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Ensiling melon biomass with different levels of inclusion of ground corn

Ensilagem da biomassa do meloeiro com diferentes níveis de inclusão de farelo de milho

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Highlights

Gas production varied from 0.24 to 2.05% among all silages. The highest lactic acid content was with 1000 g kg^{-1} of fruit with 22.1 g kg^{-1} of DM. The highest pH value was in silages with 0 g kg^{-1} of fruit with 7.95.

Abstract

The aim of this study was to evaluate silages produced with different mixtures of melon biomass and different levels of ground corn. The experiment was conducted under a completely randomized design in factorial scheme (3×4) , with four replications. The first factor consieted of three mixtures of plant (branch and leaf) and fruit (melon; scraps) on natural matter (NM) as follows: 0, 100 and 1000 g kg-1 fruit. The second factor consisted of the use of different amounts of ground corn (0, 50, 100 and 200 g kg-1). Experimental silos with capacity for 5 kg and density of 500 kg $m³$ were used. After 90 days, the silos were opened and the evaluations were carried out. The data were subjected to analysis of variance and the means were analyzed through the Tukey's test (fermentative losses, chemical composition, aerobic stability), Scott-Knott test (microbiology and organic acids) and regression, and were compared with a significance level of p<0.05. The best dry matter results were obtained in silages with 0, 100 and 1000 g kg⁻¹ fruit combined with 200 g kg⁻¹ ground corn, which averaged 289.4, 290.4 and 264.1 g

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kg⁻¹, respectively. Quadratic behavior was observed for effluent losses in silages with 100 g kg⁻¹ fruit presenting a minimum value of 46.8 e and a maximum 56.2 kg t^{-1} NM. Regarding the mold population count, the highest values were obtained in silages with 100 g kg⁻¹ fruit and 50 g kg⁻¹ ground corn. Silages with 100 and 1000 g kg⁻¹ fruit in melon biomass and 200 g kg⁻¹ ground corn presented superior quality indicators, characterizing these biomass mixtures as the most suitable for silage making*.* **Key words:** *Cucumis melo* L. Fruit. Quality. Moisture*.*

Resumo

Objetivou-se com esse trabalho avaliar as silagens produzidas com diferentes misturas da biomassa do meloeiro aditivadas com diferentes níveis de farelo de milho. O experimento foi realizado com delineamento inteiramente casualizado em esquema fatorial (3 × 4), com quatro repetições. O primeiro fator correspondeu de três misturas com base na matéria natural (MN) entre planta (rama mais folha) e o fruto (melão; refugo) da seguinte forma: 0fruto, 100 e 1000 g kg-1 fruto. O segundo fator correspondeu da utilização do farelo de milho (0, 5, 100 e 200 g kg-1, na MN). Foram utilizados silos experimentais com capacidade de 5 kg com densidade de 500 kg m-3, após 90 dias os silos foram abertos e foram realizadas avaliações. Os dados foram submetidos à análise de variância e as médias foram analisadas através do teste de Tukey (perdas durante a fermentação, composição química, estabilidade aeróbia), Teste de Scott-Knott (microbiologia e ácidos orgânicos) e regressão, sendo comparados com nível de significância de p<0,05. Para matéria seca os melhores resultados foram obtidos nas silagens com 0, 100 e 1000 g kg⁻¹ fruto adicionada de 200 g kg⁻¹ de farelo de milho com 289,4, 290,4 e 264,1 g kg⁻¹ respectivamente. Foi observado comportamento quadrático para perdas por efluentes em silagens de 100 g kg-1 de fruto com valor mínimo de 46,8 e e máximo de 56,2 kg t-1 MN. Quanto à contagem da população de fungos, os maiores valores foram obtidos nas silagens com 1000 g kg⁻¹ de fruta e 50 g kg⁻¹ de milho moído. As silagens produzidas com 100 e 1000 g kg⁻¹ de frutas na biomassa de meloeiro e com 200 g kg-1 de milho moído apresentaram indicadores de qualidade superiores, tornando essas misturas de biomassas as mais indicadas para produção de silagem*.* **Palavras-chave:** *Cucumis melo* L. Fruto. Qualidade. Umidade.

Introduction

Farmers use several alternatives to face the seasonal changes in pasture production throughout the year in regions of dry climate. Silage is one of the most common forms of forage conservation used in animal feeding, mainly because it conserves the feed with high moisture content, using simpler machinery and presenting lower costs when compared to other techniques (G. Santos et al., 2017).

The improper disposal of materials from the agro-industry, produced on a large scale almost everywhere in Brazil, is a problem that calls for attention in terms of environmental pollution, as well as soil contamination for subsequent crops (Li et al. 2021; Demartelaere et al., 2021). In this context, several studies have been proposed in order to provide a proper destination for these materials, such as being used as alternative feed sources to conventional ones in animal feeding (Stella et al., 2016).

The melon plant (Cucumis melo L.) produces one of Brazil's most exported fresh fruits (Melo et al., 2020), generating a large amount of biomass after the commercial fruit is harvested.

