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Pineapple and citrus silage as potential feed for small ruminant diets: fermentation characteristics, intake, nutrient digestibility, and aerobic stability¤

Ensilaje de piña y cítricos como posible fuente de alimento en dietas de pequeños rumiantes: características de fermentación, consumo, digestibilidad de nutrientes, y estabilidad aeróbica

Ensilagem de abacaxi e cítricos como possível fonte de alimento em dietas para pequenos ruminantes: características de fermentação, o consumo, a digestibilidade de nutrientes e estabilidade aeróbia

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Summary

Background: fruit by-products represent a feed resource for ruminants. However, preservation is needed to increase its life span. **Objectives:** to evaluate the fermentative characteristics, intake, digestibility and aerobic stability of fruit by-products. **Methods:** pineapple and citrus residues were fermented for 0, 4, 7, 11, 29 and 65 days (d). Samples from each by-product and fermentation period were analyzed for pH, microbial succession, chemical composition, and fermentation products. Crossbred rams were used to determine dry matter (DM) and crude protein (CP) intake and digestibility. Dietary treatments consisted of 100% tropical grass hay (TGH) and 20% substitution of TGH with pineapple (PS) or citrus silage (CS). Aerobic stability of PS and CS after 29 or 65 d of fermentation was determined during 5 d. **Results:** final pH at 65 d was 3.21 and 3.32 for PS and CS, respectively. During the entire fermentation for both silages, population of enterobacteriaceae was not detected, while lactic acid producing bacteria, yeast and molds showed typical microbial growth. After 65 d fermentation, lactic acid was the main product associated with the fermentation process (1.0 and 1.7 g/kg for PS and CS respectively). Concentrations of acetic acid were 0.38 in PS and 0.36 g/kg in CS. Rams consumed 98 and 85% of the DM offered as PS or CS, respectively. The DM and CP intakes and digestibility were similar among treatments. Both fermented fruit by-products were unstable upon aerobic exposure, PS after 1 d when fermented 29 d and CS after 3 d when fermented 65 d. **Conclusions:**

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results indicate that pineapple and citrus by-products could be preserved as silage and included in sheep diets at 20% substitution of TGH without adverse results; however, they are susceptible to aerobic deterioration.

Key words: *anaerobic fermentation, fruit by-products, organic residues, sheep.*

Resumen

Antecedentes: los subproductos de fruta representan una fuente de alimento para los rumiantes, sin embargo su preservación es necesaria para aumentar su vida útil. **Objetivos:** evaluar las características fermentativas, consumo, digestibilidad y estabilidad aeróbica de subproductos de frutas. **Métodos:** residuos de piña y cítricos se fermentaron durante 0, 4, 7, 11, 29 y 65 días (d). Muestras de cada subproducto y período de fermentación se analizaron para determinar pH, sucesión microbiana, composición química, y productos de fermentación. Carneros mestizos se utilizaron para determinar el consumo y digestibilidad de materia seca (MS) y proteína bruta (PB). Los tratamientos consistieron en: 100% heno de gramínea tropical (HGT); 20% sustitución de HGT con ensilaje de piña (EP) o ensilaje de cítricos (EC). La estabilidad aeróbica del EP y EC después de 29 o 65 días de fermentación se determinó durante 5 d. **Resultados:** el pH final al día 65 fue de 3,21 y 3,32 para EP y EC, respectivamente. Durante toda la fermentación y para ambos ensilajes, no se detectaron poblaciones de enterobacteriaceae, mientras que las bacterias productoras de ácido láctico, levaduras y hongos mostraron un crecimiento microbiano típico. Después de 65 d de fermentación, el ácido láctico era el producto principal asociado con el proceso de fermentación (1,0 y 1,7 g/kg para EP y EC, respectivamente). Las concentraciones de ácido acético fueron 0,38 g/kg en EP y 0,36 g/kg en EC. Los carneros consumieron 98 y 85% de la MS ofrecida como EP o EC, respectivamente. El consumo y la digestibilidad de MS y PB fueron similares entre los tratamientos. Ambos subproductos de fruta fermentados fueron inestables a la exposición aeróbica, el EP después del primer día cuando se fermenta 29 d y el EC después de 3 d cuando se fermenta 65 d. **Conclusiones:** los resultados indican que los subproductos de piña y cítricos podrían ser preservados como ensilaje y que podrían ser incluidos en las dietas de ovejas a 20% de sustitución de HGT sin resultados adversos, sin embargo, son susceptibles al deterioro aeróbico.

