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SCIENTIFIC NOTE

Effect of refused melon fruit inclusion on sorghum ensilage

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ABSTRACT

Ensilage of refused fruit with forage is a viable approach to increase resource use in ruminant feed. The use of refused melon (*Cucumis melo* L. subsp. *melo* var. *inodorus* H. Jacq.) fruit (RMF), ensiled at 0%, 2%, 4%, 6%, or 8% of natural matter with sorghum (*Sorghum bicolor* (L.) Moench), was assessed in terms of chemical-bromatological composition and fermentative profile. Experimental silos filled with mixtures and hermetically sealed were used. After 34 d of fermentation, the silos were opened, and samples were analyzed. Dry matter concentration shows a quadratic increase ($P < 0.05$) with RMF inclusion in sorghum silage rising from 30.83% to 32.08% at 4% of RMF inclusion, followed by a reduction in the subsequent levels. There was a linear increase ($P < 0.05$) in ether extract, total digestible nutrients, digestible energy, metabolizable energy, and in vitro digestibility of DM and organic matter, increasing respectively 3.96%, 5.32%, 0.25 Mcal kg^{-1}, 0.19 Mcal kg^{-1}, 1.89% and 0.74% from 0% to 8% of RMF inclusion. There was a linear reduction ($P < 0.05$) of neutral detergent fiber, cellulose, hemicellulose, and lignin with RMF inclusion. We also observed a linear increase ($P < 0.05$) in ammoniacal N, acetic acid, propionic acid, butyric acid, and lactic acid concentrations in silage with the addition of RMF. The inclusion of RMF improved the chemical-bromatological composition and fermentative profile of sorghum silage, and an inclusion level of 8% is recommended.

Key words: *Cucumis melo* subsp. *melo* var. *inodorus*, dry matter loss, fermentative profile, mixed silage, sorghum, *Sorghum bicolor*.

INTRODUCTION

The characteristic seasonality of tropical semiarid regions remains a challenge for ruminant production systems. Forage conservation via ensilage is a potential strategy to overcome feed shortages in the dry season. However, it is important to develop alternatives to enrich silages and take advantage of available resources for use in forage conservation.

Forage sorghum is one of the main grasses used in ensilage due to its high DM production and good adaptation to hot and dry climates (Moura et al., 2016; Martini et al., 2019). However, at harvest, DM concentration is usually exceeded, and high DM and low soluble carbohydrate levels can result in poor quality silage (Kung Jr. et al., 2018). In this context, additives that increase the content of soluble carbohydrates, without increasing DM of the ensiled mass, may be important alternatives.

In Northeast Brazil, more than 550 000 t of melons were produced in recent years (Kist et al., 2021). Considering that about 10% of the fruits produced are discarded by the industry (Costa et al., 2011), it is estimated that 55 000 t were refused melon fruit (RMF). The composition of this by-product has been described as follows: 8.16 ± 1.73% DM, containing 12.96 ± 2.12% crude protein, 48.71 ± 4.98% non-fibrous carbohydrates, 31.35% pectin (Lima et al., 2011; de Lima et al., 2012; Valadares Filho et al., 2018). Even with an unwanted size or shape for the industry, RMF still has high levels of nutrients such as soluble solids in the juice and pulp (Gómez-García et al., 2020), proteins, minerals, fibers, and oils in the peel and seeds (Silva et al., 2020).

There are few studies on RMF use as an additive for ensilage. However, the inclusion of other fruits has shown increases in total digestible nutrients (Ülger et al., 2020), fermentation profile improvement (Silva et al., 2011; Morales-Querol et al., 2019), and increases in the richness and diversity of microorganisms (Forwood et al., 2019) in forage silages. Thus, the objective of this research was to evaluate the effects of increasing RMF inclusion levels on chemical-bromatological composition, fermentative profile, in vitro digestibility, and energy content of sorghum silage.

