

## Elephant grass clones for silage production

Rerisson José Cipriano dos Santos<sup>1\*</sup>, Mário de Andrade Lira<sup>2,§</sup>, Adriana Guim<sup>1</sup>, Mércia Virgínia Ferreira dos Santos<sup>1</sup>, José Carlos Batista Dubeux Junior<sup>1</sup>, Alexandre Carneiro de Leão de Mello<sup>1</sup>

<sup>1</sup>UFRPE – Depto. de Zootecnia, R. Dom Manuel de Medeiros, s/n, Dois Irmãos – 52171-900 – Recife, PE – Brasil.

<sup>2</sup>Instituto Agrônomo de Pernambuco, Av. General San Martin, 1371, Bongi – 50761-000 – Recife, PE – Brasil.

\*Corresponding author <rerissonzte@yahoo.com.br>

Edited by: Concepta Margaret McManus Pimentel

Received December 08, 2011

Accepted July 02, 2012

**ABSTRACT:** Ensiling warm-season grasses often requires wilting due to their high moisture content, and the presence of low-soluble sugars in these grasses usually demands the use of additives during the ensiling process. This study evaluated the bromatological composition of the fodder and silage from five *Pennisetum* sp. clones (IPA HV 241, IPA/UFRPE Taiwan A-146 2.114, IPA/UFRPE Taiwan A-146 2.37, Elephant B, and Mott). The contents of 20 Polyvinyl chloride (PVC) silos, which were opened after 90 days of storage, were used for the bromatological analysis and the evaluation of the pH, nitrogen, ammonia, buffer capacity, soluble carbohydrates, and fermentation coefficients. The effluent losses, gases and dry matter recovery were also calculated. Although differences were observed among the clones ( $p < 0.05$ ) for the concentrations of dry matter, insoluble nitrogen in acid detergents, insoluble nitrogen in neutral detergents, soluble carbohydrates, fermentation coefficients, and *in vitro* digestibility in the forage before ensiling, no differences were observed for most of these variables after ensiling. All of the clones were efficient in the fermentation process. The IPA/UFRPE TAIWAN A-146 2.37 clone, however, presented a higher dry matter concentration and the best fermentation coefficient, resulting in a better silage quality, compared to the other clones.

**Keywords:** *Pennisetum purpureum*, grass silage, fermentation process, fodder, quality

### Introduction

Brazil has a great potential for in-pasture beef and dairy cattle production due to its large land area and favorable climatic conditions, yet, one of the characteristics of tropical forage is a high production per unit area but a reduced nutritive value (Rodrigues et al., 2004; Melo et al., 2005).

Silage production is a viable option to obtain forage during periods of reduced forage growth and quality. The use of tropical forage, such as elephant grass (*Pennisetum purpureum* Schum.), may be a viable alternative for silage production because it has a high productivity and animal acceptance. During the recommended harvest period (50–60 days of regrowth), elephant grass presents high moisture content. In general, warm-season grasses also have low soluble carbohydrates and high buffering capacity. As a result, fermentation process is difficult, resulting in poor quality silage (Rodrigues et al., 2005). Thus, ensiling practices such as wilting and use of additives to increase dry matter concentration may be used to improve the fermentation process and silage quality of warm-season grasses (Teixeira et al., 2008). If additive nutritive value is low, however, silage nutritive value will be reduced (Pires et al., 2009). In addition, production costs must be kept low in order to make additive use a viable option.

Based on these aspects, Silva et al. (2008) stated that the selection of *Pennisetum* sp. clones containing over 30 % of dry matter can create another alternative for the use of this grass as silage. Silva et al. (2008) evaluated

elephant grass clones obtained by self-pollination and inter- and intraspecific crosses with millet and observed that the highest dry matter concentration was presented by the interspecific hybrids, which display the highest dry matter concentration, with average values between 26.1 % and 27.5 % on the 60<sup>th</sup> day of growth.

The current study evaluated the bromatological composition of forage and silage using five *Pennisetum* sp. clones in the 'Zona da Mata Seca' region of the state of Pernambuco, Brazil.

