

Evaluation of Different Levels of Addition of Souari Nut Peel (*Caryocar brasiliense*) on the Quality of the Mombasa Grass Silage

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Abstract: The objective of this study was to evaluate the effects of the addition of souari nut peel on the fermentative characteristics and chemical composition of the Mombasa grass silage. A completely randomized design with five levels of addition (0%, 5%, 10%, 15% and 20% in natural matter basis) of souari nut peel with three replicates was applied in the study. The silos were opened at 55 d after starting the ensiling process. The data were analyzed by means of linear/quadratic regression analysis, according to the best fit of the data and the probability values to 5%, using the Statistical Analysis System (SAS, 2002). There were a linear decreasing effect on the neutral detergent fiber (NDF), ash and increasing values for the effluent in the silage. Quadratic effect was observed for crude protein (CP), lignin and pH. For the other factors dry matter (DM), acid detergent fiber (ADF) and gases there was no influence on the addition of souari nut peel. The 15% addition level of souari nut peel increased CP content, decreased NDF and pH, which was recommended to improve the fermentation profile of Mombasa grass silage.

Key words: Additive, effluent production, fermentation, quality.

1. Introduction

The souari nut (*Caryocar brasiliensis*) is a fruit of the cerrado biome of Brazil, which is high in nutrients, widely used in pharmacological products, in human food and its by-products are valued in animal feeds. In the industries of products derived from souari nut, such as pulp preserves, souari nut oil and souari nut candy, the peel is considered waste, it is used crushed in animal feed [1]. The peel has significant amounts of total dietary fiber, carbohydrates, magnesium, calcium, manganese and copper [2]. The peel has 37% of total carbohydrates and 5% of crude protein (CP) in its composition, it is possible to use it in the diet of ruminants [3].The utilization of souari nut peel in animal feed consists of economic, social and ecological activity of great potential for the rural properties. It allows nutritional enhancement in silage by the addition of essential micronutrients in animal metabolism, such as boron, copper, manganese, zinc and iron [4], and allows the mitigation of organic waste released into the environment, which would lead to contamination of the soil with effluents. In addition, finding alternative feeds for substitution or reduction in grain supply is a necessity for reducing costs with feedstuffs. However, there is a lack of information on the use of souari nut peel in silage. The low CP content could limit the feed utilization of ruminants, fundamentally the evaluation of the effects on forage fiber degradation [5]. Mombasa grass is a tropical grass with a high yield of 33 ton/ha of dry matter (DM), producing 11% of the annual value in the dry season [6, 7]. Mombasa grass is used in rotational grazing, as it improves animal production. However, it can be preserved for later supply to

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ruminants. The ensiling process without additives may have unsatisfactory results due to high humidity and the low soluble carbohydrate contents that can actively interfere in the fermentative processes, it prevents the pH decrease and, thus, develops secondary and undesirable fermentations for the conservation of the forage [8].

Due to the low pH and moisture content, 3.97 and 8.43%, present in the souari nut peel, the fermentation profile would be favorable, due to the rapid stabilization of the anaerobic environment, recommended to maintain good silage conservation [4]. The objective of this study was to evaluate the effects of the addition of souari nut peel on the fermentative characteristics and chemical composition of the Mombasa grass silage.

2. Materials and Methods

2.1 Experimental Site

The experiment was carried out in the cattle sector of the Federal Goiano Institute Campus Morrinhos, located on the highway BR153, KM633 State of Goiás, Brazil. According to Köppen, the climate is type Aw, hot and humid, with an average annual rainfall of 1,500 mm, presenting a rainy season in summer and being dry in winter.

Souari nut peel (*C. brasiliense*) was chopped, left with about 2 cm particle and transferred to pre-drying in the sun for 2 d.

Mombasa grass was harvested at 120 cm height and 60 d after sowing. At the moment, the grass had the highest amount of DM, with the highest amount of soluble carbohydrates, considered ideal characteristics for suitable fermentation [9]. For the cutting, a forage harvester was used at 50 cm of ground height, in order to obtain a greater amount of leaves and a smaller amount of stem, thus avoiding a very fibrous bulk. The grass was subjected to the same cut process, resulting in particles of approximately 2 cm.

