

## Palm kernel meal as additive in the elephant-grass silage\*

*Farelo de dendê como aditivo em silagens de capim elefante*

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### RESUMO

Objetivou-se com este estudo avaliar o efeito da adição de farelo de dendê (BP) como aditivo na silagem de capim-elefante. A composição química e fermentação foram analisadas em delineamento experimental inteiramente casualizado, com seis tratamentos, sendo um sem BP (C) e seis repetições, em que as médias dos resultados dos dias de fechamento e abertura foram analisadas por análise de regressão. As amostras foram coletadas no momento da ensilagem e após 190 dias de armazenamento para avaliação da matéria seca, proteína bruta, carboidratos (total, não fibrosos, solúvel em água, celulose e hemicelulose), cinzas, lignina e extrato etéreo. Na fase aeróbia, foram coletadas amostras para pH, nitrogênio amoniacal, fungos e leveduras. O capim-elefante do experimento apresentou valores de 13,9% de MS e 8,25% de PB, enquanto a BP tem 77,2% de MS e 16,9% de PB. A inclusão de BP inibiu o crescimento de fungos e leveduras. O aumento do teor de BP diminuiu a concentração de NH<sub>3</sub>-N. Maiores concentrações de BP tornaram as silagens mais estáveis, tendo sua quebra em 72 horas, enquanto que a estabilidade de C foi de 19 horas. As silagens com concentrações superiores a 15% BP não apresentaram variações significativas no pH, na fase aeróbica. A inclusão de farelo de palma, em concentrações de 10% a 15% pode ser usado em silagem de elefante, inibindo fermentação indesejável e tornando-as mais estáveis. Concentrações acima deste valor podem afetar o valor nutritivo da silagem pelo alto teor de lignina do aditivo.

**Palavras-chave:** estabilidade aeróbia, *Elaeis guineensis*, fermentação, *Pennisetum purpureum*

### SUMMARY

The objective of this study was to evaluate the effect of adding palm kernel meal (BP) as an additive in elephant grass silage. The chemical qualitative and fermentation were analysed in a completely randomized design with six treatments, one with no BP (C) and six replications, where the averages of the results of days of closing and opening were analysed by regression analysis. We collected samples at the time of ensiling and after 190 days of storage for evaluation of dry matter, crude protein, carbohydrates (total, non-fibrous, water-soluble, cellulose and hemicellulose), ash, lignin and ether extract. In aerobic phase, we collected samples for pH, ammoniacal nitrogen, fungi and yeasts. The elephant grass of this experiment showed values of 13.9% DM and 8.25% CP while the BP has 77.2% DM and 16.9% CP. The inclusion of BP had inhibited the growth of fungi and yeasts. The increase in the content of BP decreased the concentration of NH<sub>3</sub>-N. Higher concentrations of BP in silage were more stable, and the stability breaks in 72 hours, while the stability of C was 19 hours. Concentrations above 15% BP had no significant variations in pH in the aerobic phase. The inclusion of palm kernel meal at concentrations 10% to 15% can be used in silage of elephant grass, inhibiting undesired fermentation and making them more stable. Concentrations above this value may affect the nutritive value of silage by high lignin content of the additive.

**Keywords:** aerobic stability, *Elaeis guineensis*, fermentation, *Pennisetum purpureum*

## INTRODUCTION

One of the biggest problem faced by farmers is the seasonality forage at certain times of the year, where the level of animal productivity decreases due to feed deficiencies of good nutritional value.

The grasses of the genus *Pennisetum* present high production of herbage mass per hectare during the period of higher precipitation of water. This causes excess production and most often occurs underutilized grass. The ensiling process is to take advantage of this strategy over and use it, regardless of the purpose: the production of milk, meat or as a supplement in the diet during critical periods caused by adverse weather.

However, this process requires some features that should be considered to be a minimum of losses during its making. The dry matter content (DM) has great influence in the chemical reactions that occur during storage, affecting thus the nutritional value of the silage (BARNETT, 1954). McDonald et al. (1991) considers a content of about 25% of DM that does not harm the fermentation processes. The practice of including additives absorbing moisture appears as an option to assist in biochemical interactions resulting from ensiling process.

Currently, the use of agro-industrial by-products appears as viable option for the farmer, especially for the cost and benefit of its use. In Pará, the annual yield of palm oil is about 27 thousand cubic meters of palm oil for biodiesel (EMBRAPA, 2010), and as a by-product is extracted palm kernel meal that has been used in animal feed due to its availability and low cost. However, there is little scientific information about its characteristics as food and its

effect on ensiling. There are few international studies that have used palm kernel meal in silage, and in Brazil there is no study on the subject, being necessary research that will enable their characterization and definition.

The objective of this work was to evaluate the effect of adding increasing concentrations of palm kernel meal (*Elaeis guineensis*) as additives sequestering moisture silage of elephant grass (*Pennisetum purpureum*).