### Materials and Methods

The study was performed in Itambé, Pernambuco State, Brazil (7°25' S; 35°06' W, 190 m a.s.l.). The soil of the study area was a Ferric Luvisol under a semi-deciduous tropical forest and a gently undulating terrain.

The experiment was conducted using a randomized block design with four replications. The treatments consisted of two elephant grass clones (IPA/UFRPE Taiwan A-146 2.114 and IPA/UFRPE Taiwan A-146 2.37) that were obtained from the breeding program of IPA-UFRPE, one hybrid of *Pennisetum purpureum* with Pearl millet IPA HV 241, and two other clones of elephant grass (Elephant B and Mott). The soil of the experimental area was mechanically prepared by plowing and harrowing. The plants were planted in plots with an area of 25 m<sup>2</sup> (5 m × 5 m) with 9 m<sup>2</sup> (3 m × 3 m) of utile area in four blocks. The rows were spaced by 1 m, and the planting occurred on 16 Jun. 2007.

Elephant grass for silage production was harvested at 56 days of regrowth, on 4 Aug. 2008, after a staging cut. The wilting practice was not used, and no additives were used in any of the treatments. Silos remained

<sup>§</sup>UFRPE – Depto. de Zootecnia, R. Dom Manoel de Medeiros, s/n – Dois Irmãos – 52171-900 – Recife, PE – Brasil.

sealed for 104 days prior to opening. During ensiling and after the silo was opened, 500 g of the silage was collected and placed in a dryer with a forced ventilation at 55 °C for pre-drying and further determination of the chemical composition.

Plants were cut at 5 cm above the ground and chopped in a stationary machine that was regulated for particle sizes between 2 and 3 cm. Afterwards, the material was homogenized and compressed in experimental silos of PVC that were 20 cm in diameter and 60 cm in height. Before filling, each silo was weighed, and 1 kg of washed sand was added to the bottom (sealed with wood) to absorb and quantify the effluents. The sand was separated from the silage using a plastic screen, and the fodder was manually compressed with the aid of a wood pendulum. The upper part of the silo was then sealed with plastic and adhesive tape.

The density of the ensiled mass was determined by the relationship between the liquid weight of the silage and the internal volume of the experimental silo. The losses were measured according to the following two equations, as previously described by Santos et al. (2006):  $Gases = (FWi - FWf)/(FMi \times DMi) \times 100$ , where FWi = the full silo weight after sealing (kg); FWf = the full silo weight after opening (kg); FMi = the fodder mass after sealing (kg) and DMi = the fodder dry mass content after sealing and  $Effluents = [(EWf - TS) - (EWi - TS)]/FMi \times 100$ , where E = the effluent production (kg per 100 kg of MV); EWf = the weight of the empty silo plus sand after opening (kg); TS = the tare of the silo; EWi = the weight of the empty silo plus sand after sealing (kg); TS = the tare of the silo and FMi = the fodder mass after sealing (kg).

The dry matter recovery was determined using the following equation, as previously described by Santos et al. (2006):  $DMR (\%) = [(GMfo \times DMfo)/(SMi \times DMSi)] \times 100$ , where GMfo is the fodder green mass (kg) at the moment of ensilage; DMfo is the fodder dry mass (%) at the moment of ensilage; SMi is the silage mass (kg) after opening the silos and DMSi is the silage dry matter (%) after opening the silos.

Silages of the upper (top 9 cm) and lower (bottom 9 cm) portions of the silos were discarded. The remaining silage was homogenized, stored in plastic bags, and frozen. A sample of each silage was weighed and dried with forced ventilation at 55 °C for 72 hours. The samples were removed from the dryer, weighed, ground using a "Thomas-Wiley" stationary mill, and passed through a 1-mm sieve. The analyses of the dry matter (DM), crude protein (CP = Kjeldahl N x 6.25) and ash (ASH) were conducted according to the AOAC (1990).