MATERIAL AND METHODS

A completely randomized design with five treatments and four replicates was used, totaling 20 experimental units. A control treatment was considered with silage exclusively made from forage sorghum (0% inclusion) and four levels of refused melon fruit (RMF) inclusion (2%, 4%, 6%, and 8%) based on the natural matter of feeds. Sorghum (*Sorghum bicolor* (L.) Moench) 'BR 700' at the grain maturation stage of milky/pasty, aged approximately 100 d, cultivated in Governador Dix-Sept Rosado, Rio Grande do Norte (RN), Brazil, were used. The refused melon (*Cucumis melo* L. subsp. *melo* var. *inodorus* H. Jacq.) fruits were obtained from traders of the Brazilian Food Company in Mossoró, Rio Grande do Norte, Brazil. A compilation of chemical-bromatological composition of the RMF is available in Table 1.

All feeds were ground into 1.0 to 2.0 cm thick particles and mixed in proportions described in the experimental design. Subsequently, the mixtures were ensiled aiming to reach a density of 500 kg m^{-3} for 34 d in PVC (polyvinyl chloride) silos measuring 50 cm in length and 10 cm in diameter, with a capacity of approximately 4 kg. The silos had a Bunsen valve for eliminating gases and a layer of towel paper at the bottom for absorbing liquids. After opening the silos, homogenized samples of the material were collected and subdivided for chemical-bromatological, digestibility, and fermentation profile analyses.

Concentrations of DM, crude protein (CP), ethereal extract (EE), and mineral matter (MM) were determined following the respective methods (934.01, 976.05, 963.15, and 942.05) of the AOAC (2016). Concentrations of ash and protein-free neutral detergent fiber (NDFap), acid detergent fiber (ADF), cellulose (CEL), hemicellulose (HEM), and lignin (LIG) were analyzed according to Van Soest et al. (1991). Soluble carbohydrates (SCHO) were determined according to the methodology of Bailey (1967).

Subsamples were submitted to in vitro digestibility tests of DM (IVDDM) and organic matter (IVDMO) according to Tilley and Terry (1963) with the aid of a Daisy^{II} incubator (ANKOM Technology, Macedon, New York, USA), following adaptations proposed by Holden (1999). Ruminal inoculum used was collected through a cannula for an adult bovine female with a body weight of 650.0 kg, fed daily with forage sorghum silage and concentrated ration. Procedures involving animals in this work were approved by Ethics Committee in Use of Animals of Universidade Federal Rural do Semi-Árido (UFERSA) with protocol nr 23091.010626/2019-23.

Non-fibrous carbohydrates (NFC) were estimated according to Mertens (1997). Total digestible nutrients (TDN), digestible energy (DE), and metabolizable energy (ME) were estimated using the models and submodels proposed by Detmann et al. (2016). Based on the difference between the initial and final weight of

Table 1. Chemical-bromatological composition of refused melon fruits. SEM: Standard error of the mean; n: number of observations.[1]Composition was obtained through a compilation of literature data (Lima et al., 2011; de Lima et al., 2012; Valadares Filho et al., 2018) and unpublished data from the present research group.

Component	Mean	SEM	n
Dry matter (DM), %	7.35	0.42	8.00
Mineral matter, %DM	11.62	0.52	8.00
Crude protein, %DM	14.33	0.60	8.00
Ether extract, %DM	6.18	1.34	8.00
Neutral detergent fiber, %DM	24.02	1.54	4.00
Acid detergent fiber, %DM	20.31	1.82	3.00
Lignin, %DM	4.22	1.64	2.00
Total carbohydrates, %DM	67.72	2.00	8.00
Non-fibrous carbohydrates, %DM	47.84	2.25	4.00

closed silos and towel papers, we quantified the losses by gases (GL) and by effluents (EL), respectively, according to Jobim et al. (2007). The DM recovery (DMR) was calculated following the methodology proposed by Jobim et al. (2007).

Fresh subsamples of silages were submitted to manual pressing for liquid extraction. In the liquid obtained, pH and ammoniacal N in proportion to total N ($N\text{-}NH_3$) were analyzed as recommended by Mizubuti et al. (2009). Concentrations of acetic acid (AA), propionic acid (PA), butyric acid (BA), and lactic acid (LA) in the silage were determined by gas chromatography, following the methodology proposed by Erwin et al. (1961).

