Nutritional evaluation and digestibility of pearl pineapple crop waste silage in different particle sizes

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ABSTRACT
The cultivation of pearl pineapple, produced for sale in natura, generates a large amount of cultural remains that have the potential for use in feeding ruminants in the form of silage. The experiment aimed to evaluate the nutritional value and in vitro digestibility of pearl pineapple crop residue silage in different particle sizes. A completely randomized design was used in a 2 x 4 factorial scheme with two particle sizes (20 and 50 mm) and 4 fermentation times (30, 60, 90 and 120 days after ensiling) with 4 replications. At each fermentation time, 8 samples were collected to determine dry matter (DM), mineral matter (MM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HEM), total digestible nutrients (TDN), total carbohydrates (TC), non-fibrous carbohydrates (NFC) and pH values. The *in vitro* digestibility of dry matter (IVDDM), neutral detergent fiber (IVDNDF) and acid detergent fiber (IVDADF) were determined from a test carried out in an Ankom® Ruminal Fermenter ("Daisy-II Fermenter"), in a 2 x 2 factorial scheme, with 2 fermentation times (30 and 90 days) and two particle sizes. The means of observed data for each treatment were subjected to analysis of variance and compared using the Tukey test, at a 5% probability level. There was no interaction (P>0.05) between the contents of DM, OM, CP, NDF, ADF, HEM, MM, TDN, CT and NFC, except for the content of EE and pH (P<0,05). It was found that the EE content increased by 42% when the silage remained stored for 60 days with particle sizes of 50 mm. There was an interaction (P<0,05) at 90 days of fermentation in relation to the two particle sizes, when the pH values were 4.19 for 20 mm and 4.15 for 50 mm (P<0,05). IVDADF increased by 38,11% when the silage remained stored for 90 days with particle sizes of 50 mm (P<0,05). It was concluded that the chopping of the material could be done according to the availability of the type of forage harvester. Regarding digestibility, it is recommended to open the silos after 60 days of fermentation so that the pH reaches the recommended parameters.

Keywords: Pineapple culture, Alternative food, *Ananás comosus L. Merril*, Roughage, Animal nutrition.

1 INTRODUCTION

Brazil is the third country with the world's largest pineapple production, with 1,637,126 tons, behind the Philippines and Costa Rica; with the states of Pará, Paraíba and Minas Gerais standing out in Brazilian production (CONAB, 2020; FAO, 2022). Therefore, pineapple production is of great economic interest, being cultivated in several regions of the country (SOUZA; COUTINHO; TORRES, 2010).

The increase in productivity and the improvement in the quality of the fruit makes it possible to produce more fruits per hectare, consequently a greater production of crop residues. The state of Minas Gerais stands out as the third largest pineapple producer in the country, where in 2019, production reached 179,3 thousand tons in 6000 ha of planted area (SEAPA, 2020), of which about 95% of production is in the Triângulo Mineiro region (IBGE, 2017).

Another highlight is the planting of the main varieties for industry and table, respectively, the *Smooth Cayenne pineapple (*Hawaiian) and the Pearl pineapple (IBGE, 2017). Production is predominantly carried out by small farmers, in areas smaller than five hectares, on average, without irrigation, with few management practices and largely destined to the Pérola variety (SOUZA et al., 2007; RODRIGUES et al., 2010).

This is due to the cultivar produced, the Pearl pineapple, which, due to the characteristics of its plant, provides greater planting density. As a result, these regions produce a large surplus of cultural remains. In this context, the fruit grower has been looking for new ways to use these remains, to minimize environmental impacts and prevent diseases in the crop.

The crop remains of the pineapple plant are a source of forage of limited use in the places where it is grown, however, it has the potential to increase animal production (MARIN et al., 2002). Feed is the cost that most burdens livestock production. Thus, the use of alternative foods is increasingly employed in the current scenario. Increased productivity requires greater use of feed inputs to cover the critical periods of the annual forage production cycle and better expression of cattle genetic potential. The use of silage from pearl pineapple crop remains becomes, therefore, a viable alternative aimed at reducing feed costs as well to minimize environmental contamination, since the large amount of plant waste produced is large (SANTOS et al., 2010).

