

Animal Research Paper

Cite this article: Santos RD, Neves ALA, Pereira LGR, Sollenberger LE, Muniz EN, Souza EYB, Sobral AJS, Costa NV, Gonçalves LC (2020). Performance, agronomic traits, ensilability and nutritive value of pearl millet cultivar harvested at different growth stages. *The Journal of Agricultural Science* 1–8. <https://doi.org/10.1017/S0021859620000222>

Received: 22 March 2019

Revised: 11 February 2020

Accepted: 16 March 2020

Key words:


Digestibility; drought resistance; forage; semi-arid; water-use efficiency

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Performance, agronomic traits, ensilability and nutritive value of pearl millet cultivar harvested at different growth stages

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R.) is an important crop for rainfed production systems and can play a significant role as a feed source for ruminants owing to its high yield and drought tolerance. It is well-established that the maturity stage can influence the chemical composition as well as the nutritional value of crops traditionally used for silage production, although quantitative evidence that this occurs with pearl millet under rainfed conditions is lacking. The current research assessed the agronomic characteristics, ensilability, intake and digestibility of a Brazilian pearl millet cultivar (IPA BULK1-BF) harvested at four different growth stages. Forage was harvested at 35, 50, 65 and 80 days after sowing and ensiled under laboratory and farm conditions. Apparent digestibility of the silages was determined using 24 male lambs. The results showed that dry matter (DM) and panicle and stem proportions increased with the advancement maturity. The silage evaluations showed that DM, total and non-fibrous carbohydrates and lignin concentrations increased, while crude protein, ADF and *in vitro* DM digestibility decreased with the increase in plant maturity. Additionally, the fermentation characteristics were improved with the increasing maturity. The digestion study showed that intake of DM and N as well as digestibility of DM and fibre fractions decreased, while lignin intake increased. The results obtained for the production of dry and digestible DM, the ratio of plant fractions and fermentation parameters indicate the possibility of harvesting pearl millet forage after 50 days after sowing for silage production in the Brazilian semi-arid region.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an annual tropical grass that can be utilized for grain or forage production. It is highly drought tolerant and resistant to many diseases affecting other crops traditionally used for silage production (Mason *et al.*, 2015). The interest in annual forages (such as millet) has increased considerably over the years with the occurrence of constant droughts in various regions of the world, and these severe climate events have led to an urgent necessity of identifying efficient water-use plants that are adapted to the recent climate changes. It is believed that the increase in the use of such rain-fed crops may help to achieve sustainable agricultural development in areas that are more prone to extreme weather (FAO, 2008). The use of forage millet in Brazil has been mainly concentrated on the central-west and southern regions. However, forage millet cultivars adapted to Brazilian north-eastern conditions (semi-arid) have also been developed, including the IPA BULK1-BF cultivar (Agronomic Institute of Pernambuco (IPA), 1999). Several studies were conducted to evaluate the field performance of IPA BULK1-BF cultivar (Bezerra *et al.*, 2011; Costa and Priesnitz, 2014; Santos *et al.*, 2015), and it was found that they could serve as a feed source for livestock during the dry season. However, knowledge of the phenological stages at harvest and performance of IPA BULK1-BF is still lacking. The stage of maturity at harvest is a major factor to determine the nutritive value of a silage. The effects of plant maturity on silage fermentation and animal performance have been investigated in corn and sorghum crops (Bernard and Tao, 2015; Peyrat *et al.*, 2015), but the relative scarcity of data on pearl millet for silage production suggests a need for additional studies in this area of research.

As a part of an overall strategy to deal with this issue, the current study evaluated agronomic characteristics of the IPA BULK1-BF pearl millet cultivar harvested at four different growth stages and assessed its potential application in silage production. Finally, the impact of these forages on intake and digestibility in lambs was quantified.

Table 1. Meteorological data during the experimental period

Month/year	RD	Rain (mm)	Temperature (°C)			Evaporation (mm)	RH (%)	Wind (m/s)	SR (MJ)
			Max.	Min.	Mean				
June/2012	9	31.6	28.0	19.6	22.8	103.5	81.1	1.2	16.6
July/2012	13	43.3	26.1	18.7	21.6	100.2	80.7	1.2	15.2
August/2012	12	32.2	26.4	17.9	21.2	116.7	77.4	1.3	14.7
September/2012	0	0.0	27.8	17.5	21.8	82.5	70.2	1.2	15.3

RD, rainfall occurrence in days; RH, relative humidity; SR, solar radiation; wind average speed at 2 m height.

Materials and methods

Experiments location and general information

The experiment was conducted from June to October 2012 at the Semi-Arid Experimental Station of the Brazilian Agricultural Research Corporation (Embrapa) in the municipality of Nossa Senhora da Glória, Sergipe State, Brazil (10°13'S, 37°25'W, 291 m asl). The soil type in this region is a eutrophic red-yellow podzol (Santos *et al.*, 2013), with an average depth of 1.5 m. The climate is typically semi-arid with an annual rainfall of 710 mm and average maximum and minimum temperatures of 32 and 20°C, respectively. Precipitation in the region is low, erratic, and the balance between rainfall and evaporation rate can be negative in some months based on meteorological data from a weather station located about 400 m from the experimental site (Table 1 and Fig. 1). Seed of the pearl millet cultivar (IPA BULK1-BF) was supplied by the pearl millet breeding programmes of IPA.