The total DM losses were determined by the difference between DM amount of forage silage at the silo closing and the amount of DM in the forage recovered, discounting the loss by effluent and gases. Samples were also collected for the determination of pH and DM. The pH and DM were determined according to AOAC [10].

Table 1 shows the average chemical composition of Mombasa grass and souari nut peel to percentage of DM, ash, lignin, CP, neutral detergent fiber (NDF) and acid detergent fiber (ADF).

The materials were ensiled in plastic buckets (silos) with a capacity of 15 L, equipped with a valve type Bünsen adapted in its lid, to allow measuring the loss of gases through fermentation. To determine the production of effluents, 4 kg of dry sand were placed on the ground of each silo, separated from the residue by a cloth. The compaction was by trampling, averaging 13.82 kg of natural matter within each silo. After the silos were filled, they were sealed with adhesive tape, weighed and stored in a covered area at room temperature for 50 d. The loss of DM in the silage through gases and effluents was quantified by gravimetry [11]. The following equation was used to determine the effluent loss:

Item	Souari nut peel	Mombasa grass
DM (%)	23.74	16.08
NDF ¹	68.84	65.00
ADF ¹	48.7	50.91
CP ¹	7.29	12.15
Lignin ¹	28.35	36.00
Ash ¹	3.46	13.09

 Table 1
 Chemical composition of Mombasa grass and souari nut peel, used in silage.

DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein.¹ % DM.

 $E = [(BW_{\text{final}} - Tb) - (BW_{\text{initial}} - Tb)]/MF \times 1,000 \quad (1)$

where, *E*: effluent production (kg/ton of the DM); BW_{final} : empty bucket weight + sand weight at opening (kg); *Tb*: tare of the bucket (kg); BW_{initial} : empty bucket weight + sand weight at closing (kg); *MF*: matter of forage at closing (kg).

The losses in the form of gases were determined by the difference between the amount of DM of the forage at the silo closing, and the amount in the bucket at the time of the opening. Eq. (2) is as follows:

 $G = (BW_{initial} - BW_{final})/(FDM_{initial} \times FDM\%) \times 10,000$ (2) where, *G*: gas losses (%DM); $BW_{initial}$: full bucket weight at closing (kg); BW_{final} : weight of the bucket filled in the opening (kg); $FDM_{initial}$: DM forage in the closing (kg); FDM%: DM content of forage at closing (%).

The samples were also checked for NDF assayed with heat stable amylase and expressed exclusive of residual ash (aNDFom-NDF), ADF expressed exclusive of residual ash (ADFom-ADF) [12], acid method of fiber analysis and lignin processed by the sulfuric acid method [13], after sequential extractions with neutral detergent followed by acid detergent [12]. In the aNDFom-NDF analysis, a thermostable α -amylase was used without sodium sulfite, using an Ankom® fiber extractor [14].

The experimental treatments were divided as follows: T1—Mombasa grass silage, T2—95% Mombasa grass silage + 5% souari nut peel, T3—90% Mombasa grass silage + 10% souari nut peel, T4—85% Mombasa grass silage + 15% souari nut peel, T5—80% Mombasa grass silage + 20% souari nut peel. The completely randomized design with three replicates was used.

2.2 Statistical Procedures and Model Evaluation

The data submitted to analysis of variance of the regression were obtained using the procedure PROC REG of the statistical program SAS® 9.2 [15]. The significance level of 5% was adopted, where the responses of the variables in relation to the different

levels of substitution were evaluated in linear, quadratic and cubic regression.

3. Results and Discussion

The bromatological compositions of Mombasa grass and souari nut peel, used in silage are shown in Table 1. The DM content was not influenced (p > 0.05) by souari nut peel levels (p > 0.05), with a mean value between treatments of 16.08%. In spite of the high DM content in souari nut peel (23.74%), it was not sufficient to improve this silage variable, and McDonald *et al.* [9] recommended around 25% of DM. Probably due to the high moisture content of the grass at the time of silage, losses in effluent form were considerable and the solids were removed present in the silage [16].