Data were submitted to ANOVA using the SAS PROC GLM procedure. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects. The model only included level of RMF effect: $Y_{ij} = \mu + F_j + \varepsilon_{ij}$, where Y_{ij} is dependent variable observed in replicate i^{th} ($n = 4$) and treatment j^{th} ($n = 5$); μ is population average or overall constant; F_j is effect of treatment j^{th} (level of RMF inclusion); and ε_{ij} is unobserved random error. Linear and quadratic effects were considered significant at P values < 0.05 and respective regression models were presented. Tukey test at 5% probability was used to compare the means of variables with significant effects.

RESULTS

The mean concentrations of DM, CP, and NFC showed a quadratic increase ($P < 0.05$) as a function of the increase in RMF inclusion in sorghum silage. Silage DM content obtained the highest concentration (32.08%) with 4% of RMF inclusion and showed a reduction with subsequent levels. Silage CP content had a significant increase from 4% inclusion level and reached the highest concentration (8.92% DM) with 8% RMF inclusion. The maximum concentration of NFC (35.49% MS) was obtained with 4.75% inclusion of RMF (Table 2).

Table 2. Effects of refused melon fruit inclusion levels on DM, mineral matter (MM), crude protein (CP), ethereal extract (EE), neutral detergent fiber ash and protein-free (NDFap), acid detergent fiber (ADF), cellulose (CEL), hemicellulose (HEM), lignin (LIG), soluble carbohydrates (SCHO), non-fibrous carbohydrates (NFC), in vitro digestibility of DM (IVDDM), in vitro digestibility of organic matter (IVDOM), total digestible nutrients (TDN), digestible energy (DE), and metabolizable energy (ME) of sorghum silage. SEM: Standard error of the mean; L: linear effect; Q: quadratic effect; [abc]Means with different letters on the same line differ by Tukey's test at 5% probability; [1]$Y = 30.86 + 0.46x - 0.08x^2$ ($r^2 = 0.72$); [2]$Y = 7.80 + 0.06x$ ($r^2 = 0.70$); [3]$Y = 6.95 - 0.07x + 0.04x^2$ ($r^2 = 0.91$); [4]$Y = 2.90 + 0.77x$ ($r^2 = 0.93$); [5]$Y = 51.10 - 2.54x$ ($r^2 = 0.80$); [6]$Y = 42.25 - 7.03x + 0.59x^2$ ($r^2 = 0.87$); [7]$Y = 32.97 - 1.06x$ ($r^2 = 0.73$); [8]$Y = 14.73 - 0.18x$ ($r^2 = 0.91$); [9]$Y = 6.56 - 0.10x$ ($r^2 = 0.79$); [10]$Y = 31.41 + 1.72x - 0.18x^2$ ($r^2 = 0.89$); [11]$Y = 53.33 + 0.36x$ ($r^2 = 0.59$); [12]$Y = 56.10 + 0.08x$ ($r^2 = 0.88$); [13]$Y = 66.09 + 1.09x$ ($r^2 = 0.90$); [14]$Y = 2.83 + 0.04x$ ($r^2 = 0.92$); [15]$Y = 2.37 + 0.04x$ ($r^2 = 0.92$).

