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Chemical and microbiological changes and aerobic stability of marandu grass silages after silo opening[1]

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ABSTRACT - This trial had the objective of characterizing the microbial population and evaluating the aerobic stability of Marandu grass silages with pelleted citrus pulp (PCP). The collected forage was submitted to the following treatments: Silage of Marandu grass; silage of Marandu grass + 50 g/kg PCP and silage of Marandu grass + 100 g/kg PCP on natural matter basis. Metal cylindrical containers with 80 cm of height and 50 cm of diameter were used as silos during assays of microbiological dynamics and chemical changes of silages in anaerobiosis. Evaluations were performed on days 0, 2, 4 and 6 after silos were opened. The aerobic stability was evaluated by change in temperature, using approximately three kilograms of silage inside styrofoam boxes that were placed inside a climatic chamber. A completely randomized experimental design and split plot arrangement were used in the two assays, with five replications. Treatments were the plots and time was the subplots. *Bacillus* and enterobacteria were present on the Marandu grass silages with 0 g/kg PCP, which also showed pH increase throughout the feedout phase. Yeast was detected on the silages that were added with PCP. A trend of increasing temperature with extension of the aeration time was observed mainly in the silages containing 100 g/kg PCP. Isolated yeast strains showed lactate assimilation. Silages were found to be unstable due to the silo opening, both by bacterial or yeast development, which reduced the nutritional value.

Key Words: additive, *Brachiaria brizantha*, losses, spoilage microorganisms

Alterações químicas e microbiológicas de silagens de capim-marandu após a abertura dos silos

RESUMO - Esta pesquisa foi realizada com os objetivos de caracterizar a microbiologia e avaliar a estabilidade aeróbia de silagens de capim-marandu contendo polpa cítrica peletizada (PCP). A forragem colhida foi submetida aos seguintes tratamentos: silagem de capim-marandu; silagem de capim-marandu + 5% PCP e silagem do capim-marandu + 10% de PCP com base na matéria natural. As alterações químicas e microbiológicas foram feitas aos 0, 2, 4 e 6 dias após a abertura dos silos (tambores de metal com 80 cm de altura e 50 cm de diâmetro). Na avaliação da estabilidade aeróbia por meio da alteração da temperatura, 3 kg de silagem foram colocados em caixas de isopor, que foram armazenadas em câmara climática. Nos dois ensaios realizados, utilizou-se delineamento inteiramente ao acaso com cinco repetições, em esquema de parcelas subdivididas, de modo que os níveis de PCP na silagem corresponderam às parcelas e o tempo, às subparcelas. As silagens sem polpa apresentaram desenvolvimento de bacilos e enterobactérias e aumento do pH no decorrer do desabastecimento dos silos. A presença de leveduras foi detectada nas silagens contendo o aditivo e aumentou do primeiro ao sexto dia de aeração. A digestibilidade *in vitro* da matéria seca (DIVMS) reduziu com o aumento dos tempos de aeração. A temperatura nas silagens com 10% de PCP aumentou durante a aerobiose. Com a abertura dos silos, as silagens apresentaram-se instáveis, seja pelo desenvolvimento de bactérias seja pelo desenvolvimento de leveduras, o que reduziu seu valor nutritivo.

Palavras-chave: aditivo, *Brachiaria brizantha*, microrganismos espoliadores, perdas

Introduction

The anaerobic environment of a silo responsible for forage conservation becomes aerobic when the silo is opened. In such conditions, organisms that are latent in the absence of oxygen multiply rapidly, and silage is thus degraded. This phenomenon is evidenced by the development of undesirable microorganisms (bacteria and molds),

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increase in temperature and decrease in feed carbohydrate levels (Pahlow et al., 2003).

During ensilage of tropical grasses, the use of fermentation stimulating agents, such as citrus pulp, has led to the occurrence of yeast and filamentous fungi. Some well-fermented silages with additives and presenting high lactic acid levels and remaining sugars seem to be subject to fast deterioration by these microorganisms. The reason for such instability in silage supplemented with additives is probably related to the greater contents of energy sources for microorganisms involved in aerobic degradation (Kung et al., 2003).