The inclusion of souari nut peel negatively influenced the NDF values (p < 0.05), as the silages with 15% of souari nut peel resulted in 60.76% of NDF, lower value among the other treatments. In spite of the high NDF content in souari nut peel (68.85% DM), the NDF content in the Mombasa grass (65% DM), together with the high humidity, contributed to the reduction of the total NDF content, mainly due to the fermentation process. The high moisture content favors the presence of undesirable organic acids (butyric and acetic), which occur in the silage due to the presence of fungi, also responsible for the dark color in the first 5 cm of the silo surface, especially in the treatments with a higher content of the souari nut peel [9]. This fact is caused by the acid hydrolysis of the hemicellulose, thus decreasing the NDF content. Pereira et al. [17] also recorded reduction of the NDF fraction after the fermentation period in elephant grass silages. The NDF corresponds to the percentage of the neutral detergent insoluble fiber, mainly composed of cellulose, hemicellulose and lignin. In Fig. 1, the regression equation and coefficient of determination (r^2) of NDF to Mombasa grass silage with different levels of souari nut peel addition are present. A diet with high levels of NDF in ruminants will affect the

intake of DM because the fibers cause a ruminal filling sensation and, consequently, a reduction in animal performance, since the animal feels satiated before supplying the energy demand [16]. The ADF was not influenced by the levels of souari nut peel (p > 0.05), with a mean value of 50.64%. The absence of alteration in the value of ADF in silage can be justified by the similarity with the ADF value of the Mombasa grass and souari nut peel, which was 53.03% and 48.70%, respectively. Similar results were obtained by Bernardino *et al.* [18] with elephant grass silage and different levels of coffee husk, who obtained an average of 53.6% of the ADF. These values are considered high and decrease the nutritive value of the silage. The ADF fraction of feeds

includes cellulose and lignin as primary components, with varying amounts of ash and nitrogen compounds.

The souari nut peel has high content of DM, low fiber content, lignin and ash, which are considered characteristics for proper fermentation, improving the quality of the silage with its addition. Quadratic effect (p < 0.05) was observed for CP. The highest estimated value of CP was 10.76% of DM, at the 17.09% level of souari nut peel addition (Fig. 2). The souari nut peel has a protein content of 7.29% DM, increasing the protein percentage in the silage only with the Mombasa grass (8.23% DM). However, a limit was observed for the addition of souari nut peel, caused by high moisture content with the treatment with 20% souari nut peel.



Fig. 1 Regression equation and coefficient of determination (r^2) of neutral detergent fiber (NDF) of Mombasa grass silage with different levels of souari nut peel.



Level of the addition of souari nut peel (%)

Fig. 2 Regression equation and coefficient of determination (r^2) of crude protein (CP) of Mombasa grass silage with different levels of souari nut peel.

Proteins are of fundamental importance in metabolism to meet the requirements of growth, gestation and production in animals [19]. According to Van Soest [12], the minimum requirement for ruminal microorganisms is 7% CP and, therefore, the exclusive use of souari nut peel with levels of 17.09% exceeds the minimum requirement for cattle. However, it is not possible to provide an exclusive diet with only souari nut peel. It is not possible to make a balanced diet for ruminants without added souari nut peel to silage.

Lignin presented quadratic effect (p < 0.05), the estimated value with the lowest percentage of lignin, was 37.24% DM at the 12.75% level of souari nut peel addition (Fig. 3). This result is probably attributed to the low lignin content in souari nut peel (28.36%) compared to the grass (40.41%). The cell wall of forages is made almost entirely by lignin and cellulose, and is less digestive by the microorganisms of the rumen.

Lignin in ruminant nutrition has a negative influence on the digestibility of other nutrients, because it is indigestible and for the formation of a physical barrier that prevents the access of microorganisms that digest nutrients in the ruminal environment [13, 20, 21]. There was a linear decreasing effect of inclusion of souari nut peel (p < 0.05) for ash (Fig. 4). This fact was due to the low ash content of the souari nut peel (3.46% DM), which resulted in a decrease in its amount in the silage. Modification in the silage fermentation process occurs, with the addition of *Lactobacillus plantarum*, which decreased the ash content in Mombasa grass silage [22].