Agronomic characteristics

Treatments were the four harvesting intervals (35, 50, 65 and 80 days after sowing) replicated five times in a randomized complete block design (20 plots). In this experiment, plants were harvested when at least 60% of them reached the Zadoks's scale of 55, 65, 75 and 85, respectively, which correspond to the four harvesting intervals investigated in this study (35, 50, 65 and 80 days) (Zadoks *et al.*, 1974). Plots measured 10.5 m² (5 × 2.1 m), with seeds sown manually at a depth of 3 cm in four rows (on 0.70 m centres). The sowing date was 13 June 2012. The soil at the site had the following properties: pH (water): 5.8; phosphorus (P): 0.0071 cmol_c/dm³; potassium (K): 0.32 cmol_c/dm³; aluminium (Al): 0.05 cmol_c/dm³; hydrogen (H) + Al: 1.89 cmol_c/dm³; calcium (Ca): 1.4 cmol_c/dm³; magnesium (Mg): 0.74 cmol_c/dm³ and organic matter (OM): 10.54 g/kg. All plots were randomly allocated and fertilized at seeding with 150 kg N/ha as urea and with 300 kg P/ha and 41 kg K/ha in a mixed fertilizer according to soil test recommendations. Each treatment comprised c. 32 plants/m², achieved by thinning plots 15 days after emergence. Additionally, it was applied 1 kg/ha of the pre-emergent herbicide atrazine.

Plants were harvested according to each treatment, which were represented by the days 35, 50, 65 and 80 after planting. Harvests were made manually and taken at 5 cm above ground level. Only the two central rows in each plot were harvested, with the remainder being discarded. The harvested crop from each plot was collected and weighed to estimate fresh biomass (yield/ha). After chopping (average of 1.5 cm long) a representative sample from

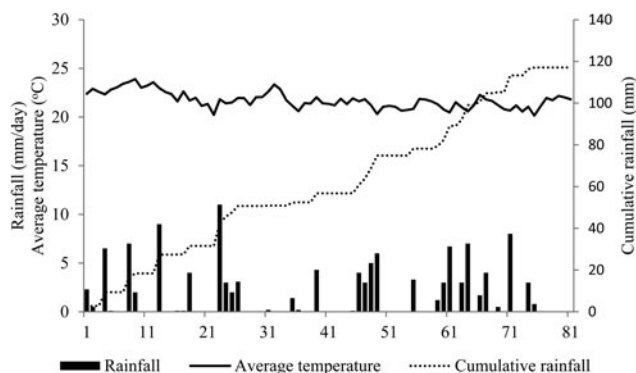


Fig. 1. Rainfall distribution (mm) and average temperature (°C) at the experimental station of Embrapa Semi-Arid from June to September 2012.

each plot, a 400-g sub-sample was oven-dried at 55°C for 48 h to determine dry matter (DM) concentrations and biomass yield of the four treatments.

The agronomic characteristics studied included: plant height; population density; extent of lodging; DM partitioning of plant organs (panicle, stem and leaf); DM yield (DMY) (t/ha) and digestible DM yield (DDMY) (t/ha). The height of ten randomly selected plants within each plot was determined by measuring from the ground level to the top of the panicle using a tape measure. These plants were then separated into panicles, stems and leaves, with the mass of each of these fractions being determined after oven-drying at 65°C for 72 h. The whole plants and their respective fractions (panicles, stems and leaves) were not chopped, and thus the time of drying and the temperature required to process them were higher than those employed to analyse the material used to determine DM concentrations and biomass yield.

In the current study, scores of lodging were estimated from the percentage area of the plot that was lodged. These scores were determined from an angle of 10° taken from the perpendicular, such that stems scored as 10 were considered as lodged and stems scored as 90 were not lodged in the plots. Lodging for each plot was then calculated as: (% plot area lodged × angle of lodging from vertical)/90 as described by Bell and Fischer (1994). The DDMY was estimated by multiplying the *in vitro* DM digestibility from each repetition by its respective DMY.

Ensiling procedures

At harvest, a silage harvester was used to chop plants within each treatment to an average of 1.5 cm long and transferred into plastic barrels with a capacity of 250 kg (large scale silos used in the animal feeding trial described below). Representative herbage

samples from each plot were also packed manually into polyvinyl chloride mini-silos (four mini-silos \times five replications for a total of 20 mini-silos; 10.5 cm diameter \times 35.5 cm high, capacity of 2.5 kg and average density of 813.7 kg/m³) using a wooden pestle (Sebastian *et al.*, 1996). The mini-silos and the large scale silos were thoroughly washed and sanitized before ensiling to avoid contamination of the silage fermentation. As gas release would be possible from both types of silos (mini-silos and the large scale silos), care was taken to seal them appropriately with plastic lids followed by storage at room temperature.

