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## Silages of pearl millet submitted to nitrogen fertilization

### Silagens de milho submetidas à adubação nitrogenada

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#### ABSTRACT

*This study aimed to evaluate the fermentation characteristics, losses and the chemical composition of two pearl millet genotypes silages submitted to nitrogen fertilization. The experimental design was a completely randomized blocks in a split plot scheme 2x5 (two nitrogen genotypes doses x five doses of nitrogen), with four replicates. Nitrogen doses were 0, 20, 40, 60, 80kg ha<sup>-1</sup> and the pearl millet genotypes were the variety ADR300 and the hybrid ADR7010. The hybrid ADR 7010 showed average lactic acid content higher than the variety ADR 300, at all doses of N, recording values ranging from 4.09 to 10.46dag kg<sup>-1</sup>. There was an interaction between nitrogen doses and genotypes for the neutral detergent fiber, which ranged from 51.81 to 63.63dag kg<sup>-1</sup> of dry matter. Dry matter recovery decreased linearly with increasing nitrogen doses only for hybrid ADR7010, the same did not happen for the ADR300. The nitrogen fertilization does not favor the fermentation characteristics and increases DM losses of the hybrid ADR7010.*

**Key words:** chemical composition, fermentation characteristics, lactic acid, semi-arid.

#### RESUMO

*Objetivou-se avaliar o perfil fermentativo, as perdas e a composição bromatológica de silagens de dois genótipos de milho, submetidos à adubação nitrogenada. O delineamento utilizado foi em blocos casualizados em um esquema de parcelas subdivididas 2x5 (dois genótipos x cinco doses de nitrogênio), com quatro repetições. As doses de nitrogênio foram 0, 20, 40, 60, 80kg ha<sup>-1</sup> e os genótipos de milho foram a variedade ADR300 e o híbrido ADR7010. O híbrido ADR 7010 apresentou teores médios de ácido láctico superiores aos da variedade ADR 300 em todos os níveis de N, registrando valores que variaram de 4,09 a 10,46 dag kg<sup>-1</sup>. Houve interação entre os níveis de adubo e os genótipos para a fibra em detergente neutro, que variaram de 51,81 a 63,63dag kg<sup>-1</sup> de matéria seca. A recuperação de matéria seca diminuiu*

*linearmente com o aumento das doses de nitrogênio somente para o híbrido ADR7010, o mesmo não acontecendo para a ADR300. A adubação nitrogenada não favorece o perfil fermentativo e incrementa as perdas de matéria seca do híbrido ADR7010.*

**Palavras-chave:** composição bromatológica, perfil fermentativo, ácido láctico, semiárido.

#### INTRODUCTION

The pearl millet is grass specie has been widely used by farmers as an alternative to reach the animal requirements in the critical period of the year, due to the fact that it is the forage more resistant to drought and is adapted to low soil fertility, presents fast growth and high yields of phytomass by unit area (AMARAL et al., 2008). It is a culture that shows good development in regions with low rainfall profile, so, it has been widely used for the production of grass silage during periods of late season and in regions that are characterized by having problems with dry periods or drought (GUIMARÃES JR. et al., 2010).

The nitrogen is considered one of the most important nutrients for the growth of several plant species because it can provide an increase in productivity of forage grass and in the amount of crude protein (CP) per hectare. But the responses to nitrogen depend on each forage species, being attributed to genetic constitution, soil and climatic conditions (DIAS et al., 2000).

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The efficiency of the silage conservation system should not be defined only by the nutritional value of silage, but also by the losses that occur from harvest to animals feeding (NEUMANN et al., 2007). The silage quality is directly related to the material which it is originated from and to the conditions under which it was ensiled (LOURES et al., 2003). In this way, changes occurring owing fertilization can provide changes in silage quality.

Therefore, this study aimed to evaluate the fermentation characteristics and the chemical composition, as well as to quantify the losses and dry matter recovery of two pearl millet genotypes silage submitted to nitrogen fertilization.