During fermentation, the degradation of structural and non-structural carbohydrates and the factors affecting the availability of these fractions for microorganism metabolism must be considered. The fate of pectin differs from that of other non-structural carbohydrates during ensilage. The major part of sugars originated from non-structural carbohydrates are fermented when ensiled. Nevertheless, approximately 90% of the pectic uronic acids were not fermented in alfalfa silage studies after 90 days (Ben-Ghedalia et al., 1991). According to Blanco et al. (1999), some yeast synthesize the enzyme polygalacturonase and are able to hydrolyze pectic substances using products as carbon source in the metabolism.

The objective of this work was to characterize the microorganism population and evaluate the aerobic stability of Marandu grass ensiled with pelleted citrus pulp as fermentation stimulating agent.

Material and Methods

The experiment was conducted at the FCAV/UNESP facilities, located at Jaboticabal, state of São Paulo, Brazil (21º15'22" S and 48º18'58" W), with an altitude of 595 m. The climate is Cwa – humid subtropical with dry winter through the international Koppen's system. Average maximum and minimum temperatures are 22.3ºC and 15.17ºC. The average precipitation is approximately 1,400 mm, and 85% of the rain is concentrated from October until March.

The soil was fertilized with nitrogen-based fertilizer in order to obtain adequate yield of pasture to be ensiled. Therefore, 65 kg N/ha was applied during November 2001, just after the matched cut of the forage.

The grass was harvested on January 17th, 2002, with 55 days of vegetative growth and chopped with a conventional forage harvester to a 3 cm theoretical length of cut.

The harvested silage was submitted to the following treatments: silage of Marandu grass immediately after cut; silage of Marandu grass + 50 g/kg of pelleted citrus pulp (PCP) and Marandu grass silage + 100 g/kg PCP as natural matter basis. Pelleted citrus pulp was added to and completely homogenized with the forage just before filling the silos.

Metal containers with 80 cm of height and 50 cm of diameter were used as silos. Forage was compacted by human treading, adjusting layers with 20 cm of thickness, achieving a density of 900 kg/m^3. After the last forage additions, silos were closed with plastic canvas, sealed with plastic adhesive tape and stored in a sheltered place at room temperature.

Silos were opened after 178 days to evaluate chemical the composition and microbiological dynamics of silages from each treatment using total microorganism counts at 0, 2, 4 and 6 days after breaking the seal. Silos were thus managed so that time zero was the opening. A layer of approximately 15 cm was taken at each two days to collect samples for analysis.

Immediately after silos were opened, approximately 3 kg of silage were sampled and put in seven-liter styrofoam boxes, which were taken to the climatic chamber at 25 ± 1ºC to evaluate the aerobic stability. Silage temperature was measured at 0, 24, 48, 72, 96, 120, 144 and 168 hours after placing the boxes in the climatic chamber, using a thermometer inserted 10 cm in the center of the silage mass. Environment temperature was monitored with the thermostat from the refrigerator and with suspended thermometers inside the chamber. The aerobic stability was considered as the time elapsed so that the temperature of the feed increased two degrees when compared to the environment temperature, after the silo was opened.

During silo feedout, samples were collected and divided into three portions. The first fraction was used in chemical analysis. Dry matter (DM) and *in vitro* dry matter digestibility (IVDMD) were estimated using method described by Tilley & Terry (1963). The second fraction from each sample was used for juice extraction through hydraulic press. Ammonia nitrogen levels (N-NH_3) were determined in the extracted juice and pH was determined through pH meter (Silva, 1998). The last fraction was used in the microbiological analysis.