Mini-silos were opened following 90 days of ensiling, with forage samples (15 g) from both mini-silos and plastic barrels being homogenized for 1 min in 500 ml of distilled water to measure the pH using a pH meter. Aqueous extracts (10 ml) were acidified with 50 μ l of 9.77 mol/l of sulphuric acid (H₂SO₄) (Kung and Ranjit, 2001) and frozen before analysis. Thawed extract samples were centrifuged for 15 min at 10 000 \times g at 4°C and analysed for acetic, propionic, lactic and butyric acids using a Varian high performance liquid chromatography system as described by Adams *et al.* (1984). Organic acids were separated using an Aminex HPX-87H column (300 \times 7.8 mm) with a mobile phase of 0.013 M H₂SO₄ at a flow rate of 0.5 ml/min. Organic acids were quantified using an ultraviolet detector set at 210 nm.

Ammonia was determined using a phenol-hypochlorite reaction, as described by Weatherburn (1967). Finally, silage sub-samples (500 g) were oven-dried at 60°C for 72 h, ground through a 1 mm screen using a Wiley mill and stored at room temperature until further analysis.

Intake and digestibility measurements

All lambs were cared for in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) (2008) and approved by the Ethical Committee on Animal Use (CEUA) of the Embrapa Semi-Arid (Permit Number: CEUA/CPATSA/002-2012). Apparent nutrient digestibility of silages was measured using 24 Santa Inês male lambs (initial body weight (BW): 24 kg \pm 1.6 kg) over a 21-day period. Lambs were blocked by weight and assigned randomly to one of four treatments. The first 17 days were used to adapt lambs to the diets in individual metabolic cages equipped with a poly-ethylene sieve tray to separate faeces from urine. Lambs were fed pearl millet silage only (without concentrate) twice daily at 07.30 and 16.30 h in a manner that assured 0.15 orts at the morning feeding. Water and a trace mineralized salt mixture were available to lambs *ad libitum*.

Apparent digestibility was determined over 5 days, with lambs being fed pearl millet silage *ad libitum* as described by Silva and Leão (1979). During these 5 days, total faeces, feed and orts of each lamb were measured and sampled daily. Samples of the 5 days were mixed, sub-sampled (400 g fresh faeces, 400 g fresh feeds and 400 g fresh orts per lamb) and stored at -20°C until analysed. The total urine output of each animal was collected daily into plastic containers containing 100 ml of hydrochloric acid (HCl) with 2N concentration to prevent fermentation, degradation and N losses. During the 5-day collection phase, subsamples (10% from the total urine volume) were collected in the morning and stored at -20°C until further analysis.

Chemical analysis

Ground samples were analysed for DM and OM as described by the Association of Official Analytical Chemists (AOAC) (2005)

(methods 942.05 and 934.01). A Leco combustion N analyser was used to measure N concentration. Crude protein (CP) was calculated as N \times 6.25. Both neutral detergent fibre (NDF), which was determined by using heat stable α -amylase, and sodium sulphite (ash free), and acid detergent fibre (ADF) were quantified as described by AOAC (2005) (methods 2002.04 and 973.18) using an Ankom Fibre Analyser. Concentration of hemicellulose was determined by subtracting ADF from NDF. Ether extract (EE) was determined as described by AOAC (2005) (method 920.39) using an Ankom Fat Extractor.

Total carbohydrate (TC) and non-fibrous carbohydrates (NFC) were calculated as described by Sniffen *et al.* (1992): TC_{g/kg DM} = 100 - (CP + EE + ash) and NFC_{g/kg DM} = 100 - (CP + EE + ash + NDF).

In vitro DMD analysis of fresh forage and silage was conducted in 100 ml serum bottles and examined in a single run for each forage/silage with triplicate bottles being used per sample. Plant material (0.5 g) was incubated with 10 ml of rumen fluid mixed with 40 ml of McDougall's buffer (McDougall, 1948) for 48 h at 39°C. Samples were subsequently incubated with 0.1N HCl and 2 g/l pepsin for further 48 h (Tilley and Terry, 1963). Equal volumes of rumen fluids were collected immediately after feeding from three rumen-fistulated bulls fed a mixture of the four treatments silages. After stirring the three samples, the combined ruminal fluid was used in the IVDMD assay as described above.

Statistical methods

Experiments were analysed by a mixed model approach with maturity stage as a fixed effect, random effects of blocks (agronomic and silage quality trials) and lambs (digestibility study) and random residual error using the MIXED procedure of SAS Version 9.1 statistical program (SAS 2002). The model used was: $Y_{ij} = \mu + T_i + B_j + E_{ij}$, where Y_{ij} is the observation, μ the overall mean, T_i the treatment, B_j the block/animal and E_{ij} the residual error. When the fixed effect of plant maturity was significant, the means were compared using Fisher's protected LSD tests (i.e., the DIFF option of the LSMEANS statement). Polynomial contrasts were used to determine linear and quadratic effects of maturity stage. Significance was declared at $P < 0.05$.

