University of São Paulo
"Luiz de Queiroz" College of Agriculture

Microbiology and fermentative losses of rehydrated whole plant silage harvested at high maturity

Victor Federico Leal dos Anjos

Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

Piracicaba
2023

Victor Federico Leal dos Anjos
Animal Scientist

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **LUIZ GUSTAVO NUSSIO**

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1. Reidratação 2. Colheita tardia 3. Perdas fermentativas 4. MS elevada I. Título

"Stability does not exist."

Flávio Augusto

To my mom, to my brothers, relatives, friends and to me

I DEDICATE

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RESUMO

Microbiologia e perdas fermentativas de silagem de planta inteira reidratada colhida em maturidade avançada

A silagem de milho planta inteira (SMPI) é o volumoso mais utilizado na dieta de bovinos de corte e leite no Brasil. O uso de tecnologias na confecção de silagem vem aumentando nos últimos anos, a fim de garantir uma colheita mais eficiente e um bom padrão fermentativo para os silos. Entretanto, em algumas regiões do país o déficit de chuvas gera estresse hídrico nas plantas, alterando a qualidade química e perfil de carboidratos disponíveis para a fermentação. Em contrapartida a presença de chuva no momento da colheita também pode gerar atrasos. Em muitos, casos a colheita de plantas de milho com matéria seca elevada gera perdas fermentativas consideráveis da silagem. Reidratadar a planta, segundo alguns pesquisadores e técnicos de campo, pode apresentar melhorias na conservação de plantas com MS elevada. As vantagens e a forma que deve ser feita reidratação de grãos de milho objetivando maior aproveitamento de amido é bem descrita na literatura. Porém, não existem estudos avaliando como a reidratação de SMPI pode melhorar em quesitos fermentativos o ambiente do silo. Nesse sentido, o objetivo deste estudo foi avaliar as perdas e o perfil fermentativo de silagens com MS acima do recomendado e silagens colhidas com MS alta e que receberam adição de água com o intuito de reduzir a MS para o padrão recomendado. Foram realizadas 5 colheitas com intervalos de 7 dias cada uma, onde na primeira colheita a massa foi ensilada sem adição de água (37,5%), nas demais colheitas os materiais foram ensilados com a MS respectiva a colheita (40,7; 46,9, 49,5 and 54,2%) e ensilados com a adição de água (34,9; 35,5; 34,0; 33,7%) para que a MS retornasse próximo à encontrada na primeira colheita. Os materiais foram ensilados em silo experimentais (baldes de 20 L) contendo 2 kg de areia no fundo separada por tela e pano afim de mensurar a produção de efluentes (PE) e equipados com tampas para total vedação. O tempo de armazenamento foi de 90 dias. Os tratamentos foram arranjados em esquema fatorial com quatro repetições (2*4+1) sendo 2 – com e sem adição de água; 4 – MS no momento da colheita e 1 o tratamento controle positivo. Os dados foram analisados utilizando o procedimento MIXED do SAS. Foram avaliadas as perdas fermentativas, pH, ácido lático, ácidos graxos voláteis, etanol, microrganismos, estabilidade aeróbica (E.A), densidade, distribuição média de partículas, capacidade de retenção de água (CRA) e bromatológica. O ph dos materiais que receberam a adição de água foram menores em comparação aos tratamentos sem adição de água e não diferiram do controle positivo. A produção de ácidos lático e a contagem de bactérias fermentadoras de ácidos lático (BAL) também foram maiores nos tratamentos reidratados. Não houve diferença entre nenhum dos tratamentos para clostridios. Quanto mais elevada a MS no momento da colheita, maior foi a adição de água, o que levou a maiores perdas por efluentes, o tratamento com DAE 135 e 36,8% de MS obteve as maiores perdas por efluentes. A densidade também foi alterada na reidratação, as plantas com maior matéria seca foram diminuindo linearmente a densidade, enquanto os tratamentos reidratados mantiveram densidade inicial, apenas o tratamento com DAE 135 e 36,8% de MS se diferenciou. Os tratamentos reidratados na média também apresentaram maior E.A, mas nenhum tratamento apresentou boa E.A. Os matérias com MS elevada foram perdendo CRA. Em conclusão, os resultados sugerem que adicionar água pode ser uma boa estratégia para reduzir as perdas e melhorar o padrão fermentativos e plantas de milho colhidas com MS elevada. Entretanto nem toda planta possui a capacidade de reter a água adicional, materiais com MS acima de 55% apresentaram maior produção de efluentes, o que gera maiores perdas e maior risco ambiental.

Palavras-chave: Reidratação, Colheita tardia, Perdas fermentativas, MS elevada

ABSTRACT

Microbiology and fermentative losses of rehydrated whole plant silage harvested at high maturity

The whole plant corn silage (WPCS) is the most used roughage in the diet of beef and dairy cattle in Brazil. The use of technologies in the making of silage has been increasing in recent years in order to ensure a more efficient harvest and a good fermentation standard for the silos. However, in some regions of the country, the rainfall deficit generates water stress in plants, altering the chemical quality and profile of carbohydrates available for fermentation. On the other hand, the presence of rain at the time of harvesting can also cause delays. In many cases, harvesting maize plants with high dry matter leads to considerable fermentation losses in the silage. Rehydrating the plant according to some researchers and field technicians can improve the conservation of plants with high DM. The advantages and the way that corn grain rehydration should be done in order to take better use of the starch is well described in the literature. However, there are no studies evaluating how SMPI rehydration can improve the fermentative aspects of the silo environment. In this sense, the objective of this study was to evaluate the losses and fermentative profile of silages with DM above the recommended level and silages harvested with high DM and which received water addition in order to reduce the DM to the recommended standard. Five harvests were performed at 7-day intervals each, where in the first harvest the mass was ensiled without adding water (34.7%), in the other harvests the materials were ensiled with the respective DM for the harvest (40.7; 46.9, 49.5 and 54.2%) and ensiled with the addition of water (34.9; 35.5; 34.0; 33.7%) so that DM returned close to that found in the first harvest. The materials were ensiled in experimental silos (20 L buckets) containing 2 kg of sand at the bottom separated by a screen and cloth in order to measure the production of effluents (EL) and equipped with lids for complete sealing. The storage time was 90 days. The treatments were arranged in a factorial scheme with four replications (2*4+1) being 2 – with and without water addition; 4 – DM at the time of harvest and 1 the positive control treatment. The data were analyzed using the SAS MIXED procedure. Fermentative losses, pH, lactic acid, volatile fatty acids, ethanol, microorganisms, aerobic stability (A.E), density, mean particle distribution, water holding capacity (WHC) and bromatological were evaluated. The pH of the materials that received the addition of water were lower compared to the treatments without the addition of water and did not differ from the positive control. Lactic acid production and lactic acid fermenting bacteria (LAB) counts were also higher in rehydrated treatments. There was no difference between any of the treatments for clostridia. The higher the DM at the time of harvest, the greater the addition of water, which led to greater losses by effluents, the treatment with DAE 135 and 36.8% DM obtained the highest losses by effluents. Density was also altered during rehydration, plants with higher dry matter linearly decreased density, while rehydrated treatments maintained initial density, only the treatment with DAE 135 and 36.8% DM differed. The average rehydrated treatments also showed higher A.E., but no treatment showed good A.E. Those with high DM were losing WHC. In conclusion, the results suggest that adding water can be a good strategy to reduce losses and improve the fermentative pattern and maize plants harvested with high DM. However, not every plant has the capacity to retain additional water, materials with DM above 55% showed greater production of effluents, which generates greater losses and greater environmental risk.

