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Effect of pineapple peel addition on sorghum ensilage

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Abstract

Silage mixed with forage and byproducts is an alternative nutritional strategy for ruminant production. This study aimed to characterize chemical–nutritional aspects, fermentative profile, and digestibility of sorghum (*Sorghum bicolor* L. Moench) silage with different contents of pineapple (*Ananas comosus* L. Merr.) peel inclusion at 0%, 20%, 40%, 60%, or 80% of fresh matter. The experimental design was completely randomized with five replicates per treatment. Mixed feeds were ensiled in experimental silos for 65 days, subsequently opened, and samples were analysed. The increase in the proportions of pineapple peel caused a linear increase in dry matter, crude protein, mineral matter, ether extract, soluble carbohydrate, cellulose, lignin, and *in vitro* digestibility of dry matter in sorghum silage. The mean concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) had a negative quadratic relationship with the inclusion of pineapple peel, reducing from 0% to 40% (-2.56% for NDF and -3.14% for ADF) followed by stabilization at subsequent contents. Silage pH was not influenced, however, acetic and propionic acids increased linearly, while butyric acid decreased linearly with pineapple peel inclusion. The highest losses in gases and effluents were obtained in silage with 0% pineapple peel inclusion (1.77% of dry matter and 4.06 kg t^{-1} of dry matter), which resulted in lower dry matter recovery (93.56%). Adding pineapple peel to sorghum benefits composition, fermentation, *in vitro* digestibility, and decreases silage losses. An inclusion of 40–80% sorghum can be recommended.

Keywords: *Ananas comosus* L. Merr., byproducts, forage conservation, fermentation, mixed silage
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Introduction

Global concerns regarding sustainability have led agribusinesses to assess residual product destinations and improve strategies for using natural resources. Millions of tons of byproducts are still generated by the fruit production and processing industry, even with a reduction of more than 80% in the world production of the waste of primary fruit from 2010 to 2019 (FAOSTAT, 2022). Encouraging results have been obtained using byproducts in ruminant feeding in search of a better use of these resources (Riestra *et al.*, 2014; Maneerat *et al.*, 2015; Oliveira *et al.*, 2018; Kyawt *et al.*, 2020). However, the seasonal availability of agro-industrial residues makes it essential to understand possible strategies for their conservation.

The pineapple crop generates a substantial number of byproducts per hectare that can be preserved and used in ruminant feeding (Idayanti *et al.*, 2022). Pineapple waste has a nutritional composition similar to tropical forages, with 6–8% crude protein, 41.92% carbohydrates, and 54.8% neutral detergent fibre (López-Herrera *et al.*, 2014; Idayanti *et al.*, 2022). High concentrations of residual soluble sugars in pineapple peel (PP) should contribute to improving the silage fermentation process. Additionally, PP presents a lignocellulosic structure with high fermentability and kinetic behaviour that facilitates the hydrolysis of hemicellulose by anaerobic microorganisms, especially in acidic conditions (Ning *et al.*, 2016; Dahunsi *et al.*, 2022).

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Silage is the dietary forage base of ruminant animals in bovine milk production systems (Grant & Adesogan, 2018) and is an essential productive strategy in tropical regions. Under these conditions, sorghum is one of the main forage alternatives for ensiling. It has high productivity and produces good-quality silage, provided it is appropriately managed (Moura *et al.*, 2016). Plant residues have been investigated to circumvent management problems and processing on sorghum ensiling, and changes in the fermentation profile and increased diversity of microorganisms during processing have been reported (Forwood *et al.*, 2019). These results have different implications for the silage products, which may vary, depending on the type of waste used.

It can be inferred that the production of mixed silage from agro-industrial residues and tropical forages enables the use and conservation of byproducts and can benefit the silage produced. However, it is necessary to characterize the product of the interaction between these feeds after the ensiling process. In this context, the objective of the present research was to evaluate the effect of PP inclusion in forage sorghum ensilage on the composition, digestibility, processing losses, and fermentative profile of the silage.

Materials and Methods

This experiment was carried out in the municipality of Rio Branco, Acre, Brazil (9°57'23.6" S 67°52'09.0" W). The climate in this area is hot and humid, of the Amazonian type. A completely randomized experimental design was used with 0%, 20%, 40%, 60%, and 80% inclusions of pineapple peel in forage sorghum, based on fresh matter, with five replicates per treatment, totalling twenty-five experimental units.