For the effluent parameter (kg/ton) there was a linear and positive effect of souari nut peel levels (p <0.05), as observed in Fig. 5. This response was obtained by non-pre-weeding of the forage, recommended by some authors, with the purpose of reducing the moisture content and avoiding the high effluent production in the silage. These values are considered high and harmful to the good fermentation of the silage, because it increases the amount of undesirable microorganisms, such as heterofermentative bacteria, of the genus Clostridium, reducing the quality of the silage [23]. Effluent production is influenced by the DM content of the ensiled crop, silo type, degree of compaction and the physical processing of the forage, such as pre-wilting. Silva et al. [24] observed that pre-wilting with Tifton grass decreased compaction, avoiding the 85 fermentation and production of lactic acids. Pre-wilting is a technique that could have been used in this experiment to reduce the moisture content of the grass. The high moisture content and consequently of effluent leads to undesirable fermentations and higher losses with gases, which was also observed in this study.



Fig. 3 Regression equation and coefficient of determination (r^2) of lignin of Mombasa grass silage with different levels of souari nut peel.

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Level of the addition of souari nut peel (%)

Fig. 4 Regression equation and coefficient of determination (r^2) of ash of Mombasa grass silage with different levels of souari nut peel.



Level of the addition of souari nut peel (%)

Fig. 5 Regression equation and coefficient of determination (r^2) of effluent of Mombasa grass silage with different levels of souari nut peel.

Silage gas losses were not influenced by the levels of souari nut peel addition (p > 0.05), with a value of 2.77%. The gases produced are the result of the high moisture content in the grass, which favors the development of the heterofermentative bacteria of the genus *Clostridium* sp. increasing the butyric fermentation. This value was close to the study of Zanine *et al.* [25], with high effluent production in the same treatment with lower gas loss, using wheat bran in Mombasa grass silage. In an experiment with addition of different additives in elephant grass silage Andrade *et al.* [26] observed an average of 4% of gas losses.

According to Morais [27], silage quality can be affected by extrinsic factors, such as climatic conditions and epiphytic microbiota, and may be controlled in part by man. The intrinsic factors, according to Van Soest [12], as soluble carbohydrates, buffering power, nitrates and other nitrogenous substances, consequently, are not controlled by human action. In this way, the discrepant values for losses with gases can have a high coefficient of variation by the intrinsic factors.

DM recovery was not influenced by souari nut peel levels (p > 0.05), with an average of 88.03%. This is probably due to the values of gas and effluent losses,



Fig. 6 Regression equation and coefficient of determination (r^2) of pH of Mombasa grass silage with different levels of souari nut peel.

which can be observed in Fig. 3. However, silages that had higher losses due to effluent had a lower gas production, thus equalizing a balance of losses and adjustment in DM recovery. Zanine *et al.* [25] added different levels of wheat bran in elephant grass silage and had 86.76% silage DM recovery value, which was higher than reported in this study. Thus, the type of additive may influence the final recovery of DM. Gas loss during the fermentation process is directly related to silage DM recovery [28].

The pH variable was influenced in a quadratic way by souari nut peel levels (p < 0.05). The lowest estimated value for pH was 4.7, for the 14.56% level of souari nut peel addition (Fig. 6).

The values obtained for pH are due to the high moisture content of the Mombasa grass, which produced high effluent and low DM content (16.99%), as recommended by McCullogh [29], in the range of 28%-34% and avoided rapid pH reduction. Faria *et al.* [30] evaluated the inclusion of citrus pulp in elephant grass, with pH values of 4.07-4.46, similar to those of the present study. The ideal pH for well-preserved silage is between 3.8 and 4.2 [31]. It is wrong to consider only the pH value of the silage as a quality parameter, although it is possible to have good quality

silages with pH values slightly above this range [32].

4. Conclusions

The inclusion of souari nut peel (*C. brasiliense*) with 15% added in the silage of Mombasa grass (*Panicum maximum*), which increased the crude protein content and decreased the neutral detergent fiber and pH contents, is the recommended level to improve the silage fermentation profile.

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