Palabras clave: *fermentación anaeróbica, ovejas, residuos orgánicos, subproductos de frutas.*

Resumo

Antecedentes: os subprodutos da agroindústria de frutas são uma fonte de alimento para os ruminantes, mas sua preservação é necessária para aumentar a vida útil. **Objetivos:** avaliar as características fermentativas, consumo, digestibilidade e estabilidade aeróbia dos subprodutos de frutas. **Métodos:** resíduos de abacaxi e frutas cítricas foram fermentados durante 0, 4, 7, 11, 29 e 65 dias (d). Amostras de cada subproduto e os períodos de fermentação foram analisadas para: pH, sucessão microbiana, composição química, e produtos de fermentação. Um quadrado latino 3 x 3, com nove carneiros mestiços foi usado para determinação de consumo e digestibilidade da matéria seca (MS) e proteína bruta (PB). Os tratamentos dietéticos utilizados foram: 100% feno de capim tropical (FCT) e 20% de substituição do FCT com silagem de abacaxi (SA) ou silagem de cítricos (SC). A estabilidade aeróbia de SA e SC depois de 29 ou 65 d de fermentação foi determinada durante 5 d. **Resultados:** o pH final (65 d) foi de 3,21 e 3,32 para o SA e SC, respectivamente. Durante a fermentação para as duas silagens, a população de enterobactérias não foi detectada. Enquanto a bactérias produtoras de ácido láctico, leveduras e fungos as silagens mostraram um crescimento microbiano típico. Depois de 65 d de fermentação, o ácido láctico era o produto principal associado com o processo de fermentação (1,0 e 1,7 g / kg para SA e SC, respectivamente). As concentrações de ácido acético foram 0,38 g / kg em SA e 0,36 g / kg em SC. Os carneiros consumiram 98 e 85% da MS oferecida como SA ou SC, respectivamente. O consumo e a digestibilidade da MS e PB foram semelhantes entre os tratamentos. Os dois subprodutos de frutas fermentados foram instáveis após a exposição aeróbia, a SA depois de 1 d, quando foi fermentada 29 d e a SC depois de 3 d, quando foi fermentada 65 d. **Conclusões:** os resultados indicam que os subprodutos de abacaxi e cítricos poderiam ser preservados como silagem e serem incluídos em dietas de ovinos em 20% de substituição do FCT sem resultados adversos, ainda que, tem que ter cuidado porque as silagens são susceptíveis à deterioração aeróbia.

Palavras chave: *fermentação anaeróbica, ovelhas, resíduos orgânicos, subprodutos de frutas.*

Introduction

Tons of by-products are generated as a result of processing fresh fruits, such as pineapple (*Ananas comosus*) and orange (*Citrus sinensis*) for juice and canned fruit. These by-products, rich in fermentable sugars, organic acids, and fiber, have high digestibility potential (Jetana *et al.,* 2009; Migwi *et al.,* 2001). These characteristics make fruit by-products a potential feed resource for small ruminants. Due to the high water content of these by-products, preservation is necessary to increase their life span and reduce their potential for environmental contamination.

Preservation of various types of organic by-products by anaerobic fermentation represents a simple, low cost alternative that can be conducted by the farmer (Scerra *et al.*, 2001). However, there is scarce information available on anaerobic fermentation as a method to preserve fruit residues for potential use in diets for small ruminants. Feedstuffs preserved by anaerobic fermentation deteriorate once exposed to aerobic conditions (Danner *et al.*, 2003). Thus, evaluation of a new fermented by-product should include not only its ability to ferment but also its aerobic stability, which is indicative of its preservation during the feeding phase.