The forage sorghum plants (*Sorghum bicolor* L. Moench) were harvested in the maturation phase of milky/pasty grain, 100 days after sowing. Pineapple (*Ananas comosus* L. Merr.) peel was collected in the fruit pulp industry of the municipality. Both feedstuffs were chopped into particles of approximately 1.5 cm and mixed in the proportions established in the experimental design.

Twenty-five experimental silos of polyethylene buckets of 3.5 L of capacity were used. Paper towels with registered weight were placed at the bottom of containers to capture effluents during fermentation. Respective mixtures were placed in silos and compacted, aiming to reach a density of 500 kg m^{-3}. The silos were hermetically sealed with plastic bags, weighed, and stored in a protected place. Bunsen valves were attached to silos to release fermentation gases. The fermentation period was 65 days.

Losses during the ensiling process were estimated according to method proposed by Jobim *et al*. (2007). Before opening, the silos were weighed to measure post-fermentation gas losses (GL) by the difference between final and initial weights. Subsequently, the silos were opened, and homogenized subsamples were collected for further analysis. Effluent losses (EL) were estimated by the difference between final and initial paper towel weights. Dry matter recovery (DMR) was calculated from the proportion of dry matter (DM) remaining after ensiling:

$$\text{DMR} = 100 - (\%\ \text{DM at closing} - \%\ \text{DM at opening}) \quad (1)$$

Dry matter, mineral matter (MM), crude protein (CP), and ether extract (EE) content were determined according to the respective methods (934.01, 942.05, 976.05, and 963.15 of AOAC, 2016). Neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose (CEL), hemicellulose (HEM), and lignin (LIG) were analysed according to the methods of Van Soest *et al*. (1991). The soluble carbohydrates (SCHO) were determined according to the method of Bailey (1967). Total digestible nutrients (TDN) were calculated according to the method of Detmann *et al*. (2016).

A fraction of the silage was placed in a manual press for juice extraction and pH determination. Organic acids such as acetic (AA), propionic (PA), butyric (BA), and lactic (LA) acids in silage juice extract were estimated using gas chromatography, according to the method of Erwin *et al*. (1961). Ammoniacal nitrogen ($N\text{-}NH_3$) was estimated according to the method of Mizubuti *et al*. (2009).

An *in vitro* dry matter digestibility test (IVDMD) was performed according to the method developed by Tilley & Terry (1963) and adapted by Holden (1999). Ruminal content inoculum was obtained from Holstein cattle of 700 kg body weight equipped with a ruminal cannula that were fed with sorghum silage and concentrate feed in the proportion, 80:20. Ruminal inoculum collections occurred three hours after feeding the donor animal. Procedures using animals were approved by the Ethics Committee on the Use of Animals of UFERSA under protocol nº: 23091.010626/2019 23.

The data were subjected to analysis of variance with orthogonal polynomial contrasts for linear and quadratic effects using the SAS PROC GLM procedure. The model included content of PP effect:

$$Y_{ij} = \mu + P_j + \varepsilon_{ij}, \quad (2)$$

where Y_{ij} = dependent variable observed in i^{th} replicate (n = 5) and j^{th} treatment (n = 5); μ = the grand mean or overall constant; P_j = effect of j^{th} treatment (content of PP inclusion); and ε_{ij} = unobserved random error. Linear and quadratic effects were considered significant at P <0.05, and the Tukey test was used to compare means.

Results and Discussion

The sorghum ensiling process with PP addition generally provided silages with different compositions and fermentation profiles (*P* <0.05). There was a linear increase (*P* <0.05) in DM, CP, MM, EE, SCHO, CEL, LIG, and IVDMD concentrations with an increase in the proportion of PP in the silage (Table 1). Concentrations of HEM decreased linearly (*P* <0.05) with increasing PP contents.

The mean of NDF, ADF, and TDN concentrations was adjusted (*P* <0.05) to quadratic polynomial models in response to PP increases in sorghum silage. The NDF content showed a marked reduction (2.57%) from control (0% PP) to the 40% PP inclusion content, followed by stabilization at subsequent contents (60 and 80%). A similar response was observed with ADF means. However, the most accentuated reduction (3.05%) occurred from control to the 20% PP inclusion content. The inverse was observed with TDN concentrations, where there was a marked increase (0.88%) from control to the 20% PP inclusion content, followed by stabilization at subsequent contents.