Samples were prepared for analysis by diluting 25 g of silage (natural matter) in 225 mL of sterile saline (8.5 g NaCl/liter of distilled water). After mixing, 10 mL of the extract was further diluted to 10^{-1} to 10^{-8} and plated in media specific for each studied microorganism.

Bacillus was evaluated according to Speck (1976), using nutrient agar (Difco) and anaerobic incubation for three days at 30ºC. Enterobacteria were evaluated in Violet Red Bile Agar (Oxoid) under anaerobiosis for three days at 35ºC, according to Jonsson (1991). Total yeast counting was performed according to Van der Walt & Yarrow (1984). Samples were incubated for three days at 28ºC in YN medium (Difco), acidified with lactic acid (pH 4.0). Mold counting was performed using the same medium and temperature, but samples were incubated for 6 days. Yeast and molds were separated based on the physical appearance of colonies; yeast form unicellular colonies whereas fungi are filamentous multicellular organisms.

After yeast count, colonies that were evidently distinct according to morphological criteria were categorized (Table 1). In the present study, six wild yeast strains were categorized, named M01, M02, M03, M04, M05 and M06. Cultures were transferred into GYMP slants (Difco) and kept under refrigeration.

Table 1 - Morphological colony characteristics used in the evaluation of isolated strains

Characteristic	Description
Size	diameter (mm)
Texture	mucoid, viscous, bright, opaque
Color	white, cream, pink, purple, etc.
Surface	smooth, rough, complex
Border	smooth, wavy, dented, filiform, rhizoid
Elevation	convex, conic, flat, elevated, drop, umbilicated

Refrigerated stock cultures in GYMP slants were used in the following tests, according to Van der Walt & Yarrow (1984):

a) Test of carbon source assimilation in 10 x concentrated liquid media. Glucose, fructose, xylose, L-arabinose and lactate were used as carbon sources;

b) Fermentation test using glucose, fructose, xylose, L-arabinose and lactate as carbon source.

A split-plot design in a completely randomized design with four repetitions was used. Treatments were the parcels and time was the sub-parcel. Data were analyzed by ANOVA (SAS, 1999). Means were compared by Tukey test (P<0.05).

Results and Discussion

Enterobacteria were detected in the silage containing 0 g/kg PCP and increased after silo opening (P<0.05), but this behavior was not similar to that observed in silages containing 5 and 100 g/kg PCP. *Bacillus* growth in the silages was similar to the behavior seen for enterobacteria (Table 2). There was an increase in *Bacillus* growth during silo feedout in the silage containing 0 g/kg PCP; *Bacillus* were not seen in the other treatments (P<0.05).

Silages containing 50 and 100 g/kg PCP showed yeast growth (Table 3), which increased during silo feedout (P<0.05). Mold growth was only detected in silages containing 100 g/kg PCP when the silos were opened (2.4 log cfu/g silage).

The mean pH of silages without PCP (5.2) possibly favored the enterobacterium growth. Besides, the reduction of the water activity (a_w) of silages added with PCP may be inhibiting the development of this kind of bacteria, since gram negative microorganisms prefer an environment with high a_w levels (0.98).

According to Ostling & Lindgren (1995), during aerobic deterioration of silages, enterobacteria have the opportunity to restart their growth significantly. Lindgren et al. (1985) studied the aerobic deterioration of grass silages and grass silages mixed with legumes and reported an increase in the number of enterobacteria after air exposure, which was even stronger at temperatures around 30ºC and in the silo surface when compared to samples collected 30 cm deeper. The authors concluded that silages sampled on the surface of the silos showed higher pH levels and lower lactic acid levels, comprising a better environment for the growth of such bacteria.

As for *Bacillus* growth, this may also result from the pH influence on the bacterial growth, since *Bacillus* do not tolerate the slightly acid substrate (pH<4.2) characteristic of silages with additives.