Keywords: Rehydration, Late harvest, Fermentative losses, High DM

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1. INTRODUCTION

Brazil has the largest commercial cattle herd in the world, with more than 187 million heads, but it is only the second largest producer of beef (IBGE, 2006). This fact is due to the fact that most animals are raised in a grazing system and, therefore, are old at slaughter (ABIEC, 2022). However, the number of feedlots has been growing in Brazil, increasing the demand for preserved forages for use in finishing diets (Oliveira and Millen, 2014).

Corn (*Zea mays L.*) is one of the most important crops in the world, with a wide range of uses, including food, feed and biofuel production. However, the success of maize cultivation is directly related to favorable climatic conditions, since the plant is susceptible to abiotic stresses, such as heat stress. Temperature is a crucial environmental factor that influences plant growth, development and productivity. Climate change and extreme events, such as heat waves, have become increasingly frequent, negatively affecting agricultural crops, including maize.

In this sense, corn silage is the forage most used by feedlots in Brazil (Oliveira and Millen, 2014) and dairy cows (Bernardes and Rego, 2014), and the area cultivated with corn in Brazil represents 20% of the world's area of corn and it is estimated that approximately 20% is harvested as corn silage (Lima et al., 2022). In addition to being a nutritionally rich food to work in ruminant diets, corn has favorable aspects for adequate fermentation to occur during the ensiling process.

Heat stress has a significant impact on the physiology and metabolism of maize plants. At high temperatures, a series of physiological and biochemical responses occur that can compromise the proper growth and development of the crop. Studies have shown that heat stress affects the photosynthetic rate, transpiration, synthesis and accumulation of carbohydrates, in addition to influencing the activity of enzymes related to the metabolism of nitrogen and other essential nutrients for maize (Jones et al., 2010 ; Li et al., 2014; Wahid et al., 2007).

The effect of heat stress on the maize plant can also directly affect the quality of silages produced from this crop. Silages are a common form of forage conservation for animal feed, the chemical composition and fermentation stability of maize silages are influenced by plant growth conditions. High temperatures during maize cultivation can lead to changes in the nutritional composition of plants, increasing the carbon:nitrogen ratio, reducing the soluble sugar content and increasing fiber levels (Zhang et al., 2012; Bernardes et al., 2016; Dunière et al., 2013).

Understanding the effects of heat stress on fermentation and composition of corn silages is critical for proper crop management, especially in regions where heat waves are frequent. Knowledge about these effects allows the development of management strategies and conservation techniques that minimize quality losses and ensure the availability of quality forage for animal feed. Therefore, it is necessary to investigate the impact of heat stress on fermentation and composition of corn silages under different climatic conditions and to propose adaptive solutions.

The soluble carbohydrate content, buffering capacity and DM content are the main factors to be considered for a good fermentation (Kaiser, 2002). However, abiotic stressors can affect maize plant structure, dry matter, protein, fiber and starch content which can affect the quality of maize silage fermentation. Within these abiotic factors, thermal stress and water deficit in the reproductive stages of the plant are known to reduce plant moisture and consequently water activity and the growth of lactic acid-producing bacteria, in addition to hindering compaction (DA SILVA et al., 2016; Oliveira, 2020). Water deficit reduces the efficiency of photosynthesis and evapotranspiration of corn plants, thus affecting the plant's biochemical processes (Nematpour et al., 2020; Baghdadi et al. 2021; Farhadi et al., 2022).

According to Neuman et al. (2011) the DM content is the factor that has the greatest impact on the final quality of the silage. For the development of the microorganisms responsible for the preservation of the silage, the availability of metabolic water is necessary (Ditchfield, 2000), which is measured by the water activity (Aw). A drop in Aw can result in a lower pH (Lindgren, 1999), while a higher Aw leads to the growth of toxins (Garcia, 2004), clostridium (McDonald et al., 1991) and salmonella (Ditchfield, 2000).

Several studies show that harvesting should occur between 30 and 35% DM (Nussio et al., 1999; Gordon et al., 1968). Management factors, such as delays in harvesting due to difficult access to machinery or intentional search for greater starch accumulation, can lead to late harvests. Increased DM results in increased pH (Kung et al., 2018), reduction of lactic acid fermenting bacteria (LAB) (Whiter and Kung, 2001), of lactic and acetic acid (Der Bedrosian et al., 2012), density (Muck and Holmes, 2000) and increased DM losses (Borreani et al., 2018).

When the DM content exceeds the indicated limits, the use of inoculants alone is not efficient in preserving the material. Weiss (2003) recommends that for high DM silages, a finer cut of the fibrous fraction, more broken grains, additives that can reduce damage to the fermentation process and improve the aerobic stability of the silage. Still according to

Weiss (2003) the addition of water at the time of ensiling can improve aspects of compaction and mass density.

Studies carried out in Brazil have significantly contributed to the understanding of the effects of heat stress on the corn plant and its consequences on silages. In a study conducted by Silva et al. (2018), it was found that heat stress negatively affected dry matter production and protein content of corn plants, resulting in silages with lower nutritional value. Likewise, Silva et al. (2016) found that high temperatures during corn cultivation reduced the soluble sugar content and increased fiber levels, compromising the quality of the silages produced.

In addition to Brazilian research, international studies have also contributed to knowledge about the effect of temperature and heat stress on corn plants and their implications on silages. In a study carried out by Li et al. (2015), in China, it was observed that heat stress resulted in a reduction in the activity of enzymes related to the fermentation of corn silage, negatively affecting the fermentative quality and stability of the silage. Likewise, Han et al. (2019), in South Korea, reported that high temperatures during maize cultivation caused changes in the chemical composition of plants, resulting in silages with lower nutritional value.

According to Thomas et al. (2017), in a study carried out in the United States of America. The researchers evaluated the effect of heat stress on the nutritional composition of maize plants and on their fermentation. The results indicated that heat stress reduced the starch contents and increased the proportion of fiber in the plant, which negatively affected the fermentation stability of the produced silages.

Given these results, it is clear that heat stress has a significant impact on the corn plant, affecting its growth, development, chemical composition and quality of the silages produced. Therefore, it is essential to adopt management measures that minimize the negative effects of heat stress, such as the choice of cultivars that are more tolerant to heat, proper irrigation management and adjustment of planting periods.