	Refused melon fruit levels (%)						P-value	
Variables	0	2	4	6	8	SEM	L	Q
DM, %[1]	30.83^{abc}	31.33^{ab}	32.08^{a}	30.20^{bc}	29.86^{c}	0.22	0.0444	0.0078
MM, %DM[2]	7.78^{c}	7.95^{bc}	7.98^{ab}	8.08^{ab}	8.14^{a}	0.03	0.0160	0.3426
CP, %DM[3]	6.85^{c}	7.11^{c}	7.43^{b}	7.48^{b}	8.92^{a}	0.17	0.3340	0.0005
EE, %DM[4]	2.85^{d}	4.39^{c}	5.40^{b}	6.11^{ab}	6.81^{a}	0.32	< 0.0001	0.0042
NDFap, %DM[5]	51.65^{a}	45.53^{b}	44.34^{c}	43.33^{d}	42.37^{e}	0.75	< 0.0001	< 0.0001
ADF, %DM[6]	44.46^{a}	25.58^{b}	25.31^{b}	24.13^{b}	22.28^{b}	1.90	< 0.0001	0.0001
CEL, %DM[7]	33.32^{a}	30.42^{b}	30.16^{c}	30.04^{c}	29.33^{d}	0.32	< 0.0001	0.0002
HEM, %DM[8]	14.77^{a}	14.29^{b}	14.09^{cb}	13.90^{c}	13.56^{d}	0.10	0.0001	0.2536
LIG, %DM[9]	6.60^{a}	6.26^{b}	6.23^{b}	6.05^{bc}	5.83^{c}	0.06	0.0183	0.7142
SCHO, %DM	14.75	14.91	14.69	15.27	15.42	0.08	0.7329	0.1284
NFC, %DM[10]	31.05^{c}	35.02^{a}	34.86^{a}	35.01^{a}	33.77^{b}	0.36	< 0.0001	< 0.0001
IVDDM, %[11]	53.08^{c}	54.55^{ab}	54.16^{b}	54.43^{ab}	54.97^{a}	0.16	0.0105	0.1655
IVDOM, %[12]	56.11^{d}	56.27^{cd}	56.46^{bc}	56.64^{ab}	56.85^{a}	0.06	0.0113	0.6723
TDN, %DM[13]	65.96^{d}	68.34^{c}	69.44^{b}	70.43^{ab}	71.28^{a}	0.44	< 0.0001	0.0033
DE, Mcal kg^{-1} DM[14]	2.82^{d}	2.92^{c}	2.97^{b}	3.01^{b}	3.07^{a}	0.02	< 0.0001	0.0154
ME, Mcal kg^{-1} DM[15]	2.36^{d}	2.46^{c}	2.51^{b}	2.55^{b}	2.60^{a}	0.02	< 0.0001	0.0154

The increase in RMF inclusion resulted in a linear increase ($P < 0.05$) in MM, EE, TND, IVDDM, IVDOM, DE, and ME of sorghum silage. From the control to the maximum level (8%) of RMF inclusion, the EE concentration increased from 2.85% to 6.81% of DM. There was a decreasing linear effect ($P < 0.05$) of RMF inclusion on NDFap concentration and its constituents CEL, HEM, and LIG (Table 2). Means of ADF concentrations showed a quadratic decrease ($P < 0.05$) in relation to RMF increment in silage, reducing at 44.46% to 25.58% from the control to 2% RMF inclusion and remaining constant at other levels.

There was no effect ($P > 0.05$) of RMF inclusion on pH, GL, EL, and DMR in sorghum silage (Table 3). However, the contents of N-NH$_3$, AA, AB, and AL increased linearly ($P < 0.05$) with respective increasing 1.91%, 1.31%, 0.40%, and 0.38% from level 0% to 8% of RMF inclusion in silage. The AP content of silage linearly reduced from 3.03% at level 0% to 2.91% at level 8%.

Table 3. Effects of refused melon fruit inclusion levels on hydrogen potential (pH), ammoniacal nitrogen (N-NH$_3$), acetic acid (AA), propionic acid (PA), butyric acid (BA), lactic acid (LA), effluent losses (EL), gases losses (GL), and DM recovery (DMR) of sorghum silage. SEM: Standard error of the mean; L: linear effect; Q: quadratic effect; ^{abc}Means with different letters on the same line differ by Tukey's test at 5% probability; $^1Y = 5.79 + 0.39x$ ($r^2 = 0.88$); $^2Y = 3.65 + 0.14x$ ($r^2 = 0.95$); $^3Y = 2.95 - 0.14x + 0.02x^2$ ($r^2 = 0.67$); $^4Y = 0.18 + 0.11x$ ($r^2 = 0.41$); $^5Y = 4.93 + 0.06x$ ($r^2 = 0.65$).