The neutral detergent fibers (NDFs), acid detergent fibers (ADF), and lignin contents were determined according to the methodology described by Van Soest et al. (1991). Sequential analyses of NDF and other fiber components, such as the insoluble nitrogen in the neutral detergent (NDIN) and the insoluble nitrogen in the acid detergent (ADIN), were conducted. The

hemicellulose content was calculated as the difference between the NDF and ADF. *In vitro* dry matter digestibility (IVDMD) of the grasses and silages was determined using the DAISY apparatus with the two-stage procedure (*i.e.*, a 48 h incubation with rumen fluid followed by a 24 h incubation with 6 M HCl and pepsin), as previously described by Holden (1999). Rumen fluid was obtained from a fistulated goat 2 h after the morning feeding. The goat was fed a high forage diet that consisted mainly of elephant grass that was cut at 60 days of regrowth. The silage pH was determined by extracting a 25 g aliquot from each silage sample in 100 mL of distilled water for 2 h. The extract was then filtered through four layers of cheesecloth, and the pH was determined using a portable pH meter.

The ammoniacal nitrogen to total nitrogen ratio was determined according to Preston's methodology (1986). The buffer capacity was determined in pre-dried samples, as previously described in Playne and McDonald (1966), and the soluble carbohydrates were determined according to the method of Yemm and Willis (1954), modified by Bezerra Neto and Barreto (2004). Soluble carbohydrates were extracted with a solution of 80 % alcohol in a reaction containing acid solution that was previously prepared using anthrone. The samples were evaluated using a spectrophotometer. A glucose solution was used as a control.

The evaluation of the forage fermentation coefficient was performed according to the methodology of Weissbach and Honig (1996), which evaluates three variables: the soluble carbohydrate concentrations, buffer capacity and dry matter content. This methodology uses the following equation:  $FC = DM + 8 \times SC/BC$ , where FC = the fermentation coefficient; DM = the dry matter; SC = the soluble carbohydrates and BC = the buffer capacity.

Data were submitted to PROC MIXED of the SAS software (2012) to perform the variance analysis. The data were expressed as the means and were compared using Tukey's test at 5 % probability.

## Results and Discussion

The elephant grass clone, Taiwan A-146 2.37, had a higher dry matter concentration ( $p < 0.05$ ), as compared to the Mott, Elephant B, and Taiwan 2.114 clones. The Taiwan 2.114 clone exhibited the lowest dry matter concentration among the clones tested. Bilal (2009) carried out a silage research using dwarf elephant grass (Mott) and observed at 60 days of regrowth 18 % of dry matter concentration. No additives were used and the silage presented poor fermentation compared to treatments using additives. In this study, however, the Taiwan A-146 2.37 clone, which is also a dwarf type of elephant grass, displayed the highest dry matter concentration among the clones evaluated. Dwarf clones are more likely to become dehydrated, as compared to tall elephant grass, and require less wilting time.

Forages with greater potential for ensiling must have an adequate chemical composition for fermentation and increased productivity. Cunha et al. (2011) also evaluated the dry matter yield of the same clones used in the current study and reported the following values: 6,258 kg ha<sup>-1</sup> for Taiwan A-146 2.37, 4,952 kg ha<sup>-1</sup> for Taiwan A-146 2.114, 5,756 kg ha<sup>-1</sup> for Elephant B, 6,873 kg ha<sup>-1</sup> for HV 241 and 4,465 kg ha<sup>-1</sup> for Mott. Silva et al. (2011a) evaluated five dwarf elephant grass clones. Average dry matter yield of these clones was 5,521 kg ha<sup>-1</sup>, at 60 days of regrowth. Therefore, dwarf elephant grass clones with a high dry matter concentration are suitable for ensiling.