Due to the high production of melon for the market, there is a considerable availability of residual biomass from this crop, composed of parts of the plant (twigs and leaves) and remaining fruits (melon). This biomass has high levels of carbohydrates, but also has a high moisture content. To improve the fermentation process, it is possible to combine this biomass with suitable additives This procedure can facilitate an improvement in the silage fermentation process and enable its use as an alternative source of food for ruminants (Nascimento et al., 2023).

In order to improve the nutritional value, additives have been added to high moisture silages, such as absorbent additives, which are usually sources of carbohydrates and are used to increase the silage DM content, stimulating the fermentation process in the silage mass (R. R. Ferreira et al., 2017). This tends to reduce silage moisture, reduce losses due to effluents and gases during the fermentation process, thus ensuring adequate fermentation, as well as being easy to manipulate, available and inexpensive to purchase (Troni et al., 2016).

Ground corn is an excellent additive precisely because it absorbs excess moisture from the ensiled material, reducing effluent losses and making the environment less favorable for yeast development, thus increasing dry matter levels and improving the quality of the melon biomass (A. P. M. D. Santos et al., 2018). According to Bezerra et al. (2016), it has a high nutritional value, as well as being a highly energetic source, with carbohydrates that are quickly fermented in the rumen and are easily available on the market.

Thus, ensiling melon biomass with the addition of ground corn may be a viable alternative for use in animal feeding, but it is necessary to assess the quality indicators of these silages. Therefore, the aim of this study was to evaluate silages produced with different mixtures of melon biomass and ground corn.

Material and Methods

Experimental protocol

The melon biomass was obtained from a melon-producing rural property located in the *Vale do Gurgueia* region in the Southern Piauí. The material was harvested from the field during the melon season, which goes through July, August, September and October, 85 days after planting (DAP) and after three commercial melon harvests. The material was analyzed at the Animal Nutrition Laboratory and at the Microbiology Laboratory of CPCE/UFPI, both located in Bom Jesus, Piauí, Brazil.

The experiment was conducted in a completely randomized design with factorial scheme (3 x 4) with four replications. The first factor corresponded to three mixtures of melon biomass natural matter including plant (branch plus leaf) and fruit (melon scrap, after harvest) as follows: 0, 100 and 1000 g kg^{-1} fruit. The second factor was the addition of ground corn in the following proportions: 0, 50, 100 and 200 g kg-1.

The evaluations were carried out when the biomass was collected, where the green forage mass (GFM) was measured using the PVC pipe square method [0.5m x 0.5m (0.25m²) framel, and the weight obtained was converted to t ha⁻¹. After collection, the material was fractionated according to the treatments and taken to the oven to determine the dry matter.

The biomass was processed in a forage chopper and crusher machine (GTM-2001sb GARTHEN®) adjusted to cut particles to a size of 2.5 cm. After being chopped, the mixtures were mixed manually and the ground corn was added at the moment of ensiling according to the treatments and placed in experimental silos made of PVC with capacity for 5 kg, with a Bunsen valve adapted to its lid, to allow the gases of fermentation to escape. One kilogram of sand was deposited at the bottom of each silo, separated from the forage by a layer of cotton fabric, making it possible to measure the amount of effluent retained. All material was compacted to reach a density of approximately 500 kg $m⁻³$.

In order to characterize the quality of the silage after 90 days of storage, the following parameters were analyzed: fermentation losses; buffer capacity and soluble carbohydrates; pH and ammonia nitrogen (N-NH₂); microbiological dynamics; volatile fatty acids; chemical composition and aerobic stability.

Silage fermentation parameters

To determine the chemical composition of the silage, dry matter (DM, method no. 934.01), mineral matter (method no. 981.10), crude protein (CP, method no. 920.39) and ether extract (EE, method no. 920.29) contents were determined according to Association of Official Analytical Chemists [AOAC] (1990). While the analyses to determine neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (ADF) were carried out according to Van Soest et al. (1991).

The concentration of total soluble carbohydrates (TSC) was obtained through the concentrated sulfuric acid method described by Dubois et al. (1956), with adaptations by Corsato et al. (2008). Soluble carbohydrate concentrations were measured by reading absorbance at 490 nm using D-glucose as standard (Dubois et al., 1956). The proportion of TSC, in g/100 ml, was calculated based on the solution and then adjusted based on the dry matter of each sample used. All the analyses were carried out at the Animal Nutrition Laboratory (LANA) at CPCE/UFPI. The chemical composition of the material before ensiling is shown in Table 1.

Chemical composition (dry matter basis) of melon biomass before ensiling

ANDF: neutral detergent insoluble fiber; ADF: acid detergent insoluble fiber; pH: hydrogen potential; N-NH₂: ammonia nitrogen based on total N; SC: soluble carbohydrates; Buffer capacity unit: mg NaOH 100 g⁻¹ DM.

The experimental silos were weighed after closure and after opening to determine dry matter (DM) losses in the form of gases and effluents, and dry matter recovery (DMR) according to the equations described by Zanine et al. (2010).

Gas losses were obtained from the difference in weight of the dry forage mass.