## MATERIALS AND METHODS

The experiment was conducted at the Estação Experimental Pendência, of Empresa Estadual de Pesquisa Agropecuária da Paraíba S.A. (EMEPA), located in Agreste region of Paraíba, Microregion of Western Curimatá, municipality of Soledade. The precipitation volume during the experimental period (May to July 2010) was 96mm.

The soil analysis of the experimental area was conducted in the laboratory of Federal University of Paraíba, presenting the following chemical attributes (profiles 0 to 20cm): pH (H<sub>2</sub>O): 6,32; P: 1,63; K<sup>+</sup>: 159 (mg dm<sup>-3</sup>); H<sup>+</sup>+Al<sup>3+</sup>: 1,49; Al<sup>3+</sup>: 0,0; Ca<sup>2+</sup>: 5,85; Mg<sup>2+</sup>: 1,10 (cmol<sub>c</sub> dm<sup>-3</sup>), base saturation (V): 83.87dag kg<sup>-1</sup> and organic matter (OM): 8.60g kg<sup>-1</sup>.

At the stage of pearl millet cultivation, it was used split-plot design with them hole plots arranged in a randomized complete-block design, with the plots represented by the dosages of nitrogen (0, 20, 40, 60, 80kg ha<sup>-1</sup>), and the subplots represented by pearl millet genotypes (variety ADR300 and hybrid ADR7010), with four replications. The spacing used in the culture was 0.5m between rows. The sowing of millet was held on May 4, 2010. At 15 days after emergence (DAE) it was performed the thinning, to obtain 15 plants/linear meter.

The nitrogen fertilization with ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) was performed, manually, directly beside the rows of plants, being the dosages of 20 and 40kg ha<sup>-1</sup> applied once, at 14 days after sowing (DAS), and the remaining dosages in installments, half of them applied at 14 days and half at 28DAS. At the time of sowing, it was performed the phosphate fertilization with 50 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, using simple superphosphate.

In this experiment, which corresponds to the evaluation of the silages, all the material for each combination of the factors studied in the four blocks

was cut for ensilage, and replicates were established by experimental silos. Thus, the experimental design was completely randomized in a 2x5 factorial scheme, with two pearl millet genotypes (variety ADR300 and the hybrid ADR7010) and five nitrogen dosages (0, 20, 40, 60 and 80kg ha<sup>-1</sup>) with four replicates.

The harvest of plants was done 64DAS, when the grains were in early-dough stage. In the plots of each treatment, the millet was cut to a height of 10cm from the ground and chopped into particles of approximately 2cm and ensiled in PVC silos with 15cm diameter and 30cm height, equipped with Bunsen valve, to exhaust gases. At the bottom of the silos, it was placed 1.50kg of sand to capture the effluent. The compaction of the material allowed approximately 3kg of fresh forage per silo. The usable area of silo was 15cm diameter and 28cm height.

After 30 days, the silos were opened, and then it was collected two samples of each experimental unit, in order to perform the analysis at the Laboratory of Animal Nutrition of the Center of Agrarian Sciences at the Federal University of Paraíba (UFPB).

At opening time, it was eliminated the top and bottom portions of each one, equivalent to approximately 5cm each. The central portion was homogenized and a sample of approximately 200g for the determination of pH and ammonia nitrogen was used. The analysis of pH and ammonia-N were conducted according to the methodology described by BOLSEN et al. (1992). For analysis of organic acids, 10g of sample was diluted in 90mL of distilled water and filtered through Whatman filter (KUNG JR. & RANJIT, 2001).

Also at the opening moment, another sample of approximately 200g was collected from each silo. These samples were pre-oven-dried at 65°C for 72h and ground in a Willey knife mill with a 1mm sieve for determination of the chemical constituents.