Taiwan A-146 2.37 had the lowest insoluble nitrogen in the acid detergent (ADIN) content. However, Taiwan A-146 2.37 did not differ from Mott, Taiwan A-146 2.144 or HV 241 ( $p > 0.05$ ). These clones were similar ( $p > 0.05$ ) to Elephant B, which showed a higher ADIN value; in addition, the clones exhibited similar protein concentrations. Therefore, the difference that was observed in the ADIN values between the clones reflected the decreased availability of this nutrient for use by the animal. This variable denotes the nitrogen that is bound to the cell wall of the silage, which renders it unavailable throughout the digestive tract of the animal. However, the observed values do not compromise the quality of the forage. Similar research with tall elephant grass cv. Napier that was cut at 60 days of regrowth has reported an ADIN of 20.1 %, which corresponds to 8.1 % of total N (Pinho et al., 2008; Pires et al., 2009).

The clone Taiwan A-146 2.37 clone exhibited the highest FC ( $p < 0.05$ ), when compared to the other clones (Table 1). The amount of soluble carbohydrates that are needed to obtain a satisfactory fermentation in the ensilage process depends on the dry matter concentration and the buffer capacity of the crop. Forages with insufficient fermentable substrates or rather low DM content present values for the FC  $< 35$ , indicating the

need for the direct addition of sugar, such as molasses, or the addition of enzymes that release extra sugar from the forage (Oude Elferink et al., 2000). Therefore, all of the clones tested would require additives for satisfactory fermentation. However, Taiwan A-146 2.37 displayed the highest chance of fermentation success during the ensilage process without the use of additives because it had the highest dry matter content compared to the other clones during the developmental stage that was studied.

The Taiwan A-146 2.114 and Elephant B clones showed the lowest and highest IVDMD, respectively ( $p < 0.05$ ) (Table 1). A higher digestibility of the dwarf clones was expected because they usually have a higher leaf-to-stem ratio. However, we did not calculate this ratio due to the age of the plants at harvest. At 54 days of regrowth, however, the tall and dwarf elephant grass have similar nutritive value. Differences for the leaf-to-stem ratio will likely be observed at maturity.

The silages obtained from the Taiwan A-146 2.37 clone had a higher ( $p < 0.05$ ) DM concentration than the other clones (Table 2). The average DM concentration (23.7 %) presented by the Taiwan A-146 2.37 silage closely approximated the value (25 % of DM) that has been recommended by McDonald et al. (1991) as the condition necessary for minimizing effluents losses inside the silo and for maintaining the nutrients of the ensiled material. This finding was confirmed by the highest ( $p < 0.05$ ) DM recovery rate and by the numerically lowest amount of effluent that was shown by Taiwan A-146 2.37 (Table 3).

In the current study, the CP, NDF, ADF, cellulose, lignin, NDIN, ADIN, ASH, residual soluble carbohydrates and IVDMD contents of the silages did not differ ( $p > 0.05$ ). Furthermore, the values were similar to those that have been previously reported in the literature for elephant grass silage (Ferrari Junior and Lavezzo, 2001; Pinho et al., 2008). Forage mass density observed at the silo sealing determines the amount of

Table 1 – Chemical composition of the *Pennisetum* sp. clones before ensiling, on the 56<sup>th</sup> day after sowing.

Variables <sup>†</sup>	Clones					CV %
	Mott	Elephant B	Taiwan A-146 2.114	HV 241	Taiwan A-146 2.37	
DM	20.7 b <sup>†</sup>	20.0 b	17.7 c	21.7 ab	25.7 a	3.3
CP	10.4 a	10.1 a	10.8 a	12.2 a	11.7 a	9.6
ASH	12.3 a	9.3 a	11.4 a	11.1 a	10.0 a	13.5
NDF	64.7 a	66.7 a	61.7 a	64.9 a	65.5 a	5.3
ADF	38.4 a	41.2 a	36.5 a	41.9 a	39.7 a	9.8
HEMICEL	26.3 a	25.5 a	25.2 a	23.0 a	24.3 a	15.1
LIGNIN	8.8 a	10.0 a	8.7 a	10.5 a	10.6 a	7.6
ADIN	5.1 ab	7.0 a	4.9 ab	5.6 ab	3.6 b	20.5
NDIN	4.8 b	5.8a	4.9 b	4.7 b	4.4 b	5.1
BC	30.7 c	26.3 b	33.5 c	22.0 a	27.7 b	10.0
WSC	4.1 c	13.8 a	5.1 c	11.6 ab	10.6 b	13.7
FC	21.9 c	24.3 bc	19.3 d	25.4 b	29.0 a	5.3
IVDMD	54.7ab	57.3 a	48.7 b	53.0 ab	53.3 ab	6.7