Table 2 - Enterobacterium and *Bacillus* growth (log cfu/g silage) during silo feedout in Marandu grass silage added with pelleted citrus pulp (PCP)

Silage (g/kg PCP)	Time (days)				CV (%)
	0	2	4	6	2.62
	Enterobacteria				
0	3.9Ab	4.0Ab	4.1Ab	4.5Aa	
50	<2.0Ba	<2.0Ba	<2.0Ba	<2.0Ba	
100	<2.0Ba	<2.0Ba	<2.0Ba	<2.0Ba	
	Bacillus				
0	<2.0Ab	2.1Ab	2.8Bb	3.2Ba	
50	<2.0Aa	<2.0Aa	<2.0Aa	<2.0Aa	
100	<2.0Aa	<2.0Aa	<2.0Aa	<2.0Aa	
CV (%)	1.56				

Means with same capital letters in the columns and small letters in the rows are not different (P>0.05) by Tukey test.

Table 3 - Yeast and mold growth (log cfu/g silage) during silo feedout in Marandu grass silages added with pelleted citrus pulp (PCP)

Silage (g/kg PCP)	Time (days)				CV (%)
	0	2	4	6	3.03
	Yeast				
0	<2.0Ba	<2.0Ba	<2.0Ba	<2.0Ba	
50	2.0Ba	2.3Aa	2.2Ba	2.3Ba	
100	2.4Ab	2.4Ab	2.7Aa	2.8Aa	
	Molds				
0	<2.0Ba	<2.0Aa	<2.0Aa	<2.0Aa	
50	<2.0Ba	<2.0Aa	<2.0Aa	<2.0Aa	
100	2.4Aa	<2.0Ab	<2.0Ab	<2.0Ab	
CV (%)	1.77				

Means with same capital letters in the columns and small letters in the rows are not different (P>0.05) by Tukey test.

Woolford (1990) reported that *Bacillus* was firstly believed to have a secondary function when compared to yeast in silage deterioration. Nonetheless, many studies have evidenced that these microorganisms exert a function that is far more important than previously believed for the same forage species. Many studies conducted in North America and Europe have shown that bacteria from the genus *Bacillus* are in the silages at the final phases of deterioration, and when yeasts have consumed lactic acid and feed, pH levels are high. Nevertheless, tropical environments enable favorable conditions for the development of this kind of bacterium, since forages are characterized by a fermentation process in which pH values stabilize over 4.5.

The presence of *Bacillus* changes significantly the chemical composition of silages, especially in protein content, since the majority of *Bacillus* species is proteolytic. The most important species involved in aerobic deterioration is *Bacillus cereus* (Granum, 1997). According to McDonald et al. (1991), the loss of aminoacids in silages indicates the production of nitrogen compounds such as polyamines. Steidlová & Kalac (2002) evaluated the production of polyamines in corn silages produced in farms and reported that tyramine, cadaverine and putrescine added up to 90% of the total of polyamines.

In a review, Charmley (2001) reported that fermentation products have determined a decrease in ingestion when cultures are ensiled, and in some studies, the decrease in intake is mainly related to the nitrogen fractions. Nussio et al. (2002) have alerted that recent studies have used supplementation with specific aminoacids in order to compensate the low protein levels in the food, instead of feeding a supplement with high protein level.

The yeast growth in the present study was similar to results reported in literature; immediately after oxygen enters the silo, there is an intense activity of aerobic microorganisms, especially yeast, beginning the deterioration process (Lindgren et al. 1985; Jobim et al., 1999; Guim et al., 2002; Ashbell et al., 2002). The yeast population may increase steeply from 2.0 log cfu/g silage to 12.0 log cfu/g silage in only three days of aeration (Pitt et al. 1991). Silages with yeast population above 5.0 log cfu/g silage are highly susceptible to deterioration (Woolford, 1990); therefore, silages in the present study may be classified as non-susceptible to fast deterioration if only the number of yeast present in the feed is considered.