Results

Agronomic traits

Plant height, stem, leaves and panicles proportion increased linearly ($P < 0.001$) with the increasing plant maturity. At the same plant density for all treatments, DM yield and digestible DM yield increased linearly ($P < 0.001$) with the increase in plant maturity (Table 2).

Silage quality

Increasing plant maturity resulted in a linear increase ($P < 0.001$) in DM, OM, TC, NFC and lignin concentrations, while the CP and ADF concentrations decreased linearly (Table 3). There was a significant positive quadratic relationship between hemicellulose ($P = 0.004$) and NDF concentration ($P = 0.005$) with the age of harvest. However, the relationship was negative for IVDMD ($P = 0.011$) (Table 3). A larger variation in fermentation products was detected among treatments, with a linear effect for silage pH and concentrations of acetic and butyric acids ($P < 0.001$) being

Table 2. Performance, phenological traits and fermentation products concentrations (g/kg DM) of silages produced from cultivar IPA BULK1-BF harvested in different days after planting

Variable	Days after planting				SEM	P-Value	
	35	50	65	80		Linear	Quadratic
Plant height (cm)	112	200	221	222	3.6	<0.001	<0.001
Plant density (1000 plants/ha)	265	278	257	258	9.8	0.431	0.736
DM yield (t/ha)	2.3	8.1	10.6	13.7	0.69	<0.001	0.065
Digestible DM yield (t/ha)	1.5	4.7	5.9	7.1	0.78	<0.001	0.029
Lodging score	2.5	1.9	3.4	1.1	1.11	0.639	0.773
DM partitioning of plant organs (t/ha)							
Panicles	0	0.5	1.4	2.4	0.15	<0.001	0.175
Stems	1.0	5.8	7.2	9.4	0.50	<0.001	0.027
Leaves	1.3	1.8	2.0	1.8	0.19	0.039	0.053
Fermentative silage parameters							
pH	5.85	4.61	3.71	3.50	0.150	<0.001	0.005
Lactic acid (g/kg DM)	0.0	0.48	0.87	0.79	0.78	<0.001	0.003
Acetate (g/kg DM)	0.11	0.39	0.31	0.26	0.032	<0.001	<0.001
Propionate (g/kg DM)	0.37	0.39	0.31	0.13	0.039	<0.001	0.006
Butyrate (g/kg DM)	0.81	0.32	0.0	0.0	0.071	<0.001	0.005
NH ₃ -N (g/kg TN)	48	30	18	18	3.0	<0.001	0.011

DM, dry matter; SEM, standard error of mean; TN, total nitrogen.

Table 3. Chemical composition (g/kg DM) of silages produced from cultivar IPA BULK1-BF harvested in different days after planting

Variable	Days after planting				SEM	P-Value	
	35	50	65	80		Linear	Quadratic
DM (g/kg)	90	147	210	245	5.6	<0.001	0.071
Chemical composition (g/kg DM)							
Organic matter	850	895	920	931	6.2	<0.001	0.005
CP	124	116	94	71	3.4	<0.001	0.029
NDF	567	600	610	588	9.5	0.059	0.005
ADF	397	377	364	343	7.8	<0.001	0.932
Hemicellulose	171	223	246	246	8.5	<0.001	0.004
TC	664	743	807	840	9.6	<0.001	0.005
NFC	96	144	198	252	8.9	<0.001	0.674
EE	62	36	19	20	9.4	0.073	0.051
Lignin	24	35	51	56	2.6	<0.001	0.392
IVDMD (proportion of DM)	0.68	0.60	0.54	0.52	0.12	0.034	0.011

DM, dry matter; IVDMD, *in vitro* dry matter digestibility; SEM, standard error of mean.

observed in the current study. A quadratic effect was observed for lactic acid concentration ($P=0.003$), with the silage produced from plants harvested at 65 days after planting exhibiting the highest values. A quadratic effect ($P<0.001$) was also observed for propionic acid and ammonia-N concentrations, with the lowest values noted in silages made of plants harvested at 65 and 80 days after sowing (Table 2).

Digestion study

Intake of DM, OM, CP, NDF and ADF decreased linearly ($P<0.005$), while NFC and lignin intake increased quadratically ($P<0.05$) in animals fed silages produced from plants harvested at later maturity stages (Table 4). Digestibility of all variables decreased linearly ($P<0.05$), except for NFC, which exhibited

Table 4. Intake (g/kg BW^{0.75}), total apparent and true digestibility (fraction) of dietary components and nitrogen balance in lambs fed silage produced from cultivar IPA BULK1-BF harvested in different days after planting