<sup>†</sup>Means followed by the same letter in the row are not different (Tukey's test,  $p > 0.05$ ); <sup>†</sup>DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), HEMICEL (hemicelluloses), LIG (lignin), ADIN (the percentage of insoluble nitrogen in acid detergent to the total nitrogen), NDIN (the percentage of insoluble nitrogen in neutral detergent to the total nitrogen), BC (buffer capacity, in HCl/100 g of DM), ASH (ashes), WSC (soluble carbohydrates), FC (fermentation coefficient) and IVDMD (*in vitro* dry matter digestibility).

Table 2 – Chemical composition of the silages from *Pennisetum* sp. clones on the 56<sup>th</sup> day after sowing.

Variable <sup>†</sup>	Clones					CV %
	Mott	Elephant B	Taiwan A-146 2.114	HV 241	Taiwan A-146 2.37	
DM	18.7 b <sup>†</sup>	19.0 b	16.6 c	19.2 b	23.7 a	4.2
CP	9.5 a	10.1 a	10.0 a	10.4 a	12.2 a	13.9
NDF	55.8 a	59.6 a	56.7 a	59.6 a	60.3 a	7.8
ADF	34.2 a	36.7 a	31.5 a	36.0 a	33.3 a	6.9
HEMICEL	21.6 a	22.8 a	25.2 a	23.6 a	27.0 a	16.2
LIG	9.1 a	9.0 a	8.9 a	9.6 a	9.8 a	10.8
ADIN	6.1 a	5.3 a	4.4 a	5.3 a	4.5 a	17.1
NDIN	4.1 a	3.7 a	3.7 a	3.8 a	4.1 a	12.7
ASH	10.1 a	9.2 a	9.0 a	10.5 a	10.2 a	9.8
WSC	0.6 a	1.1 a	0.6 a	0.7 a	0.8 a	18.7
IVDMD	55.6 a	55.4 a	52.2 a	58.4 a	55.3 a	7.4
Silage Density	582.9 a	583.8 a	594.7 a	603.9 a	543.4 a	7.1

<sup>†</sup>Means followed by the same letter in the row were not different (Tukey's test,  $p > 0.05$ ); <sup>†</sup>DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), HEMICEL (hemicelluloses), LIG (lignin), ADIN (percentage of insoluble nitrogen in acid detergent to the total nitrogen), NDIN (percentage of insoluble nitrogen in neutral detergent to the total nitrogen), ASH (ashes), WSC (soluble carbohydrates), and IVDMD (*in vitro* dry matter digestibility).

Table 3 – Mean values for pH, effluents, gas loss, and ammoniacal nitrogen matter recovery of the silages of *Pennisetum* sp. clones.

Clones	pH	Effluents	Gases	Dry Matter Recovery	Ammoniacal Nitrogen
		kg t <sup>-1</sup> of MV	% DM	%	% NH <sub>3</sub> /NT
Mott	4.0 a <sup>†</sup>	27.4 a	1.3 a	81 ab	1.31 a
Elephant B	4.0 a	29.4 a	1.3 a	82.5 ab	0.9 a
Taiwan A-146 2.114	4.5 a	31.3 a	1.6 a	80.2 ab	2.7 a
HV 241	4.2 a	28.3 a	1.1 a	76.5 c	2.8 a
Taiwan A-146 2.37	4.2 a	25.2 a	0.4 a	88 a	1.8 a
CV (%)	10.4	39.5	63.4	5.8	29.8

<sup>†</sup>Means followed by the same letter in the column are not different (Tukey's test,  $p > 0.05$ ).

residual oxygen. Herbage dry matter concentration affects directly this response variable. In this study, silage density did not differ among clones and varied from 583 (104.9 kg DM m<sup>-3</sup>) to 604 (151.2 kg DM m<sup>-3</sup>) kg m<sup>-3</sup> of fresh forage. Holmes and Muck (1999) suggested 225 kg DM m<sup>-3</sup> for silage density in order to obtain a satisfactory fermentation.