G = (FSc – FSo) ÷ *(FMc × DMc)* × 100

Where:

G: losses through gas (dag/kg DM);

FSc: weight of full silo at closure (kg);

FSo: weigh of full silo at oppening (kg);

FMc: forage massa at closure (kg);

DMc: forage dry matter content at closure (dag/kg).

Effluent losses were calculated using the following equation, based on the

difference in sand weight and related to the mass of fresh forage at closure.

$$
E = [(ESo - ST) - (ESc - ST)] \div FMc \times 100
$$

Where:

E: effluent yield (kg ton of silage);

ESc: weight of empty silo + weight of sand at closure (kg);

ESo: weight of empty silo + weight of sand at oppening; (kg);

ST: silo tare;

FMc: forage mass at closure (kg).

The following equation was used to estimate dry matter recovery:

$$
DMR = (FMo \times DMo) \div (FMc \times DMc) \times 100
$$

Where:

DMR: dry matter recovery (dag kg);

FMo: forage mass at openning (kg);

DMo: forage dry matter content at openning (dag kg);

FMc: forage mass at closure(kg);

DMc: forage dry matter content at closure (dag kg).

Buffer capacity (BCAP) was determined according to the methodology of Mizubuti et al. (2009) by using 10 to 20 g of silage macerated with 250 ml of distilled water. Then, the macerate was titrated to pH 3.0 with 0.1N HCl to release bicarbonates, such as carbon dioxide. It was then titrated to pH 6.0 with 0.1N NaOH, and the volume of NaOH used to change the pH from 4.0 to 6.0 was recorded. BCAP was calculated through the equation:

$$
BCAP = 0.1 \times (Vs - Vb) \times 100/DSW
$$

Where,

BCAP = buffer capacity in e.mg NaOH 100 g DM;

0.1 = NaOH Normality;

Vs = volume of NaOH used to change sample pH from 4.0 to 6.0;

Vb = volume of NaOH used to change blank pH from 4.0 to 6.0;

DSW = weight of dry sample = [(sample weight \times DM) \div 100].

The pH values and ammonia nitrogen concentration $(N-NH₃)$ of the silage were determined when the silos were opened. The pH in distilled water was determined in duplicate by taking 25 g samples of silage from each treatment and adding 100 ml of water. After 1 hour, the pH was read according to the methodology described by Bolsen et al. (1992), by a potentiometer. To determine the N-NH₃ content in the samples, the methodology described by Bolsen et al. (1992) was used, where 200 mL of 0.2 N H₂SO₄ solution was added to 25 g of fresh sample.

The microbiological assessment of the population of lactic acid bacteria, enterobacteria, molds and yeasts was carried out according to the methodology described by González and Rodrigues (2003). A 25 g sample of fresh silage was collected and mixed with 90 ml of distilled water, and the mixture blended for approximately 1 minute. Then, 1 ml of the mixture was taken and pipetted with the appropriate dilution (10-1 to 10-9). Plating was carried out in duplicate for each culture medium. Populations were determined using the selective anaerobic medium culture technique:

1° Rogosa Agar medium for counting lactic acid bacteria, after incubation for 48 hours in oven at 37° C;

2° BDA Agar (Potato Dextrose Agar) acidified with 1% tartaric acid, to count molds and yeasts, after 48 hours of incubation at room temperature;

3° Brilliant Green Bile Agar medium for counting enterobacteria, after incubation for 24 hours at 35° C.

Petri dishes with 30 to 300 CFU (colony forming units) were considered susceptible to counting, and the averages of the selected dilution plates were considered. The differentiation between yeasts and molds was made by the physical structure of the colonies, which was visually noticeable, as yeasts are unicellular and molds multicellular.

To determine the concentrations of organic acids (lactic, acetic, propionic and butyric), 10g of each silage was weighed, mixed with 90 ml of distilled water, homogenized in a blender for 1 min and then filtered through a 0.22 um pore PVDF syringe filter. Subsequently, a 10-ml sample was taken from the filtrate, placed in tubes to be centrifuged, added 1.0 ml of metaphosphoric acid and two drops of 50% sulfuric acid, and the solution formed was centrifuged for 15 minutes at 13,000 rpm. After this process, the supernatant was collected in splendor tubes and frozen to determine the concentration of organic acids using the high-performance liquid chromatography technique (HPLC; SHIMADZU, SPD-10A VP) (Siegfried et al., 1984). The HPLC apparatus was equipped with an Ultraviolet Detector using an Aminex HPX-87H column (BIO-RAD, CA, USA) with the mobile phase containing 0.005 M sulfuric acid, flow rate of 0.6 ml/min and wavelength of 210 nm.

When the silos were opened, the mass of silage was homogenized and 2.5 kg of silage was returned to the original silos for evaluation of stability in aerobiosis. The silage samples were exposed to air at a controlled ambient temperature (25 °C), similar to the assessments carried out by Johnson et al. (2002). Room temperature was controlled using an INCOTERM® room thermometer, and the internal temperature of the silage was measured using an INCOTERM® digital skewer thermometer by inserting the stainless steel tip into the center of the material, while the surface temperature was measured using a BENETECH® digital infrared thermometer with laser sight (-50 °C to 420 °C).