## MATERIAL AND METHODS

The experiment was conducted between May 2012 and June 2013 at the Federal University of Pará (UFPA), in the Faculty of Veterinary Sciences, located in the city of Belém/PA. The field phase trial lasted 240 days and this is understood by cutting levelling, preparation of experimental silos and silo opening. The forage used was the elephant grass (*Pennisetum purpureum* cv Cameron), harvested with 1.20m high with about 45 days, the height of harvesting was based on studies from Wadi et al. (2004) which demonstrates that this height have the nutritional equilibrium with dry matter production. The grass was allocated in experimental silos in plastic buckets of 15kg. Compression was achieved using iron rods, and to quantify the mass was determined the volume of each silo experimental deducting the space occupied by sand and screen. After compaction of the forage, the silos were sealed with plastic caps and taped all around.

Table 1 shows the chemical composition of Palm kernel meal used in the experiment as an additive for elephant grass silage.

Table 1. Chemical composition of palm kernel meal found in Pará

Compounds	Palm kernel meal
DM (g.100g <sup>-1</sup> )	77.2
CP (g .100gDM <sup>-1</sup> )	16.9
ADF (g .100gDM <sup>-1</sup> )	48.9
NDF (g .100gDM <sup>-1</sup> )	71.5
EE (g .100gDM <sup>-1</sup> )	1.7
Ash (g.100DM <sup>-1</sup> )	4.6
Cel (g.100DM <sup>-1</sup> )	41.2
Hem (g.100DM <sup>-1</sup> )	22.6
Lig (g.100DM <sup>-1</sup> )	7.7
ADIN (g. N total <sup>-1</sup> )	3.4
CHO (g .100gDM <sup>-1</sup> )	76.7
NFC (g .100gDM <sup>-1</sup> )	12.9
WSC (g .100gDM <sup>-1</sup> )	0.9

DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, EE = ether extract, Cel = cellulose, hemicellulose = Hem, Lig = lignin, ADIN = acid detergent insoluble nitrogen, CHO = carbohydrates, NFC = non-fiber carbohydrates, WSC = water soluble carbohydrates. Source: Analysis of field data, 2012

Each silo behaved between 13-15kg of elephant grass (with or without additives), with screen and 2kg of sand, with the compression of  $668.31 \pm 37.70\text{kg/m}^3$  of natural raw.

Treatments were composed of increasing concentrations of palm kernel meal based on natural raw elephant grass. The treatments were:

C: 0 % (no added palm kernel meal - control); BP5%: 95% elephant grass, 5% palm kernel meal; BP10%: 90% elephant grass, 10% palm kernel meal; BP15%: 85% elephant grass, 15% palm kernel meal; BP20%: 80% elephant grass, 20% palm kernel meal; BP25%: 75% elephant grass, 25% palm kernel meal.

To determine the loss of dry matter (LDM) we used an adaptation to the

equation described by Jobim et al. (2007), the loss of gasses (LG) were estimated by the difference in gross weight of the experimental silos, silage and on the opening date. The determination of gas loss was calculated by the equation described by Siqueira et al., (2007). The determination of effluent production (EP) was calculated by the equation described by Siqueira et al. (2007).

Representative samples were taken from the elephant grass, palm kernel meal and from each treatment before the closing of the silos, and on opening day, for chemical, qualitative analyses. On days 0, 3, 6 and 9 were analysed for microbiological aerobic and aerobic stability.

Samples were pre-dried in a forced circulation oven at 55°C for three days (to stabilize its weight) and milled after sieve 1mm in a Wiley mill type. The content of dry matter (DM) and crude protein (CP), ash and ether extract (EE) were analysed according to Association of Official Analytical Chemists (1995). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by sequential method by Van Soest et al., (1991) using the apparatus analyser fiber without adding  $\alpha$ -amylase and sodium sulphite, lignin was obtained by extraction with 72% H<sub>2</sub>SO<sub>2</sub> and subsequently the measurement was taken of this lignin to obtain values of cellulose, hemicellulose and lignin, with the ashes corrected. Compounds acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996).

The water soluble carbohydrates (WSC) were extracted with 80 % ethanol and determined by the anthrone method (DISCHE, 1962). The results were expressed in g.100gDM<sup>-1</sup>. The total carbohydrate (CHO) were determined by the following equation:  $100 - (\%CP$

+ %EE + %Ash), according to Sniffen et al. (1992). The non-fibrous carbohydrates were determined by the difference of the CHO and NDFap, where NDFap is NDF corrected for ash and protein.

In addition to the analyses described, aliquots of aerobic treatments were sampled to obtain the aqueous extract for pH and ammoniacal nitrogen (NH<sub>3</sub>-N), according to the methodology described by Tabacco et al. (2009).

After the opening of the experimental silos were removed from each sample unit to be packaged in smaller silos. On days 0; 3; 6 and 9 were sampled for microbial count.