The potentially digestible nutrient set is represented by the organic content present in DM, and an increase in this content is desirable for animal feed. However, a limit of 35% of DM should be considered to facilitate compression and allow the anaerobic fermentation process in forage ensiling (Kung Jr. *et al.*, 2018). In this case, the DM increments obtained did not exceed the recommended limit even with the maximum inclusion of PP.

A reduction in NDF and ADF concentrations was related to the dilution effect with PP inclusion in sorghum silage. The magnitude of this effect was attenuated after 40%, indicating that it is a limit for dilution of cell wall constituents with PP addition. This response can be explained by slightly lower concentrations of NDF and ADF in PP than in forage sorghum. Some studies report NDF and ADF concentrations of 54.8 and 20.8% for PP (López-Herrera *et al.*, 2014), and ~63.3 and 36.11% for sorghum (Valadares Filho *et al.*, 2018), respectively. As they are components of lower digestibility, the response to NDF and ADF was inversely reflected in TDN concentration.

An increase of CP in silage DM also represents a benefit of PP inclusion in sorghum ensiling. One of the main nutritional limitations of tropical forages for ruminants is their low protein content, especially during the dry season (Souza *et al.*, 2010; Franco *et al.*, 2021). Concentrations lower than 7–8% CP in feed DM can restrict microbial growth and reduce fibre degradation capacity, making protein supplementation essential under these conditions (Detmann *et al.*, 2014). Considering these factors, it can be inferred that the 40% PP inclusion raised the CP content of sorghum silage to a desirable content for use in ruminant feed, increasing from 6.07 to 8.53% in DM. Low concentrations of CP in pineapple waste have been reported (López-Herrera *et al*., 2014; Idayanti *et al*., 2022). However, the residue used in the present research was composed of PP which had a protein content similar to sorghum, considering the compilation presented in Table 1.

Most observed variations in silage composition may be associated with a dilution effect by efficiency increase in ensiling process, where there is a decrease in the proportion of primarily fermented components in relation to the rise in concentration of preserved compounds. This effect may explain the significant increase in CP, EE, MM, LIG, and CEL associated with the reduction in HEM and the slight variation (β = 0.0002) of SCHO with PP inclusion in sorghum silage.

Researchers have indicated PP as an important fermentation substrate for several applications (Raiane *et al.*, 2016; Aruna, 2019; Maria Monteiro Vieira *et al.*, 2021), mainly due to the chemical structure of the LIG-CEL-HEM matrix with highly hydrolyzable polymeric chains, and high fermentability of hemicellulose under acidic conditions (Dahunsi *et al.*, 2022). Available for fermentation in an anaerobic environment, HEM is reduced to simple sugars by microorganisms, and this action is limited to the beginning of the fermentation process due to the acidification of the medium, which causes a decline in microbial enzymatic activity (Ning *et al.*, 2016). Numerically different concentrations of HEM and SCHO in the pre-ensilage and ensiled materials (Table 1) indicate that these compounds were extensively metabolized during the ensiling process, and that the more fermentable lignocellulosic matrix of PP contributed to improving the preservation of the other constituents.

The effects of PP inclusion in sorghum silage can be considered positive because of the increase in protein, mineral, lipid, and soluble carbohydrate contents, and the reduction in the fibrous constituents of silage to some extent. These responses can be attributed to PP chemical–nutritional characteristics associated with an adequate ensiling process. Observed responses of DM concentrations reinforce this assertion.

The sum of CP, MM, EE, SCHO, and NDF constituents in silages with 0, 20, 40, 60, and 80% PP resulted in 66.68, 68.25, 67.74, 68.17, and 68.84% DM, respectively, indicating that there were components conserved in silage DM that were not determined in our study. Unidentified constituents can be classified as resistant non-structural carbohydrates, mainly starch and fructans, or even other polysaccharides such as galactans, β-glucans, and residual pectic substances in the ensiled material (Hall, 2003).