Silages with 0 g/kg PCP showed no yeast growth, which might be explained by the lack of substrate for the microorganisms, so that PCP supplementation may guarantee nutrient for their assimilation and, consequently, later growth, either by the higher lactic acid levels in the supplemented silages or by the presence of pectin. Walker (1998) and Blanco et al. (1999) reported that *Candida* and *Cryptococcus* species use pectic substances efficiently as carbon source in the cellular metabolism, and, according to Woolford (1990), these are the two main genus involved in the aerobic deterioration process.

Another explanation for the absence of yeast in the control silages would be the presence of acetic and propionic acids that are produced during the fermentation process and also after the breakage of the seal. According to Corlett Jr. & Brown (1980), different acids may exert an inhibitory or lethal effect on the microbe cell, because in optimum substrate condition and close to neutrality, the internal pH of the cell is close to 7.0. Nevertheless, such value is extremely affected by external changes and the microbe inhibition is due to the hydrogen ion concentration (free H^+) or by the toxicity of the non-dissociated acid. Walker (1998) reported that non-dissociated organic acids (acetic, propionic) are easily soluble in the yeast cell membrane, releasing H^+ and consequently inhibiting the microorganism (Figure 1).

In order to keep an adequate internal pH, the yeast cell exports H^+ that is stored inside the cell using the ATPase enzyme and consuming energy during the process. The energy spent in this process results in reduced cell growth or even death, since ATPase is the most important enzyme in yeast cells, responsible for the control of cell pH, nutrients and ion transport (Walker, 1998).

It is worth noting that the research studies in the past few years have been carried out using silages with high levels of nutrients (Kung & Ranjit, 1998; Higginbotham et

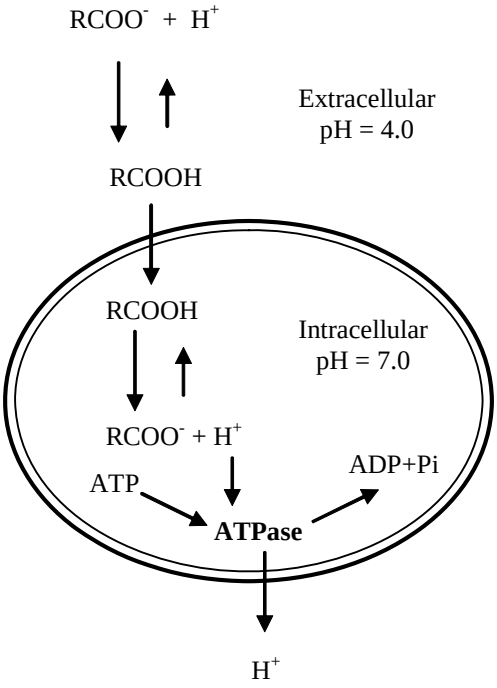

Figure 1 - Fate of organic acid in an environment of low pH and in the presence of microbe cells. Adapted from Davidson (1997).

al., 1998; Driehuis et al., 2001). Such studies have evaluated bacteria such as *Lactobacillus buchneri* and *Propionibacterium acidipropionici*, which are able to produce not only lactic acid during the fermentation process, but also acetate and propionate by means of the heterolactic fermentation way route. In such way, these bacteria may reduce the presence of microorganisms (mainly yeast) that cause deterioration after silo opening.

The absence of yeast in the non-added silages may also be explained by the microorganism-microorganism interaction. According to Woolford (1990), one of the genuses that increase during aerobic deterioration is *Streptomyces*. Walker (1998) reported that the presence of these bacteria inhibits the yeast growth because they produce compounds such as cycloheximides, which are lethal to microbe cells.

As for the occurrence of filamentous fungi, it can be inferred that the presence of oxygen on the upper part of the silo caused this behavior. The difficulty in compacting this area during ensiling favored oxygenation, and consequently, the occurrence of molds.

According to Muck et al. (1991), molds have slower growth when compared to yeast, and usually they present lower population during storage due to their higher susceptibility to the absence of oxygen.