The evaluation of aerobic stability was carried out over 5 days in an airconditioned room at a temperature of 25 ± 1°C, with readings of the internal and surface temperatures of all silos being taken every 4 hours, over a period of 96 hours. Aerobic stability was calculated as the time (hours), that the silages presented an increase of 2°C in relation to the ambient temperature, and pH at intervals of 24 hours. For pH analysis, subsamples of 25 g of silage were collected, which 100 mL of distilled water were added and kept at rest for two hours and then a digital pH meter was read (Bolsen et al., 1992). When the silages lost aerobic stability, a sample was taken to count microorganisms according to the methodology described by González and Rodrigues (2003).

Statistical analysis

The data was subjected to analysis of variance using the following model:

$$
Yijk = \mu + \tau i + \gamma j + (\tau \gamma) ij + \varepsilon ijk
$$

where: Y*ijk* = observation referring to the different mixtures of melon biomass *i* with ground corn *j*; μ = general constant; *τi* = effect of the different mixtures of melon biomass *i*; $i = 1, 2, 3$ (0 fruit, 100 fruit, 1000 g kg⁻¹ fruit); *γj* = the effect of ground corn (0, 50, 100 and 200 g kg-1 ground corn); (*τγ*)*ij* = interaction between the different mixtures of melon biomass *i* with additives *j*; *εijk* = random error associated with each mixture of melon biomass with additives.

The means were compared through the Tukey's test (fermentative losses, chemical composition and aerobic stability)

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and Scott-Knott test (microbiology and organic acids) between melon biomass mixtures; and analyzed by regression for ground corn levels up to first and second degree polynomial models. For the total microbial count, it was expressed as colony forming unit (CFU) and transformed into a logarithm in base 10, using the average values to perform the statistical analysis. The data was analyzed using the SISVAR software version 5.0 (D. F. Ferreira, 2011), and 0.05 probability was adopted in all analyses.

Results and Discussion

Fermentation losses

It was observed an effect of interaction (P<0.05) between the different biomass materials and ground corn levels on effluent and gas losses, as well as on pH and N-NH₃ (Table 2), while there were isolated effects of the different mixtures and levels of ground corn on dry matter recovery (DMR) (P<0.01).

Effluent losses in silages with 100 g kg⁻¹ fruit showed quadratic behavior with a minimum value of 46.8 kg t-1 NM and maximum value of 56.2 kg t-1 AFNM for silages with 200 and 50 α kg⁻¹ of ground corn, respectively. The reduction in effluent production may be associated with the addition of fruit, which reduces the pH of silages and availability of soluble carbohydrates that serve as substrate for the production of lactic acids, resulting in loss reduction (Gandra et al., 2018).

Gas production showed a linear decreasing effect in all silages as the levels of ground corn increased. The silage with 1000 g kg^{-1} fruit showed greater gas loss in all levels of ground corn. The use of additives in the ensiling resulted in a significant increase in the dry matter content of the ensiled material (Table 3), in addition to promoting moisture retention and reducing gas production. A relatively low dry matter content favors the development of bacteria from the genus *Clostridium* and other aerobic microorganisms, which are accumulated for the occurrence of secondary fermentation (Silva et al., 2018). According to R. Muck (1996), the production of gas from the ensiled mass is a secondary production, which means unwanted fermentation.

Losses, pH and N-NH3 of melon biomass silages with different mixtures and addition of ground corn

NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. Means followed by the same letter in the column do not differ significantly according to the Tukey's test at 5% probability.

Regression equations: Effluent: Y_{100FMB} = 51.13 + 1.5072x - 0.0860x²; Gases: Y = 5.38 - 0.1492x; DM recovery: Y_{GC} = 70.58 + 1.9685x - 0.0620x²; pH: Y_{0FMB} = 7.96 - 0.1798x; Y_{100FMB} = 7.55 - 0.1680x; N-NH₃: Y_{0FMB} = 0.9375 - 0.0747x + 0.002350x²; $Y_{100FMB} = 0.47 + 0.0267x - 0.001891x^2; Y_{1000FMB} = 1.025 - 0.0896x + 0.002441x^2.$

The different biomass mixtures provided silages with low average DMR values. These losses are closely related to the low concentrations of dry matter which resulted in a reduction in DMR, and that result can be verified through the reduction of losses in the form of gases and effluents. According to Machado et al. (2012) DMR can be acceptable in ranges that vary between 800 and 990 g kg-1, since in cases of undesirable fermentations, values lower than 800 g kg^{-1} mean that significant losses occur through the production of heat inside the silo, production of $CO₂$ and organic acids such as butyric acid and ethanol, which does not preserve the ensiled material and also due to poor compaction of the material at the moment of ensiling. The results found in this research corroborate the findings of the same authors mentioned above.