No differences ( $p > 0.05$ ) were observed between the silages for the variables of the pH, ammoniacal nitrogen, effluents, and gases (Table 3). The studied silages revealed satisfactory pH values (3.8 – 4.2), except for Taiwan A-146 2.114. However, the pH cannot be used as the sole fermentative indicator, as wilted silage often displays a high pH. In addition, other factors, such as the ammoniacal N, lactic acid, butyric acid, and homo-/heterofermentative characteristics, are important factors to measure fermentation quality; indeed, these variables should be used as indicators to measure fermentation quality (Jobim et al., 2007). In the current study, all of the clones exhibited less than 3 % of ammoniacal N. The ammoniacal N is a product of clostridial fermentations, and the ammonia content must be less than or equal to 12 % of the total nitrogen for grass (McDonald et al., 1991). Silage studies evaluating elephant Grass harvested between 60 and 70 days of regrowth, without wilting

or additive use in the control treatments, presented ammoniacal N less than 6 % (Teixeira et al., 2005; Ferreira et al., 2009; Cavali et al., 2010; Monteiro et al., 2011). These values are similar to the values observed in the current study. Other studies, however, found ammoniacal N greater than 12 % (Pinho et al., 2008; Ferreira et al., 2010; Tales Rego et al., 2010).

No differences were observed ( $p > 0.05$ ) for the production of effluents and gases. No difference may be due to effluent and gaseous losses during the first days of fermentation. Rezende et al. (2008) have shown that elephant grass cut at the 70<sup>th</sup> day of regrowth exhibits effluent and gaseous losses of 24.4 kg t<sup>-1</sup> of fresh matter and 1.3 % DM, respectively, which are similar values to those found in the present study. Silva et al. (2011b), however, observed effluent and gaseous losses (66 kg t<sup>-1</sup> of fresh matter and 6.8 % on dry matter basis, respectively), for elephant grass silage when the forage was harvested at 50 days of regrowth. Dry mass recovery (DMR) is a measure of the ensilage efficiency because it indicates the amount of dry matter that is recovered from the original amount that was deposited in the silo. Therefore, it is possible to use the DMR to confirm that the Mott, Elephant B, Taiwan A-146 2.114 and Taiwan A-146 2.37 clones produced silages with satisfactory fer-

mentation due to less DM loss during the ensiling process. Overall, the Taiwan A-146 2.37 clone exhibited the highest number of desirable characteristics for silage production.

### Conclusions

The dwarf elephant grass, IPA/UFRPE Taiwan A-144 2.37, had the highest DM concentration and the best fermentation coefficient, indicating a better potential for quality silage production. Except for Taiwan A-146 2.114 and IPA HV 241, all elephant Grass clones presented an efficient fermentative process, despite their low dry matter concentration.

### Acknowledgments

This work has been financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of Ministério de Educação do Brasil. The authors would like to thank the Instituto Agrônômico de Pernambuco for providing the installations and the clones that were used in this study.