Variable	Days after planting				S.E.M.	P-Value	
	35	50	65	80		Linear	Quadratic
Intake (g/kg BW ^{0.75} per day)							
DM	84.4	64.3	49.1	45.1	5.30	<0.001	0.150
Organic matter	80.7	70.0	70.3	68.7	1.73	0.004	0.019
CP	11.3	7.8	6.9	6.3	0.31	<0.001	0.004
NDF	52.5	52.5	46.3	45.3	1.23	0.001	0.665
ADF	31.2	33.5	28.2	26.1	0.82	<0.001	0.101
TC	64.9	59.2	61.7	60.6	1.75	0.185	0.198
NFC	12.4	6.7	15.4	15.3	0.91	0.004	0.005
EE	4.5	2.9	1.8	1.9	0.37	<0.001	0.034
Lignin	1.6	3.7	2.5	2.9	0.27	0.026	0.014
Digestibility (kg/kg)							
DM	0.69	0.60	0.55	0.52	0.040	0.007	0.475
Organic matter	0.81	0.75	0.80	0.69	0.020	0.003	0.305
CP	0.76	0.68	0.74	0.56	0.031	0.002	0.156
NDF	0.78	0.74	0.75	0.62	0.023	0.005	0.082
ADF	0.77	0.72	0.73	0.56	0.026	<0.001	0.019
TC	0.82	0.75	0.80	0.70	0.019	0.005	0.352
NFC	0.91	0.85	0.95	0.88	0.023	0.937	0.932
EE	0.89	0.86	0.80	0.64	0.050	0.001	0.204
Nitrogen balance (g/day)							
Nitrogen intake	27.4	20.1	17.5	16.9	2.42	0.08	0.07
Nitrogen faecal	6.4	6.5	4.6	7.3	1.41	0.92	0.74
Nitrogen urinary	0.6	0.7	0.6	0.6	0.38	0.88	0.79
Nitrogen absorbed	20.4	12.9	12.3	9.0	2.13	0.06	0.25

SEM, standard error of mean; BW, body weight.

an increase in its digestibility as the maturity stages of the plants advanced (Table 4).

Discussion

Agronomic traits

Significant differences were observed among treatments for plant height, DM and DDM yield, panicles and stem proportions with respect to the different ages of harvest. All these parameters increased significantly with the delay in harvesting, as they showed the highest values at 80 days after planting, likely due to the completion of the required time for the plants to finalize their phenological development. The delay in the harvest date from 35 to 80 days after sowing resulted in an increase of nearly 2.4 cm per day in plant height, and this result is similar to the average plant height (2.8 cm per day) reported for three Pakistani cultivars of pearl millet grown in the Pakistani hot desert climate (Bukhari *et al.*, 2011).

The increase in DM and digestible DM yields associated with the increasing plant maturity is consistent with previous research

won silage crops (Marsalis *et al.*, 2010; Atis *et al.*, 2012; Aoki *et al.*, 2013). For example, Monks *et al.* (2005) reported that DM yield ranged from 2.3 to 11.5 t/ha for pearl millet harvested at 44 or 144 days after sowing and grown in a Brazilian sub-tropical region. The increase in DM yield associated with the increasing plant maturity is likely caused by the increase in stem and panicle proportions, which can reduce the leaf ratios as the plants age (Fig. 2).

Silage quality

The stage of maturity at harvest is a major factor to determine the nutritive value of silages (Johnson *et al.*, 1999). Previous research (Johnson *et al.*, 2002) has demonstrated that DM concentration of silages increases with the advance in maturity. In the current study, increasing plant maturity resulted in a 2.4% increase in DM concentration per week. In general, the nutritive value of forages declines dramatically with the increase in maturity, mainly due to the increase in concentrations of NDF and ADF and a decrease in CP (Blaser *et al.*, 1986).

Hassanat *et al.* (2007) reported that IVDMD of pearl millet stover decreased with the increasing plant maturity and was

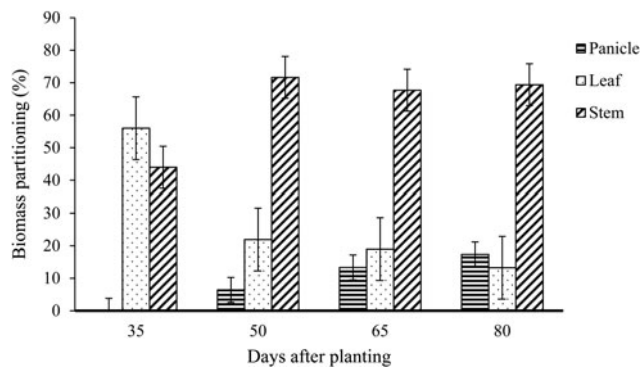


Fig. 2. Participation of morphological components (%) in the total biomass of cultivar IPA Bulk1BF harvested in different days after planting.