Microbiological analyses were carried out at the Microbiology Laboratory of the Federal University of Pará. Therefore, were weighed 25g silage and added 225mL of saline peptone at 0.1% for counting fungi and yeast according to Normative Instruction 62 (BRASIL, 2003). Dilutions were made from 10<sup>-1</sup> to 10<sup>-7</sup> and from these dilutions, the seedlings performed in triplicate in petri dishes with culture medium potato dextrose agar with correction for pH 4, by means of tartaric acid and soon after incubated aerobically for 72 hours at room temperature.

To determine the aerobic stability from the time of opening of the silo at the 190th day we inserted into a data logger inside the mass on each bucket to determine the temperature, and four devices were placed near the buckets to evaluate ambient temperature for ten days.

The aerobic stability was considered as the time required to achieve mass 2°C above room temperature (KUNG JUNIOR et al., 1984). The data loggers were programed for analysis of temperature in every 30 minutes.

The chemical (DM, ASH, CP, ADIN, CHO, NFC, WSC, EE, cellulose,

hemicellulose and lignin) and fermentation (density, EP, LDM, LG, pH and NH<sub>3</sub>-N) were analysed in experiments randomized design with six treatments and six replications where the average results of the day of closing and opening day were analysed by regression analysis using the software SAS (STATISTICAL ANALYSIS SYSTEM, 2001).

The experiment used a split-plot, so that treatments (concentrations of palm kernel meal) were randomized to the plots and evaluation times (0; 3; 6 and 9 days in aerobic environment) subplots. Data were analysed statistically by analysis of variance procedures suggested by Steel et al. (1997) with dismemberment of the regression curves for treatment in the experiments in split plot when plots are measured in time , using software SAS (Statistical Analysis System, 2001).

## RESULTS AND DISCUSSION

The Chemical characteristics before ensiling are shown at Table 2 and Table 3 demonstrates the chemical characteristics of the treatments at the opening of the silos

The inclusion of BP increased significantly (P < 0.05) for DM, CP, NDF, ADF, Lig, NIDA and EE.

BP notoriously had lower density and elephant grass in this experiment, to obtain the 25% dry - matter claimed by McDonald (1981), it was necessary to include 19.26% of BP, yielding a density of 642.02kg/m<sup>3</sup>. The DM content in treatments with concentrations less than 19.25% of BP are below the 25% recommended by McDonald et al. (1991) as a prerequisite for silage effluent has minimum loss and hence the retention of nutrients occurs in silage.

In CP, NDF and ADF decreased sharply on values found in elephant grass (Table 2) compared with the levels found in the silage at the opening (Table 3). Unlike what happened with the values of NIDA, given that the levels increased significantly after the fermentation period.

The increase of the BP content conserved CP present in the silage more efficiently in the treatment BP5%, BP10% and BP15%, which preserved 97%, 85% and 83% of CP, respectively, the C treatment, and BP20% BP25% preserved only 77%, 75% and 73% respectively, it is assumed that these losses were digestible protein, as ADIN representing the indigestible protein showed higher values. The high values of  $\text{NH}_3\text{-N}$ , also may explain the decrease of the values of CP, where the ammonia is a by-product of proteolysis caused by unwanted organism present in moist environments.

The increase of both Lig, NDF and ADF in the opening of the silos were given the loss of soluble components DM in the effluent, which increases the levels of these components (McDonald, 1981).

Reducing the amount of digestible protein in the substrate due to the change in  $\text{NH}_3\text{-N}$  is one of the determining factors for the increase indirect ADIN. Another key factor in achieving this effect was warm naturally occurring due metabolism of the silage, since this procedure slightly decreases the digestibility by increasing the levels of NIDN and ADIN (Van SOEST, 1994). The increase in the content of ADIN is not desirable because the nitrogen attached to the ADF is not used by ruminal bacteria.

Increasing concentrations of the additive, the levels of WSC decreased in treatments because the BP has lower concentration of WSC that the elephant

grass. However, the values were well below those found in the opening, which demonstrates that the WSC was consumed by the microorganisms which developed during the fermentation process.

The values of WSC differed in the time of ensiling (Table 2), which is justified by the lower concentration of WSC in BP, however, at the end of the fermentation period, no significant differences were found (Table 3). The highest values of WSC in silages with lower concentrations of BP explain why the pH found in these treatments were lower than that found in treatments with higher concentrations of BP because the WSC is an essential nutrient for the development of lactic acid bacteria, which produce lactic acid, so the treatments with higher concentrations of WSC had lower pH compared to treatments with lower levels of WSC. As included 1% BP, lost about 0.43% of WSC.

WSC values were lower in treated inclusions with higher BP concentrations (Table 2). At the opening of the silos values WSC showed no significant differences (Table 3). Table 3 presents the results of chemical composition of the silages after 190 days of fermentation. In the variables DM, CP, NDF, ADF, Lig, and NIDA observed difference among treatments ( $P < 0.05$ ). The maximum DM were found in the treatments with greater inclusion of BP. The levels of CEL, HEM, WSC, ASH and EE showed no significant difference ( $P > 0.05$ ) between treatments.