In Brazil, rehydration of whole corn plant silage is performed without evidence of improvement in the process. It is not known whether the exogenous water really improves the fermentation process and guarantees the good conservation of the material, in addition to not knowing whether the plant has the capacity to absorb the added water, preventing leaching. The production of effluents can generate nutritional and environmental damage (Gebrehanna et al, 2014; Deans and Svoboda, 1992; Cropper and DuPoldt, 1995). Therefore, the hypothesis of this study is that rehydration of harvested silages with high

DM content can improve fermentative aspects, but that rehydration should be done up to a certain DM level due to the material's ability to retain additional water.

1.1 Objectives

This work aimed to evaluate the losses, fermentative and microbiological quality of silages that received water addition during ensiling under high DM content in relation to those that were ensiled with the original DM content at harvest.

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2. MICROBIOLOGY AND FERMENTATIVE LOSSES OF REHYDRATED WHOLE PLANT SILAGE HARVESTED AT HIGH MATURITY

Abstract

Whole corn silage is the most used source of roughage for cattle in Brazil. Harvest delays due to climatic effects or in cases of plants that suffered water stress directly influence the fermentation quality of whole plant corn silages (WPCS). The objective of this work was to evaluate the rehydration of maize silages harvested with high dry matter (DM) content in comparison with materials ensiled with DM for the respective harvest. Five harvests were performed at 7-day intervals each, where in the first harvest the mass was ensiled without adding water (37.5%), in the other harvests the materials were ensiled with the respective DM for the harvest (44.2; 49 .8, 54.1 and 59.2%) and ensiled with the addition of water (37.9; 37.7; 37.2; 36.8%) so that DM returned close to that found in the first harvest. The measure was taken in days after emergence (DAE) as follows: 107; 114; 121; 128; 135. The treatments were organized in a completely randomized design in a factorial arrangement (2*4+1). The pH of the rehydrated treatments was lower and remained similar to the control treatment. Higher lactic acid production and higher lactic acid fermenting bacteria counts were observed in rehydrated silages. The advance of DM reduced the water retention capacity of the plants, the treatments with DAE 135 and 36.8% had greater loss per effluent. Treatments without rehydration showed less aerobic stability. In conclusion, the rehydration of maize plants harvested with dry matter can be an alternative to improve the fermentative aspects and reduce losses.

Keywords: Rehydration; Late Harvest; Fermentative losses, high DM.

2.1 Introduction

The corn for silage is the most used crop for dairy cattle (Bernardes and Rego, 2014) and beef cattle (Pinto and Millen, 2016). In addition to being a nutritionally rich food to work with in ruminant diets, corn has favorable aspects for adequate fermentation to occur during the ensiling process. As the exposed equation the soluble carbohydrate content, buffering capacity and DM content are the main factors to considered for a good fermentation (Kaiser, 2002). Still according to Neuman et al. (2011) the DM content is the factor that has the greatest impact on the final quality of the silage. For the development of microorganisms responsible for preserving silage, the availability of metabolic water is necessary (Ditchfield, 2000), which is measured by water activity (Aw).

Several studies show that harvesting should occur between 30 and 35% DM (Nussio et., 1999; Gordon et al., 1968). Management factors, such as delays in harvesting due to difficult access to machinery or intentional search for greater starch accumulation, can lead to late harvests.

When the DM content exceeds the indicated limits, the use of inoculants by itself is not efficient in preserving the material. Weiss (2003) recommends that for high DM silages a finer chopping of the fibrous fraction should be done, more grain should be broken, additives should be used that can reduce damage to the fermentation process and improve the aerobic stability of the silage. Still according to Weiss (2003) the addition of water at the time of ensiling can improve aspects of compaction and mass density.

In Brazil, rehydration of whole corn plant silage is performed without evidence of improvement in the process. It is not known whether the exogenous water actually improves the fermentation process and guarantees the good conservation of the material, in addition to not knowing if the plant has the capacity to absorb the added water, preventing leaching. The effluents production can generate nutritional and environmental damage (Gebrehanna et al, 2014; Deans and Svoboda, 1992; Cropper and DuPoldt, 1995). Therefore, the hypothesis of this study is that the rehydration of silages harvested with high DM content can improve the fermentative aspects, but that rehydration must be done up to a certain DM level due to the material's ability to retain additional water.

Our hypothesis is that plant hydration can generate a favorable environment for microbial growth beneficial for good fermentation. The objective of this work was to evaluate the fermentative losses, microbial population and the quality of rehydrated silages after harvesting with high DM content.

2.3 Material and Methods

2.3.1 Local, hybrid, planting and harvesting

The experiment was carried out at the Faculty of Animal Science and Food Engineering (FZEA) of the University of São Paulo (USP), Pirassununga, São Paulo, Brazil (latitude 21° 59' 46" South, Longitude: 47° 25' 36" West and 627 meters of altitude). The climate in the region is tropical climate with dry season, reaching an average temperature of 22 º C and an average rainfall of 1,394 mm/year.

The hybrid BM3066 (Biomatrix Seeds, Brazil) with “Roundup Ready” biotechnology offering resistance to glyphosate, sown on November 7, 2019, aiming for 72 thousand plants per hectare. Fertilizations were sown with 47kg ha^{-1} of N, 94 kg ha^{-1} of P2O5 and 47 kg ha^{-1} of K2O and coverage (13 days after sowing) with 135 kg/ha of N and 45 kg ha^{-1} K2O. In addition, weed control was done with Atrazine WG 1.25 kg ha^{-1} with 4 L ha^{-1} of Glyphosate. An

insecticide was also applied with 200g of emamectin benzoate and fungi control with 200g of Picoxystrobin per ha.

Corn plants were harvested on five consecutive dates, with seven-day harvest intervals, presented as days after emergence (DAE 107; DAE 114; DAE 121, DAE 128 and DAE 135), resulting in different DM contents (34 .7%, 40.7%, 46.9%, 49.5% and 54.2%) consecutively (Figure 1) nutritional values at the time of harvest are shown in table 1. Treatments were divided into two groups (with and without rehydration) and one treatment with typical MS harvest (34.7%) was performed. The first group (without rehydration) was composed of the following MS: 40.7%; 46.9%; 49.5% and 54.2%. The other group (with rehydration) received the addition of water to return DM close to that of the first crop (DAE-107; 34.7%), obtaining the following DM: 34.9%; 35.5%; 34.0%; 33.7% (Figure 1). The material without rehydration was weighed and quantified how much water should be added for the DM to return to 34.7%. After that, the water was incorporated into the material in a plastic container until homogeneity was guaranteed. After being rehydrated, the material was added to the experimental silos (plastic buckets).

