorghum, adapted from Monção et al. (2019, 2020). Row spacing was defined according to the study of May et al. (2016).

Biomass sorghum was planted in 2018 with seeds donated by Embrapa Maize and Sorghum. Before planting, the soil was prepared by plowing and harrowing (twice). During the planting phase, NPK fertilizer (4-14-08) was applied as recommended by the soil analysis for sorghum crop. Supplemental



Source: Instituto Nacional de Meteorologia (INMET, 2019).

Figure 1 - Climatic data during the experimental period.

irrigation during the experiment was given according to soil moisture level. Weeds and insects were controlled by manual weeding and insecticides applied by a tractor-mounted sprayer, respectively. The evaluation of fresh forage productivity and DM content of the ensiled material was performed after each cut for different maturity and row spacings.

At each row spacing and maturity at harvest, forage was manually harvested (25% total area) and ground using a tractor-mounted harvester model JF-90 Z10 (JF Agricultural Machinery, SP, Brazil) and a New Holland TL 75 tractor (New Holland Agriculture®, Paranavaí - PR, Brazil). Experimental PVC silos of known weight measuring 50 cm length and 10 cm diameter were used for silage production. The bottom of the silos contained 10 cm of dry sand (400 g), which was separated from the forage by foam to allow the measurement of effluents. After complete homogenization, without using microbial inoculant, the resulting material was deposited into the silos and compacted using a wooden plunger. For each treatment, silage density was quantified (550 kg of natural material m⁻³), and approximately 4 kg of the chopped fresh forage was ensiled as recommended by Ruppel et al. (1995). After filling with forage, silos were closed with PVC lids fitted with Bunsen-type valves, sealed with adhesive tape, and weighed. The silos were stored at room temperature and opened 65 days after ensiling.

Dry matter losses in the form of gases and effluents were quantified by differences in weight according to Jobim et al. (2007). Effluent losses were calculated according to equation 1, as follows:

$$E = (Wop - SWen) / (GREM) \times 1000, \quad (1)$$

in which E = effluent production (kg/t of green mass), Wop = set weight (full bucket + lid + wet sand + foam) at silo opening (kg), SWen = set weight (full bucket + lid + dry sand + foam) at the time of ensiling (kg), and GRME = green forage mass ensiled (kg).

Gas losses (G; % DM) were calculated according to equation 2:

$$G = [(Wen - SWen) * DMen] - [(Wop - SWen) * DMop] \times 100 / [(Wen - SWen) * DMen], \quad (2)$$

in which Wen = weight of the full bucket at ensiling (kg), SWen = set weight (empty bucket + lid + dry sand + bag) at ensiling (kg), DMen = forage DM at ensiling, Wop = weight of the full bucket at silo opening (kg), and DMop = forage DM content at silo opening. The DM recovery for each silo was

calculated based on the initial and final weights and DM contents of forages and silages, according to Jobim et al. (2007).

Plastic buckets containing 2.0 kg of silage sampled from each mini silo were placed in a room at ambient temperature (24.5–25.5 °C) to evaluate aerobic stability. Silage temperature was monitored every 30 min with the aid of a temperature data logger inserted into the center of mass. Ambient temperature was also measured every 30 min with the aid of a data logger placed near the buckets. Aerobic stability was calculated as the time taken by silage upon exposure to air to show a 2 °C increase in temperature above room temperature (Moran et al., 1996).

The silage juice was analyzed for pH, ammoniacal nitrogen (N-NH₃), and organic acids (Pryce, 1969). The pH was measured using a potentiometer (DM-22, Digimed, São Paulo, SP, Brazil), and ammoniacal nitrogen (N-NH₃) was determined according to technique described by Noel and Hambleton (1976). Volatile fatty acid contents were estimated by gas chromatography-mass spectrometry (GCMS; GCMS QP 2010 plus, Shimadzu®, Kyoto, Japan) with a capillary column (Stabilwax, Restek®, Bellefonte, USA; 60 m, 0.25 mm ø, 0.25 µm crossbond carbowax polyethylene glycol) according to the manufacturer's recommendations.