Increases in IVDMD coefficients with PP inclusion in sorghum ensiling are related to a combined increase of highly degradable constituents; an increase in lignin concentrations could have limited the magnitude of this response. However, the linear regression model for LIG showed a low slope value ($\beta = 0.001$), indicating a slight increase in this constituent and a low negative impact on IVDMD. In addition, as previously discussed, PP has a lignocellulosic structure with high fermentability (Dahunsi *et al.*, 2022), which may have contributed to the greater *in vitro* digestibility of DM.

EL during ensiling reduced linearly (P <0.05) with the increase in proportion of PP in sorghum silage. GL reduced quadratically (P <0.05) with PP inclusion in the silage, with minimal losses (0.78%) observed at the 60% PP inclusion. There was a marked increase (2.0%) in DM recovery from the control to the 40% PP inclusion, followed by stabilization at subsequent percentages.

High-quality silages present negligible losses during processing and good recovery of ensiled DM. Increments in DMR, associated with reductions in EL and GL, indicate an improvement in the sorghum ensiling process with PP inclusion. The improvement in the fermentation process can explain these responses. In summary, pH was maintained while LA concentrations increased, contributing to reducing losses and consequently greater preservation of DM with PP addition. Furthermore, the lower moisture content in PP contributed to drier silage that lost less liquid on compaction.

Given the benefits reported on the composition and the reduction on losses with the mixture between PP and forage sorghum, we used the data to perform a composite response surface analysis to associate TDN, DMR, and PP inclusion. Canonical analysis of the response surface based on coded data showed a predicted value at a stationary point: 72.16% of PP inclusion, setting TDN and DMR at 58.88% and 97.55%, respectively. Based on this analysis, we can infer that proportions of up to 70% PP and 30% sorghum are indicated to improve composition and DM recovery of mixed silage.

There was no influence (P >0.05) of PP inclusion on silage pH. However, $N\text{-}NH_3$ content showed a quadratic increase (P <0.05), rising from the control to the 40% PP, followed by a reduction in subsequent contents (Figure 1). AA and PA increased linearly (P <0.05), while butyric acid decreased linearly (P < 0.05) with PP inclusion in sorghum silage. The lactic acid concentrations were adjusted to a quadratic polynomial model (P <0.05) in response to PP inclusion, with a marked increase (3.09%) from the control to the 40% PP inclusion, followed by stabilization at other percentages.

The pH content (3.61) was below that normally found in forage silage (4.3–4.7), even in the control silage. This can be explained by a high content of SCHO in sorghum, associated with the low buffer capacity of forage (Kung Jr. *et al.*, 2018). An increase in LA of silage indicates an increase in SCHO concentrations in sorghum mixed with PP. Rodrigues *et al*. (2020) estimated that each percentage unit of SCHO in sorghum DM raised 0.37 units of LA in silage DM until a plateau of 8.42% of LA was obtained with 12.54% of SCHO, which coincides with pH stabilization at ~3.62. With a mixed silage, the average pH (3.60) was close to the maximum pH reduction proposed by Rodrigues *et al*. (2020). However, this limit was reached at concentrations of LA of ~6.07%. The pH was maintained even with an LA increase due to the pH range being close to the dissociation power limits of this acid (pKa 3.86).

The reduction of BA content to almost undetectable contents with PP inclusion in sorghum silage indicates low impairment by clostridial fermentation, mainly associated with increased DM content (Kung Jr. *et al.*, 2018). In addition, the maximum $N\text{-}NH_3$ of 6.51% was lower than the recommended content (10–15%), indicating that there was little enzymatic and microbial proteolytic action in the silage (Kung Jr. *et al.*, 2018). The benefits of PP inclusion with sorghum on the fermentative profile of silages occur mainly after the 40% inclusion. This inference can be illustrated by high concentrations of LA and reductions in $N\text{-}NH_3$ and BA contents.

Table 1 Average content of dry matter (DM), crude protein (CP), mineral matter (MM), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), soluble carbohydrates (SCHO), cellulose (CEL), hemicellulose (HEM), lignin (LIG), total digestible nutrients (TDN) and *in vitro* dry matter digestibility (IVDMD), dry matter recovery (DMR), losses by effluents (EL), and gas losses (GL) of sorghum silage with pineapple peel (PP)