The EE showed little increase in their values in silages compared with the forage (Table 2).

Table 2. Density and chemical composition of silage at the time of ensiling

Variable	BP	BP5%	BP10%	BP15%	BP20%	BP25%	Equation	R <sup>2</sup>
DEN (kg/m <sup>3</sup> )	722,6	701,3	671,5	646,3	638,4	629,4	Y = 716,87 - 3,8866x	0,83
DM (g.100g <sup>-1</sup> )	13,9	16,9	17,0	22,0	25,7	29,2	Y = 13,15 + 0,6153x	0,98
CP (g.100gDM <sup>-1</sup> )	8,25	8,56	10,9	12,0	13,7	15,4	Y = 7,8 + 0,2898x	0,98
NDF (g.100gDM <sup>-1</sup> )	70,25	70,87	74,27	74,34	74,34	75,33	Y = 70,67 + 0,205x	0,82
ADF (g.100gDM <sup>-1</sup> )	41,26	44,26	44,96	45,10	46,83	47,42	Y = 42,211 + 0,2209x	0,89
Cel (g.100gDM <sup>-1</sup> )	39,2	41,3	43,18	43,8	43,7	45,0	Y = 1,331 - 0,1565x	0,68
Hem (g.100gDM <sup>-1</sup> )	28,0	27,5	25,9	25,3	24,6	24,2	Y = 1,989 - 0,1615x	0,48
Lig (g.100gDM <sup>-1</sup> )	3,04	5,01	5,19	5,78	6,04	6,11	Y = 0,4132 + 2,9562x	0,75
ADIN (%N-total)	2,48	2,82	3,12	3,36	3,68	3,88	Y = 2,5219 + 0,0561x	0,97
CHO (g.100gDM <sup>-1</sup> )	85,1	85,4	83,4	82,6	80,7	79,4	Y = 89,925 - 0,2484x	0,83
NFC (g.100gDM <sup>-1</sup> )	21,8	17,7	17,5	16,8	14,9	15,0	Y = 20,362 - 0,2463x	0,84
WSC (g.100gDM <sup>-1</sup> )	7,36	4,27	3,69	2,79	2,46	1,95	Y = 6,1406 - 0,1909x	0,93
Ash (g.100gDM <sup>-1</sup> )	6,50	5,91	5,54	5,29	5,42	5,10	Y = 6,2487 - 0,0497x	0,62
EE (g.100gDM <sup>-1</sup> )	1,64	1,62	1,65	1,69	1,79	1,84	Y = 1,5943 + 0,0089x	0,85

All results showed P < 0.05; Legend: DEN = density, DM = dry matter, CP = crude protein, Cel = cellulose, hemicellulose = Hem, Lig = lignin, ADIN = acid detergent insoluble nitrogen, CHO = carbohydrate total, NFC = non-fiber carbohydrates, WSC = water soluble carbohydrates, ash and EE = ether extract. Source: Analysis of field data, 2012

Table 3. Chemical characteristics of the treatments at the opening of the silos

Variable	BP	BP5%	BP10%	BP15%	BP20%	BP25%	Equation	R <sup>2</sup>
DM (g.100g <sup>-1</sup> )*	15,2	16,8	17,6	21,8	25,6	31,8	Y = 15,443 + 0,3102x	0,41
CP (g.100gDM <sup>-1</sup> )*	6,37	8,45	9,28	9,96	11,6	10,9	Y = 7,0776 + 0,1885x	0,61
NDF (g.100gDM <sup>-1</sup> )*	64,9	71,9	76,7	74,7	76,1	77,2	Y = 69,653 + 0,3923x	0,58
ADF (g.100gDM <sup>-1</sup> )*	43,9	46,8	52,4	50,9	52,9	53,3	Y = 45,514 + 0,3624x	0,64
Cel (g.100gDM <sup>-1</sup> )	41,04	43,34	47,06	45,03	46,2	47,1	NS	--
Hem (g.100gDM <sup>-1</sup> )	23,8	25,1	24,3	23,8	23,2	23,9	NS	--
Lig (g.100gDM <sup>-1</sup> )*	3,38	5,43	5,54	5,87	6,08	6,20	Y = 0,2342 + 0,3523x	0,78
ADIN (% N total)*	12,2	12,5	14,1	15,4	15,9	16,8	Y = 9,8671 + 0,2542x	0,19
WSC (g.100gDM <sup>-1</sup> )	0,78	1,11	0,60	0,94	0,83	0,77	NS	--
Ash (g.100gDM <sup>-1</sup> )	7,23	7,40	7,21	7,27	7,60	7,37	NS	--
EE (g.100gDM <sup>-1</sup> )	2,00	2,03	2,60	2,37	2,72	2,67	NS	--

\*P < 0.05, NS - Not significant, DM = dry matter, CP = crude protein, Cel = cellulose, hemicellulose = Hem, Lig = lignin, ADIN = acid detergent insoluble nitrogen, WSC = water soluble carbohydrates, ASH = ash, EE = ether extract. Source: Analysis of field data, 2012.