	Refused melon fruit levels (%)						P-value	
Variables	0	2	4	6	8	SEM	L	Q
pH	3.84	3.91	3.96	4.01	4.11	0.02	0.1256	0.6095
N-NH$_3$, %total N^1	5.68^d	6.72^c	6.93^c	7.23^b	7.59^a	0.15	< 0.0001	0.0023
AA, %DM^2	3.65^d	3.99^c	4.18^c	4.71^b	4.96^a	0.11	0.0005	0.4834
PA, %DM^3	3.03^a	2.61^c	2.69^{bc}	2.86^{abc}	2.91^{ab}	0.04	0.0052	0.0028
BA, %DM^4	0.18^b	0.40^{ab}	0.48^{ab}	0.61^a	0.58^{ab}	0.05	0.0337	0.1992
LA, %DM^5	4.91^c	5.08^{bc}	5.14^{ab}	5.16^{ab}	5.29^a	0.03	0.0357	0.4980
GL, %DM	6.01	4.51	4.62	4.65	3.38	0.32	0.4810	0.9783
EL, kg t^{-1} DM	1.20	0.77	0.71	1.50	1.59	0.16	0.3027	0.1425
DMR, %MS	92.82	94.47	94.25	93.88	94.85	0.34	0.4474	0.6909

DISCUSSION

The response observed for DM content of sorghum silage with RMF is related to fruit physical characteristics. Due to the high moisture levels of fruits, a gradual reduction in DM was expected with increasing inclusion levels. However, the softness of the fruits resulted in greater densification of the mixture and a greater filling of the experimental silos at lower levels of RMF inclusion. According to Tomich et al. (2004), materials with higher moisture content are easier to compress. This may explain the increase in DM at 2% and 4% inclusion levels, followed by reduction at subsequent levels. The approximate residual density was 154.15, 156.65, 160.40, 151.00, and 149.30 kg DM m^{-3} in silos with 0%, 2%, 4%, 6%, and 8% of RMF inclusion, respectively.

The sorghum plant normally has a high concentration of DM due to a high stem proportion, especially in more advanced maturation stages (Veriato et al., 2018). This did not happen in the present study, since sorghum-only silage presented DM content within the indicated range (30% to 35%). However, the results indicate that the inclusion of RMF may be an important alternative to correct sorghum DM and improve silage density.

Even with a reduction in DM and a consequent increase in moisture, EL and DMR were not influenced by RMF addition. Furthermore, considering that 28% to 35% of DM has been recommended for adequate compaction and fermentation of silages (Kung Jr. et al., 2018), all levels resulted in an appropriate DM of the silage. However, sorghum silage with 4% RMF provided a maximum DM concentration.

In general, the increase in MM, CP, and EE of the silage obtained with increasing the RMF proportion can be considered positive. As fruits with skin, pulp, and seeds were used, seeds may have largely contributed to increased lipid and protein contents of the silage. Lipid concentrations between 25% and 31% and protein concentrations between 20% and 27% of DM have been reported for melon seeds (da Cunha et al., 2020; Rolim et al., 2020). Melon has about 7.48%, and 3.33% more CP and EE respectively in DM compared to sorghum silage (0% RMF inclusion), considering the whole fruit composition with 14.33% CP, and 6.18% EE (Table 1).

From a nutritional viewpoint, the concentration of N compounds is a limiting factor for the adequate activity of rumen microorganisms, regarding the potentially digestible fibrous energy substrate use (Cabral et al., 2014). In addition, CP concentrations below 70 to 80 g kg^{-1} DM in forage can result in lower microbial growth by ruminants (Figueiras et al., 2016). Thus, increasing the CP level of sorghum silage with RMF inclusion, in addition to allowing a reduction in protein supplements in the ration, should benefit digestibility and intake of silage by ruminants. Part of these responses can be reinforced by the results of increase in IVDDM and IVDOM observed.