### References

- Association of Official Analytical Chemist [AOAC]. 1990. Official Methods of Analysis. 15ed. AOAC, Washington, DC, USA.
- Bezerra Neto, E.; Barreto, L.P. 2004. Methods for Chemical Analysis in Plants. Imprensa Universitária da UFRPE, Recife, PE, Brazil (in Portuguese).
- Bilal, M.Q. 2009. Effect of molasses and corn as silage additives on the characteristics of mott dwarf elephant grass silage at different fermentation. *Periods Pakistan Veterinary Journal* 29: 19-23.
- Cavali, J.; Pereira, O.G.; Valadares Filho, S.C.; Porto, M.O.; Fernandes, F.E.P.; Garcia, R. 2010. Mixed sugarcane and elephant grass silages with or without bacterial inoculants. *Revista Brasileira de Zootecnia* 39: 462-470.
- Cunha, M.V.; Lira, M.A.; Santos, M.V.F.; Freitas, V.E.; Dubeux Junior, J.C.B.; Mello, A.C.L.; Martins, G.R.M. 2011. Association between the morphological and productive characteristics in the selection of elephant grass clones. *Revista Brasileira de Zootecnia* 40: 482-488 (in Portuguese, with abstract in English).
- Ferreira, A.C.H.; Neiva, J.N.M.; Rodrigues, N.M.; Campos, W.E.; Borges, I. 2009. Nutritional evaluation of pineapple industry by-product as additive on elephant grass silage. *Revista Brasileira de Zootecnia* 38: 223-229 (in Portuguese, with abstract in English).
- Ferreira, A.C.H.; Neiva, J.N.M.; Rodrigues, N.M.; Lopes, F.C.F.; Lôbo, R.N.B. 2010. Intake and digestibility of elephant grass silages with the different levels of acerola industry by-product. *Revista Ciência Agrônômica* 41: 693-701 (in Portuguese, with abstract in English).
- Ferrari Junior, E.; Lavezzo, W. 2001. Quality of elephant grass silage (*Pennisetum purpureum* Schum.) wilted or adding cassava meal. *Revista Brasileira de Zootecnia* 30: 1424-1431 (in Portuguese, with abstract in English).
- Holmes, B.J.; Muck, R.E. 1999. Factors affecting bunker silo densities. Madison, University of Wisconsin, 7p. Available at: <http://www.uwex.edu/ces/crops/uwforage/BunkDens3.PDF> [Accessed Jul. 14, 2012]
- Holden, L.A. 1999. Comparison of methods of *in vitro* matter digestibility for ten feeds. *Journal of Dairy Science* 2: 1791-1794.
- Jobim, C.C.; Nussio, L.G.; Reis, R.A.; Patrick, S. 2007. Methodological advances in evaluation of preserved forage quality. *Revista Brasileira de Zootecnia* 36: 101-119 (in Portuguese, with abstract in English).
- McDonald, P.; Henderson, A.R.; Heron, S.J.E. 1991. *The Biochemistry of Silage*. Chalcombe, Marlow, England.
- Melo, R.; Nornberg, J.L.; Rocha, M.G.; David, D.B. 2005. Productive and qualitative characteristics of corn hybrids for silage. *Revista Brasileira de Milho e Sorgo* 4: 79-94 (in Portuguese, with abstract in English).
- Monteiro, I.J.G.; Abreu, J.G.; Cabral, L.S.; Ribeiro, M.D.; Reis, R.H.P. 2011. Elephant grass silage additives with alternative products. *Acta Scientiarum. Animal Sciences* 33: 347-352 (in Portuguese, with abstract in English).
- Oude Elferink, S.J.W.H.; Driehuis, F.; Gottschal, J.C.; Spoelstra, S.F. 2000. Silage fermentation processes and their manipulation. p. 180. In: Mannelje, L. 't., ed. *Silage making in the tropics with particular emphasis on smallholders*. FAO, Rome, Italy.
- Pinho, B.D.; Pires, A.J.V.; Ribeiro, L.S.O.; Carvalho, G.G.P. 2008. Elephant Grass silage with cassava meal. *Revista Brasileira de Saúde e Produção Animal* 9: 641-651 (in Portuguese, with abstract in English).
- Pires, A.J.V.; Carvalho, G.G.P.; Garcia, R.; Carvalho Junior, J.N.D.; Ribeiro, L.S.O.; Chagas, D.M.T. 2009. Elephant grass ensiled with coffee hulls, cocoa meal and cassava meal. *Revista Brasileira de Zootecnia* 38: 34-39 (in Portuguese, with abstract in English).
- Playne, M.J.; McDonald, P.T. 1966. The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture* 17: 264-268.
- Preston, T.R. 1986. *Better Utilization of Crop Residues and by Products in Animal Feeding: research guidelines; a practical manual for research workers*. FAO, Rome, Italy.
- Rêgo, M.M.T.; Neiva, J.N.M.; Cavalcante, M.A.B.; Candido, M.J.D.; Clementino, R.H.; Restle, J. 2010. Bromatological and fermentative characteristics of elephant grass silages with the addition of annato by-product. *Revista Brasileira de Zootecnia* 39: 1905-1910 (in Portuguese, with abstract in English).
- Rezende, A.V.; Gastaldello Jr., A.L.; Valeriano, R.A.; Casali, A.O.; Medeiros, L.T.; Rodrigues, R. 2008. Use of different additives in elephant grass silage. *Ciência Agrotécnica* 32: 281-287 (in Portuguese, with abstract in English).
- Rodrigues, A.L.P.; Sampaio, I.B.M.; Carneiro, J.C.; Tomich, T.R.; Martins, R.G.R. 2004. *In situ* degradability of dry matter of tropical forages obtained at different times of cut. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 56: 658-664 (in Portuguese, with abstract in English).
- Rodrigues, P.H.M.; Borgatti, L.M.O.; Gomes, R.W.; Passani, R.; Meyer, P.M. 2005. Addition of increasing levels of citrus pulp on fermentative quality and nutritive value of silage of elephant grass. *Revista Brasileira de Zootecnia* 34: 1138-1145 (in Portuguese, with abstract in English).