Fermentation characteristics, losses and microbial count of elephant grass silages treated with different concentrations of BP after 190 days of fermentation are shown in Table 4. Thus the regression analyses showed significance for the

content of LG and Fun estimating quadratic equations. Variables LDM, EP, pH and NH<sub>3</sub>-N varied significantly in linear equations. Yeasts in the regression was not significant (P > 0.05).

Table 4. Characteristics fermentative, losses and microbial count of elephant grass silages treated with different inclusion levels of palm kernel meal (BP) at the opening of the silos

Variable	BP	BP 5%	BP 10%	BP 15%	BP 20%	BP 25%	Equation	R <sup>2</sup>
LDM(g.100gDM <sup>-1</sup> )*	9,83	7,73	5,97	5,46	4,95	3,61	Y = 9,7968 - 0,2375x	0,74
LG(g.100gDM <sup>-1</sup> )*	4,40	4,00	5,64	5,16	2,82	3,67	Y = 4,2534 + 0,1289x - 0,0068x <sup>2</sup>	0,29
EP (Kg.ton NM <sup>-1</sup> )*	44,6	42,0	27,3	28,1	10,4	5,31	Y = 47,056 - 1,6601x	0,69
pH*	3,63	4,24	4,39	4,71	4,81	4,88	Y = 3,8519 + 0,0473x	0,98
NH <sub>3</sub> -N (% of total N)*	11,7	12,6	11,4	5,67	4,41	1,93	Y = 13,677 - 0,4558x	0,97
Fun(log CFU.g <sup>-1</sup> )*	2,03	3,04	3,17	3,34	1,84	1,00	Y = 2,0775 + 0,2326x - 0,0113x <sup>2</sup>	0,75
yeast(log CFU.g <sup>-1</sup> )	1,81	2,62	2,52	2,47	2,88	1,00	NS	NS

\*P < 0.05; NS - Not significant

NM = Natural matter, CFU = colony formation unit, LDM = Loss of dry matter, LG = Loss gases, EP = Production effluent NH<sub>3</sub>-N = ammoniacal nitrogen, Fun = fungi, and yeasts. Source: Analysis of field data, 2012.

There was a quadratic response for all treatments for the variables Yeasts, Fun and Temperature. When using the BP, it was noted linear decrease in the production of NH<sub>3</sub>-N. On the last day of aerobic conditions (day 9), it was noted that the yeast count had the highest value in the treatment without the additive followed by treatment with less inclusion of BP.

The addition of the BP yielded smaller DM losses, this was because BP has greater DM value and lignin, which according to Jones & Jones (1995) the ability to retain moisture additives absorbent may vary with the type of material used, noting that materials with higher lignification have higher water retention ability. However, according to these authors, though highly absorbent, these additives reduce the nutritive value of silage.

The pH of the treatment at the time of opening of the silo increased according to the concentration of the BP, values

ranged from 3.63 in C to 4.88% in BP25%, in addition, showed a good appearance and smell. According to Van Soest (1994) in silage with high DM content, the pH is less important, good quality can be obtained even at higher pH. Whittenbury et al. (1967) demonstrated that, evaluating the pH isolated, it becomes a less important parameter, because to consider a good quality silage, the pH should decrease rapidly to avoid the immediate production of NH<sub>3</sub>-N and butyric acid. In silage, low content of ammoniacal nitrogen (NH<sub>3</sub>-N), less than 10 % of total nitrogen (TN), indicates that the fermentation process did not result in excessive breakdown of protein into ammonia (VAN SOEST, 1994). In contrast, NH<sub>3</sub>-N concentration exceeding 15% NT means the breakdown of proteins was high, that silage can be rejected by the animals, resulting in low consumption. The concentration of NH<sub>3</sub>-N also indicates the activity of

bacteria of the genus *Clostridium*, because this compound is produced in small quantities by other microorganism and enzymes from plants (McDonald, 1981). The values found in this experiment were considered good, values below 10 % were observed in treatments with higher inclusions of BP (15%, 20% and 25%).

The higher values of NH<sub>3</sub>-N in the treatments with lower concentrations of BP demonstrated that even with low pH did not inhibit the bacteria of the genus *Clostridium*, which have their optimal growth in moisture content above 72 % and a pH of around 5, but it is possible their growth in silage having problem to stabilize (McDonald et al., 1991).

Count yeast, filamentous fungi, temperature, pH and NH<sub>3</sub>-N in the elephant grass silages with increasing inclusion of palm kernel meal after silo opening are shown at Figure 1.

The C treatment also had the highest values for filamentous fungi and pH (Figure 1). The variable pH, it was found that the treatments with inclusion of BP higher than 15% did not change significantly ( $P > 0.05$ ) during aerobic conditions.