Lipids are an excellent source of energy for ruminants; however, according to dose, this nutrient can interfere with the degradability, and rumen fermentation (Macheboeuf et al., 2008). However, these effects vary depending on several factors, and still present inconsistent results and poorly understood mechanisms of action (Cobellis et al., 2016). For feedlot animals, dietary levels between 8% and 10% of total fatty acids have been used to maintain the nutrient balance (Palmquist and Mattos, 2006). Considering these factors, the increase of 3.96% of EE in DM of silage with maximum RMF inclusion indicates an increase in energy density within an adequate range for use in ruminant diets. In view of this result, further studies are recommended to evaluate RMF not only as rich source of non-starch carbohydrates but also as a considerable source of lipids for ruminants.

The linear increase in TDN, DE, and ME with RMF inclusion indicates an increase in the caloric value of silages. These results are related to responses obtained in the chemical composition of silages, mainly due to EE, CP, and NFC increases. The NFC content is also related to increased caloric value of silages. This fraction includes high-energy polysaccharides of the feed, such as starch, pectin, and sugars. Melon has high concentrations of sucrose and pectin (Burger et al., 2006; Gómez-García et al., 2020).

The reduction of NDF, ADF, CEL, HEM, and LIG with RMF inclusion in sorghum silage is related to dilution effects due to the low concentrations of fibrous carbohydrates and the high content of NFC in RMF (Pinto et al., 2019). In the same way, the high levels of SCHO present in melon could result in a residual increase in this nutrient in silages; however, the SCHO level was not influenced by RMF inclusion. Soluble carbohydrates are the main fermentation substrates in ensilage and may have been consumed as the RMF inclusion levels increased. This is supported by the linear rise in the proportions of organic acids (AA, PA, and LA) in response to an RMF increase in silage.

The fermentation profile responded positively to RMF inclusion in sorghum silage, based on the maintenance of the pH and the increase in organic acids (AA, PA, and LA) of silages with RMF inclusion. In addition, BA levels below 1% of DM indicate a weak clostridial fermentation in silages (Kung Jr. et al., 2018). The inclusion of RMF raised AA to moderately high levels (~ 4%), which can be considered a positive factor since AA has an antifungal action contributing to aerobic stability of the silage (Kung et al., 2018). A moderate increase in AA may be related to secondary metabolism that converts AL to AA, mainly attributed to *Lactobacillus buchneri*, which is considered normal and does not negatively affect animal intake (Muck et al., 2018).

The $N\text{-}NH_3$ increase may represent greater enzymatic and microbial proteolysis (Rooke and Hatfield, 2015); however, the residual CP content of silages was not compromised and increased with RMF inclusion. In addition, the highest concentration of $N\text{-}NH_3$ (7.59% of total N), obtained with the highest RMF inclusion (8%), was below the indicative level of quality (< 10% of total N) for silages (Kung Jr. et al., 2018).

The absence of microbial parameters and aerobic stability evaluations may represent limitations of the present study. However, responses of fermentation profile and DM content (above 28%) are an indication of adequate microbial action and good conditions for oxidative stability of the mixed silages, which allows validating conclusions. This can be reinforced through the water activity (a_w) estimated for the silages produced. Following model ($a_w = 1 - c/m$) proposed by Greenhill (1964) with adaptations indicated by Jobim et al. (2007), where c is a constant with a value of 0.08 suggested by the authors for moist feeds rich in soluble sugars, and m is the moisture content of the feed; we found 0.88, 0.88, 0.88, 0.89, and 0.89 for a_w of silages with 0%, 2%, 4%, 6%, and 8% of RMF, respectively. Most bacteria and fungi that pose a risk to public health have their development and production of toxins restricted at a_w greater than 0.90 (Jobim et al., 2007).

CONCLUSIONS

The addition of refused melon fruit (RMF) improved the chemical-bromatological composition, digestibility, and fermentative profile of sorghum silage without influencing losses during ensiling. Levels up to 8% based on natural matter can be recommended.

Author contributions

Conceptualization: L.P. Methodology: L.P. Formal analysis: P.dO. Investigation: P.dO. Resources: L.A., S.F., D.dL.J. Data curation: T.dA. Writing-original draft: P.dO. Writing-review & editing: T.dA. Visualization: L.A., S.F., D.dL.J. Supervision: P.L. Project administration: P.L. All co-authors reviewed the final version and approved the manuscript before submission.

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