Silage samples were oven-dried at 55 °C. A portion of the pre-dried material was ground in a Willey knife mill to pass a 1-mm screen for chemical composition analysis, and remaining samples were ground to pass a 2-mm screen for the *in situ* degradability assay. Samples were analyzed for DM (INCT-CA G-001/1 and G-003/1), crude protein (INCT-CA N-001/1), ether extract (INCT-CA G-005/1), ash (INCT-CA M-001/1), neutral detergent fiber (NDF; INCT-CA F-002/1), and acid detergent fiber (INCT-CA F-003/1); indigestible neutral detergent fiber (iNDF; INCT-CA F-008/1) with corrections for ash (INCT-CA M-002/1) and protein (INCT-CA N-004/1), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), lignin (INCT-CA F-005/1), and non-fibrous carbohydrates, following the methodology of Detmann et al. (2012). The content of total digestible nutrients (TDN) was estimated according to NRC (2001).

Four rumen-cannulated crossbred steers with an average weight of 500±70 kg were used for the *in situ* ruminal degradation kinetics of DM and NDF of biomass sorghum silages. The animals received 4.0 kg of concentrate in two equal amounts in the morning and afternoon, in addition to biomass sorghum silage diets. The *in situ* degradability assay was performed using 7.5 × 15 cm non-woven fabric bags (100 g m⁻²; Pore size 60 microns), according to Casali et al. (2009); the number of samples was based on sample size to bag surface area ratio of 20 mg DM cm⁻² (Nocek, 1988).

The samples were placed in the ventral sac of the rumen for 0, 3, 6, 12, 24, 48, 72, 96, 120, and 144 h in bags attached to a nylon cord. Zero-time bags were not incubated in the rumen but were washed in running water similarly to the incubated bags. All samples were removed and washed in cold water to stop fermentation. Subsequently, the samples were oven-dried at 55 °C for 72 h, cooled in a desiccator, and weighed. The obtained residues were analyzed for DM and NDF contents according to Detmann et al. (2012). The disappearance percentage was calculated from the proportion of food remaining after incubation.

Data were adjusted to a non-linear regression model using the Gauss-Newton method in SAS software (Statistical Analysis System, version 9.0) according to the equation proposed by (Ørskov and McDonald, 1979):

$$Y = a + b(1 - e^{-ct}), \quad (3)$$

in which Y = disappearance (%) at time t; a = intercept of degradation curve when t = 0, which corresponds to the rapidly soluble fraction of the analyzed constituent; b = fraction of the constituent that is slowly degradable; a + b = potential degradation of the nutritional component analyzed when time is not a limiting factor; c = fractional degradation rate of disappearance of fraction b in rumen; and t = incubation time. Once calculated, the coefficients a, b, and c were applied to the equation proposed by Ørskov and McDonald (1979):

$$ED = a + (b \times c/c + k), \quad (4)$$

in which ED = effective ruminal degradation of the analyzed nutritional component and k = passage rate. Estimated rumen passage rates (2, 5, and 8% h⁻¹) were assumed as suggested by the AFRC (1993). The DM and NDF disappearances at time zero (fraction a) were used to estimate the lag time (LT) according to Goes et al. (2017), in which parameters "a", "b", and "c" were obtained by the Gauss-Newton algorithms:

$$LC = [-\ln (a'-a-b)/c] \quad (5)$$

Data were submitted to analysis of variance using the IML, MIXED, and REG procedures of SAS. The UNIVARIATE procedure was used to detect outliers and examine the normality of the residues. Data on fermentative profile and chemical composition were analyzed according to the model:

$$Y_{ijk} = \mu + E_i + B_j + e_{ij} + IC_k + E_i \times IC_j + e_{ijk}, \quad (6)$$

in which Y_{ijk} = observed response for row spacing (plot) k of maturity at harvest (subplot) i in block j; μ = overall mean; E_i = effect of row spacing i, with i = 1, 2, and 3; B_j = effect of block j, with j = 1, 2, 3, 4, 5, 6, 7, and 8; e_{ij} = experimental error associated with plots (assumed to be normally distributed with zero mean and unit variance); IC_k = effect of maturity at harvest k, with k = 1, 2, 3 and 4; $E_i \times IC_j$ = effect of the interaction between the i-th level of row spacing and the k-th level of maturity at harvest; and e_{ijk} = experimental error associated with all observations (Y_{ijk}) assumed to be normally distributed with zero mean and unit variance.

If the F test was significant, the means for row spacing and interactions were compared by Tukey's test. Maturities at harvest were compared by partitioning the sum of the squares into orthogonal linear contrasts and quadratic effects, with subsequent adjustments to the regression equations. For all statistical procedures, $\alpha = 0.05$ was the maximum tolerable probability of type III error.