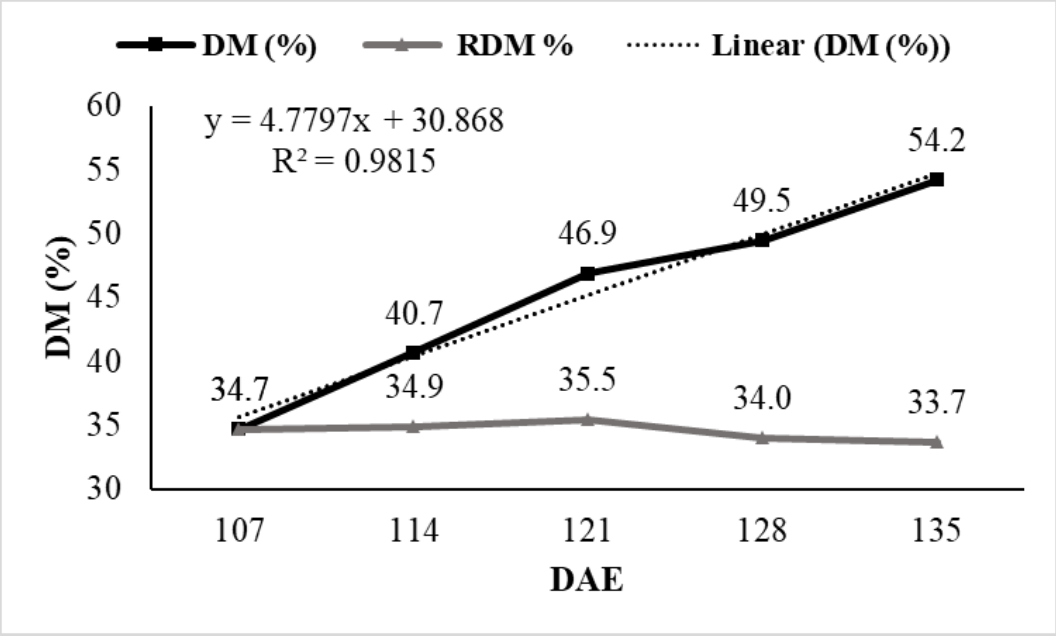

Figure 1 - Plant Dry Matter at the time of harvest and after rehydration. **DM** – dry matter. **RDM %** - DM of rehydrate materials. **DAE** – days after emergency.

Table 1 - Particle size distribution, Water Holding Capacity (WHC), Chemical composition based on the DM of the plants at the time of Harvest.

	Treatment				
Item	**DM 34.7%**	**DM 40.7%**	**DM46.0%**	**DM49.5%**	**DM54.2%**
	Chemical composition				
DM,[1] %	34.7±0.86	40.7±0.84	46.9±0.79	49.5±0.80	54.2±0.68
CP,[2] % DM	8.77±0.19	8.41±0.18	9.92±0.15	9.9±0.15	11.46±0.16
EE,[3] % DM	2.69±0.23	2.8±0.24	2.38±0.24	1.27±0.37	2.73±0.24
NDF,[4] % DM	49.04±1.02	50.57±1.11	56.04±1.14	49.45±1.27	42.46±1.18
ADF,[5] % DM	28.07±2.06	35.96±2.14	30.77±2.08	29.28±2.31	32.71±2.24
ASH, % DM	3.85±0.78	2.62±0.41	2.96±0.43	2.93±0.76	3.23±0.81
	Particle Size Distribution				
>19-mm, %	17.50±1.9	15.15±1.8	14.19±2.2	18.78±3.2	21.13±2.8
8-mm, %	50.40±2.4	45.73±3.6	47.38±3.5	54.33±4.1	49.3±3.5
4-mm, %	25.80±1.7	25.14±1.8	26.48±1.7	20.40±2.4	23.03±2.1
Pan, %	6.30±0.7	13.98±1.4	11.95±1.1	6.49±0.7	6.54±0.6
MPS[6], mm	10.41±0.05	8.89±0.04	9.14±0.04	10.92±0.05	10,66±0.05
	Water Holding Capacity				
	2.77±0.23	2.4±0.22	1.66±0.36	1.24±0.12	2.06±0.09

[1]Dry matter; [2]Crude protein; [3]Ethereal extract; [4]Neutral detergent fiber; [5]Acid detergent fiber; [6]Mean Particle Size length as described by Kononoff et al. (2003).

The corn was harvested manually at about 30 cm from the ground and, subsequently, the plants were transported wrapped in a plastic tarp to avoid loss of morphological components, and then they were chopped using a forage shredder. (TRAPP® Trf 70) adjusted to obtain a theoretical cut size of 10 mm, the average particle size and sieve distribution are described in table 1..

Plastic buckets with a capacity of 20 liters, previously weighed to obtain the tare, were used as experimental silos. The buckets were filled with 2 kg of dry sand (an oven at 55º for 3 days) placed at the bottom of the buckets and covered with a fine screen and cotton cloth to

avoid contamination of the ensiled mass with sand. The function of the sand was to absorb the effluent produced, since one of the foundations of the research was to investigate the percolation of liquids in the silo when water is added to the forage mass.The treatments consisted of a control treatment (DM - 34.7%), four treatments with DM corresponding to the harvest season (DM - 40.7%; 46.9%; 49.5% and 54.2%) and four treatments with the addition of water or rehydrated (DM - 34.9%; 35.5%; 34.0% and 33.7%), totaling nine treatments.

The compaction of the mass in the silos was done with the feet, previously cleaned with 70% alcohol. Layers approximately 10 cm thick were established, and each layer was compacted. The pressure exerted on the forage was similar in order to reach a packing density of 650 kg/m3 and making it possible to observe differences in densities between the driest materials and those that will receive the addition of water, as well as highlighting possible differences in the characteristic's physics of silages The sets of buckets were weighed before and after filling with silage, sealed with adhesive tape and stored at room temperature.

2.3.2 Evaluation of fermentation losses and density

After 90 days of ensiling, the experimental silos were opened. For this purpose, the silos were weighed to determine the losses and the adhesive tape was removed. A top layer of 5 cm was discarded. The silage contained in the silos was carefully removed so that there was no loss or contamination of the sand contained at the bottom of the buckets. Silage samples were taken for further analysis. The depth and diameter of the buckets were measured to obtain the area of the experimental silos. With the quantification of the weight, the volume was measured, making it possible to know the density of the silos.

To quantify the losses in the silage fermentation process, was used the methodology described by Jobim et al. (2007) and Mari (2003). Total DM losses were computed through differences in DM before ensiling and after opening the silos in relation to the amount of ensiled forage. For this, the following formula was used:

$$\textbf{DM losses} = \frac{[(DMi - DMf)]}{DMi} * 100$$

Which in:

DM Losses= Total DM Loss;

DMi= Initial DM amount (Silo weight after filling – empty set weight without forage * DM content of ensiled forage) in kg

DMf= Final MS amount. Silo weight.

Effluent losses were calculated by the difference in weight of the sand before ensiling and when the silos were opened, using the following equation:

$$EL = \frac{WE * 1000}{Mi}$$

Which in:

EL = effluent losses;

WE = weight of effluent (weight of dry sand – weight of sand after ensilage) and (kg);

Mi = amount of ensiled forage green mass (kg).