highly correlated with ADF and lignin contents. Although the reduction in IVDMD was associated with the increase in lignin concentrations in the current study, it was found that plant maturity did not affect NDF concentrations despite the linear decrease observed for ADF. Similar results were found by Morales *et al.* (2014) who reported a reduction in ADF and IVDMD associated with increasing concentrations of lignin in pearl millet evaluated in four phenological stages (leaf 7th, flag leaf, boot stage and flowering). A possible explanation for those results is that increasing proportions of grains are typically observed in later maturity stages of pearl millet crops, and this increased proportion of grains may weaken the relationship between plant maturity and digestibility of the whole plant silage. Khan *et al.* (2007) studied maturity effects in maize, sorghum and pearl millet silage quality. Concentrations of NDF and ADF in the whole plant decreased as maturity proceeded from pre-heading to milk stage maturity. Despite the decline in NDF and ADF contents, *in situ* DM digestibility of the whole plant decreased progressively from early (63.0%) to late (54.1%) maturity. These data highlight the importance of assessing grain development and stover quality in conjunction with maturity management of pearl millet silages. Identification of pearl millet hybrids that maintain stover quality while increasing grain proportion at advanced stages of maturity is a logical strategy for improving pearl millet silage quality.

The highest levels of pH (5.9) and ammonia-N (4.8 g/kg TN) observed in silages produced from pearl millet harvested at 35 days after sowing are in agreement with the levels frequently observed in high pH silages, in which secondary fermentations usually lead to an increase in ammonia-N (McDonald *et al.*, 1991). The larger amount of ammonia-N in silages produced from plants harvested at 35 days of sowing is a reflection of its higher moisture and CP contents at the time of harvest. According to Ferraretto *et al.* (2015), ammonia-N and soluble CP are good indicators of an efficient *in vitro* digestibility of starch present in the whole plant of corn silages. A reduction in butyric acid concentration with the increase in plant maturity was expected owing to an increase in the DM content of the mature forage, as was observed for silages made of plants harvested at 80 days after sowing. The silages produced from plants harvested after 65 days after sowing had the highest lactic acid concentrations and the lowest pHs as compared to plants harvested in other maturity stages. Despite the contribution of all acids formed during fermentation, lactate plays a critical role in the reduction of silage pH due to its low dissociation constant

(Moisio and Heikonen, 1994). This is mainly relevant as indicative of silages made with plants less than 35 days after planting, which have a low lactic acid content and consequently low silage stability. As shown by Ferreira *et al.* (2013), a higher lactic acid production leads to a lower rate of DM loss in silages that undergo active lactic acid fermentations. On the other hand, acetic and butyric fermentations are undesirable and need to be avoided as they favour DM losses in the form of gases released from the fermentative process.

Digestion study

Increased plant maturity resulted in decreased DM intake due to a decrease in digestibility, and this finding is corroborated by previous studies that investigated cereal crop silages (Helander *et al.*, 2015; Khan *et al.*, 2015). Lower intake of silages produced from plants harvested at later stages of maturity could be caused by higher stem proportions and more lignified stems, which in turn may result in a higher NDF and lignin concentrations in the silages. Overall, decreased digestibility of pearl millet silage decreased DM intake, and this agrees with the assumption that decreased digestibility decreases intake potential (Illius and Jessop, 1996). A reduction in DM digestibility with an increasing plant maturity was expected due to a reduction in plant cell wall digestibility. This observation was confirmed in the current study as shown by the linear decrease in NDF and ADF digestibility followed by the subsequent increase in lignin intake as the plant maturity increased. In fact, Davis *et al.* (2014) demonstrated that DM digestibility was correlated with consumption, digestible DM intake and BW gain in multiple regression models.

The decrease in CP concentration with the increasing plant maturity has been observed in previous studies (Khan *et al.*, 2007; Guimaraes *et al.*, 2014) and can be associated with a higher proportion of stems in the plants, which could ultimately reduce the protein solubility. It is also important to emphasize that the CP concentrations were higher than 60 g/kg DM for all treatments, a level that is considered suitable to sustain optimal microbial activity for an efficient ruminal fermentation (Van Soest, 1994). A decrease in CP concentration had no effect on CP intake, but the lambs examined in this study underwent a decrease in N intake. Regardless, N intake and N absorbed were on average larger than those recorded for Sipli lambs (8.7 and 4.5 g/day) fed pearl millet silage in Pakistan, as demonstrated by Khan *et al.* (2011). It should be mentioned that the positive N balance and the lack of body reserve mobilization observed in all treatments suggest an adequate digestibility of dietary protein.

Therefore, the current study showed that the pearl millet cultivar IPA BULK1-BF has the potential to yield forage within a wide harvest window, which ranges between 50 and 80 days after sowing. According to the digestion and *in vitro* digestibility studies, the nutritive value of pearl millet silage decreases as the plant maturity advances. In contrast, the fermentation parameters were improved with the increasing plant maturity. The possibility of harvesting pearl millet forage in any phenological stage between 50 and 80 days after sowing allows for the implementation of harvest management strategies that reduce the risk of losses caused by droughts while ensuring forage availability for ruminant feeding programs in arid regions. However, the current study has limitations that need to be addressed in future research, which include investigations on key ensilage metrics of the cultivar IPA BULK1-BF (such as DM loss, the chemical composition of pre-ensiling) as well as the microbial dynamics and its impact

on silage quality and ruminal fermentation. Additionally, investigations involving yield, DM intake and digestibility of IPA BULK1-BF compared with competing forages (e.g., sorghum, corn) cultivated in a similar fertilization scheme are warranted in future studies in order to harness the full potential of the inclusion of pearl millet in ruminant feeding programs.