The ruminal degradability assay was conducted in a split-plot randomized block design with 12 treatments (plots) and 10 incubation times (subplots) and four blocks. Animals were blocked by body weight. The following statistical model was used:

$$Y_{ijk} = \mu + T_i + B_j + e_{ij} + P_k + T_i \times P_{ik} + e_{ijk}, \quad (7)$$

in which Y_{ijk} = observed response for time (P) in the subplot k of the treatment (T) in block j; μ = overall mean; T_i = effect of treatment i, with i = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12; B_j = effect of block j, with j = 1, 2, 3, and 4; e_{ij} = experimental error associated with plots (assumed to be normally distributed with zero mean and unit variance); P = effect of incubation time k, with k = 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; TP_{ik} = effect of the interaction between the i-th level of treatment and the k-th incubation time; and e_{ijk} = experimental error associated with all observations (Y_{ijk}) assumed to be normally distributed with zero mean and unit variance.

If the F test was significant, the means for treatments were compared by the Scott-Knott test. The incubation times were compared by partitioning the sum of the squares into orthogonal linear contrasts and quadratic effects, with subsequent adjustments to the regression equations. For all statistical procedures, $\alpha = 0.05$ was the maximum tolerable probability of type III error.

3. Results

pH responded quadratically to maturity at harvest ($P < 0.01$), with a minimum at 131 days; a similar effect was found for N-NH₃ ($P < 0.01$) with a minimum at 134 days (Table 1). Gas and effluent losses decreased linearly with maturity at harvest ($P < 0.01$). Dry matter recovery increased by 0.1909% for every 1-day increase in maturity at harvest. There was a significant interaction between row spacing and maturity at harvest of biomass sorghum on the loss of aerobic stability ($P < 0.01$). Aerobic stability decreased linearly (0.46 h/day) in sorghum planted at 45-cm spacing, whereas it responded quadratically to maturity at harvest in plants spaced 70 cm apart, with a minimum point at 124 days.

On the other hand, aerobic stability increased linearly with maturity at harvest (0.4 h/day) in sorghum planted at 90-cm spacing.

The concentrations of malic, succinic, and acetic acids (Table 2) responded quadratically to maturity at harvest, reaching their minimums at 129, 108, and 98 days of maturity, respectively. Lactic acid content also responded quadratically to maturity at harvest, with a maximum point at 98 days of maturity. The concentration of ethanol in biomass sorghum silage responded quadratically to maturity at harvest ($P < 0.05$), with a minimum point at 98 days of maturity. Row spacing affected the concentrations of malic ($P = 0.03$) and succinic ($P < 0.01$) acids and ethanol ($P < 0.01$), with the highest means for plants spaced 70 cm apart.

Dry matter ($P < 0.01$) and total carbohydrate ($P < 0.01$) contents increased linearly with maturity at harvest (Table 3). Ash content reduced by 0.0485% ($P < 0.01$) and crude protein content reduced by 0.0468% ($P < 0.01$) for every 1-day increase in maturity at harvest. Ether extract content responded quadratically to maturity at harvest ($P < 0.01$) with a minimum at 117 days of maturity, whereas non-fibrous carbohydrates content ($P < 0.01$) reached its maximum at 142 days of maturity. Neutral detergent fiber corrected for ash and protein content ($P < 0.01$), lignin ($P = 0.01$), and iNDF ($P < 0.01$)

Table 1 - pH, ammoniacal nitrogen (N-NH₃), and losses during fermentation of biomass sorghum silage managed at different maturities at harvest and row spacings

Item	Spacing (cm)	Maturity at harvest (days)				SEM	P-value			
		70	100	130	160		IdL	IdQ	Spa	Id × Spa
pH ¹	45	3.45	3.15	2.55	3.30	0.06	<0.01	<0.01	0.59	0.18
	70	3.50	3.13	2.33	3.38					
	90	3.40	3.23	2.30	3.33					
N-NH ₃ (%TN) ²	45	11.34	3.20	3.24	4.26	0.42	<0.01	<0.01	0.15	0.13
	70	11.11	3.10	3.72	3.30					
	90	11.85	2.41	4.58	4.78					
Gas losses (% DM) ³	45	18.69	14.47	8.00	6.93	2.53	<0.01	0.18	0.58	0.79
	70	13.45	10.96	9.44	8.17					
	90	15.92	10.63	6.67	7.87					
Losses effluents (kg fresh mass/t) ⁴	45	40.47	41.25	36.18	37.46	2.56	<0.01	0.29	0.52	0.54
	70	47.97	44.50	34.22	36.62					
	90	42.72	44.83	33.41	36.42					
DM recovery (%) ⁵	45	76.04	78.62	80.43	95.23	2.53	<0.01	<0.01	0.39	0.54
	70	76.30	78.91	87.80	97.20					
	90	80.23	78.31	83.99	93.63					
Aerobic stability (h) ⁶	45	168 A	138 A	126 A	126 A	11.22	0.01	<0.01	0.05	<0.01
	70	168 A	96 B	102 A	120 A					
	90	108 B	120 AB	114 A	150 A					