In this context, pineapple crop residues could be used as food in the form of silage, even contributing to lower the cost of feed for dairy cows. The ensiling process follows the same procedure as for the corn plant, and trench or surface silos can be used. Silage has been used empirically, and studies are needed in order to enable its rational use as roughage, so that its use by the animal is more efficient, which basically depends on the knowledge of the chemical composition and the digestibility of its nutrients. Thus, the present study aimed to evaluate the nutritional value and in *vitro* digestibility of silage from pearl pineapple crop residues in different particle sizes.

2 MATERIALS AND METHODS

The work was carried out at the Animal Nutrition Laboratory and at the Animal Unit of Digestive and Metabolic Studies and in the Dairy Cattle Sector of the Faculty of Agricultural and Veterinary Sciences, Jaboticabal Campus, State University of São Paulo (FCAV/UNESP), from July 2012 to July 2013.

The pearl pineapple crop remains were chopped in a mobile forage chopper with precision hydraulic drive for cutting height (JF92 Z10), equipped with 10 knives for cutting according to the adjustment. The material was ensiled in artificial silos consisting of double plastic bags with 50 cm x 80 cm in size and weight of around 30 kg, and 32 artificial silos were prepared, 16 with particle sizes of 20 mm and 16 with particle sizes of 50 mm; with scheduled opening times every 30 days.

The artificial silos were kept in a closed environment, free of moisture, covered with dark tarpaulin, labeled with data such as fermentation days, particle sizes and opening dates; being placed in order of withdrawal for the collection of samples, thus avoiding the incidence of light in the other silos. Every 30 days, 4 silos with particle sizes of 20 mm and another 4 with particles of 50 mm were opened until completing 120 days. Samples of about 500g were taken from the central part of the silo, properly packed in a plastic bag, identified and frozen for further bromatological analysis.

At each collection period, about 50 g of fresh sample of each treatment was taken and sent to the laboratory of the State University of Minas Gerais, Frutal Campus, for pH determination, according to the methodology described by Silva and Queiroz (2009).

At the end of the 120-day period, silage samples from Pérola pineapple crop residues were thawed, and 300 g were used for bromatological analysis. The samples were pre-dried in an oven with forced air ventilation at 55°C for 72 hours and ground in a Willey mill in sieves with 1 mm meshes, stored, and identified in plastic jars. Then, they were sent to the Animal Nutrition Laboratory of the Faculty of Agrarian and Veterinary Sciences, Jaboticabal Campus, State University of São Paulo (FCAV/ UNESP) for bromatological analyses, and the contents of dry matter, mineral matter, crude protein, ether extract according to AOAC (1990), neutral detergent fiber, acid detergent fiber according to Van Soest et al. (1991) and hemicellulose were determined by the difference between the fiber contents in Neutral detergent and acid detergent fiber.

The total carbohydrate values were determined according to the methodology described by Sniffen et al. (1992), in which: CHOT = 100 - (CP + EE + ASH) and non-fibrous carbohydrates (NFC) were calculated according to Mertens (1997), where NFC = 100 – (NDF + CP + EE + ASH). The total digestible nutrients were obtained by the formula TDN = 87.84 - (0.7 x ADF), where ADF is acid detergent fiber (RODRIGUES, 2010).

For the bromatological analyses, a completely randomized design was used, in a 4 x 2 factorial scheme, with 4 fermentation times (30, 60, 90 and 120 days of fermentation) and 2 particle sizes (20 and 50 mm) with 4 replications.

To obtain the ruminal fluid inoculum, a castrated male bovine, without a defined breed standard, cannulated in the rumen, weighing approximately 500 kg, was used as a rumen donor animal. During the adaptation period, the animal was kept in confinement, receiving daily 15 kg of silage from pearl pineapple crop remains, 2 kg of concentrate, divided into two meals, and water at will. After the 15-day adaptation period, before the first meal of the morning, the ruminal content was collected, filtered in cotton fabric, and the ruminal liquid was transported in thermoses containing water previously heated to 39°C, to the incubation site.