Small ruminant production in the tropics often depends on low-quality grasses (low crude protein and high neutral detergent fiber), which is usually aggravated by their scarce availability during the dry season (Pitman, 2001). This situation demands the search for alternative resources (Rodríguez, 1996) to supply nutrient requirements without affecting animal performance.

The objective of the present study was to evaluate fermentation characteristics and aerobic stability of pineapple and citrus by-products preserved as silage. Effects of replacing tropical grass hay (TGH) with 20% pineapple or citrus by-product silages on dry matter (DM) and crude protein (CP) intake and digestibility in rams were also evaluated.

Materials and Methods

By-product silage preparation

Preparation and characterization of the fermentation processes were conducted in the Animal Nutrition Laboratory at the University of Puerto Rico, Mayagüez Campus. Pineapple and citrus by-products were collected from processing plants the same day they were generated. Residues consisted of fruit parts remaining after processing pineapples and oranges for juice or canned fruit (pineapples). The pineapple by-product consisted of skin and crown pieces, while the citrus by-product was composed of skin, remnants of pulp, and seeds. By-products were fermented in 15 polyvinyl chloride (PVC) laboratory micro-silos (3 kg capacity). The micro-silos (equipped with gas-releasing valves) were manually packed and kept at room temperature (28 ˚C to 30 ˚C) until further chemical and biological analysis. In addition, by-products were fermented in 19 L containers (equipped with gas-releasing valves) for at least 30 d before the feeding trial. Fresh samples (n = 3) from each by-product were collected and analyzed for initial chemical and biological characteristics before ensiling.

Microbiological and chemical evaluation of silages

Triplicate silos of each by-product were opened after 0, 4, 7, 11, 29, and 65 d of fermentation. Samples were analyzed for pH and microbial succession, as described by González and Rodríguez (2003). Prepared extracts were analyzed for organic acids (lactic, acetic, propionic, and butyric) produced during the by-product fermentation periods by a commercial laboratory (Dairy One Forage Lab, Ithaca, NY, USA). Ammoniacal nitrogen (NH_3-N) content was determined by an oxidation method (Strickland and Parson, 1972) to calculate NH_3-N/ total nitrogen (TN).

Samples were oven dried (55 ºC), ground to pass through a 1-mm screen, and analyzed for DM (AOAC, 1990) to determine the nutrient content of fresh and fermented by-products. Nitrogen concentration was determined by the Kjeldhal

method (AOAC, 1990) and CP was calculated as N multiplied by 6.25. Cell wall components (neutral detergent fiber, NDF; and acid detergent fiber, ADF) were determined sequentially via the crucible method (Van Soest *et al*., 1991). Content of water-soluble carbohydrates (WSC) was determined by the colorimetric method, as described by Dubois *et al*. (1956).

Voluntary intake and nutrient digestibility

Nine crossbreed rams (BW = 22.7 ± 3.3 kg) were randomly assigned to treatments and confined to individual metabolism crates. Fermented by-products substituted 20% of the estimated DM intake of TGH, which was presumed to be 3% of animal body weight (BW) on a DM basis.

The TGH was mechanically shredded to a particle size of approximately 5 cm before being fed to animals. Dietary treatments consisted of TGH (T1), TGH+PS (T2), and TGH+CS (T3). Soybean meal was also provided, increasing dietary CP contents to 8%. Hay and soybean meal were offered mixed while fermented by-products were separately but simultaneously offered. The diets were offered during three periods, including 8 d devoted to adapt animals to the feed and management routine and 5 d for total collection of feces.

During collection, offered feed and orts were sampled and total production of feces was collected and weighted. Samples (10% aliquot) of feed, orts, and feces were collected daily from each ram and assayed for DM and CP as previously described.

Aerobic stability of fermented by-products

The aerobic stability of PS and CS was evaluated after 29 and 65 d of fermentation. Samples of each fermented by-product (1 kg) were placed in plastic bags into styrofoam containers and exposed to room temperature air (27 ˚C to 30 ˚C) for 5 d. A thermometer was placed at the center of each silage mass to monitor temperature every 6 h during the first 48 h and every 8 h thereafter. Fermented by-products were considered spoiled when the air-exposed silage temperature reached two degrees above room temperature. Samples of fermented by-products were collected after 0, 1, 3, and 5 d of aerobic exposure to determine pH, total bacteria (TB), yeast, and mold (YM) populations, as described by González and Rodríguez (2003).