TN - total nitrogen; DM - dry matter; SEM - standard error of the mean; IdL - linear effect; IdQ - quadratic effect; Spa - row spacing; Id × Spa - interaction between maturity and row spacing.

Means followed by different letters in the column (spacing effect) differed by Tukey's test ($P < 0.05$).

Regression equations:

$$^1 \hat{y} = 7.50 - 0.0789X + 0.0003X^2, R^2 = 0.6744.$$

$$^2 \hat{y} = 41.25 - 0.5908X + 0.0022X^2, R^2 = 0.8773.$$

$$^3 \hat{y} = 21.629 - 0.0922X, R^2 = 0.897.$$

$$^4 \hat{y} = 50.51 - 0.0934X, R^2 = 0.6544.$$

$$^5 \hat{y} = 61.741 + 0.1909X, R^2 = 0.8886.$$

$$^6 \hat{y}_{45} = 192.4 - 0.46X, R^2 = 0.8076; \hat{y}_{70} = 476.9 - 6.21X + 0.025X^2, R^2 = 0.9318; \hat{y}_{90} = 77.0 + 0.4X, R^2 = 0.6897.$$

* Significant by the t test ($P < 0.01$).

Table 2 - Concentration of organic acids and ethanol from biomass sorghum silage managed at different maturities at harvest and row spacings

Item (% DM)	Spacing (cm)	Maturity at harvest (days)				Mean	SEM	P-value			
		70	100	130	160			IdL	IdQ	Spa	Id × Spa
Malic acid ¹	45	21.20	6.27	4.95	7.38	9.95 AB					
	70	19.26	7.73	8.05	7.47	10.62 A	1.88	<0.01	<0.01	0.03	0.06
	90	10.97	4.57	4.02	9.00	7.14 B					
Succinic acid ²	45	0.78	0.26	0.34	0.37	0.44 B					
	70	1.03	0.34	0.54	0.56	0.90 A	0.03	<0.01	<0.01	<0.01	0.07
	90	0.85	0.28	0.28	0.46	0.46 B					
Lactic acid ³	45	1.34	1.55	1.46	1.36						
	70	1.38	1.59	1.48	1.15		0.09	0.06	0.01	0.66	0.47
	90	1.34	1.67	1.25	1.18						
Acetic acid ⁴	45	0.36	0.25	0.53	0.70						
	70	0.36	0.25	0.53	0.70		0.05	<0.01	<0.01	0.88	0.44
	90	0.51	0.23	0.48	0.69						
Butyric acid	45	<0.01	UN	UN	UN						
	70	<0.01	UN	UN	UN		<0.01	0.16	0.28	0.68	0.96
	90	0.01	UN	UN	UN						
Ethanol ⁵	45	0.65	0.45	0.73	1.68	0.87 B					
	70	0.68	0.52	0.87	1.83	0.97 A	0.05	<0.01	<0.01	<0.01	0.07
	90	0.54	0.36	0.52	1.79	0.80 B					

DM - dry matter; UN - undetectable; SEM - standard error of the mean; IdL - linear effect; IdQ - quadratic effect; Spa - row spacing; Id × Spa - interaction between maturity and row spacing.

Means followed by different letters in the column (spacing effect) differed by Tukey's test (P<0.05).

Regression equations:

$$^1 \hat{y} = 61.865 - 0.8827*X + 0.0034*X^2, R^2 = 0.9579.$$

$$^2 \hat{y} = 3.0544 - 0.0434*X + 0.0002*X^2, R^2 = 0.8636.$$

$$^3 \hat{y} = 0.3549 + 0.0217*X - 0.0001*X^2, R^2 = 0.7978.$$

$$^4 \hat{y} = 1.0683 - 0.0156*X + 0.00000005*X^2, R^2 = 0.8509.$$

$$^5 \hat{y} = 3.2629 - 0.0587*X + 0.0003*X^2, R^2 = 0.9982.$$

* Significant by the t test (P<0.01).

increased linearly with maturity at harvest. Acid detergent fiber responded quadratically to maturity at harvest, with a maximum at 114 days of maturity. Total digestible nutrients reached its minimum at 119 days of maturity.