The *in vitro* digestibility of dry matter (IVDDM), neutral detergent fiber (IVDNDF), and acid detergent fiber (IVDADF) were determined from an assay performed in an Ankom® Rumen Fermenter ("Daisy-II Fermenter"). Incubation was done using silage samples from pearl pineapple crop residues with 2 fermentation times, 30 and 90 days in two particle sizes, 20 and 50 mm.

0.500 grams of samples were weighed in each F57® digestion bag, then sealed and placed in the digestion jars (up to 25 bags per jug), containing previously prepared solution. In each jar, 1600 mL of a previously heated buffer solution (39ºC) were added, consisting of a mixture of two solutions A and B, in a 5:1 ratio, respectively. Solution A consisted of: 10 g L^{-1} of KH_2PO_4; 0,5 g L^{-1} of $MgSO_4.7H_2O$; 0,5 g L^{-1} of NaCl; 0,1 g L^{-1} of $CaCl_2$ and 0,5 g L^{-1} of reactive urea-grade. Solution B consisted of: 15 g L^{-1} of Na_2CO_3 and 1,0 g L^{-1} of $Na_2S.9H_2O$. Then, the rumen fluid inoculum was added to each jar (400 mL per jar). The samples were incubated for 48 hours, after which a second stage was performed with the addition of 8 g of pepsin and 40 mL of HCl 6N in each jar, keeping the system heated for another 24 hours, with the temperature being controlled and constantly checked by digital thermometers.

After the incubation period of the samples, the bags were washed in running water and taken to an oven at 65ºC for 72 hours, to determine the DM content of the samples in an oven at 105ºC. For IVDNDF and IVDADF, the bags were dried in an oven at 65ºC, weighed and then taken to the Ankom® Fiber Analyzer. for the determination of NDF and ADF levels in a sequential manner.

The following formula was used to calculate the percentages of IVDDM, IVDNDF, and IVDADF: IVD, % = (W3 – W1) X 100/W2, where: W1 = tare weight of the filter bag; W2 = sample weight; W3 = final weight of the filter bag, after 24 h of digestion with pepsin + hydrochloric acid.

For *in vitro* digestibility, a completely randomized design was used, in a 2 x 2 factorial scheme, with 2 fermentation times (30 and 90 days of fermentation) with 2 particle sizes (20 and 50 mm) with 5 replications. The means of the data were submitted to analysis of variance and compared using the Tukey test, at the level of 5% probability, using AgroEstat, v.1.1.0.701, (BARBOSA and

MALDONADO Jr., 2014). The mathematical model used for the chemical composition and *in vitro digestibility was:* $\mathbf{Y}_{ijk} = \mathbf{m} + \mathbf{TF}_i + \mathbf{TP}_k + (\mathbf{TF\ TP})_{ik} + \mathbf{e}_{ijk}$, where: $\mathbf{m}$ = general mean; $\mathbf{TF}_i$ = effect, the ith fermentation time; **TP**k = effect of k-th particle size; $(\mathbf{TF\ TP})_{ik}$ = effect of the interaction between the i-th fermentation time and the k-th particle size; $\mathbf{e}_{ijk}$ = experimental error.

3 RESULTS AND DISCUSSION

Table 1 shows the chemical composition of pearl pineapple silage with 4 fermentation times and 2 particle sizes.

It was observed that there was no interaction (P>0,05) between the contents of dry matter, organic matter, crude protein, neutral detergent fiber, acid detergent fiber, hemicellulose, mineral matter, total digestible nutrients, total carbohydrates, and non-fiber carbohydrates, except for the contents of ether extract (P<0,05) and pH (P<0,05), in relation to fermentation time and particle size.

Table 1 - Average values in Dry Matter (DM), in percentage, of Organic Matter (OM), Ether Extract (EE), Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Hemicellulose (HEMI), Mineral Matter (MM), Total Digestible Nutrients (TDN), Total Carbohydrates (TC), Non-Fiber Carbohydrates (NFC) and pH, of pearl pineapple crop residue silage. Frutal-MG. 2012/2013.