Variables	Pre-ensiled		Pineapple peel contents (%)					SEM	P-Value	
	Sorghum*	PP*	0	20	40	60	80		L	Q
DM, %[1]	24.11 ± 2.24	14.70 ± 6.26	30.38^{d}	32.46^{c}	32.85^{c}	33.62^{b}	34.56^{a}	0.29	< 0.0001	0.0040
CP, %DM[2]	6.84 ± 1.00	6.22 ± 2.61	6.07^{e}	7.91^{d}	8.53^{c}	8.96^{b}	9.33^{a}	0.24	< 0.0001	< 0.0001
MM, %DM[3]	6.41 ± 0.62	4.64 ± 0.74	6.88^{d}	7.28^{c}	7.40^{c}	7.79^{b}	8.05^{a}	0.08	< 0.0001	0.8704
EE, %DM[4]	3.04 ± 0.64	1.25 ± 0.50	2.19^{c}	2.76^{b}	2.83^{ab}	2.84^{ab}	2.92^{a}	0.06	< 0.0001	< 0.0001
NDF, %DM[5]	63.35 ± 1.68	49.89 ± 4.76	50.12^{a}	48.86^{b}	47.55^{c}	47.13^{d}	47.10^{d}	0.24	< 0.0001	< 0.0001
ADF, %DM[6]	36.11 ± 1.44	24.81 ± 4.98	43.18^{a}	40.13^{b}	40.04^{b}	39.23^{c}	39.13^{c}	0.30	< 0.0001	< 0.0001
SCHO, %DM[7]	10.84 ± 0.96	5.10 ± 0.90	1.43	1.43	1.44	1.44	1.44	0.00	0.0268	0.5513
CEL, %DM[8]	31.18 ± 1.69	21.52 ± 9.31	32.17^{d}	32.30^{c}	32.39^{b}	32.48^{a}	32.52^{a}	0.03	< 0.0001	0.0059
HEM, %DM[9]	26.81 ± 1.45	23.74 ± 5.40	13.94^{a}	13.83^{a}	13.67^{b}	13.65^{b}	13.61^{b}	0.03	< 0.0001	0.0113
LIG, %DM[10]	5.70 ± 1.04	11.25 ± 5.43	6.41	6.43	6.46	6.47	6.51	0.01	0.0042	0.8359
TDN, %DM[11]	-	-	56.88^{b}	57.76^{a}	57.84^{a}	57.87^{a}	57.92^{a}	0.09	< 0.0001	0.0002
IVDMD, %[12]	-	-	53.17^{b}	54.46^{b}	53.70^{b}	55.69^{a}	56.28^{a}	0.27	< 0.0001	0.2662
DMR, %DM[13]	-	-	93.56^{b}	95.09^{a}	95.56^{a}	95.51^{a}	95.70^{a}	0.18	< 0.0001	< 0.0001
EL, kg t^{-1} DM[14]	-	-	4.03^{a}	3.70^{b}	3.46^{bc}	3.26^{cd}	3.17^{d}	0.07	< 0.0001	0.0213
GL, %DM[15]	-	-	1.77^{a}	1.02^{b}	0.97^{b}	0.78^{b}	0.86^{b}	0.08	< 0.0001	0.0002

* Means obtained through literature compilation (Elizondo-Salazar & Campos-Granados, 2014; López-Herrera *et al.*, 2014; Riestra *et al.*, 2014; Raiane *et al.*, 2016; Valadares Filho *et al.*, 2018; Dahunsi *et al.*, 2022; Pereira *et al.*, 2022)
SEM = standard error of the means; L = linear effect; Q = quadratic effect
[abcde] Means with different letters on the same line differ by Tukey's test at 5% probability
[1]Y = 30.87 + 0.05x (r^2 = 0.88); [2]Y = 6,64 + 0,04x (r^2 = 0,86); [3]Y = 6.91 + 0.01x (r^2 = 0.95); [4]Y = 2,4 + 0,008x (r^2 = 0,64); [5]Y = 50.18 - 0.09x + 0.0006x^2 (r^2 = 0.99); [6]Y = 42.88 - 0.1x + 0.0009x^2 (r^2 = 0.90); [7]Y = 1.43 + 0.0002x (r^2 = 0.22); [8]Y = 32.20 + 0.004x (r^2 = 0.91); [9]Y = 13.91 - 0.004x (r^2 = 0.78); [10]Y = 6.41 + 0.001x (r^2 = 0.34); [11]Y = 56.97 + 0.03x - 0.0003x^2 (r^2 = 0.90); [12]Y = 53.17 + 0.04x (r^2 = 0.62); [13]Y = 93.68 + 0.07x - 0.0006x^2 (r^2 = 0.94); [14]Y = 3.96 - 0.01x (r^2 = 0.85); [15]Y = 1.71 - 0.03x + 0.0003x^2 (r^2 = 0.94)