The rapidly soluble fraction of DM (fraction "a"), degradation rate of the insoluble fraction "c", potential degradability, and effective degradability decreased (P<0.01) as the maturity at harvest increased from 70 to 160 days. Lag time increased (P<0.01) with maturity at harvest (Table 4).

There was no interaction (P = 0.86) between maturity at harvest and row spacing on the parameters of ruminal degradation of NDF. Means for standardized potentially degradable insoluble fraction (fraction Bp) of NDF were higher in sorghum planted at 45 and 70 cm row spacings than at 90-cm spacing. The fraction Bp reduced by 0.1471%, while the indigestible fraction of NDF increased by 0.164 for every 1-day increase in maturity at harvest (Table 5). There was no effect of row spacing (P = 0.21) and maturity at harvest (P = 0.06) of biomass sorghum on the degradation rate of fraction Bp "c", with a mean of 1.43%/h. Lag time and effective degradability (k = 5%/h) of NDF responded quadratically to maturity at harvest, with minimum points at 92.18 and 114.96 days, respectively.

4. Discussion

This research was carried out to determine the ideal maturity at harvest of biomass sorghum BRS 716 planted in three row spacings for silage production in the semiarid region. The proportion of cell content to cell wall components varies as plants reach maturity, with increases in cell wall components of low moisture content (Wilson, 1994). It justifies the increase in DM content and

Table 3 - Chemical composition of biomass sorghum silage managed at different maturities at harvest and row spacings

Item (% DM)	Spacing (cm)	Maturity at harvest (days)				SEM	P-value			
		70	100	130	160		IdL	IdQ	Spa	Id × Spa
Dry matter ¹	45	16.56	19.18	22.59	27.03					
	70	16.91	19.72	22.14	26.91	0.79	<0.01	0.01	0.19	0.83
	90	16.04	18.42	20.38	27.06					
Ash ²	45	9.71	7.53	8.26	4.73					
	70	9.34	7.48	7.57	4.68	0.34	<0.01	0.04	0.32	0.44
	90	10.08	6.89	7.70	4.18					
Crude protein ³	45	10.00	8.45	5.69	5.51					
	70	10.17	7.04	6.06	5.33	0.33	<0.01	<0.01	0.24	0.06
	90	9.77	7.95	6.12	6.19					
Ether extract ⁴	45	3.71	1.53	2.18	2.64					
	70	4.48	1.89	1.50	2.97	0.34	<0.01	<0.01	0.11	0.27
	90	4.41	2.13	3.08	3.07					
Total carbohydrates ⁵	45	76.58	82.49	83.87	87.55					
	70	76.02	83.59	84.86	87.63	0.57	<0.01	<0.01	0.14	0.44
	90	75.75	83.02	83.10	87.31					
Non-fibrous carbohydrates ⁶	45	15.95	16.12	18.88	20.04					
	70	13.99	19.67	23.60	21.22	1.25	<0.01	<0.01	0.11	0.10
	90	14.11	19.85	19.35	20.11					
NDFap ⁷	45	60.62	66.37	64.99	67.51					
	70	62.02	63.93	61.26	66.41	1.32	<0.01	0.91	0.24	0.45
	90	61.64	63.17	63.75	65.85					
Acid detergent fiber ⁸	45	40.90	47.05	46.94	46.71					
	70	37.35	44.44	43.44	46.00	1.67	<0.01	0.01	0.10	0.92
	90	40.58	44.73	46.27	45.97					
Lignin ⁹	45	7.28	10.62	11.08	12.38					
	70	7.01	9.65	8.55	9.98	1.24	0.01	0.05	0.23	0.69
	90	8.10	8.79	9.81	11.17					
iNDF ¹⁰	45	7.54	15.45	16.93	18.40					
	70	9.56	14.10	16.42	18.21	1.16	<0.01	<0.01	0.22	0.71
	90	10.82	16.50	16.64	19.41					
Total digestible nutrients ¹¹	45	50.08	45.51	46.66	47.92					
	70	51.28	47.03	47.83	49.05	0.95	0.13	<0.01	0.12	0.93
	90	50.66	47.72	48.74	49.73					

DM - dry matter; NDFap - neutral detergent fiber corrected for ash and protein; iNDF - indigestible neutral detergent fiber; SEM - standard error of the mean; IdL - linear effect; IdQ - quadratic effect; Spa - row spacing; Id × Spa - interaction between maturity and row spacing.