Measurements of dry matter (DM), ether extract (EE), and crude protein (CP) was determined by using the methods described by SILVA & QUEIROZ (2002). The content of neutral detergent fiber (NDF) was evaluated according MERTENS (2002), using an Ankom fiber analyzer. In the NDF, the samples were treated with alpha-amylase heat-stable without the use of sodium sulfite. The determination of soluble carbohydrates (SC) was performed according to DERIAZ (1961). It was calculated the concentration of total carbohydrates and of the non-fibrous carbohydrates, according to SNIFFEN et al. (1992). The main values of the chemical composition of the genotypes before ensiling can be observed in table 1.

Table 1 - Average values of chemical composition of plant genotypes used for ensilage.

Genotype	N doses (kg.ha <sup>-1</sup> )	DM	CP	NDF	EE	NFC	SC
----- dag kg <sup>-1</sup> DM -----							
ADR 300	0	24.74	6.80	57.49	2.56	25.57	13.26
	20	21.64	7.70	62.80	2.63	17.54	11.76
	40	20.53	7.28	63.89	2.61	16.43	14.17
	60	19.94	8.69	61.81	2.55	17.91	11.31
	80	21.36	5.85	60.31	2.56	22.47	14.07
ADR 7010	0	22.27	6.08	57.36	2.55	24.56	11.67
	20	19.38	6.76	56.63	2.60	25.82	13.39
	40	22.35	6.13	58.21	2.59	24.61	15.67
	60	22.74	6.12	52.30	2.55	29.54	17.17
	80	22.45	6.13	56.60	2.56	25.33	18.31

(DM): dry matter, (CP): crude protein, (NDF): neutral detergent fiber, (EE): ether extract, (NFC): non-fiber carbohydrates, (SC): soluble carbohydrates.

The losses of dry matter (DM) in form of gas and effluent, and the dry matter recovery (DMR) were estimated according to the equations described by ZANINE et al. (2010).

The results were submitted to analysis of variance, using the F test ( $\alpha=0.05$ ) to compare the means between genotypes, using the program SAEG, version 8.0 (UNIVERSIDADE FEDERAL DE VIÇOSA, 2000). When there was interaction, data were unfolded to evaluate effect of genotypes in each nitrogen doses and effect of doses in each genotype. For the effect of fertilizer, it was performed linear regression analysis. The criteria for selecting the models were the coefficients of determination, as well as the significance of the regression parameters tested by Student's *t* test ( $\alpha=0.05$ ).

## RESULTS AND DISCUSSION

There was difference for the value of DM ( $P<0.05$ ) between the genotypes. The variety ADR 300 presented higher value of DM than the hybrid ADR 7010 (Table 2). The values for DM decreased quadratically ( $P<0.05$ ) with the increasing of nitrogen doses. Concerning the values of CP and EE, there were not found difference ( $P>0.05$ ) among the genotypes studied.

There was an interaction ( $P<0.05$ ) between doses of fertilizer and genotypes for the NDF (Table 3). The NDF content increased linearly with the increasing of nitrogen dosages to the variety ADR 300 and decreased linearly to the hybrid ADR 7010. The values found are lower than those noticed by AMARAL et al. (2008) and GUIMARÃES JR. et

al. (2008) which studied cultivars of millet, and found values of NDF ranging from 60.15 to 68.63 and 70.50 and 71.2 dag kg<sup>-1</sup> DM, respectively. The low values of NDF observed in this study may be due to drought stress suffered by the culture, due to low rainfall observed during the experimental period. This stress undergone by plant caused to accelerate its cycle, staying with a smaller size, i.e., producing a smaller amount of fibers, because there was not a further expansion of the stem, resulting in greater panicle's participation in DM.

There was an interaction ( $P<0.05$ ) between doses of fertilizer and genotypes for the content of NFC. NFC values decreased linearly with increased dosages of nitrogen to the variety, the opposite happening for hybrid in which the values increased linearly with the increasing of nitrogen. The hybrid ADR 7010, when not fertilized, had lower amount of non-fibrous carbohydrates when compared to others. This can be explained by NDF values reported in the same table. The amount of NDF is inversely proportional to the value of NFC and, as the NDF values in the hybrid ADR 7010 decreased with the increasing in the doses of nitrogen.