2.3.3 Laboratory analysis

For aqueous extract samples (25 g) were homogenized with 225 mL of distilled water in a blender for 1 minute (Kung Jr. 1996). The aqueous extract was filtered through a sieve and cloth to obtain the liquid fraction. The pH was measured with a digital potentiometer (DM 20 pH meter, Digimed Analytica, São Paulo, Brazil). To determine the concentration of the final fermentation products, a gas chromatograph with a mass detector (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) and a capillary column were used. (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm, i.d. 0.2590 m), lactic acid was determined by colorimetry (Pryce, 1969). The dry matter content corrected for volatiles (DMcorr) was calculated using the equation of Weissbach (2009): DMcorr (% as fed) = oven DM (% as fed) + n-alcohols (% as fed) + 2,3-butanediol (% as fed) + 0.95 × volatile fatty acids (% as fed) + 0.77 × 1,2-propanediol (% as fed) + 0.08 × lactic acid (% as fed).

A 1 mL aliquot of the aqueous extract was collected using a micropipette and added to a test tube containing 9 mL of peptone water at 0.1% concentration. From this, it was possible to perform the dilutions expressed as logarithms in base 10 (10-1 to 10-6) for each replicate of the treatments. All material used for plating was autoclaved to avoid contamination. The in-depth plating of the dilutions (pour plate) constituted in quadruplicate, in disposable petri plates containing the culture media: Lactobacilli MRS Agar + antifungal natamycin (0.25 g/L), for counting lactic acid bacteria (LAB); Malt Extract Agar, for fungus and yeast counting; Reinforced Clostridial Medium plus cycloserine-D, for clostridial count; Plate Count Agar (PCA) for aerobes. The preparation of the media proceeded according to the manufacturers' recommendations and the amounts were calculated so that each plate received 20 mL of medium. When necessary, microwave preheated distilled water was used to dissolve the media.

Plating was performed in a hood with a ventilation curtain to prevent the entry of microorganisms. Before plating, the hood received ultraviolet light for 15 minutes for sterilization. The plates were incubated in BOD (Biochemical Oxygen Demand) ovens, during the following incubation times: LAB for 48 hours at an average temperature of 30ºC; Clostridia at 37ºC for 120 hours; Fungi and Yeasts at 30ºC for 48 hours; Bacilli at 34ºC for 72 hours. The ovens were sterilized with 70% alcohol before receiving the plates.

To determine the WHC, 2.5g of fresh silage were soaked in 250 mL of distilled water for 16 ± 24 hours. After this period, the mixture was filtered through a sintered glass crucible (porosity 2) and the sides of the glass were carefully washed. The sample was decanted for 10 minutes and then weighed (Giger – Riverdin, 2000; Table 1).

To determine the aerobic stability, the time required for the silage temperature to exceed 2 °C above the ambient temperature was defined (Kung et al., 2000). Samples (3 kg) were placed in plastic buckets and allocated for 10 days in a room with natural ventilation and without exposure to the sun. Every 15 minutes, the temperature of the silage and the environment was measured using dataloggers (ELITECH® Model RC-5). The mean particle size (Table 1) was determined using the Penn State Particle Size Separator (Kononoff et al., 2003).

Fresh samples at the time of ensiling and silage samples after opening the silos were collected to determine the bromatological components. For quantification of dry matter (DM) a forced air circulation oven was used at 55 °C for 72 h and after that the samples were ground in a Wiley hammer mill with a 1 mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Definitive DM, ether extract (EE) and ash concentration followed the recommendations according to the Association of Official Analytical Chemicals (AOAC, 1990), methods 934.01, 924.05 and 920.39, respectively. The crude protein (CP) content of silages was obtained by the Dumas combustion method (Leco 2000, Leco Instruments Inc). To determine the fibrous fraction, NDF and ADF, it was performed using the method by Van Soest et al. (1991), sodium sulfite and α-amylase were used based on the sequential method proposed by ANKON Fiber Analyser. The methodology of Dubois et al. (1956) to obtain the value of total soluble carbohydrates. For non-fiber carbohydrates, the following formula was used: NFC = 100 – (NDF + CP + EE + ASH). (Table 1).

2.3.4 Statistical analyses

Data were analyzed using a completely randomized design in a factorial scheme with additional treatment (2 × 4+ 1). The breakdown would be: 2 rehydration conditions (with and without the addition of water), 4 stages of evolution of culture maturity (40.7%; 46.9%, 49.5%

and 54.2% DM) + 1 positive control, represented by the harvest with 35% DM, with 4 replications for each treatment.

All data were submitted to the Shapiro-Wilk test (Shapiro-Wilks, 1965) to verify normality and remove outliers. The homogeneity of variances was verified using the Levene test (Levene, 1960). Mean values were compared using orthogonal contrasts (control vs treatment). Treatment means were obtained by Tukey's test ($P < 0.05$).

Variables were analyzed considering a model including treatments (DM and water addition) as fixed effects, silos (experimental unit in the RANDOM SAS option) and residual random error (NIID) of (0, σ 2) as random effect. All statistical analyzes were performed using the PROC MIXED procedure of SAS 9.4 software (SAS Inc., Cary, NC).

2.4 Results

The maturation evolution of the maize crop expressed in days after emergence (DAE) correlated with the DM content of the plant at harvest (R^2= 0.99; Figure 1). The addition of water in the mass with the objective of reaching 34.7% DM was effective at the time of ensiling, in general the rehydrated materials reached the rehydration objective.

Orthogonal contrasts are presented in Table 2. The variables EL, CP and NDF were significant at 99% confidence interval ($P<0.0001$). DMl, AE, Density, LAB and Yeast were significant at 95% ($P<0.05$). In the other variables, there was no significance between the control and the means of the other treatments. One can notice the effect of rehydration and advancing DM influencing the control differing from the other means.

There was no interaction ($P>0.05$) for fermentation products as shown in Table 3. There was rehydration effect and DM for LA. The increase in the DM content of the plant at harvest time affected the ET and ACP variables. For the microorganisms LAB and AER there was interaction between the factors (table 4). Rehydration and DM also influenced AER microorganisms, rehydration reduced the population of AE microorganisms, while MS increased AER. The same occurred for LAB. DM advancement was significant ($P<0.05$) for fungi and yeasts. Note that the addition of water tended ($P=0.07$) clostridia. Treatments that had added water showed a reduction ($P < 0.001$) in pH when compared to treatments ensiled with DM without correction (table 3).

Table 2 - Orthogonal contrasts between the control treatment (34.7% DM) and the means of the other treatments.