Regression equations:

$$^1 \hat{y} = 8.3364 + 0.1098 * X, R^2 = 0.9746.$$

$$^2 \hat{y} = 12.966 - 0.0485 * X, R^2 = 0.8312.$$

$$^3 \hat{y} = 12.79 - 0.0468 * X, R^2 = 0.9004.$$

$$^4 \hat{y} = 13.476 - 0.1873 * X + 0.0008 * X^2, R^2 = 0.8811.$$

$$^5 \hat{y} = 69.666 + 0.1119 * X, R^2 = 0.8969.$$

$$^6 \hat{y} = -2.0096 + 0.3137 * X - 0.0011 * X^2, R^2 = 0.9998.$$

$$^7 \hat{y} = 58.592 + 0.0463 * X, R^2 = 0.7455.$$

$$^8 \hat{y} = 20.228 + 0.3747 * X - 0.0013 * X^2, R^2 = 0.9279.$$

$$^9 \hat{y} = 5.3552 + 0.036 * X, R^2 = 0.8925.$$

$$^{10} \hat{y} = 4.502 + 0.0935 * X, R^2 = 0.8742.$$

$$^{11} \hat{y} = 65.947 - 0.3111 * X + 0.0013 * X^2, R^2 = 0.8437.$$

* Significant by the t test (P<0.01).

Table 4 - Ruminal kinetics of dry matter from biomass sorghum silage managed at different maturities at harvest and row spacings

Item (% DM)	Spacing (cm)	Maturity at harvest (days)				SEM	P-value			
		70	100	130	160		IdL	IdQ	Spa	Id × Spa
Fraction A ¹	45	16.81	18.54	15.95	13.85	1.18	<0.01	0.11	0.27	0.30
	70	18.76	17.80	18.45	15.63					
	90	20.56	16.92	15.83	14.91					
Fraction B	45	47.97	50.12	48.32	53.32	3.14	0.07	0.32	0.42	0.13
	70	54.45	54.36	43.85	50.53					
	90	53.85	48.95	46.90	42.00					
Degradation rate of fraction B "c" (%/h) ²	45	3.00	2.00	1.00	1.00	<0.10	<0.01	0.05	0.44	0.07
	70	2.00	1.00	1.00	1.00					
	90	2.00	1.00	2.00	2.00					
Colonization time (h) ³	45	3.93	6.70	8.69	7.50	1.43	<0.01	0.34	0.31	0.32
	70	5.37	6.26	9.54	11.24					
	90	6.23	7.63	6.54	6.79					
Potential degradability ⁴	45	64.78	68.66	64.27	67.17	1.77	<0.01	0.44	0.28	0.08
	70	73.21	72.16	62.30	66.16					
	90	74.41	65.86	62.73	56.91					
Effective degradability (k = 5%/h) ⁵	45	34.33	30.27	24.54	22.54	1.28	<0.01	0.09	0.80	0.12
	70	32.93	29.89	28.07	23.14					
	90	35.31	26.70	26.71	24.53					

DM - dry matter; k - passage rate (AFRC, 1993); SEM - standard error of the mean; IdL - linear effect; IdQ - quadratic effect; Spa - row spacing; Id × Spa - interaction between maturity and row spacing.

Regression equations:

$$^1 \hat{y} = 21.767 - 0.0411X, R^2 = 0.97.$$

$$^2 \hat{y} = 0.0273 - 0.0001X, R^2 = 0.76.$$

$$^3 \hat{y} = 3.0103 + 0.0361X, R^2 = 0.90.$$

$$^4 \hat{y} = 76.838 - 0.0887X, R^2 = 0.8453.$$

$$^5 \hat{y} = 41.161 - 0.1113X, R^2 = 0.96.$$

* Significant by the t test (P<0.01).