After exposure of the silage to air, there are important indicators of deterioration of the ensiled mass: increase in temperature, decrease in soluble carbohydrate levels, low lactic acid concentration, and increase in pH (Pitt et al., 1991; Higginbotham et al., 1998).

The digestibility was influenced by PCP addition so that higher digestion levels were seen in silages containing this additive. During silo feedout, the silage digestibility decreased ($P<0.05$), evidencing a decrease in the nutritional value due to feed deterioration (Table 4).

According to McDonald et al. (1991), energy losses by aerobic deterioration after silo opening may be over 15%. In the present study, IVDMD was reduced in 5% from silo opening until the sixth day of aeration, evidencing that the silo feedout is an important cause of nutrient loss during the ensiling process. Nussio et al. (2002) reported that after silo opening, the instability must be considered as an additional means of loss of dry matter and energy and, as such, it should be added to inherent losses of the production system.

The silages presented different pH levels when silos were opened (Figure 2), so that PCP decreased pH. After the seal was broken, silages with 0 and 50 g/kg PCP had an increase in pH ($P<0.05$). The treatment with 100 g/kg PCP showed a slight decrease after two days of unsealing, but it was stable afterwards (4 and 6 days).

pH increase in silages containing 0 and 50 g/kg PCP may be related to lactic acid use by deteriorating microorganisms. Nevertheless, the pH behavior that was seen in silages with 100 g/kg PCP was not expected, since these silages theoretically have higher carbohydrate and lactic acid levels to be consumed when compared to the other silages. Probably, the high density of this treatment (260 kg DM/m^3) has lead to low porosity, preventing silage oxygenation on deeper layers of the silo. Nevertheless, the growth of the

Table 4 - Dry matter (DM), pH, ammonia nitrogen ($N\text{-}NH_3$) and *in vitro* digestibility of dry matter (IVDMD) levels during silo feedout of Marandu grass silages added with pelleted citrus pulp (PCP)

Silage (g/kg PCP)	DM (g/kg)	pH	$N\text{-}NH_3$ (g/kg total N)	IVDMD (g/kg DM)
0	205C	4.9A	228A	423C
5	233B	4.5B	139B	547B
10	265A	4.1C	87C	631A
CV (%)	3.7	2.9	26.1	3.7
		Time (days)		
0	234B	4.5B	152C	551A
2	254A	4.7AB	156C	541A
4	252A	4.8AB	243B	540A
6	248AB	4.9A	319A	523B
CV (%)	3.2	3.0	10.2	4.9

Means with same superscript letter in columns are not different ($P>0.05$) by Tukey test.

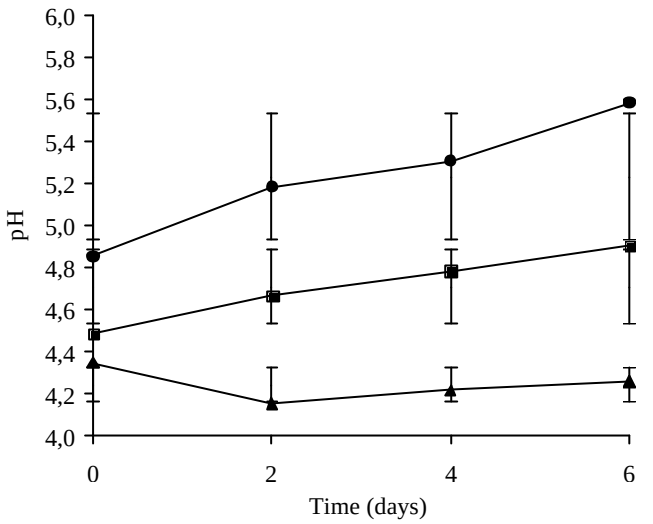

Figure 2 - pH levels of Marandu grass silages added with 0 (◆), 50 (■) and 100 (▲) g/kg of pelleted citrus pulp during silo feedout.

main deteriorating microorganism, yeast, has increased during silo feedout. Ashbell & Lisker (1988) studied the aerobic deterioration of corn silages in bunker silos and reported similar results, so that deeper layers presented lower pH values when compared to exposed layers.