Experimental design and statistical analysis

Microbiological and chemical data of each by-product were analyzed separately in a completely randomized design (Lyman and Longnecker, 2001). Dry matter and CP intake and digestibility were analyzed as a 3 x 3 Latin Square with three animals per treatment in each of the three periods. A general linear model was used (SAS, 1990) and treatment means were compared with the Bonferroni t-test (significant differences at $p<0.05$). Aerobic stability data of fermented by-products were analyzed as a completely randomized design in a 2 x 4 factorial arrangement of treatments (29 or 65 d fermentation periods; 0, 1, 3, and 5 d of aerobic exposure) for pH and microbial succession, while temperature data involved 2 fermentation periods x 15 temperature readings. Data were statistically analyzed using the PROC MIXED procedure of SAS (SAS, 1990). When significant differences were detected, the LSMEANS statement was used to separate multiple means with the PDIFF option (SAS, 1990).

Results

Microbiological and chemical characterization of fresh by-products

Both fresh by-products (pineapple and citrus) showed acidic or slightly acidic pH (Table 1). Despite the high acidity in fresh pineapple by-products, moderate populations of enterobacteria (ENT) and yeasts and molds (YM) with increased lactic acid-producing bacteria (LAPB) were observed. There were moderate populations of LAPB and YM, while ENT colonies were not detected in the fresh citrus by-products.

Dry matter content of pineapple by-product was 129.6 g/kg, while citrus by-products had 242.8 g/kg DM. Both by-products had an optimal content of water-soluble carbohydrates (WSC; > 40 g/kg DM), which facilitates fermentation, and low CP content (< 60 g/kg DM). In addition, fresh pineapple and citrus by-products had 433.7 and 162.8 g NDF/kg

DM, respectively and 218.9 and 137.2 g ADF/kg DM, respectively.

Microbiological and chemical characterization of pineapple silage

Acidity of the pineapple by-product varied throughout the fermentation process. Enterobacteriaceae colonies were not detected ($p<0.05$) at any time during fermentation, which was not the case for the fresh residue. The LAPB and YM populations did not change statistically throughout the fermentation. In addition, by-product chemical composition did not show defined change patterns during fermentation.

Lactic acid content of fermented pineapple by-product was higher ($p<0.05$) than that of fresh residue after the fourth day of fermentation and remained relatively stable thereafter. Acetic acid concentration followed a similar pattern, being the highest at the fourth day of fermentation and then remaining relatively constant. Propionic and butyric acids were not detected throughout the fermentation process. The NH_3-N/TN ratio remained nearly constant until day 7 and decreased slowly thereafter.

Table 1. Fermentation-period effects on pH, microbial population, chemical composition, and fermentation end-products from pineapple and citrus by-products.