High pH values were found in silages with 0 and 100 g kg⁻¹ fruit. However, the best results were observed in silages with 100 g kg-1 fruit due to the high soluble carbohydrate content of the fruit (Table 1). Properly fermented silages have a pH between 3.8 and 4.2 (McDonald et al., 1991). However, the pH of silages with 200 g $kg⁻¹$ ground corn showed results close to the ideal when compared to the range normally found for grass silages with adequate fermentation, which present pH between 4.3 and 4.7 (Kung

et al., 2018). On the other hand, the high pH values observed in this study in silages with 0 and 100 g kg-1 fruit can be explained by the fact that the plant branch (leaf + stem) has a high buffer capacity (22.62 e.mg NaOH 100 g DS, Table 1). Buffer substances correspond to anions (organic acid salts, sulphates, orthophosphates, nitrates and chlorides) and plant proteins which will affect the fermentation profile of silages (Macêdo et al., 2021).

The silage without melon fruit showed a quadratic effect for N-NH₂ with minimum point between 100 and 200 g kg-1 ground corn; while the silage with 100 g kg-1 melon had a quadratic effect with minimum point at 200 g kg-1 ground corn; and the silage with 1000 g kg⁻¹ of melon had a quadratic effect with a minimum point between 100 and 200 g kg⁻¹ of ground corn. The N-NH₃ values obtained in the present study demonstrate that the inclusion of ground corn inhibited the growth of proteolytic microorganisms, such as bacteria of the genus *Clostridium,* which produce butyric acid. Acidic conditions (pH<4.6), reduced moisture content and supply of soluble carbohydrates are the main factors that lead to a decrease in $N-NH₂$ production (Mcdonald et al., 1991). Therefore, all silages analyzed were considered goodquality silages, presenting levels below the limit of 100 g kg⁻¹ TN (McDonald et al., 1991).

Chemical composition of melon biomass silages with different mixtures and the addition of ground corn

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NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. Means followed by the same letter in the column do not differ significantly according to the Tukey's test at 5% probability.

Regression equations: DM: Y_{oFMB} =20.66 + 0.3914x; Y_{100FMB} = 18.84 + 0.4484x; Y_{1000FMB} = 16.27 + 0.5828x; MM: Y_{oFMB} = 7.20 + 0.1584x - 0.0086x²; Y_{100FMB} = 7.19 + 0.0544x; Y_{1000FMB} = 8.2828 - 0.3311x + 0.0158x²; CP: Y_{oFMB} = 6.03 + 0.08094x; Y_{100FMB} = 5.87 + 0.1218x; Y_{1000FMB} = 6.24 + 0.0483x; NDF: Y_{оFMB} = 62.00 + 1.113x - 0.0531x2; Y_{100FMB} = 69.91 - 0.4344x; Y_{1000FMB} = 62.63 - 1.8064x + 0.055548x²; ADF: Y_{оғмв} = 40.77 - 0.441686X; Y_{100ғмв} = 42.963182 + 0.112182 - 0.034836X²; Y_{1000ғмв} = 33.737500 - 0.432857x; EE: Y_{оFMB} = 8.2424 - 0.3211x + 0.0128x²; Y_{100FMB} = 7.10 + 0.1484x - 0.0346x²; Y_{1000FMB} = 8.20 + 0.1484x - 0.0346x²; SC: Y_{oFMB} = 5.84 + 0.3998x - 0.0145x²; Y_{100FMB} = 6.93 - 0.0040x; Y_{1000FMB} = 8.06 + 0.1762x.

Chemical composition of the silages

There was a significant effect of interaction (P<0.05) between the different mixtures of biomass and levels of ground corn on the contents of dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF), ether extract (EE) and soluble carbohydrates (Table 3).

There was an increasing linear effect on the DM content with the addition of ground corn, with the highest values being observed when 200 g kg^{-1} of ground corn

were added in the biomass mixtures of 0 and 100 g kg^{-1} of melon, obtaining 289.4 and 290.4 g kg⁻¹ DM, respectively. According to Oliveira et al. (2010), the recommended DM content for silages should be between 260 and 380 g kg-1. The incorporation of ground corn resulted in an increase in dry matter (DM) content due to its high concentration of this component and its moisture retention capacity, as mentioned by Shinohara et al. (2021) and Paula et al. (2020).

The MM content showed quadratic behavior, with a minimum value of 68.9 g kg-1 DM for 0 fruit in silages with 200 g kg-1 ground

corn and 64.8 g kg-1 DM in silages with 1000 g kg-1 fruit and 100 ground corn. There was a linear decreasing effect of ground corn addition on MM content in the silage with 100 g kg⁻¹ melon. It may be associated with the effect of its dilution on the DM produced by this mixture, together with ground corn, which has a low quantity. This is the result of the production of effluent in silages, due to the greater possibilities of losses, resulting in losses of organic material (Brant et al., 2017) through leaching. This is within the range recommended by Bertoncelli et al. (2017), who states that the content should be between 6% and 9%. Low mineral matter content indicates better preservation of the forage, since when fermentation is insufficient, there is loss of organic matter, increasing its relative content in the DM. While higher MM levels in the silage, cause decrease in energy content (Ortiz et al., 2021).

Regarding the crude protein content, there was an increasing linear effect of all melon biomass mixtures according to the addition of ground corn, showing values between 60.1 and 66.8 g kg^{-1} DM, with the highest CP content observed in silages with the 100 and 1000 q kg⁻¹ fruit biomass. In general, an increase in crude protein (CP) content in the silage was observed in comparison to the original ensiled material, indicating that CP losses were minimal, which is surprising for materials with high moisture content. The presence of soluble carbohydrates (Table 1) and the addition of ground corn probably provided favorable fermentative conditions, which is a reflection of lower losses due to the higher dry matter content (Lira et al., 2018). According to Bueno et al. (2020) proteolysis tends to be higher in silages with low dry matter content.