The microorganism that initiate the attack on organic acids and are the most responsible for aerobic deterioration of silage are yeasts (PAHLOW et al., 2003). Many species of yeasts degrade lactic acid, causing the pH of the silage and provided conditions for other spoilage microorganism develop (McDonald et al., 1991). This explains the faster growth of the yeast population at the beginning of the aerobic process.

Regression analysis of yeast counts (yeast), filamentous fungi (Fun), temperature, pH and ammonia (NH<sub>3</sub>-N), during aerobic conditions are shown in Figure 1. The count of yeasts and filamentous fungi showed reduced values

in silages with inclusion of more than 15% of BP (Figure 1).

Filamentous fungi grow best under aerobic conditions and higher pH (5.0 to 6.0). Thus, when the silo is opened, there is an increase in pH and oxygen concentration allowing the growth of these microorganism capable of degrading wide variety of nutrients, including structural carbohydrates and lignin. Thus, the degradation of complex compounds can release substrates for lactic acid bacteria and yeast to continue to grow (WINTERS et al., 1987).

The temperature elevation and its maintenance over time were important indicators of aerobic deterioration being due to the growth of yeasts and filamentous fungi.

Figure 2 shows the difference in temperature inside the silo and the environment and indication of the breakdown of the aerobic stability of silage elephant grass with increasing addition of BP after opening. The highest temperature during the aerobic treatments were C, and BP5% and BP10% respectively. The treatments that had broken stability later were BP15%, BP20% and BP25% % both with 72 hours, making them more stable than the others. The C treatment was the most unstable, breaking its stability at 19 hours after silo opening, followed by BP5 % (27 hours) and BP10% (29 hours).

The aerobic stability of silage can be defined as the resistance of the mass of material degradation after opening the silo. Some authors define as the time it takes for the silage to a temperature higher than 2°C above room temperature (KUNG JUNIOR et al., 1984) or the accumulation of temperature during the days when the silage is exposed to air. The rapid metabolism of yeast during the first hours and the aerobic environment facilitated the development of other spoilage microorganism caused the

temperature varied significantly in the first few hours of aerobic, in particular to treatments with concentrations less than 15% BP.

The breakdown of stability was influenced by the addition of BP silage. The highest concentration of DM treatments made silages took longer to break aerobic stability, as higher values of DM hampered the growth of

microorganism, lack of adequate moisture (BATISTA et al., 2006).

The amplitude of the temperature variation of the silo was quite high in treatments with less of 15% of BP, reaching a peak of 7.2°C above the temperature at 5% inclusion, which could infer that there was intense microbial activity at this time.