	Treatments						Treatments					
	Fermentation times, days (T)				Test F	MSD	Particle size, mm (TP)		Test F	MSD	F for interaction	
	30	60	90	120			20	50			TxTP	CV
DM	18,91a	18,16a	18,40a	19,28a	1,89NS	1,43	18,8a	18,58a	0,36NS	0,76	2,69NS	5,57
OR	94,69a	94,93a	94,93a	95,12a	0,34NS	1,37	94,60a	95,20a	2,97NS	0,72	0,32NS	1,04
EE	1,94a	2,33a	2,25a	2,06a	1,96NS	0,50	2,10a	2,19a	0,30NS	0,26	4,16*	17,04
CP	5,27a	5,43a	5,51a	5,40a	0,73NS	0,46	5,53a	5,28b	4,30*	0,24	0,82NS	6,23
NDF	52,46a	53,49a	49,91a	54,39a	2,20NS	5,09	52,96a	52,95a	0,37NS	2,69	1,45NS	7,02
ADF	20,02a	31,48a	30,40a	31,77a	2,90NS	2,85	30,81a	30,52a	0,16NS	1,51	1,03NS	6,75
HEMI	23,44a	22,01a	19,50a	22,62a	1,29NS	5,83	22,14a	21,65a	0,11NS	3,08	0,59NS	19,29
MM	5,37a	5,06a	5,07a	4,88a	0,34NS	1,37	5,40a	4,79a	2,97NS	0,72	0,32NS	19,48
Ph	3,81b	3,87b	4,17a	3,87b	17,66**	0,14	3,94a	3,92a	0,31NS	0,08	3,39*	2,75
TDN+	67,53a	65,80a	66,55a	65,60a	2,89NS	1,99	66,27a	66,47a	0,16NS	1,06	1,03NS	2,18
CHOT++	87,42a	87,17a	87,16a	87,65a	0,34NS	1,57	86,96a	87,74a	3,70NS	0,83	0,72NS	1,30
CNE+++	34,95a	33,68a	37,25a	33,26a	2,06NS	4,87	34,00a	35,56a	1,55NS	2,58	1,84NS	10,16

Means followed by the same letter in the line do not differ from each other according to Tukey's test (P>0.05). NS, *, **: not significant; significant at 5 and 1% probability by Tukey's test, respectively.
CV (%) = Coefficient of variation. MSD (%) = Minimal significant difference.
[+] Averages calculated according to RODRIGUES, 2010, where TDN = 87,84 - (0,7 x ADF), in % in DM
[++] Averages calculated according to McDOWELL et al. (1974), where CHOT = 100 - (CP + EE + MM), in % in DM
[+++] Averages calculated according to Mertens (1997), where NFC = 100 – (CP + EE + ASH), in % in DM

Pinto et al. (2005), Fagundes and Fagundes (2010) found similar results in the bromatological composition of what they called pineapple hay, which is composed of plants crushed with a forage machine and exposed to the sun for 3 days. The average of 5,95% of crude protein did not differ much from the results found in the 4 fermentation times, 2,54% of ether extract, a result 30% higher than that found at 30 days of fermentation time in the present experiment.

Acidity is an important factor in silage conservation, as it acts by inhibiting or controlling the development of harmful microorganisms, such as bacteria of the genus Clostridium. The pH value indicates whether the fermentation was satisfactory, and its determination is used in the evaluation of silage quality (PEREIRA et al., 2007).

Comparing the neutral detergent fiber and acid detergent fiber contents, averages of 52,69% and 29,16%, respectively, with the contents found for corn silage by Zanine (2007), averages of 66,11% and 32,96%, respectively, the silage of pearl pineapple crop residues showed a decrease of 13% in the acid detergent fiber content.

Muller (1978), cited by Fagundes (2010), states that the total digestible nutrient contents of tropical grasses and legumes have values around 55%, while the levels found in this experiment were, on average, around 66%, which represents an increase of 20%.

The breakdown of the interaction between fermentation time and particle sizes for the ether extract contents (Table 2) showed that the average ether extract contents were higher when the silage remained longer in fermentation, with the largest difference being 1.91 and 2,44% of ether extract (P<0.01). This difference corresponds to a 27,74% increase in 90 days compared to 60 days of fermentation with a particle size of 20 mm.

Table 2. Unfolding of the interaction between fermentation times and particle size for the ether extract of silage from pearl pineapple crop remains.