Table 5 - Ruminal kinetics of neutral detergent fiber from biomass sorghum silage managed at different maturities at harvest and row spacings

Item (% DM)	Spacing (cm)	Maturity at harvest (days)				Mean	SEM	P-value			
		70	100	130	160			IdL	IdQ	Spa	Id × Spa
Fraction Bp ¹	45	67.61	61.99	54.16	61.93	61.42 A	2.00	<0.01	<0.01	<0.01	0.08
	70	68.47	67.47	54.99	62.20	63.28 A					
	90	68.23	51.90	50.03	43.45	53.39 B					
Degradation rate of fraction B "c" (%/h)	45	2.00	1.75	1.50	1.25	0.01	0.06	0.95	0.21	0.86	
	70	1.50	1.50	1.50	1.25						
	90	1.75	1.00	1.25	1.00						
Colonization time (h) ²	45	5.30	5.60	5.00	7.58	1.10	<0.01	0.02	0.14	0.07	
	70	8.10	6.21	6.38	9.32						
	90	4.96	4.17	8.40	16.91						
Effective degradability (k = 5%/h) ³	45	31.98	27.59	20.05	24.85	1.72	<0.01	<0.01	0.13	0.42	
	70	30.25	27.02	25.47	24.46						
	90	30.41	17.07	20.04	23.41						
Indigestible fraction ⁴	45	32.39	38.00	45.83	38.07	38.57 B	1.73	<0.01	<0.01	<0.01	0.08
	70	31.53	32.53	45.00	37.80	36.71 B					
	90	31.76	48.10	49.97	56.55	46.60 A					

DM - dry matter; k - passage rate (AFRC, 1993); SEM - standard error of the mean; IdL - linear effect; IdQ - quadratic effect; Spa - row spacing; Id × Spa - interaction between maturity and row spacing.

Means followed by different letters in the column (spacing effect) differed by Tukey's test (P<0.05).

Regression equations:

$$^1 \hat{y} = 76.28 - 0.1471X, R^2 = 0.7566.$$

$$^2 \hat{y} = 23.75 - 0.4056X + 0.0022X^2, R^2 = 0.9999.$$

$$^3 \hat{y} = 50.12 - 0.5978X + 0.0026X^2, R^2 = 0.9265.$$

$$^4 \hat{y} = 11.77 + 0.164X, R^2 = 0.9760.$$

* Significant by the t test (P<0.01).

fibrous fraction as a function of maturity at harvest. Dry matter content at ensiling is one of the most important parameters for a successful fermentation process. Kung Jr. et al. (2018) reported that DM levels below 25% in sorghum silage could hinder a rapid pH decline and allow the development of undesirable microorganisms such as those of the genus *Clostridium*. The DM contents observed in this study are within the recommended range for biomass sorghum harvested after 160 days, regardless of row spacing. It is explained by the pH values, low losses of N-NH₃ and DM in the form of gases and effluents, and high DM recovery rate. According to Kung Jr. et al. (2018), silage pH values below 3.5 are considered low, although they are inhibitory of non-lactic acid bacteria. It is justified by the absence of butyric acid resulting from undesirable fermentation of bacteria of the genus *Clostridium*. Well fermented silages have N-NH₃ levels below 10% (Kung Jr. et al., 2018) as observed in silages harvested at 100, 130, and 160 days of maturity. On the other hand, N-NH₃ levels were higher than the recommended in plants cut at 70 days. This result is attributed to the low DM content at the time of ensiling (14%), which promoted proteolytic activity of bacteria of the genus *Clostridium* (Muck et al., 2018).

Borreani et al. (2018) emphasized that losses in the form of gases and effluents should not exceed 4 and 0.5%, respectively. However, fermentative losses in the present study were higher than those recommended. Higher gas losses (mainly due to CO₂ production) were due to the increase in numbers of enterobacteria and clostridia (Kung Jr. et al., 2018). Effluent losses are detrimental to the nutritional value of silage as it favors losses by leaching of nutrients produced during the process.

Aerobic stability is an indicator of longevity of the post-opening material. In this sense, sorghum planted at 90-cm spacing had better aerobic stability, i.e., remained stable longer than sorghum silages planted at 45 and 70 cm row spacings. Aerobic stability is lost upon exposure of silage to air after the end of anaerobic storage due to the deterioration of fermentation products such as organic acids (Wilkinson and Davies, 2013).