There was an interaction ( $P<0.05$ ) between the nitrogen (N) doses and the genotypes for pH, noting that the values differ only statistically ( $P<0.05$ ) in the dosages of 60 and 80 kg ha<sup>-1</sup> of N (Table 4). Due to drought stress occurred during the experimental period, the millet shortened its cycle, so, there was not enough time for the plants extend their stems and, because of this, plants presented a small height (average height of 115cm). When

Table 2 - Average values of dry matter (DM), crude protein (CP) and ether extract (EE) of two pearl millet genotypes silages under nitrogen (N) fertilization.

Genotype	DM <sup>1</sup>	CP <sup>2</sup>	EE
	dag kg <sup>-1</sup> NM	----- dag kg <sup>-1</sup> DM -----	
ADR 300	18.05a	8.34a	1.69a
ADR 7010	16.23b	7.69a	1.68a
Mean	17.14	8.02	1.69
N doses (kg ha <sup>-1</sup> )			
0	18.90	6.57	1.58
20	17.88	7.55	1.69
40	17.24	8.18	1.77
60	15.24	9.08	1.63
80	16.56	8.70	1.78
Mean	17.15	8.02	1.69
CV <sup>3</sup> (%)	23.42	34.90	7.42

Means followed by different letters in the column differ among themselves by F test ( $\alpha = 0.05$ );

$$^1\hat{Y} = 19,1391 - 0,0825x + 0,0055x^2 \quad r^2 = 0,84;$$

$$^2\hat{Y} = 6,5153 + 0,0639x - 0,0004x^2 \quad r^2 = 0,96;$$

<sup>3</sup>CV – Coefficient of variation.

subjected to higher dosages of N, plant did not grow, hence it responded by concentrating its amount of soluble carbohydrates. It is likely that, in this case, the excess of sugar has caused a reduction in the pH range for growth of some species of yeast, below the ideal range recommended by McDONALD et al. (1991), between 3.8 and 4.2, which justifies the increase in losses by gasses.

There was an interaction ( $P < 0.05$ ) between N doses and the genotypes for the content of lactic acid (LA) (Table 4). The hybrid ADR 7010 had average contents of LA superior to the ones of the variety ADR 300, at all doses of N, being not differed statistically ( $P < 0.05$ ), only in the absence of fertilization. The hybrid showed higher average content of LA ( $P < 0.05$ ), probably because it had a greater concentration of soluble carbohydrates, the main substrate used for fermentation of the bacteria which produce LA. According to McDONALD et al. (1991), content of 5.0 from 10.0 dag kg<sup>-1</sup> of LA shows silage with an adequate fermentation patterns associated.

As for the acetic acid (AA), it was observed an interaction ( $P < 0.05$ ) between the genotypes and doses of N (Table 4). Genotypes differed ( $P < 0.05$ ) only in treatment which did not receive nitrogen fertilizer. The values of AA are lower than those found by GUIMARÃES JR. et al. (2005), who reported mean values of AA of 1.04 dag kg<sup>-1</sup> DM for millet silages opened at 28 days after ensiling. Low concentrations of AA as seen in table 4, show that there was little incidence of fermentation by enterobacteria, which produce such acid.

Concerning the N-NH<sub>3</sub>, there was interaction ( $P < 0.05$ ) between genotypes and N dosages (Table 4). The means were different ( $P < 0.05$ ) only for the first two N doses (20 and 40 kg ha<sup>-1</sup>). The N-NH<sub>3</sub> values are lower than those found by GUIMARÃES JR. et al. (2005), who found results ranging from 7.27 to 7.7 dag kg<sup>-1</sup> NT. The low production of N-NH<sub>3</sub> can be explained by the rapid drop in pH, which leads to decrease in populations

Table 3 - Average values of neutral detergent fiber (NDF) and non-fiber carbohydrates (NFC) in dagkg<sup>-1</sup> DM of two pearl millet genotypes silages under nitrogen (N) fertilization.