- Santos, E.M.; Zanine, A.M.; Ferreira, D.J.; Oliveira, J.S.; Pereira, O.G.; Almeida, J.C.C. 2006. Effect of addition of cheese whey on the chemical composition, fermentation, losses and dry matter recovery in elephant grass silage. *Ciência Animal Brasileira* 7: 235-239 (in Portuguese, with abstract in English).
- SAS Institute. 2012. SAS/STAT: user's guide; version 9.1.3. SAS Institute, Cary, NC, USA
- Silva, M.C.; Santos, M.V.F.; Lira, M.A.; Mello, A.C.L.; Freitas, E.V.; Santos, R.J.M.; Ferreira, R.L.C. 2008. Preliminary tests on self-pollination and cross breed in elephant grass breeding. *Revista Brasileira de Zootecnia* 37: 401-410 (in Portuguese, with abstract in English).
- Silva, T.C.; Dantas, P.A.S.; Dórea, J.R.R.; Santos, E.M.; Zanine, A.M.; Pereira, O.G. 2011a. Microbial populations, fermentation profile and chemical composition of elephant grass silages with jackfruit. *Archivos de Zootecnia* 60: 247-255 (in Portuguese, with abstract in English).
- Silva, M.A.; Lira, M.A.; Santos, M.V.F.; Dubeux Junior, J.C.B.; Freitas, E.V.; Araújo, G.G.L. 2011b. Forage yield and nutritive value in Pennisetum clones harvested in the forest zone. *Archivos de Zootecnia* 60: 63-74 (in Portuguese, with abstract in English).
- Teixeira, F.A.; Santos, L.C.; Nascimento, P.V.; Sá, J.F.; Souza, A.O.; Veloso, C.M.; Pires, A.J.V. 2005. Ammoniacal nitrogen losses in elephant grass silage (*Pennisetum purpureum* Shum.) Meal plus Cocoa (*Theobroma cacao*). *Revista Eletrônica de Veterinária* 6: 1-6 (in Portuguese, with abstract in English).
- Teixeira, F.A.; Veloso, C.M.; Pires, A.J.V.; Silva, F.F.; Nascimento, P.V.N. 2008. Losses in silage of elephant grass added with sugar cane and cocoa meal. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 60: 227-233 (in Portuguese, with abstract in English).
- Van Soest, P.; Robertson, J.B.; Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Animal Science* 74: 3583-3597.
- Yemm, E.W.; Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* 57: 508-514.
- Weissbach, F.; Honig, H. 1996. About the prediction and control of course of fermentation in the ensilage of green fodder from extensive cultivation. *Landbauforschung Volkenrode* 1: 10-17 (in German, with abstract in English).