Financial support. This work was implemented by Fundo Setorial do Agronegócio (CTA Agro) as part of the project 'Forrageiras e alimentos alternativos do Nordeste – Tabelas de composição de alimentos e estratégias de formulação de dietas de melhor benefício bioeconômico para bovinos leiteiros (CNPq/CTA Agro No. 555708/2009-8)', made possible by the support of the Banco do Nordeste.

Conflict of interest. The authors declare no conflict of interest.

Ethical standards. Not applicable.

References

- Adams RF, Jones RL and Conway PL (1984) High-performance liquid-chromatography of microbial-acid metabolites. *Journal of Chromatography B* **336**, 125–137.
- Agronomic Institute of Pernambuco (IPA) (1999) *Milheto IPA BULK1-BF: Cultivar de Milheto Forrageiro Para Corte, Feno E Silage*, 1st Edn., Recife, Pernambuco, Brazil: IPA.
- Aoki Y, Oshita T, Namekawa H, Nemoto E and Aoki M (2013) Effect of cutting height on the chemical composition, nutritional value and yield, fermentative quality and aerobic stability of corn silage and relationship with plant maturity at harvest. *Grassland Science* **59**, 211–220.
- Association of Official Analytical Chemists (AOAC) (2005) *Official Methods of Analysis*, vol. 2, 18th Edn. Arlington, VA, USA: AOAC.
- Atis I, Konuskan O, Duru M, Gozubenli H and Yilmaz S (2012) Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. *International Journal of Agriculture and Biology* **14**, 879–886.
- Bell MA and Fischer RA (1994) Guide to plant and crop sampling: measurements and observations for agronomic and physiological research in small grain cereals. Wheat Special Report, 66.
- Bernard JK and Tao S (2015) Short communication: production response of lactating dairy cows to brachytic forage sorghum silage compared with corn silage from first or second harvest. *Journal of Dairy Science* **98**, 8994–9000.
- Bezerra JDC, Santos IAS, Almeida GAP, Pereira MS, Lima DFF and Ferreira GDC (2011) Morfoanatomia do colmo de milheto (IPA BULK 1 BF) sob condições de irrigação no Agreste Pernambucano. *Revista Científica de Produção Animal* **13**, 38–42.
- Blaser JL, Hammes RC, Fontenot JP, Bryant HT, Polan CE, Wolf DD, McClougherty FS, Kline RG and Moore JS (1986) Growth stages of plants, forage quality and animal production. Forage-Animal Management Systems. *Virginia Agriculture Experiment Station Bulletin* **9**, 86–87.
- Bukhari MA, Ayub M, Ahmad R, Mubeen K and Waqas R (2011) Impact of different harvesting intervals on growth, forage yield and quality of three pearl millet (*Pennisetum americanum* L.) cultivars. *International Journal for Agro Veterinary and Medical Sciences* **5**, 307–315.
- Costa ACT and Priesnitz R (2014) Influence of spatial arrangement of pearl millet in relation to the phenological stage and thermal units. *Global Science and Technology* **7**, 37–47.
- Davis MP, Freetly HC, Kuehn LA and Wells JE (2014) Influence of dry matter intake, dry matter digestibility, and feeding behavior on body weight gain of beef steers. *Journal of Animal Science* **92**, 3018–3025.
- FAO (2008) *Climate Change and Food Security: A Framework Document*. Rome: FAO Publications.
- Ferraretto LF, Shaver RD, Massie S, Singo R, Taysom DM and Brouillette JP (2015) Effect of ensiling time and hybrid type on fermentation profile, nitrogen fractions, and ruminal in vitro starch and neutral detergent fiber digestibility in whole-plant corn silage. *The Professional Animal Scientist* **31**, 146–152.
- Ferreira DJ, Lana RP, Zanine AM, Santos EM, Veloso CM and Ribeiro GA (2013) Silage fermentation and chemical composition of elephant grass inoculated with rumen strains of *Streptococcus bovis*. *Animal Feed Science and Technology* **183**, 22–28.
- Guimaraes KC, Costa KAP, Paludo A, Santos NF, Rossi RM and Cruvinel WS (2014) Protein fraction, degradability and digestibility of pearl millet silage at different cutting ages. *Acta Scientiarum. Animal Sciences* **36**, 33–39.
- Hassanat F, Mustafa AF and Seguin P (2007) Effect of the brown midrib trait and stage of development at harvest on cell wall composition and degradability of forage pearl millet leaves and stems. *Canadian Journal of Animal Science* **87**, 421–429.
- Helander C, Nørgaard P, Zalis K, Martinsson K, Murphy M and Nadeau E (2015) Effects of maize crop maturity at harvest and dietary inclusion rate of maize silage on feed intake and performance in lambs fed high-concentrate diets. *Livestock Science* **178**, 52–60.
- Illius AW and Jessop NS (1996) Metabolic constraints on voluntary intake in ruminants. *Journal of Animal Science* **74**, 3052–3062.
- Johnson L, Harrison JH, Hunt C, Shinnors K, Doggett CG and Sapienza D (1999) Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. *Journal of Dairy Science* **82**, 2813–2825.
- Johnson LM, Harrison JH, Davidson D, Robutti JL, Swift M, Mahanna WC and Shinnors K (2002) Corn silage management I: effects of hybrid, maturity, and mechanical processing on chemical and physical characteristics. *Journal of Dairy Science* **85**, 833–853.
- Khan SH, Khan AG, Sarwar M and Azim A (2007) Effect of maturity on production efficiency, nutritive value and *in situ* nutrients digestibility of three cereal fodders. *International Journal of Agricultural Research* **2**, 900–909.
- Khan SH, Shahzad MA, Nisa M and Sarwar M (2011) Nutrients intake, digestibility, nitrogen balance and growth performance of sheep fed different silages with or without concentrate. *Tropical Animal Health and Production* **43**, 795–801.
- Khan NA, Yu P, Ali M, Cone JW and Hendriks WH (2015) Nutritive value of maize silage in relation to dairy cow performance and milk quality. *Journal of the Science of Food and Agriculture* **95**, 238–252.
- Kung L and Ranjit NK (2001) The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *Journal of Dairy Science* **84**, 1149–1155.
- Marsalis MA, Angadi SV and Contreras-Govea FE (2010) Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates. *Field Crops Research* **116**, 52–57.
- Mason SC, Maman N and Pale S (2015) Pearl millet production practices in semi-arid West Africa: a review. *Experimental Agriculture* **51**, 501–521.
- McDonald P, Henderson AR and Heron SJE (1991) *The Biochemistry of Silage*, 2nd Edn. Marlow. Chalcombe Publications, p. 226.
- McDougall EI (1948) Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal* **43**, 99–109.
- Moisio T and Heikonen M (1994) Lactic acid fermentation in silage preserved with formic acid. *Animal Feed Science and Technology* **47**, 107–124.
- Monks PL, Ferreira OGL and Peske ST (2005) Forage yield and quality, pre and post seed harvesting, of pearl millet submitted to different cutting regimes. *Revista Brasileira de Agrociência* **11**, 227–230.
- Morales JU, Alatorre AH, Becerra JFC and Vazquez HG (2014) Nutritive characteristics of pearl millet forage in four phenological stages. *Revista Mexicana Ciencias Pecuarias* **5**, 321–330.
- National Council for the Control of Animal Experimentation (CONCEA) (2008) Procedures for the scientific use of animals. Based on the CLAUSE VII of the 1st Paragraph in Article 225 of the Brazilian Federal Constitution. Brasília, DF, Brazil: Brazilian Government through the National Council for the Control of Animal Experimentation (CONCEA) and Institutional Animal Care and Use Committees (CEUA).
- Peyrat J, Baumont R, Le Morvan A and Nozière P (2015) Effect of maturity and hybrid on ruminal and intestinal digestion of corn silage in dry cows. *Journal of Dairy Science* **98**, 8009–8017.
- Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumberras JF, Coelho MR, Almeida JA, Cunha TJJ and Oliveira JB (2013) *Sistema Brasileiro de Classificação de Solos*. Brasília, DF, Brazil: Embrapa.