Genotype	----- N doses (kg ha <sup>-1</sup> ) -----					Mean	CV <sup>5</sup> (%)
	0	20	40	60	80		
	----- NDF (dag kg <sup>-1</sup> DM) -----						
ADR 300 <sup>1</sup>	52.67b	56.10b	57.62a	59.98a	60.20a	57.31	10.12
ADR 7010 <sup>2</sup>	63.63a	60.91a	57.60a	53.82b	51.81b	57.55	10.12
	----- NFC (dag kg <sup>-1</sup> DM) -----						
ADR 300 <sup>3</sup>	29.65a	24.61a	22.90a	19.50a	20.19a	22.90	10.12
ADR 7010 <sup>4</sup>	19.78b	21.26a	24.49a	25.65a	26.50a	24.49	10.12

Means followed by same letter in column do not differ by F test ( $\alpha = 0.05$ ).

$$^1\hat{Y} = 53,5770 + 0,0932x \quad r^2 = 0,90;$$

$$^2\hat{Y} = 61,6815 - 0,1536x \quad r^2 = 0,83;$$

$$^3\hat{Y} = 28,1750 - 0,12x \quad r^2 = 0,87;$$

$$^4\hat{Y} = 19,3955 + 0,0905x \quad r^2 = 0,91;$$

<sup>5</sup>CV – Coefficient of variation.

Table 4 - Mean values of fermentation profile in silages of two pearl millet genotypes under nitrogen (N) fertilization

Genotype	N doses (kg ha <sup>-1</sup> )					Mean	<sup>6</sup> CV(%)
	0	20	40	60	80		
	pH						36,90
ADR 300	3.49a	3.50a	3.37a	3.44a	3.44a	3.44	
<sup>1</sup> ADR 7010	3.68a	3.35a	3.29a	2.98b	3.03b	3.29	
	N-NH <sub>3</sub> (dag kg <sup>-1</sup> TN)						23.93
<sup>2</sup> ADR 300	0.61b	0.94b	1.19a	1.23a	1.19a	1.19	
<sup>3</sup> ADR7010	1.96a	1.6a	1.30a	1.13a	1.44a	1.44	
	LA (dag kg <sup>-1</sup> DM)						15.43
<sup>4</sup> ADR 300	5.69a	4.47b	4.09b	7.81b	7.90b	5.69	
ADR7010	6.02a	9.62a	8.83a	10.46a	9.60a	9.6	
	AA (dag kg <sup>-1</sup> DM)						25.95
<sup>5</sup> ADR 300	0.12b	0.16a	0.32a	0.52a	0.30a	0.3	
ADR7010	0.55a	0.26a	0.31a	0.34a	0.28a	0.31	
	BA (dag kg <sup>-1</sup> DM)						15.43
ADR 300	0.01	0.01	0.01	0.01	0.01	0.01	
ADR7010	0.01	0.01	0.01	0.01	0.01	0.01	

Means followed by same letter in column do not differ by F test ( $\alpha = 0.05$ ); N-NH<sub>3</sub> - ammonia nitrogen as a percentage of total nitrogen, LA - lactic acid, AA - acetic acid, BA - butyric acid;

$$^1 \hat{Y} = 3,5895 - 0,0073x \quad r^2 = 0,78;$$

$$^2 \hat{Y} = 0,6052 + 0,0210x - 0,0001x^2 \quad r^2 = 0,98;$$

$$^3 \hat{Y} = 2,0040 - 0,0283x + 0,0002x^2 \quad r^2 = 0,95;$$

$$^4 \hat{Y} = 5,4026 - 0,0573x + 0,0012x^2 \quad r^2 = 0,75;$$

$$^5 \hat{Y} = 0,0680 + 0,0121x - 0,0001x^2 \quad r^2 = 0,72;$$

<sup>6</sup>CV = Coefficient of variation.

of proteolytic microorganisms (enterobacteria and bacteria from the *Clostridium* family).