	Orthogonal Contrasts (p-value)	Control DM 34.7%	DM 40.7%		DM46.0%		DM49.5%		DM54.2%	
Item			**NR**	**R**	**NR**	**R**	**NR**	**R**	**NR**	**R**
LA[1]	0.5093	2.3712	2.3964	4.3682	1.8416	2.3257	1.4717	2.5143	1.3668	2.4552
ET[2]	0.596	0.3124	0.6547	0.3786	0.4	0.3721	0.4963	0.4387	0.2514	0.1569
AA[3]	0.2889	0.3727	0.4255	0.5001	0.4781	0.3336	0.4728	0.4566	0.3785	0.5774
pH	0.1039	3.7175	3.7775	3.61	3.7975	3.685	3.805	3.6775	3.9725	3.7175
DML[4]	0.0014	5.65	7.6475	18.0175	8.5675	12.6225	20.0025	15.2133	18.8625	15.95
EL[5]	<0.0001	1.0183	1.2599	4.5082	0.467	4.6124	1.1147	4.6318	1.0561	7.5275
AE[6]	0.0009	85.1875	121.19	119.5	47.875	71.1875	30.75	31	26.1875	48.125
DENS[7]	0.0015	671.17	622.3	688.43	470.02	629.13	504.19	684.77	526.22	774.35
ASH	0.1895	3.2106	2.8925	3.0625	2.7091	2.5966	2.9068	3.0886	3.3528	2.4846
CP[8]	<0.0001	12.5777	9.9322	8.7591	10.1453	9.1239	9.7706	8.5836	9.7122	8.1287
NDF[9]	<0.0001	41.862	44.2771	42.8379	43.3663	45.1776	46.1773	47.6478	47.5632	46.8603
ADF[10]	0.781	26.9891	27.7635	27.2969	26.2435	28.166	22.9471	27.6696	28.2751	30.8425
LAB[11]	0.0003	5.8522	6.04	6.4885	5.061	7.0117	7.8425	7.8675	8.116	8.406
YE[12]	0.0009	1.9925	2.4203	2.5637	3.8495	4.2936	2.9153	3.9099	3.2673	3.1271
MOL[13]	0.9328	3.8194	3.1543	2.6213	4.3897	4.8601	4.3271	4.996	3.4345	3.0724
AER[14]	0.8113	3.336	4.3808	2.8966	3.0633	3.0429	2.9989	2.6778	4.0977	3.8885
CLOS[15]	0.6781	2.0354	2.034	2.5615	2.2861	2.4807	1.6765	2.1809	1.8967	2.1165

NR = not rehydrated. R = rehydrated.[1]Lactic acid. [2]Ethanol. [3]Acetic Acid. [4]Dry Matter Losses. [5]Effluent Losses. [6]Aerobic Stability. [7]Density. [8]Crude Protein. [9]Neutral detergent fiber; [10]Acid detergent fiber. [11]Lactic Acid Bacteria. [12]Yeasts. [13]Molds. [14]Aerobic spores. [15]Clostridia.

In the fermentative losses (DMl and EL) there was interaction (P<0.05) between the factors as shown in table 5. In addition to the interaction of the factors, EL was influenced by the increase in DM (P<0.001) and rehydration (P<0.001) Table 6, the treatments that received the highest amounts of water were consequently the treatments with the highest DM at the time of harvest, which led to greater losses by effluents. The positive control (34.7%) obtained lower DMl when compared to the other treatments, but did not differ from the treatment with 44.7% without DM rehydration.

Although there was no interaction for AE, there was significance for rehydrated (P =0.0191) and DM (P=<0.0001), rehydrated treatments on average had higher AE and the increase in DM reduced the stability of silages (Table 5). There was a considerable density increase in the rehydrated silages, with interaction between the factors as shown in table 7. The silos made with plants with high DM had lower density, but the addition of water at the time of ensiling reduced the effect of high DM, increasing the silo density (Table 5).

There was no interaction for most chemical components, except NDF. Increasing DM altered Ash (P=0.0021), NDF (P<0.0001) and ADF (P=0.06). The rehydration reduced the CP of the silages, the rehydrated materials showed an increase in ADF (Table 8), while NDF and ADF increased with DM advancement.

The orthogonal contrasts for WHC are shown in table 9. It is possible to see that there was no difference between the control treatment (34.7% DM) and the second DM (40.7%), but the other DMs had differences when compared to the control. Applying the regression model for WHC, it was possible to observe a tendency for quadratic behavior (R^2= 0.6757) for the variable (table 10). This is confirmed with the application of contrasts, where we observe that the plants harvested with 54.2% DM have higher values than 46.0% and 49.5% DM.

Table 3 - Fermentative and microbiological profile of silages with high DM content and rehydrated silages

	Rehydration		*SEM*	%DM				*SEM*	p-value		
Item	**NR**	**R**		**40.7%**	**46.0%**	**49.5%**	**54.2%**		**R**	**DM**	**R*DM**
Fermentation Profile											
pH	3.8381	3.6725	0.0828	3.7781	3.7913	3.8030	3.9738	0.0319	<0.0001	<0.0001	0.0126
LA[1]	1.6550 a	2.4800 b	0.4843	2.4218 b	1.8142 a	1.5519 a	1.4799 a	0.3561	<0.0001	<0.0001	0.3629
ET[2]	0.5017	0.3868	0.0582	0.5341 a	0.4827 ab	0.5206 ab	0.2495 b	0.0675	0.1439	0.0265	0.7221
AA[3]	0.4303	0.4876	0.0141	0.4212	0.4544	0.4393	0.3613	0.0151	0.6774	0.4617	0.6304
Microbiology Profile											
Lactic acid bacteria, log cfu/g	6.7650	7.4435	2.9743	6.2644	6.0664	7.8552	8.2610	4.2063	0.0075	<0.0001	0.0433
Yeasts, log cfu/g	3.1131	3.4736	0.1802	2.4920 b	4.0715 a	3.4126 ab	3.1972 ab	0.3255	0.1569	0.0016	0.424
Moulds, log cfu/g	3.8264	3.8874	0.0325	2.8878 b	4.6249 a	4.6615 a	3.2535 b	0.4601	0.8417	0.0003	0.421
Aerobic spores, log cfu/g	3.6351	3.1264	0.2086	3.6387	3.0531	2.8384	3.9931	0.2832	<0.0001	<0.0001	0.0002
Clostridia, log cfu/g	1.9733	2.3349	0.1808	2.2978	2.3834	1.9287	2.0066	0.1102	0.0744	0.2994	0.8866

[1]Lactic acid. [2]Ethanol. [3]Acetic Acid. R = Rehydrated; DM = Dry Matter; R*DM Rehydrated*Dry Matter.

Table 4 - Unfolding the MS*REHYDRATION interaction for LAB and AER.

Rehydration	%DM				Mean
	40.7%	**46.0%**	**49.5%**	**54.2%**	
			LAB[1]		
Not Rehydrated	6.0400 Ab	5.0610 Bb	7.8425 Aa	8.1160 Aa	**47.6083**
Rehydrated	6.4885 Ab	7.0117 Ab	7.8675 Aa	8.4006 Aa	**56.2952**
Mean	39.797	37.8703	61.8217	68.3181	
p-value		0.0433			
SEM		0.2165			
			AER[2]		
Not Rehydrated	4.3808 Aa	3.0633 Ab	2.9989 Ab	4.0977 Aa	**3.6351**
Rehydrated	2.8966 Bb	3.0429 Ab	2.6778 Ab	3.8885 Aa	**3.1264**
Mean	3.6387	3.0531	2.8384	3.9931	
p-value		0.0002			
SEM		0.1208			

Averages followed by different letters. lowercase horizontally (comparing %DM) and uppercase vertically (comparing rehydration). differ by the Tukey-Kramer test ($p<0.05$). [1]Lactic Acid Bacteria. [2]Aerobic Spores.