Guim et al. (2002) used the PET system to evaluate deterioration in elefante grass silage (*Pennisetum purpureum* Schum.) for a eight-day exposure period and also reported increase in pH levels, from 3.9 at silo opening to 5.8 at the eighth day of aeration. Lindgren et al. (1985) worked with grass silages in bunker silos and reported 4.0 vs 6.4; 4.0 vs 4.5 and 4.0 vs 4.0 pH values on silages at the day of opening and eight days after air exposure, for layers 0-15 cm; 40-60 cm and 140-160 cm, respectively. The same study reported a significant reduction on yeast, *Bacillus* and enterobacterium populations in the deepest layer (140-160 cm), which shows potential changes that the microorganisms might have caused to the food.

It must be noted that, besides leading to favorable niches for subsequent microorganisms, the use of lactic acid by the organisms that begin the deterioration process also reduce the nutritional value of feed. According to Chamberlain (1987), lactic acid from the silage fermentation process is the best product used in the ruminal environment, compared to other organic acids present on silage. The ruminal bacterium *Megasphaera elsdenii* produces 2 ATPs when lactic acid is fermented, which may contribute to microorganism maintenance and growth. On the other hand, ruminal microorganisms do not use acetic acid and butyric acid (Van Soest, 1994; Russell & Rychlik, 2001).

Cumulative temperature data of the first seven days after silo opening are shown in Table 5. The three silages were stable considering the parameter evaluated in the study, *i.e.*, one degree above room temperature (25ºC). Nevertheless, the temperature tends to increase with aerobic exposure, mainly on the treatment added with 100 g/kg PCP.

Silages with higher nutrient levels are less stable, which may be proved by the fact that the recovered dry matter levels (RDM) are lower in silages added with 100 g/kg PCP.

The PCP inclusion during ensiling promoted higher temperature profiles. This might have been due to the higher levels of nutrients (lactic acid and carbohydrates) available to aerobic microorganisms, mainly yeast, as previously discussed. According to Ashbell et al. (2002), the most important factors that affect the aerobic silage stability are the presence of oxygen and substrate and the temperature the feed is submitted to.

Igarasi (2002) evaluated the aerobic stability of Tanzania grass silages (*Panicum maximum* Jacq. cv. Tanzânia) through the temperature accumulated during 5 days after silo opening, and reported that the treatment added with PCP showed higher accumulated temperature than the control silage, corroborating data describe here. Balsalobre et al. (2001) reported that silages of more finely chopped Tanzania grass supplemented with PCP tended to have more aerobic stability when compared to the treatment with larger particles and without PCP.

Bernardes et al. (2007) evaluated the effects of the chemical and heterofermentative bacteria additives on the aerobic stability of the Marandu grass silage, and reported that the temperature of silages showed little changes during the six day of air exposure.

Table 5 - Increase in temperature and recovered dry mater (RDM) from Marandu grass silages added with pelleted citrus pulp (PCP)

Time (days)	Silages (g/kg PCP)			Mean	CV (%)
	0	50	100		
0	19.7Ca	19.9Aa	19.9Ca	19.8D	1.32
1	19.3Cc	21.5Ab	22.3Ba	21.0CD	-
2	19.3Cc	21.6Ab	22.6Ba	21.2CD	-
3	20.4Bc	21.9Ab	23.0Aa	21.8C	-
4	20.6Bc	21.5Ab	23.0Aa	21.8C	-
5	20.8Bc	21.6Ab	23.7Aa	22.0CB	-
6	21.4Ac	21.9Ab	24.0Aa	22.4B	-
7	21.6Ab	22.0Ab	25.0Aa	22.9A	-
Mean	20.4c	21.4b	23.0a	-	-
CV (%)	1.89	-	-	-	-
RDM (%)	91.3a	91.0a	90.4b	-	0.71

Means with same capital letters in the columns and small letters in the rows are not different (P>0.05) by Tukey test.