Muck et al. (1991) described the presence of malic and succinic acids in grass silages as typical. High concentrations of malic and succinic acids were found in the silage at 70 days of maturity, exceeding values of <0.2% for malic acid and <0.5% for succinic acid as described by Rooke and Hatfield (2003). However, these concentrations do not suggest fermentation losses, since malic acid is used by lactic acid-fermenting bacteria to generate lactate, while succinic acid is used by propionic acid bacteria to produce propionate (Borreani et al., 2018; Rooke and Hatfield, 2003). The concentrations of lactic and acetic acids were lower than those recommended by Kung Jr. et al. (2018) for corn silage (3-6 and 1-3%, respectively). However, the pH remained limiting for undesirable microorganisms regardless of the maturity at harvest. Beneficial results to the fermentation profile of silages included the low concentrations of butyric acid and ethanol, which were lower than the recommendation of <0.5% of DM (Kung Jr. et al., 2018). Butyric acid and ethanol are undesirable because they contribute to higher DM losses and energy losses (Borreani et al., 2018). The highest ethanol content was observed in the sorghum silage planted with 70-cm row spacing.

The DM content of silage is dependent on DM content of the plant at the time of ensiling. Furthermore, the DM content of silage increased with maturity at harvest. Santos et al. (2011) reported a similar response when ensiling *Brachiaria* grass at different regrowth maturity, in which the DM content ranged from 19.4 to 23.8% in plants harvested at 30 and 70 days, respectively. The same response was observed for total carbohydrates, which was mainly caused by the increase in cell wall components (Van Soest, 1994; Wilson, 1994). The decrease in ash and crude protein contents was probably due to the dilution effect, as verified by Monção et al. (2020) in a study with BRS capiaçu (elephant grass) at different maturity at harvest and Ziki et al. (2019) in a study with Sudan grass in a semiarid region. Another factor that can reduce crude protein content during the fermentation process is the degradation caused by proteolytic microorganisms (Rooke and Hatfield, 2003; Borreani et al., 2018; Kung Jr. et al., 2018). The levels of ether extract remained below 7%; therefore, they did not interfere with rumen metabolism. The response observed for non-fibrous carbohydrates is possibly associated with the dynamics of sugar utilization by microorganisms during the fermentation process (Rooke and Hatfield, 2003). Higher NDFap, acid detergent fiber, and lignin levels are attributed to cell wall development,

which is essential to provide structural support to plants. Maturity at harvest influenced DM content and plant height, which is affected by genetic and environmental factors such as the light requirement for photosynthesis (Santos et al., 2011). The cells of the phytomer divide mitotically according to the physiological maturity of the plant to expose leaf blades to light. Thus, lignin is involved in the thickening of the cell wall and provides resistance to plants due to the strong bond between lignin and hemicellulose through ester linkages (Van Soest, 1994; Wilson, 1994). This fact contributed to the increase of NDF of silages with maturity at harvest.

The degradability of biomass sorghum silage reduced, but lag time increased with maturity at harvest. Reductions in silage digestibility are detrimental to ruminants as the feed remains for a longer period in the rumen, which could limit DM intake. This is probably associated with a higher degree of lignification, as shown by the increase in iNDF. Among row spacings, the greatest ruminal degradability of NDF was observed at 70 cm. Biomass sorghum silage is a viable option to semiarid regions, where the lack of feed results in higher losses to ruminant production. The adequate fermentation pattern combined with the high productivity of plants demonstrates the potential of BRS 716 biomass sorghum to be used in regions where feed seasonality is frequent.

5. Conclusions

The BRS 716 biomass sorghum should be planted at 70-cm row spacing and harvested at 160 days for silage production based on fermentative profile, dry matter losses, and nutritional characteristics.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: V.R. Rocha Júnior, F.P. Monção and M.W.S. Cordeiro. Data curation: V.R. Rocha Júnior and F.P. Monção. Formal analysis: F.E. Queiroz, V.R. Rocha Júnior, F.P. Monção, A.S. Santos and M.W.S. Cordeiro. Funding acquisition: R.A.C. Parrella. Methodology: F.E. Queiroz, V.R. Rocha Júnior, F.P. Monção, A.S. Santos and M.W.S. Cordeiro. Project administration: V.R. Rocha Júnior and F.P. Monção. Resources: R.A.C. Parrella. Supervision: V.R. Rocha Júnior, F.P. Monção and J.P.S. Rigueira. Validation: V.R. Rocha Júnior. Writing-original draft: F.E. Queiroz, V.R. Rocha Júnior, F.P. Monção, J.P.S. Rigueira, R.A.C. Parrella, L.D.A. Rufino, A.S. Santos and M.W.S. Cordeiro. Writing-review & editing: F.E. Queiroz, V.R. Rocha Júnior, F.P. Monção, J.P.S. Rigueira, R.A.C. Parrella, L.D.A. Rufino, A.S. Santos and M.W.S. Cordeiro.

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