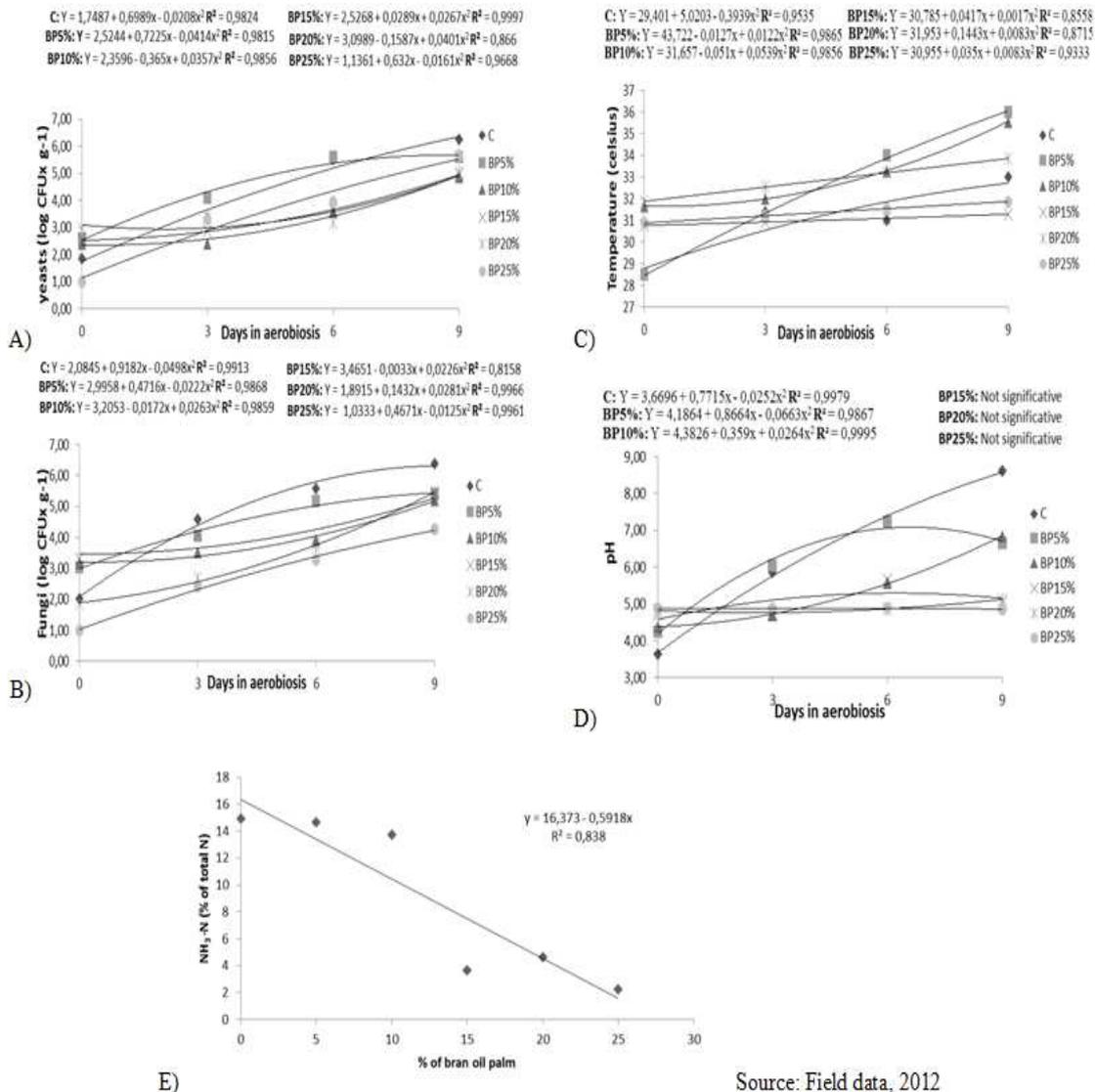
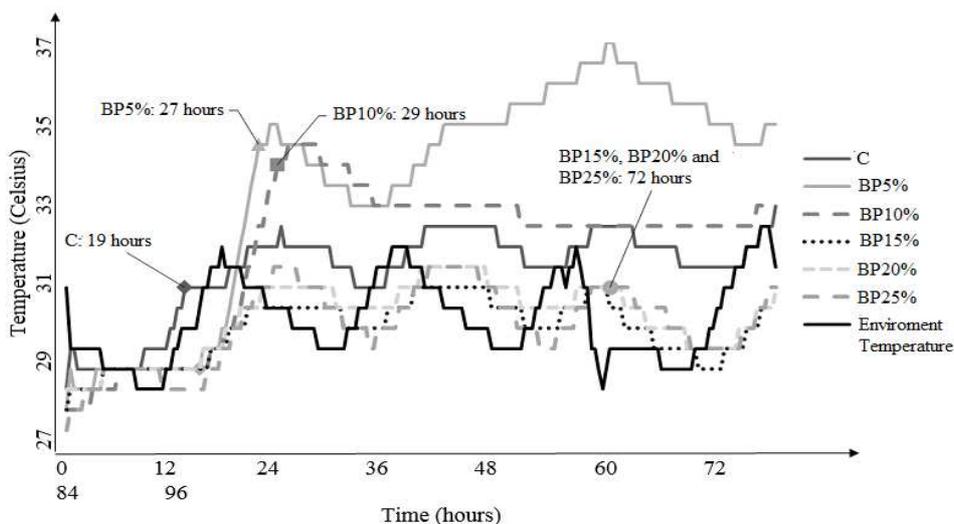


Figure 1. Count yeast (A), filamentous fungi (B), temperature (C), pH (D) and NH<sub>3</sub>-N (E) in the elephant grass silages with increasing inclusion of palm kernel meal (BP) after silo opening



Source: Field data, 2012.

Figure 2. Temperature difference silo with temperature and an indication of the breakdown of aerobic stability of elephant grass silage with increasing additions of palm kernel meal (0%, 5%, 10%, 15%; 20% and 25%) after opening the silos

Treatment with lower levels of BP had higher concentration of WSC resulting in lower pH values because the microorganism produced greater amounts of organic acids, including lactic acid serving as a substrate for spoilage microorganism during aerobic conditions, causing the pH to rise again and have a greater proliferation of fungi and yeasts, causing heat generation. It was noted that the treatments with inclusion of more than 15% BP, despite the temperature variation, showed no significant changes in pH during the aerobic period evaluated. The higher concentration of DM silages with values above 15% of BP and the absence of change in pH, inhibit the growth of bacteria of the genus *Clostridium*, microorganism responsible for the main metabolizing protein  $\text{NH}_3\text{-N}$ , and the larger was the addition of BP, the lower the concentration of  $\text{NH}_3\text{-N}$  found. The inclusion of palm kernel meal in concentrations between 10-15% can be used in elephant grass silage, inhibiting undesired fermentation, reducing losses

of DM and making them more stable. Concentrations above this value may affect the nutritive value of silage because of the high lignin content of the additive.

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## REFERENCES

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official Methods of Analysis**. 16. ed. Washington DC, 1995.
- BARNETT, A.J.G. **Silage fermentation**. London: Butterworths Sci. Publ., 1954.