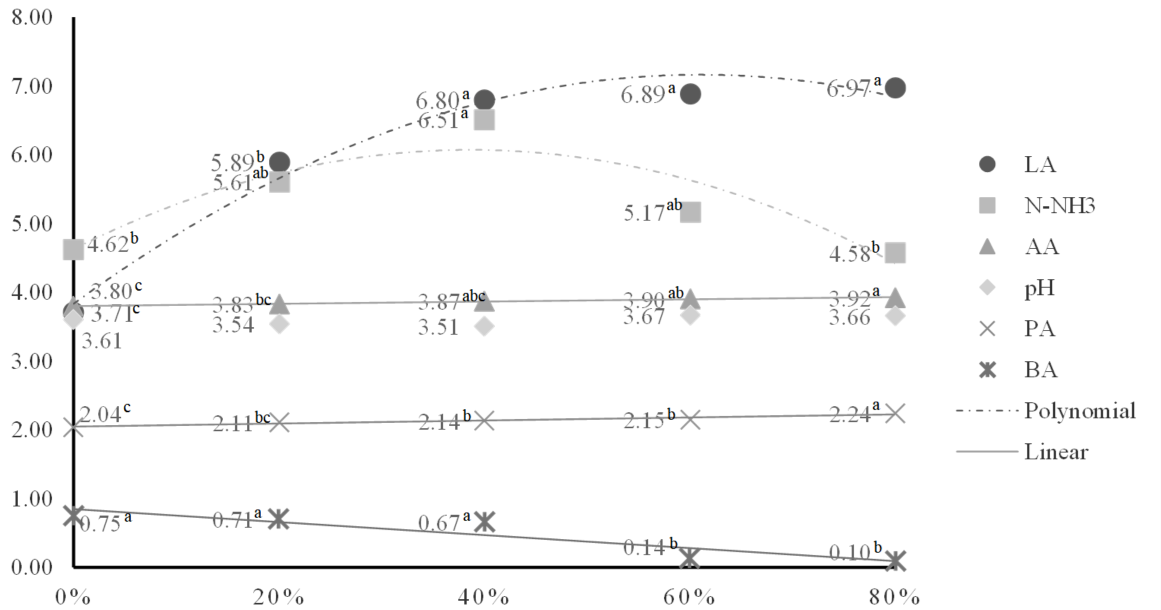

Figure 1 Fermentative profile of sorghum silage with pineapple peel inclusion
pH = potential of hydrogen (Y = 3.55)
N-NH3 (% of total N) = ammonia in total nitrogen ($Y = 4.63 + 0.07x - 0.001x^2$, $r^2 = 0.82$)
AA (% of dry matter) = acetic acid ($Y = 3.80 + 0.001x$, $r^2 = 0.55$)
PA (% of dry matter) = propionic acid ($Y = 2.05 + 0.002x$, $r^2 = 0.67$)
BA (% of dry matter) = butyric acid ($Y = 0.85 - 0.009x$, $r^2 = 0.76$)
LA (% of dry matter) = lactic acid ($Y = 3.83 + 0.1x - 0.0009x^2$, $r^2 = 0.98$)
-.-.-.- Polynomial = means with significant adjustment to polynomial quadratic model (*P* <0.05)
—— Linear = means with significant adjustment to a linear model (*P* <0.05)
[abcde] Means with different letters on the same line differ by Tukey's test at 5% probability

Conclusions

Pineapple peel mixed with forage sorghum produces good composition, fermentation, and *in vitro* digestibility of silage, indicating that it is a great strategy for using this byproduct as an alternative feed conservation for ruminants. It can be recommended that 40–80% of PP inclusion, based on fresh matter, promotes the benefits indicated in the present study.

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Authors' Contributions

SARGC: methodology, collected data, investigation, and original draft preparation. TLACA: analysed data, interpreted the results, and wrote the manuscript. LAP: conceptualization and design of the study. DMLJ, LCSLCA, and MWFP: methodology, visualization, and investigation. POL: validation, and supervision.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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