PS (mm)	T(days)				Test F	MSD (5%)
	30	60	90	120		
20	1,95aA	1,91aB	2,44aA	2,12aA	1,74NS	0,71
50	1,93bA	2,75aA	2,06abA	2,01bA	4,32*	
F-Test	0,01NS	10,52**	2,15NS	0,19NS		
MSD (5%)	0,53					

Averages followed by different letters in the columns (uppercase) and rows (lowercase) differ from each other by the Tukey test.
NS = not significant; *(P<0,05); **(P<0,01); MSD = minimum significant difference; T = Fermentation time; PS = Particle sizes.

From the point of view of ether extract content, it is interesting that the fermentation period is 90 days, when the pineapple crop remains are ensiled with particle sizes of 20 mm. If the chipping is done allowing particle sizes of 50 mm, it is interesting to keep the silo closed until the fermentation period of 60, since the increase in ether extract content from 30 to 60 days of fermentation was 42%. Considering the ether extract values at 30 days of fermentation, regardless of particle size, if there is a need to open the silo, the silage will have lower ether extract contents.

By splitting the interaction between fermentation times and particle size for pH, it was found that there was no difference (P>0,05) at 90 days of fermentation in relation to particle sizes of 20 and 50 mm. The pH was 8,04% and 6,68% higher than the values of 30 and 60 days of fermentation, returning to the same level after 120 days of fermentation (Table 3).

Possenti et al. (2005), studying the bromatological and fermentative parameters of corn and sunflower silages, observed that a stable pH is not obtained in the silages, which is due to the deficiency of soluble carbohydrates or due to the excessive moisture of the material, which can be observed in the silage of pineapple crop residues, due to the high moisture content. The appropriate pH value to promote efficient conservation of ensiled forage depends on the moisture content of the silage (CUNHA et al., 2009).

Tomich et al. (2004) reported that pH values between 3,8 and 4,2 are considered adequate for well-preserved silages, because in this range there is restriction of the plant's proteolytic enzymes and enterobacteriaceae and clostrides.

Table 3. Unfolding of the interaction between fermentation time and particle sizes for pH, of silage from pearl pineapple crop remains.

PS (mm)	T(days)				Test F	MSD (5%)
	30	60	90	120		
20	3,73bB	3,88aB	4,19aA	3,96aB	12,35**	0,21
50	3,89bA	3,85aB	4,15aA	3,78bB	8,70**	
F-Test	4,64*	0,18NS	0,27NS	5,37*		
MSD(5%)	0,15					

Averages followed by different letters in the columns (uppercase) and rows (lowercase) differ from each other by the Tukey test.
NS = not significant; *(P<0,05); **(P<0,01); MSD = minimum significant difference; T = Fermentation time; PS = Particle sizes.

In the present experiment, the pH of the silage of pineapple crop residues varied between 3,73 and 4,19 at different fermentation times. The lowest average obtained at 30 days of fermentation, with a particle size of 20 mm, is close to the ideal range of 3,8 reported by Tomich et al. (2004) and Cunha et al. (2009), but this value cannot be considered ideal, and it is recommended to wait for 60 days to open the silo. Similar values were also found by Cunha et al. (2009) when comparing silages of different proportions of pineapple and maniçoba industrial residue and Pereira et al. (2007) in the evaluation of corn silages.

3.1 IN VITRO *DIGESTIBILITY*

There was no interaction (P>0,05) between fermentation times and particle size for the in *vitro* digestibility values of dry matter (IVDDM) and neutral detergent fiber (IVDNDF), as shown in Table 4. The IVDM averages are below the values found by Lousada Júnior et al. (2006) in relation to pineapple industrial waste, which was 61,31%. This same industrial pineapple residue has a high hemicellulose content, 40,65%, in contrast to the silage of pineapple crop residues, which presented a variation of 19 to 23% in the hemicellulose content.

Table 4. Mean values, in percentage, of *in vitro* digestibility of dry matter (IVDDM), neutral detergent fiber (IVDNDF) and acid detergent fiber (IVDADF) of silage from pearl pineapple crop residues.