Item[1]	PINEAPPLE								CITRUS							
	Fermentation period[3] (days)						SEM[4]	P-value	Fermentation period[3](days)						SEM[4]	P-value
	0	4	7	11	29	65			0	4	7	11	29	65		
pH	3.7^{a}	3.2^{cd}	3.2^{d}	3.4^{b}	3.3^{c}	3.2^{d}	0.02	0.01	5.4^{a}	3.9^{b}	3.7^{bc}	3.5b^{c}	3.5^{c}	3.3^{c}	0.09	0.01
Microbial populations (cfu/g)																
Enterobacteriaceae	6.1^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0.09	0.01	ND	ND	ND	ND	ND	ND	ND	ND
Lactic acid producing bacteria	7.2	6.3	5.3	8.4	5.9	2.5	2.05	0.23	5.8^{ab}	7.7^{a}	7.5^{a}	7.2^{a}	4.6^{b}	4.4^{b}	0.8	0.03
Yeast and molds	5.3	4.6	3.4	3.6	2.9	1.5	0.85	0.15	5.3	4.1	2.6	2.7	4.0	3.2	1.29	0.68
Chemical composition (g/kg)[2]																
Dry matter	129.6^{a}	107.8^{b}	109.1^{b}	105.2^{b}	103.9^{b}	102.1^{b}	2.1	0.01	242.8^{a}	195.3^{b}	197.6^{b}	187.3b	190.1^{b}	187.3^{b}	6.2	0.01
Crude protein	38.6^{b}	41.2^{ab}	39.8^{ab}	43.7^{ab}	44.7^{ab}	50.1^{a}	0	0.02	57.8	61.9	59.6	63.9	64.6	65.1	3.5	0.68
Neutral detergent fiber	433.7^{b}	512.2^{a}	515.1^{a}	493.8^{ab}	489.7^{ab}	457.5^{b}	8.1	0.01	162.8^{b}	228.4^{a}	234.3^{a}	246.6^{a}	242.9^{a}	237.2^{a}	4.9	0.01
Acid detergent fiber	218.9^{b}	292.4^{a}	315.4^{a}	302.7^{a}	276.0^{ab}	272.1^{ab}	10.7	0.01	137.2^{c}	207.3^{b}	215.7^{ab}	213.4^{ab}	226.9^{ab}	232.5^{a}	4.4	0.01
Water soluble carbohydrates	61.1	39.1	53.0	34.7	36.3	73.1	9.7	0.08	56.8^{a}	20.9^{b}	18.8^{b}	37.1^{ab}	42.0^{ab}	54.5^{a}	4.6	0.01
Fermentation end-products (g/kg)[2]																
Lactic acid	0.01^{b}	1.00^{a}	1.05^{a}	0.89^{a}	1.03^{a}	1.01^{a}	0.4	0.01	0.02^{c}	1.13^{b}	1.17^{b}	1.01^{b}	1.2^{b}	1.7^{a}	0.4	0.01
Acetic acid	0.12^{c}	0.44^{a}	0.40^{ab}	0.32^{b}	0.33^{ab}	0.39^{ab}	0.2	0.01	0^{c}	0.18^{b}	0.20^{b}	0.24^{b}	0.27^{b}	0.36^{a}	0.1	0.01
Propionic acid	0	0	0	0	0	0.01	0	0.24	0^{b}	0^{b}	0^{b}	0.006^{ab}	0.01^{a}	0.01^{a}	0	0.01
Butyric acid	0	0	0	0	0	0.001	0	0.27	ND	ND	ND	ND	ND	ND	ND	ND
NH_3 -N/total N	2.1	2.0	2.1	1.9	1.8	1.6	1	0.34	1.4	1.3	1.3	1.3	1.3	1.2	0.1	0.39

[1]N=3; [2]Expressed on a dry matter basis; [3]Significant differences ($P<0.05$) within the same row are indicated by different letters ($^{a, b, c}$); [4]Standard error of the mean; ND= not detected.

Microbiological and chemical characterization of citrus silage

The citrus by-product pH decreased significantly ($p<0.05$) during the initial 7 d of fermentation and changed little from there until 29 or 65 d. No ENT colonies were detected either in the fresh or fermented by-product. The LAPB were dominant during the first 10 d, but decreased by day 29. Yeasts and mold populations did not change significantly during the fermentation process. Initial DM in citrus residue was higher than that of fermented residue, however it did not change significantly during fermentation. Similar to pineapple by-product, there were no evident patterns in chemical composition changes with time for citrus by-product during fermentation. The

CP and WSC contents varied somewhat during the fermentation process.

Lactic acid content was higher after 4 d of fermentation compared to that of fresh residue. Contrary to what was observed during pineapple fermentation, fermentation of citrus by-product produced more lactic acid during d 65 than at a shorter period (29 d). A low but gradually increasing content of acetic acid was detected. Propionic acid was very low, and no production of butyric acid was detected during fermentation. As with pineapple by-product, the NH_3- N/TN ratio of citrus by-product remained nearly constant during 65 d of fermentation.