Table 5 - Fermentative losses, aerobic stability and density.

Item	Rehydration		*SEM*	%DM				*SEM*	p-value		
	NR	**R**		**40.7%**	**46.0%**	**49.5%**	**54.2%**		**R**	**DM**	**R*DM**
DML[1]	13.77	15.4508	1.37	12.8325	10.595	17.6079	17.4062	2.0847	0.1768	0.0006	0.0007
EL[2]	0.9744	5.32	2.1728	2.8840	2.5397	2.8733	4.2918	0.3898	<0.0001	<0.0001	<0.0001
AE[3]	56.5000 B	67.4531 A	3.7397	120.34 A	59.5312 B	30.8750 C	37.1562 C	19.9802	0.0191	<0.0001	0.0928
DENS[4]	530.68	694.17	81.7467	655.37	549.58	594.48	650.28	25.0822	<0.0001	<0.0001	<0.0001

[1]Dry Matter Losses. [2] Effluent Losses. [3]Aerobic Stability. [4]Density. R = Rehydrated; DM = Dry Matter; R*DM Rehydrated*Dry Matter.

Table 6 - Unfolding the DM*REHYDRATION interaction for Effluent Losses.

Rehydration	%DM				Mean
	40.7%	**46.0%**	**49.5%**	**54.2%**	
Not Rehydrated	1.2599 Ba	0.4670 Bb	1.1147 Ba	1.0561 Ba	**0.9744**
Rehydrated	4.5082 Ab	4.6124 Ab	4.6318 Ab	7.5275 Aa	**5.3200**
Mean	**2.8840**	**2.5397**	**2.8733**	**4.2918**	
p-value		**<0.0001**			
SEM		**0.3665**			

Averages followed by different letters. lowercase horizontally (comparing %MS) and uppercase vertically (comparing rehydration). differ by the Tukey-Kramer test (p<0.05).

Table 7 - Unfolding the DM*REHYDRATION interaction for Density.

Rehydration	%DM				Mean
	40.7%	46.0%	49.5%	54.2%	
Not Rehydrated	622.30 Ba	470.02 Bc	504.19 Bbc	526.22 Bb	**530.68**
Rehydrated	688.43 Ab	629.13 Ac	684.77 Ab	774.35 Aa	**694.17**
Mean	**655.37**	**549.58**	**594.48**	**650.28**	
p-value		**<0.0001**			
SEM		**18.7942**			

Averages followed by different letters. lowercase horizontally (comparing %MS) and uppercase vertically (comparing rehydration). differ by the Tukey-Kramer test (p<0.05).

Table 8 - Chemical composition of silages with high dry matter and rehydrated silages.

	Rehydration		*SEM*	%DM				*SEM*	p-value		
Item	**NR**	**R**		**40.7%**	**46.0%**	**49.5%**	**54.2%**		**R**	**DM**	**R*DM**
Ash	3.4693	3.2999	0.0664	3.6446 ab	3.7460 a	2.9953 c	3.1526 bc	0.0739	0.2373	0.0021	0.4068
CP[1]	9.8901 a	8.6488 b	0.6099	9.3457	9.6346	9.1771	8.9205	0.1526	<0.0001	0.277	0.8757
NDF[2]	45.346	45.6309	0.0092	43.5575	44.2719	46.9125	47.2118	0.9085	0.1113	<0.0001	<0.0001
ADF[3]	26.3073 b	28.4937 a	1.1185	27.530 ab	27.204 ab	25.308 b	29.5588 a	0.9062	0.0079	0.006	0.1361

[1]Crude Protein. [3]Neutral detergent fiber; [3]Acid detergent fiber. R = Rehydrated; DM = Dry Matter; R*DM Rehydrated*Dry Matter.

Table 9 - Orthogonal Contrasts for Water Holding Capacity.

	Control[1]	**DM 40.7%**	**DM46.0%**	**DM49.5%**	**DM54.2%**
WHC[2] mean	2.7704	2.399	1.6578*	1.2362*	2.0646*
Orthogonal Contrasts (p-value)	-	0,0724	0,0001	<0.0001	0.0034
Orthogonal Contrasts (SD)	-	0.1174	0.3518	0.4851	0.2232
Orthogonal Contrasts (*SEM*)	-	0.0303	0.0908	0.1252	0.0576

[1]Control: forage 34.7-DM. [2]Water holding capacity.
***Indicate difference between treatment (DM) and control.**

Table 10 - Regression for water holding capacity variable.

Model	p-value	SD	*SEM*	R^2	Beta-0	Beta-1	Beta-2	Equation
Linear	0.0056	0.4507	0.1163	0.4585	4.58188	-0.0566	-	$Y = 0.4585 – 0.05655x$
Quadratic	0.0012	0.363	0.0937	0.6757	17.4389	-0.6519	0.00672	$Y = 17.43893 – 0.65188x + 0.00672x^2$

2.5 Discussion

The DM advance showed linear behavior as expected (Zopollato et al. 2009), altering chemical and fermentative components. This linear increase in DM affected the percentage of lactic acid in the silages, probably due to the reduction of soluble carbohydrates available for fermentation. Although lactic acid reduced, the silages will range in pH between 3.77 - 3.97, being slightly above that recommended by Mcdonald et al. (1991) but within that observed by Kung et al. (2018) in their metaanalysis. In our study none of our treatments had lactic acid within the optimal range, but behaved similarly to plants harvested at high DM (Kung et al., 2018). The rehydrated treatments differed from those that did not receive added water, presenting lower pH. This same behavior was observed by the microorganisms, despite the possible reduction of soluble carbohydrates, rehydration allowed a better environment for lactic fermentation to occur, a behavior proven with the reduction of pH in rehydrated silages. For good development of these microorganisms it is necessary to have water activity (Aw) in the environment (Lindgren, 1999), with increasing DM the Aw probably reduced, however when the materials were rehydrated the environment may have become more favorable for LAB growth and pH drop due to the increase in lactic acid. Although rehydration benefited LAB growth, it did not provide an environment for the growth of fungi, yeast and clostridia, however it was responsible for the reduction of aerobic spores. This is mainly due to the recovery of the density of the materials when rehydrated, since the reduction in density affects the porosity of the ensiled material (Bolsen & Bolsen, 2004). Increased DM enabled the growth of aerobes as well as pointed out by Vilela et al. (2008). Ensuring higher density promotes better conservation of soluble carbohydrates and reduces DM losses (Sucu et al., 2016). The increase in density was linear to rehydration, coinciding with the fermentative behavior of the silages. Only for ethanol was the influence of DM content found, the treatment harvested at 54.2% DM was numerically lower than the other treatments. Silages with lower density present higher consumption of soluble carbohydrates that would be destined to the fermentation of microorganisms (Senger et al., 2005; Velho et al., 2007), the treatment with 54.2% DM was the one that presented the lowest density without rehydration, which may have generated less alcoholic fermentation. It is worth remembering that ethanol contents should be between 1 and 3% DM (Kung et al., 2018), in our study no treatment had ethanol concentration higher than 1% in DM. Acetic acid was not influenced by any of the factors and was below the range observed in other works, but it is worth noting that inoculants were not applied in our study. Inoculants based on heterofermentative bacteria as L.buchneri or associated with homolactic

bacteria as L. plantarum have the ability to convert hexoses into lactic acid, acetic acid, CO2 and water (McDonald et al., 1991; Filya, 2003; Filya et al., 2006; Kleinschmit et al., 2005).