- Santos RD, Neves ALN, Pereira LGR, Sollenberger LE, Rodrigues JAS, Tabosa JN, Verneque RS, Oliveira GF, Jayme DG and Gonçalves LC** (2015) Agronomic traits, ensilability and nutritive value of five pearl millet cultivars grown in a Brazilian semi-arid region. *Journal of Agricultural Science* **1**, 1–9.
- Sebastian S, Phillip LE, Fellner V and Idziak ES** (1996) Comparative assessment of bacterial inoculation and propionic acid treatment on aerobic stability and microbial populations of ensiled high-moisture ear corn. *Journal of Animal Science* **74**, 447–456.
- Silva JFC and Leão MI** (1979) *Fundamentos de Nutrição dos Ruminantes*. Piracicaba, SP, Brazil: Livrocereis.
- Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG and Russell JB** (1992) A net carbohydrate and protein system for evaluating cattle diets: 2. Carbohydrate and protein availability. *Journal of Animal Science* **70**, 3562–3577.
- Tilley JMA and Terry RA** (1963) A two stage technique for the in vitro digestion of forage crops. *Grass and Forage Science* **18**, 104–111.
- Van Soest P** (1994) *Nutritional Ecology of the Ruminant*, 2nd Edn. New York. Cornell University Press, p. 476.
- Weatherburn MW** (1967) Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry* **39**, 971–974.
- Zadoks JC, Chang TT and Konzak CF** (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.