Intake and nutrient digestibility of fermented by-products

Table 2 presents the chemical composition of ingredients used in the experiment. As expected, both fermented fruits had lower DM (99.6 and 187.1 g DM/kg for PS and CS, respectively) than TGH (887.3 g DM/kg). Inclusion of 20% PS or CS did not affect ($p>0.05$) total DM intake of TGH plus supplemental silage (Table 3). In this experiment, PS and CS intake represented 22.9% and 19.6% of the total DM consumption, respectively. Dry matter and CP digestibility coefficients ranged from 0.52 to 0.56 and 0.43 to 0.51, respectively. No significant differences among treatments for DM and CP digestibility were observed.

Aerobic stability of fermented by-products

Pineapple by-product fermented for 65 d showed a slow acidity decrease during exposure to air compared to 29 d (Table 4). These pH changes were evident after aerobic exposure for 3 d. Proliferation of YM during the first day of aerobic exposure was greater ($p<0.05$) in PS exposed after 29 d than after fermentation for 65 d (Table 4). This 3.80 cfu/g increase in the residue fermented for 29 d could be the main cause for its faster aerobic deterioration. Total bacteria population was also greater ($p<0.05$) after d 3 of aerobic exposure for pineapple by-product fermented 29 d compared to 65 d. Temperature increase with duration of aerobic exposure was also observed for PS, regardless of fermentation length. However, fermenting PS for 65 d delayed the increase of reaching two degrees above room temperature from 1.25 d to 2.0 d.

Table 2. Chemical composition of ingredients.

Chemical composition (g/kg)[1]	Ingredients[2]				SEM[3]
	TGH	PS	CS	Soybean meal	
Dry matter	887.3[a]	99.6[c]	187.1[b]	884.0[a]	4.7
Organic matter	896.2[c]	942.8[a]	950.2[a]	928.4[b]	1.4
Inorganic matter	103.8[a]	57.2[c]	49.8[c]	71.6[b]	1.4
Crude protein	40.0[b]	53.5[b]	75.6[b]	463.7[a]	24.6
Neutral detergent fiber	745.5[a]	613.0[a]	247.1[b]	188.9[b]	28.3
Acid detergent fiber	467.8[a]	347.9[b]	241.3[b]	49.9[c]	21.0
Hemicelullose[4]	277.8[a]	265.1[a]	13.9[c]	139.0[b]	13.5

[1]Dry matter basis. [2]Means with different letters within a row indicates significant differences ($p<0.05$). [3]Standard error of the mean. [4]Calculated by difference (NDF- ADF).

Table 3. Diet intake and digestibility of DM and CP.

Item	T1	T2			T3			SEM[3]
	TGH[1]	Total	PS[2]	TGH[1]	Total	CS[2]	TGH[1]	
Dry matter								
Intake (g/d)	501.4	471.2	108.0	363.2	507.9	99.5	408.4	44.0
Digestibility	0.52	0.56			0.547			0.17
Crude protein								
Intake (g/d)	29.0	27.7	5.4	22.3	32.4	7.6	24.8	3.3
Digestibility	0.43	0.44			0.51			0.12

[1]TGH intake includes soybean meal (DM = 884.0 g/kg; CP= 463.7 g/kg DM). There was no soybean meal refusal. [2]Represents 20% of TGH substitution. [3]Standard error of the mean.

Table 4. Aerobic exposure effects on pH, microbial population, and temperature for fermented pineapple and citrus by-products.