- BATISTA, A.M.V.; GUIM, A.; SOUZA, I.S.; LIRA, K.G.; SANTOS, M.V.F. dos. DUBEX JUNIOR, J. C. B. Efeitos da adição de vagens de algaroba sobre a composição química e a microbiota fúngica de silagens de capim-elefante. **Revista Brasileira de Zootecnia**, v.35, n.1, p.1-6, 2006.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa nº62**. Brasília, 2003.
- DISCHE, Z. General color reactions. In: WHISTLER, R.L.; WOLFRAM, M.L. ed. **Carbohydrate chemistry**. New York, Academic Press, 1962. p.477-512.
- EMBRAPA. **Zoneamento agroecológico do dendezeiro para as áreas desmatadas da Amazônia Legal**. Relatório Síntese. Rio de Janeiro, 2010. 44p.
- JOBIM, C.C., NUSSIO, L.G., REIS, R.A., SCHMIDT, P. Avanços metodológicos na avaliação da qualidade da forragem conservada. **Revista Brasileira de Zootecnia**, v.36, p.101-119, 2007. Suplemento especial.
- JONES, D.I.H.; JONES, R. The effect of crop characteristics and ensiling methodology on grass silage effluent production. **Journal of Agricultural Engineering Research**, v.60, n.1, p.73-81, 1995.
- KUNG JUNIOR, L.; GRIEVE, D.B.; THOMAS, J.W.; HUBER, J.T. Added ammonia or microbial inoculant for fermentation and nitrogenous compounds of alfalfa ensiled at various percents of dry matter. **Journal of Dairy Science**, v.67, p.299-306, 1984.
- LICITRA, G.; HERNANDEZ, T.M.; VAN SOEST, P.J. Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology**, v.57, n.4, p.347-358, 1996.
- McDONALD, P. **The biochemistry of silage**. New York: John Wiley & Sons, 1981. 207p.
- McDONALD, P.; HENDERSON, A.R.; HERON, S.J.E. **The biochemistry of silage**. New York: Chalcombe Publications, 1991.
- PAHLOW, G.; MUCK, R.E.; DRIEHUIS, F.; OUDE ELFERINK, S.J.W.H.; SPOELSTRA, S. F. Microbiology of ensiling. In: BUXTON, D. R.; MUCK, R. E.; HARRISON, J.H. (Ed.). **Silage science and technology**. Wisconsin: American Society of Agronomy, Crop Science Society of America and Soil Science Society of America. 2003. p.31-94.
- SIQUEIRA, G.R.; REIS, R.A.; SCHOCKEN-ITURRINO, R. P., PIRES, A.J.V., BERNARDES, T.F., AMARAL, R.C. Perdas de silagens de cana-de-açúcar tratadas com aditivos químicos e bacterianos. **Revista Brasileira de Zootecnia**, v.36, p.2000-2009, 2007.
- SNIFFEN, C.J.; O'CONNOR, J.D.; VAN SOEST, P.J. A net carbohydrate and protein system for evaluating cattle diets: II- Carbohydrate and protein availability. **Journal of Animal Science**, v.70, p.3562-3577, 1992.
- STATISTICAL ANALYSIS SYSTEM - SAS. **The SAS System for Windows**: version 8.02. Cary: SAS Institute, 2001.

STEEL, R.G.D.; TORRIE, J.H.;  
DICKEY, D.A. **Principles and  
procedures of statistics a biometrical  
approach**. 3. ed. Boston: WCB  
McGraw-Hill, 1997. 666 p.

TABACCO, E.; PIANO, S.;  
CAVALLARIN, L.; BERNARDES,  
T.F.; BORREANI, G. Clostridia spore  
formation during aerobic deterioration  
of maize and sorghum silage as  
influenced by *Lactobacillus buchneri*  
and *Lactobacillus plantarum* inoculants.  
**Journal of Applied Microbiology**,  
v.107, p.1632-1641, 2009.

VAN SOEST, P.J.; ROBERTSON, J.B.;  
LEWIS, B.A. Symposium: carbohydrate  
metodology, metabolism, and  
nutritional implications in dairy cattle.  
**Journal Dairy Science**, v.74, n.10,  
p.3583-3597, 1991.

VAN SOEST, P.J. **Nutritional ecology  
of the ruminant**. 2.ed. Ithaca: Cornell  
University Press, 1994. 476p.

WADI A; ISHII, Y; IDOTA, S. Effects  
of cutting interval and cutting height on  
dry matter yield and overwintering  
ability at the established year in  
*Penisetum* species. **Plants Production  
Science**, v.7, p.88-86, 2004.

WHITTENBURY, R; McDONALD, P;  
BRYAN-JONES, D.J. A short review of  
some biochemical and microbiological  
aspects of silage. **Journal Science Food  
and Agricultural**, v.18, n.4, p.441-444,  
1967.

WINTERS, A.L.; WHITTAKER, P.A.;  
WILSON, R.K. Microscopic and  
chemical changes during the first 22  
days in Italian ryegrass and cocksfoot  
silages made in laboratory silos. **Grass  
and Forage Science**, v.42, p.191-196,  
1987.

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