There was no effect of any source of variation ( $P > 0.05$ ) for the values of butyric acid (BA) (Table 4). The lower values observed in this study (around 0.01 dag kg<sup>-1</sup> DM) can be explained by the rapid decrease in pH, probably due to the development of LA producing bacteria (LAB).

Table 5 shows that there was an interaction ( $P < 0.05$ ) between N doses and the genotypes for the dry matter recovery (DMR). The DMR decreased ( $P < 0.05$ ) linearly with the increasing in the dosages of nitrogen only for the hybrid ADR7010, the same is not true for the hybrid ADR300. It was observed that the variety ADR300 showed, on average, superiority in the DMR of approximately 14.8 dag kg<sup>-1</sup> compared to the hybrid ADR7010.

In the highest doses of fertilizer hybrid ADR7010 showed lower DMR 69.19 and 68.98 for N doses of 60 and 80 kg ha<sup>-1</sup>, respectively. This increased loss can be explained by higher losses gases (Table 5) and was also observed when the genotype was subjected to the highest N doses. This lower DMR can be the result of undesirable fermentations, especially

yeast, since this hybrid showed high concentrations of soluble carbohydrates (Table 1), which possibly allowed a reduction in pH values close to 3.0 (PAIVA et al. 2011). In this pH range yeasts have greater development in detriment to other microbial groups, resulting in production of ethanol and consequently increasing the losses in the silage.

Concerning the losses by gas, it is possible to say that there was interaction ( $P < 0.05$ ) between nitrogen doses and the genotypes. It is observed that the hybrid ADR7010, when subjected to dosages of 60 and 80 kg ha<sup>-1</sup> of N, presented higher losses when compared to others. Losses by gas in the hybrid may have been influenced by high concentrations of soluble carbohydrates, which favored a significant drop in pH, lower than the optimum value, stimulating secondary fermentation. Concerning the losses by effluent, it was observed no effect of any source of variation ( $P > 0.05$ ). This can be explained due to the same doses of DM.

## CONCLUSION

Spite of the increase in crude protein and decreased neutral detergent fiber content, it is



Table 5 - Average values of dry matter recovery (DMR), losses by gas (G), losses by effluent (E) of two pearl millet genotypes silages under nitrogen (N) fertilization.

Genotype	----- N doses (kg ha <sup>-1</sup> ) -----					Mean	<sup>3</sup> CV (%)
	0	20	40	60	80		
----- DMR (dag kg <sup>-1</sup> DM) -----							12,72
ADR 300	87,76a	89,86a	86,24a	86,38a	88,16a	87,68	
<sup>1</sup> ADR 7010	81,41a	83,78a	81,29a	69,19b	68,98b	76,93	
----- G (dag kg <sup>-1</sup> DM) -----							36,90
ADR 300	13,25a	10,14a	13,75a	13,61b	11,84b	12,52	
<sup>2</sup> ADR 7010	18,58a	16,20a	18,71a	35,80a	31,01a	24,06	
----- E (kg Mg <sup>-1</sup> NM) -----							27,35
ADR 300	3,45	2,92	4,51	3,15	3,40	3,49a	
ADR 7010	3,12	3,24	4,71	3,42	3,91	3,68a	

Means followed by same letter in column do not differ by F test ( $\alpha = 0.05$ );

$$^1\hat{Y} = 88,8302 - 0,2723x \quad r^2 = 0,74$$

$$^2\hat{Y} = 11,17 + 0,2723x \quad r^2 = 0,64$$

<sup>3</sup>CV – Coefficient of variation.

observed that the nitrogen fertilization does not favor fermentation and increases the losses of dry matter of hybrid ADR7010. The genotypes under study, when ensiled, result in well-fermented silage with chemical composition, which indicates an adequate nutritional value, providing a feeding alternative to the use as roughage supplementation in semiarid regions.

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