Item	Days of exposure	PINEAPPLE						CITRUS					
		Fermentation period		SED[3]	P-value			Fermentation period		SED[3]	P-value		
		29	65		F[4]	AE[5]	F x AE[6]	29	65		F[4]	AE[5]	F x AE[6]
pH	0	3.3^{C1}	3.2^{C}	0.05	0.01	0.01	0.01	3.4^{B1}	3.3	0.04	0.01	0.01	0.01
	1	3.4^{C}	3.2^{C}					3.5^{Ba2}	3.3^{b}				
	3	3.8^{Ba2}	3.5^{Bb}					3.5^{Ba}	3.3^{b}				
	5	4.2^{A}	3.7^{A}					3.8^{Aa}	3.3^{b}				
Microbial population (cfu/g)													
Total bacteria	0	0^{D}	0^{C}	0.19	0.01	0.01	0.01	0^{C}	0	0.27	0.43	0.01	0.03
	1	3.9^{C}	3.9^{B}					3.8^{AB}	2.6				
	3	5.5^{Ba}	4.0^{Bb}					2.9^{B}	2.9				
	5	6.1^{A}	5.7^{A}					4.0^{A}	2.6				
Yeasts and molds	0	2.9^{B}	1.7^{B}	0.82	0.16	0.01	0.02	4.0	3.2	0.70	0.99	0.46	0.56
	1	6.7^{Aa}	2.7^{Bb}					2.9	3.3				
	3	7.2^{A}	7.3^{A}					1.9	4.0				
	5	6.4^{A}	8.0^{A}					4.5	3.9				
Temperature (˚C)	0	21.7^{Hb}	26.7^{Ea}	0.92	0.47	0.01	0.01	25.0^{I}	26.5^{EF}	0.67	< 0.01	< 0.01	< 0.01
	0.25	23.3^{GHb}	27.0^{Ea}					26.3^{HI}	25.9^{F}				
	0.5	25.0^{G}	27.0^{E}					26.5^{HI}	26.3^{EF}				
	0.75	25.9^{FG}	27.2^{E}					26.9^{GHI}	26.1^{F}				
	1	27.8^{EF}	26.7^{E}					27.0^{GH}	25.6^{EF}				
	1.25	30.2^{DEa}	25.7^{Eb}					28.7^{FGa}	26.3^{Eb}				
	1.5	31.1^{CDa}	26.3^{Eb}					31.7^{DEa}	26.3^{EFb}				
	1.75	33.1^{ABCa}	26.9^{Eb}					35.2^{ABa}	28.2^{Eb}				
	2	34.4^{ABa}	30.2^{Db}					35.9^{ABa}	32.2^{Cb}				
	2.5	35.7^{A}	36.7^{BC}					36.7^{Aa}	33.7^{ABCb}				
	2.75	34.6^{AB}	35.9^{A}					36.7^{Aa}	30.2^{Db}				
	3.5	31.5^{ABC}	34.6^{AB}					33.2^{DE}	33.0^{CD}				
	4	31.1^{CDb}	31.7^{ABa}					34.1^{CD}	34.6^{BC}				
	4.5	32.0^{CD}	31.3^{CD}					34.4^{BC}	35.0^{AB}				
	5.0	32.1^{BCD}	30.9^{D}					30.5^{EFb}	35.0^{Aa}				

[1]Significant differences (p<0.05) within the same column are indicated by different upper case letters ($^{A, B, C}$); [2]Significant differences (p<0.05) within the same row are indicated by different lowercase letters ($^{a, b, c}$); [3]Standard error of the difference; [4]Effect of fermentation period; [5]Effect of aerobic exposure; [6]Interaction between fermentation period and aerobic exposure.

Independent of fermentation length, the CS pH remained constant during the first 3 d of aerobic exposure (Table 4). However, a greater decrease (p<0.05) in acidity was observed at the fifth day of aerobic exposure when citrus residues were fermented for 29 d compared to 65 d. No significant changes in YM and TB populations were observed for CS at either fermentation length. It took 1.5 and 2.0 d to CS exposed to the air to reach two degrees above the room temperature when fermented for 29 d (31.7 °C) or 65 d (32.2 °C), respectively. In addition, it was observed that fermentation during d 65 delayed the rise in CS temperature exposed to the air for half a day.

Discussion

The low DM content of both pineapple and citrus by-products presupposed difficulties in their preservation by anaerobic fermentation. However, results from this experiment demonstrated that 129.6 and 242.8 g/kg DM for pineapple or citrus by-products, respectively, did not preclude a favorable fermentation process. A pH below 4.2 is considered to represent acidity sufficient to avoid proliferation of undesirable microorganisms (Ting and Attaway, 1971). The acidity found in the pineapple and citrus by-products is typical of ripen fruits and results from the presence of organic acids,

mainly citric, malic, ascorbic and tartaric (Falade *et al.*, 2003; Bartolomk *et al.*, 1995). Perhaps, the initial acidity of pineapple and citrus by-products and the epiphytic population of LAPB overcame the negative effects of high-water content in the residues by initiating the fermentation process.