The silage without fruit showed quadratic effect for NDF, with a maximum point between 50 and 100 g kg-1 of ground corn. In the silage with 100 g $kg⁻¹$ melon, the effect was linearly decreasing with a minimum point with 200 g kg⁻¹ ground corn. In silage with 1000 g kq^{-1} melon, the effect was quadratic with a minimum point between 50 and 200 g kg-1 ground corn. This is the result of the lower proportion of this fraction among the forages that make up the silages (Table 1), giving an effect of diluting NDF in the silages according to the level of ground corn. NDF is an important source of nutrients for ruminants, as it stimulates rumination. However, Santin et al. (2020), mention that values above 600.0 g kg-1 DM of NDF reduce intake due to rumen fill because of its slow degradation and reduced passage rate.

In silages without and with 100 g kg⁻¹ melon, a linearly decreasing effect was observed for ADF with a minimum point of 200 g kg-1 ground corn, while in silages with 1000 g kg⁻¹ melon, the effect was quadratic with a minimum point between 50 and 100 g kg⁻¹ of ground corn. According to Neumann et al. (2017), ADF levels should not be high because it has a direct influence on the feed digestibility, as it is made up of less digestible fractions and is detrimental to the quality of the silage. According to Van Soest (1994), ADF values should be less than 400 g kg-1 DM. In our study, both NDF and ADF content reduced, indicating that the ground corn could lead to improved digestibility for the animals when they consumed melon biomass silage.

The silage without fruit showed a quadratic effect for EE, with a maximum point close to 200 g kg^{-1} of ground corn. Silages with 100 g $kg⁻¹$ melon showed a quadratic

effect with minimum point between 100 and 200 g kg-1 of ground corn, whereas silages with 1000 g kg⁻¹ melon showed a quadratic effect with minimum point between 50 and 100 g kg⁻¹ of ground corn. The EE values may have been influenced by its fat-soluble characteristic, since when evaluating different levels of ground corn, the effect of effluent losses was observed, favoring the entrainment of water-soluble compounds more easily than fat-soluble ones, explaining such dynamics, in addition to a change in the organic matter from this study. Van Soest (1994) recommended that the maximum EE content in ruminant diets should be 60 g kg-1. This value is in line with the results obtained in the present study, suggesting a favorable aspect, given that lipids are an excellent source of energy (Farias et al., 2021).

On the other hand, soluble carbohydrates showed an increasing linear effect in silages with 100% fruit according to the ground corn levels, with the highest values in relation to the melon biomass mixtures. This was related to the amount

of carbohydrates in the fruit, 174.8 g kg⁻¹ DM (Table 1), and the added ground corn. The amount of soluble carbohydrates in the plant (60 and 120 g kg^{-1}) provides favorable conditions for the establishment of homofermentative bacteria (Monção et al., 2020), promoting the preservation of the ensiled material. However, as there is a large amount of soluble carbohydrates, the production of lactic acid is very intense, which predisposes to the development of yeasts that are a problem when the silo is opened, directly relating to the aerobic stability of the silage (F. N. S. Santos et al., 2020).

Microbiology

It was observed effect of interaction (P<0.01) between the different biomass mixtures and levels of ground corn on the population count of enterobacteria, molds and yeast. However, there was no effect (P>0.05) on the count of lactic acid bacteria population (Table 4).

Population count of microorganisms when opening melon biomass silages with different mixtures and addition of ground corn

NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. Means followed by different uppercase letters in the column are different at p<0.05 for FMB. Means followed by different lowercase letters in the row are different at p<0.05 for the level of GC according to the Scott- Knott test at P<0.05.

Lactic acid bacteria had no effect of interaction, which could be explained by the competition with enterobacteria for the substrate. Enterobacteria are the main competitors of lactic acid bacteria, mainly for soluble carbohydrates (R. E. Muck, 2010). However, all silages had LAB values above the minimum of 5 log CFU g, which is ideal for silages with good fermentation (K. A. Muck & Albrecht, 1991).

Enterobacteria populations were not found in the silages with mixtures of 0, 100 and 1000 g $kg⁻¹$ fruit associated with 200 g kg⁻¹ ground corn. The absence of enterobacteria in these silages are associated to the addition of corn, which improves the fermentation pattern of the ensiled material, along with the decrease in pH, which ranged from 3.95 to 4.63 in these mixtures, and were effective in inhibiting

the proliferation of these microorganisms. However, the pH reduction influences the proliferation of undesirable bacteria, such as enterobacteria, which inhibits the activity of these microorganisms when it is below 5 (S. F. Santos et al., 2013). According to A. P. M. D. Santos et al. (2018), the use of ground corn as an absorbent additive has the function of reducing the fermentation of undesirable microorganisms, and is used as a way of minimizing losses.