The increase in linear DM altered not only the fermentative patterns such as pH, lactic acid and microorganisms, but also showed differences in losses. Between the treatment with 40.7% DM and 46.9% DM there was an increase of 11.44 percentage points, as well as differing from the control treatment (34.7% DM). DMl appear below 10% in silages (Rabelo et al., 2012; Borreani et al., 2018). It is observed that the highest losses were found in the treatments with lower density (49.5% DM and 504 Kg/ m^3; 54.2% DM and 526.22 Kg/m^3), confirming that silages with lower density tend to obtain higher DMl (Bolsen & Bolsen, 2004), in the 1980s studies were done and correlated silage density with plant moisture content (Tang, Jofriet and LeLievre, 1988), it is notable that rehydration was the predominant factor to change the density in the different treatments. Fungi and yeasts have good correlation (R^2=0.827) with DMl (Borreani et al., 2018), according to these authors, fungal counts above 6 log-10 CFU/g cause 40% DMl and mycotoxin risk Gotlieb (2016), in our study none of the treatments reached such high levels of these microorganisms. DM influenced the proliferation of these microorganisms in our study, with the control treatment being the one that showed the lowest yeast count (P=0.009). The interaction of the factors (DM and rehydration) on the DMl can be explained by the percolation of liquids when there was rehydration, effluent production is one of the causes for DMl (Gebrehanna et al., 2014). In the rehydrated treatments liquid percolation and effluent production was considerably higher, mainly due to the capacity to retain exogenous liquids, in addition to the pressure exerted in the environment (Wolford, 1978), which consequently generated higher DMl when the plants were rehydrated. The effluent losses in the rehydrated treatments were mainly due to the water-holding capacity (WHC). This variable is dependent on chemical factors of the plant, in our study the quadratic behavior observed is due to the proportion of NDF of the plant. Giger Riverdin (2000) observed that bulky feeds have better WHC when compared to concentrates, this occurs due to the proportion of NDF of these feeds (R^2=0.764). Materials with 40.7% DM, 46.9% DM and 49.5% DM had lower WHC while 54.2% DM had similar WHC to the control treatment. This same treatment (54.2% DM) had higher NDF than the others. This behavior of increasing NDF is known, at a certain stage the plant stops accumulating starch. The reduction of starch and increase of NDF influences that NDF generates lower density of food, increasing porosity and water holding capacity.

The higher the DM at the time of harvest, the greater was the amount of water added to the material for rehydration in order to return the material to 34.7% DM. This addition of water was not corresponding to the absorption of the different DM of the harvested plants, as the

chemical characteristic was changed with time, the WHC was reduced and with this, the materials had higher effluent losses (P<0.0001). The production of effluent not only generates the DMl but also generates environmental risk, effluent generated during the ensiling process has high biochemical oxygen demand, nitrogen and phosphorus. Generally the pH of this material is low (3.9 - 4.2), which in contact with water generates risks to the biome present (Deans and Svoboda, 1992). One liter of effluent is capable of depleting oxygen in 10000 liters of water (Cropper and DuPoldt, 1995) and due to the high amount of organic acids it presents corrosive aspects to steel and concrete (Bellman, 1999) which generates deterioration of the silos. Therefore, the addition of water should take into consideration the WHC of the materials, despite the improvement of fermentative aspects such as increase in LAB and consequently decrease in pH, because the percolation of the additional liquids generates risk to the environment.

The biggest factor for aerobic deterioration to occur is the presence of air in the mass, because this enables the presence and growth of unwanted microorganisms. Muck et al. (1991) emphasizes that the presence of fungi and yeast is a determining factor for aerobic deterioration to occur. None of the treatments had good aerobic stability, which according to Kung Jr. et al. (2003) is 240 hours. After this period the material that has good fermentation reaches 2ºC above room temperature. Although they did not achieve good stability, the addition of water increased the stability of materials harvested with high DM content. Perhaps because the increased density provided greater expulsion of air from the silos and consequently better fermentation. Raising temperature over short periods leads to reduced LAB (Weinberg et al., 2001) and increased proteolysis (Muck and Dickerson, 1998). Kim and Adesogan (2006) had loss of aerobic stability in materials with yeast counts above 6.26 log CFU/g. On average, the yeast count was 4.42 log CFU/g. In our experiment no treatment had higher counts than those found by these authors, the treatments with the highest yeast counts were with 35.5% and 34.0% DM with 4.29 and 3.91 log CFU/g respectively.

Among the chemical characteristics, only NDF and CP of the treatments were different from the control treatment. The ash content affected by DM (P=0.0021) is due to DM accumulation in the plant (Pinho 2011; Kayser 2021). The effect of rehydration on reducing CP content is not clear, there is variation in CP fractions as pointed out by Kayser (2021) when changing the harvest period of the corn plant, but no effect of rehydration is observed in the literature. The reduction of NDF in silages was expected due to lignification of the plant and with it an increase of ADF (Cabral et al., 2002; Vilela et al., 2008) the reduction of NDF is

explained by the greater participation of grains in the plant in the final stages (Lavezzo et al., 1997).

When observed the set of all variables it is possible to observe that the production of effluents was the one that had the greatest influence on the data. This is due to the large amount of liquid added and subsequently percolated. Highlighting the treatment harvested with 54.2% DM and rehydrated, this material despite having shown better WHC when compared to 46% DM and 49.5% DM, the amount of liquid added for it to reach 33.7% DM was high and the WHC of this material was not effective.

2.6 Conclusion

The rehydration of the whole corn plant for making silage, harvested with high DM values, proved to be an alternative to minimize losses and improve the fermentative pattern, providing a favorable environment for the growth of beneficial microorganisms for the conservation of the ensiled mass.

A point of attention was noted regarding the addition of water, drier harvested materials have less capacity to absorb the water added for rehydration, thus generating effluents. The production of effluents is not sought in the ensilage process due to the loss of nutritional quality of the material and contamination of the environment, therefore materials with more than 50% DM do not seem to be viable for rehydration, in these cases the destination of the plant can be given to grain production or snaplage.

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