TREATMENTS	IVDDM	IVDNDF	IVDADF
T(days)			
30	57,58	32,12	16,72
90	56,00	34,76	20,43
Test F	$1,00^{NS}$	4,68*	$27,30^{**}$
MSD (5%)	3,33	2,58	1,50
XS (mm)	IVDDM	IVDNDF	IVDADF
20	57,51	33,40	18,07
50	56,08	33,48	19,09
Test F	$0,84^{NS}$	$0,00^{NS}$	$2,09^{NS}$
MSD (5%)	3,33	2,58	1,50
F for interaction	IVDDM	IVDNDF	IVDADF
T x TP	$2,23^{NS}$	$2,41^{NS}$	$11,44^{**}$
CV, %	6,19	8,16	8,54

Averages followed by different letters in the columns (uppercase) and rows (lowercase) differ from each other by the Tukey test.
NS = not significant; *(P<0,05); **(P<0,01); CV = Coefficient of variation; MSD = Minimum significant difference; T = Fermentation Time; PS = Particle sizes.

Regarding the IVDADN and the IVDADF (Table 5), there was an interaction between fermentation time and particle size of the silage of pearl pineapple crop remains.

At 90 days of fermentation with a particle size of 50 mm, the IVDADF showed an increase of 18,26% compared to the same period with 20 mm of particle size. In relation to the same particle size of 50 mm, with 90 days of fermentation it showed an increase of 38,11%, in relation to the 30 days of fermentation.

Evaluating the particle size integer in relation to time, it is observed that within the days there is no difference for the particle sizes, however, when the time in days within each particle size is verified, there is an increase of 6,99% of the acid detergent fiber (IVDADF) from 30 to 90 days in the 20 mm size. For the 50 mm size, there was a 27,60% increase in acid detergent fiber (IVDADF) (Table 5).

Table 5. Breakdown of the interaction between fermentation time and particle sizes for the *in vitro* digestibility of acid detergent fiber (IVDADF), in percentage of different silages from pearl pineapple crop remains.

PS (mm)	T(days)		Test F	DMS (5%)
	30	90		
20	17,41bA	18,72aA	1,70NS	2,12
50	16,03bA	22,14aA	37,05**	
F-Test	1,88NS	11,65**		
DMS (5%)	1,5			

Averages followed by different letters in columns (uppercase) and rows (lowercase) differ from each other by Tukey's test.
NS = not significant; *(P<0,05); **(P<0,01); DMS = minimum significant difference; T = Fermentation Time; PS = Particle size.

4 CONCLUSIONS

As the particle size and fermentation time practically did not influence the nutritional composition of the silage, the material can be chopped according to the availability of the type of silage machine, in particle size with 20 mm or with 50 mm.

About the digestibility of the silage of pineapple crop residues can be used from the 30 days of fermentation, coming from both larger and smaller particles, but it is recommended to wait for the 60 days of fermentation, so that the pH reaches the recommended limits.

As it has potential for feeding dairy cows, if area is available, the cost for the dairy farmer can be minimized by obtaining silage from crop residues on the farm itself. Further research is suggested to verify the performance of dairy cows fed silage and pineapple crop remains, including economic analysis, pointing out its viability in relation to the cost per kilogram of milk produced and profitability.

The commercialization of silage from pearl pineapple crop remains closes the production cycle for the pineapple grower, minimizing the costs in the implementation of a new crop. For the farmer, cutting the cultural remains of the pearl pineapple becomes a cheap way of cleaning the area for new plantations, with an interaction between the pineapple grower and the milk producer.

This interaction has an impact on dairy farming located in the vicinity of pineapple plantations, as the production of silage from pineapple crop residues reduces the costs of animal feed, while also reducing the costs of the product to the consumer. The cutting of pearl pineapple crop residues for silage enables the control of white mealybug and fusariosis, by the less use of pesticides for its control, prevents soil compaction by the lower number of machine passes in the field, also avoiding the burning of crop residues, mitigating the environmental pollution that this practice entails.

The producer who wants to take care of his animals throughout the year will always find material available for silage, since the production of the fruit takes place almost all year round. And with the determination of the nutritional value of silage from pearl pineapple crop remains, it becomes interesting to associate another ingredient, such as citrus pulp, to increase the dry matter and protein content of the feed.

Finally, silage from pineapple crop remains is another viable alternative for feeding ruminants, as it has a low production cost and nutritional value comparable to other residues used for this purpose.

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