Regarding the mold population count, the highest values were obtained in silages with 100 g kg^{-1} fruit and 50 g kg^{-1} ground corn. This combination may have promoted greater gelatinization of the starch present in corn, which is a phenomenon that probably did not occur in silages containing 1000 g kg⁻¹ melon. During gelatinization, the protein matrix and starch granule structures are disrupted, resulting in the solubilization of starch, which can be degraded by bacterial action or solubilized by acetic acid and lactic acid. That, together with the concentration of soluble carbohydrates present in the fruits during the fermentation process, can increase the surface available for the colonization and action of fungi (Arcari et al., 2016; Ehtiati et al., 2017). In addition, molds are responsible for consuming soluble sugars and lactic acid, metabolizing cellulose and other components of the cell wall and producing mycotoxins, which can be harmful to animals (Macêdo et al., 2017). High populations of molds are undesirable for good-quality silage.

The yeast population results in silages with 1000 g kg⁻¹ fruit combined to all levels of ground corn can be explained by the fact that the fruit has a higher sugar concentration. Behling et al. (2017) reported that the presence of yeasts directly influences silage quality during fermentation by increasing the production of ethanol and CO₂, consequently leading to gas losses. Also, according to these authors, the consumption of soluble carbohydrates reduces the amount of this compound in the ensiled material, impairing the development of LAB, causing losses of dry matter and energy after opening the silo, and is also responsible for the deterioration of silage exposed to oxygen.

Organic acids

There was significant effect of interaction (P<0.05) between the different mixtures of biomass and levels of ground corn on lactic and acetic acid contents, but there was no effect (P>0.05) on propionic acid content, and there was no interaction with butyric acid. (Table 5).

The lactic acid content increased linearly with ground corn levels in silages without melon biomass; while in silages with 100 and 1000 g $kg⁻¹$ melon, the effect was quadratic with maximum points close to 200 g kg⁻¹ ground corn. The use of melon fruits favored the fermentation process in the ensiled mass and the development of lactic acid bacteria, increasing the lactic acid content in the silages. The levels of lactic acid observed in this study characterized the product (100 and 1000 g $kg⁻¹$ fruit) as good-quality silage, in accordance with the classification made by Sá et al. (2020), who classified good-quality silages those with levels above 50 g $kg⁻¹$ in the DM.

Silages without melon showed quadratic effect of ground corn addition on acetic acid content, with minimum point

between 100 and 200 g kg^{-1} of ground corn; while silages with 100 g $kg⁻¹$ melon showed quadratic effect with minimum point between 50 and 200 g kg-1 ground corn; and silages with 1000 g kg^{-1} melon biomass had no effect of ground corn addition. This result is associated with lower gas production, revealing lower activity of enterobacteria and secondary fermentations. According to Macêdo and Santos (2019) the acetic acid content may reflect the action of enterobacteria that causes high losses of dry matter during the fermentation process, as well as heterofermentative lactic acid bacteria. From the fermentation point of view, it is not recommended that the acetic fermentation predominates in the ensiled mass.

Table 5

Organic acid content (VFAs) of silages produced with different mixtures of melon biomass and addition of ground corn

continue...

continuation...

NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. Means followed by different uppercase letter in the column and lowercase in the rows are statistically different according to the Scott-Knott test at P<0.05.

Regression equations: LA: Y_{oFMB} = 1.3926 + 0.3877x; Y_{100FMB} = 2.55 + 0.7197x - 0.0155x²; Y_{1000FMB} = 11.33 - 0.1066x + 0.0324x2; AA: Y_{oFMB} = 15.86 - 1.02x + 0.0301x²; Y_{100FMB} = 12.92 - 1.039x + 0.0408x²; BA:Y_{GC} = 4.90 + 0.7413x - 0.0810x².

For butyric acid, there was an independent effect of the addition of ground corn to the silage, with the maximum point occurring between 0 and 100 g $kg⁻¹$ of ground corn. These results are related to the greater buffer capacity in relation to corn, preventing the rapid drop in the silage, and providing conditions for the development of bacteria of the genus *Clostridium,* which are responsible for the production of butyric acid to the detriment of other organic acids (Costa et al., 2022), and mainly due to the greater losses of nutrients and fermentation products observed in the form of effluent. However, with the addition of ground corn (200 g kg-1 NM) the point of maximum butyric acid concentration improved, characterizing a good-quality silage.

Aerobic stability

There was interaction (P<0.05) between the different biomass mixtures and level of ground corn on aerobic stability, pH and internal temperature of the silages (Table 6). There was no break in aerobic stability up to 96 hours in silages with 0 and 100 fruit associated with 50, 100 and 200 q kg $^{-1}$ of ground corn. These results can be explained by the absence of fermentable substrates in the silage, which inhibit or hinder the development of deteriorating aerobic microorganisms (Freitas et al., 2020).

Aerobic stability of silages produced with different mixtures of melon biomass and addition of ground corn

NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. The evaluation of aerobic stability was carried out over 5 days in an air-conditioned room at a temperature of 25 ± 1°C, with measurements being carried out at 4 four-hour intervals, over a period of 96 hours. Means followed by different uppercase letters in the column are statistically different at p<0.05 for FMB. Means followed by different lowercase letters in the row are statistically different at p<0.05 for GC to the Tukey's test at 5% probability.