Isolated yeast strains showed many different characteristics (Table 6). A slight variation in colony size was seen (1 to 4 mm) and all colonies were smooth and convex, but texture, border and color were different.

The results of carbon source assimilation (Table 7) showed that different strains present diverse results. Strains M03, M04 and M06 showed not only glucose and fructose assimilation, the two most abundant structural carbohydrates in plants, but they also assimilated arabinose, xylose and lactate.

No strain was able to ferment arabinose, xylose and lactate (Table 8); only glucose and fructose fermentation occurred, although in low levels.

Besides glucose and fructose, strains M03, M04 and M06 also assimilated arabinose and xylose. Free pentosans are present in low levels in forages, but these levels might increase by acid hydrolysis and hemicellulase activity that occur during fermentation. According to Van Soest (1994), analysis of the sugar compounds produced by hemicellulose hydrolysis evidences the presence of xylose and arabinose. Prior & Kotter (1997) reported that pentosans are converted into xylose-5-phosphate, which can be transformed into acetylphosphate during acetate or glyceraldehyde-3-phosphate during ethanol production. According to Walker (1998), pentosans metabolism in yeast depends on the species (presence of enzyme) and nutritional status of the organism. For example, glucose generally decreases yeast assimilation of other sugars.

Table 6 - Morphological aspects of strain colonies

Strain	Size (mm)	Texture	Surface	Border	Elevation	Color
M01	2-4	bright	smooth	dented	convex	beige
M02	2-4	bright	smooth	dented	convex	beige
M03	2-4	bright	smooth	dented	convex	beige
M04	1-2	opaque	smooth	smooth	convex	white
M05	2-4	bright	smooth	dented	convex	beige
M06	1-2	opaque	smooth	smooth	convex	white

Table 7 - Behavior of yeast strains in carbon source assimilation test

Strain	Carbon source				
	Glucose	Fructose	Arabinose	Xylose	Lactate
M01	+	+	-	-	+
M02	+	+	-	-	+
M03	+	+	+	+	+
M04	+	+	+	+	+
M05	+	+	-	-	+
M06	+	+	+	+	+

Assimilation: positive (+); negative (-).

Table 8 - Strain behavior in basal fermentation media

Strain	Carbon source				
	Glucose	Fructose	Arabinose	Xylose	Lactate
M01	+W	+W	-	-	-
M02	+W	+W	-	-	-
M03	+W	+W	-	-	-
M04	+W	+W	-	-	-
M05	+W	+W	-	-	-
M06	+W	+W	-	-	-

Fermentation: weak (+W); negative (-).

All strains were able to assimilate glucose, fructose and lactate. Gancedo & Serrano (1989) reported that, when the microbe cell uptakes the lactic acid, it is oxidized into pyruvate, induced by L-lactate:cytocrome *c* oxyredutase, an enzyme located on the internal mitochondrial membrane in yeast.

According to Woolford (1990), yeast population that uses lactate as energy source determines if the silage may or may not deteriorate during air exposure. Thus, a great population does not necessarily mean that there will be a fast deterioration. Lindgren et al. (1985) reported seven yeast species during aerobic exposure of grass silages in temperate climate, such that *Candida lambica*, *Candida krusei* and *Hansenula anomala* showed lactic acid assimilation during laboratory tests. Pahlow et al. (2003) reported that, from the aerobic microorganisms present during deterioration, yeast genus *Hansenula* and *Candida* are the most important because of lactate assimilation.

Conclusions

Bacillus and enterobacteria are present on Marandu grass silages without pelleted citrus pulp, which also show pH increase throughout the feedout phase. Yeasts are present on silages with pelleted citrus pulp as fermentation stimulating agent. The trend in temperature increase with extension of aeration time occurs mainly in silages containing pelleted citrus pulp.

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