However, there was break in aerobic stability in silages with 1000 g $kg⁻¹$ fruit associated to all levels of ground corn, with the silages that had the addition of ground corn (50, 100 and 200 g kg-1; 40, 24 and 36 hours, respectively) showing faster break in stability when compared to the silages without ground corn (0 g kg⁻¹; 64 hours). This is explained by the high concentration of soluble carbohydrates present in the melon fruit silages $(170.0 \text{ g kg}^{-1})$ in DM) (Table 1), and by the addition of ground corn,

which increased lactic acid and decreased acetic acid. Silages with high amounts of fermentable substrates usually present more intense aerobic deterioration, mainly with the presence of yeasts (Lima et al., 2015).

The highest pH value was observed in the silage with 0 g kg^{-1} of melon fruit without the addition of ground corn. This high value is correlated with the buffering capacity of the plant at the moment of ensiling (22.62 e.mg NaOH 100 g DM; Table 1). According to Anjos et al. (2018) the buffering power of the

plant is a limiting factor in dropping the silage pH. Some factors contribute to the higher pH values, such as: the greater production of butyric acid, which is a weaker acid (R. E. Muck, 2010), the greater buffer capacity (Fluck et al., 2017) and the lower amount of soluble carbohydrates (Brüning et al., 2017). Silages with 100 g kg⁻¹ fruit plus 100 and 200 g kg⁻¹ ground corn presented the lowest pH values, indicating that reducing the pH of silages helps preserve mass by inhibiting undesirable microorganisms responsible for silage deterioration, as bacteria consume sugars and produce lactic acid, which causes the drop in pH (Domingues et al., 2011).

The highest internal temperature values were recorded in silages without fruit and without ground corn, when there was a break in aerobic stability. Temperatures between 25 and 40°C generally favor the growth of aerobic microorganisms in the silage. According to Florentino et al. (2020) the increase in temperature in silages is determined by aerobic deterioration caused by the actions of aerobic microorganisms (enterobacteria, molds and yeasts).

There was effect of interaction (P<0.05) between the different biomass mixtures and addition of ground corn in the stability on lactic acid bacteria (LAB), enterobacteria, molds and yeasts (Table 7). The largest LAB populations were found in silages with 1000 g kq^{-1} fruit, which showed values of 5.62, 5.57, 4.92 and 5.10 log CFU g^{-1} for the addition of 0, 50, 100 and 200 g kg $^{-1}$ ground corn, respectively. This was probably due to the fruit having a higher concentration of soluble carbohydrates, which is an essential substrate for this group of bacteria to produce lactic acid even after exposure to air (Table 1). In the deterioration process, the metabolization of the lactic acid present in the silage and the residual carbohydrates results in pH raise, increase in temperature and growth of aerobic microorganisms (Woolford et al., 1982).

Population of microorganisms in silages produced with melon biomass with different mixtures added with ground corn in the aerobic stability break

NM: natural matter; SEM: standard error of the mean; FMB: fruit in melon biomass; GC: ground corn. Means followed by different uppercase letters in the column are statistically different at p<0.05 for FMB. Means followed by different lowercase letters in the row are statistically different at p<0.05 for GC, according to the Scott-Knott test at P<0.05.

Enterobacteria count showed the highest values in silages with 0 fruit and 100 g $kg⁻¹$ fruit associated with 0, 50 and 100 g kg⁻¹ ground corn, respectively. The population of enterobacteria is directly correlated with the pH of the silage (Table 4), which reduced over time of exposure to oxygen. According to Maia et al. (2021), dropping the pH can hinder the proliferation of enterobacteria, which was

observed in our study. In addition, they form acetic acid and carbon dioxide, and cause the loss of dry matter and energy.

Higher values of mold population count were observed in aerobic stability in silages with 100 g kg-1 fruit (5.18, 3.81, 3.81 and 2.58 log CFU g^{-1}) added with 0, 50, 100 and 200 g kg^{-1} ground corn, respectively (Table 7). That result was probably due to

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the lower concentration of organic acids with antifungal potential in this silage, the concentration of which was not sufficient to inhibit the growth of these microorganisms, in addition to the growth conditions being conducive to proliferation, thus reducing the aerobic stability of the silages (Arriola et al., 2021).

No yeast counts were recorded in silages with 0 and 100 g $kg⁻¹$ fruit with 0, 50 and 100 g kg-1 ground corn. The absence of yeasts after aerobic stability is possibly related to the depletion of sugars remaining after lactic fermentation in the silages produced, since carbohydrates are one of the sources of energy used by spoilage microorganisms after opening the silo, in addition to greater production of acetic acid in this silage, which suppressed the growth of these microorganisms, being an indication of high aerobic stability (Woolford, 1990).

Conclusion

Silages with 100 and 1000 g kg⁻¹ of fruits in melon biomass and 200 g kg-1 of ground corn presented superior quality indicators, characterizing these biomass mixtures as the most suitable for silage making.

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Conflicts of interest

The authors declare no conflict of interest.

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