Microbial population changes were observed in both by-products throughout the fermentation process. Enterobacteriaceae were only detected in the fresh pineapple by-product. Reduction of ENT population was previously observed when wheat straw, poultry litter, and different levels of citrus pulp were ensiled (Migwi *et al.*, 2001). This indicates that anaerobic condition, total acidity, and the presence of certain organic acids contributed to halt proliferation of these microorganisms. For the citrus by-product, absence of ENT is associated with handling at the processing plant, since the fruit is washed and disinfected prior to processing. Natural occurrence of these facultative anaerobes (McDonald *et al*., 1991) in fruits contributes to a rapid colonization and initiation of the fermentation process, resulting in good preservation of the by-products. The YM population did not vary throughout the fermentation period of both by-products. This is partially contradictory because Okine *et al*. (2007) found that anaerobic conditions and lactic acid accumulation decreased yeasts population.

Some previous research evaluating fruit by-products present similar results, but more often a different chemical composition was found (Tripodo *et al.*, 2004; Gutiérrez *et al.*, 2003). In the present case, CP, NDF and ADF contents varied during the fermentation process with no defined pattern, which could indicate an active and variable microbial ecology. It is also necessary to consider that fruit by-products are not uniform residues; they are characterized by different proportions of skin, remnants of pulp and, in some cases, pieces of crowns or seeds. This can make it difficult to define a pattern of change for chemical composition during the fermentation period. For pineapple by-products, no significant changes throughout the fermentation process were observed in WSC concentration, while a significant reduction was observed during citrus by-product fermentation. These results indicate that pineapple and citrus by-products (even with the reduction observed for CS) contain enough WSC to promote acceptable fermentation that allows preservation of the residues.

Despite the greater LAPB numbers observed in fruit by-products compared with tropical grasses (González and Rodríguez, 2003), the lactic acid concentration of the resulting silage was lower. Lower concentrations of lactic acid could result from the presence of lactate utilizing yeast. However, lactic acid was the main acid present throughout the fermentation period of the by-products, suggesting that the LAPB present were mainly homofermentative (McDonald *et al.*, 1991). The absence of propionate and butyrate in addition to the constant NH_3- N/TN ratio indicate a stable fermentation process in both by-products, free from secondary degradation of proteins. Moreover, these results suggest that undesired secondary fermentations, typical of tropical forages (González and Rodríguez, 2003), are less likely to occur during fermentation of these fruit by-products.

During the trials animals consumed 98.8% (109.3 g/d offered) and 85.8% (115.9 g/d offered) of DM from the fermented pineapple and citrus by-products, respectively, indicating good organoleptic characteristics and acceptability of both silages.

Related studies elsewhere have reported benefits of including dried citrus by-products in the digestibility of straw (Barrios- Urdaneta *et al.*, 2003). However, in the present study substitution of TGH with 20% fermented citrus or pineapple by-product did not increase DM or CP digestibility. A possible explanation for the lack of effect on digestibility of the diets is that the level of fermented by-product substitution used (20%) was not enough to improve digestibility of the nutrients considered. Experiments that evaluate intake and digestibility resulting from higher PS and CS inclusion levels in TGH-based diets would clarify this point.

Increase in pH is an indicator of microbial activity responsible for the deterioration of the fermented feedstuff, and thus of aerobic stability (Woolford, 1984). In the present study, both

fermented residues were more stable to aerobic conditions, as evidenced by pH changes after the longer fermentation period (65 d *vs.* 29 d). The type, proliferation, dynamics, and metabolic activity of microorganisms associated with aerobic deterioration (i.e., YM and bacteria) have been considered as determining factors with regard to the instability of fermented feedstuffs once exposed to air. Chemical composition and fermentation end-products (residual WSC, lactic and acetic acids) have also been linked to the proliferation and activity of these microorganisms. Likewise, yeasts that degrade lactic acid have been associated with the initiation of aerobic instability of fermented forages (Filya *et al.*, 2000). Yeast species from the genera *Candida*, *Haensenula*, and *Issatchenkia* degrade lactic acid causing pH and temperature increases and consequent deterioration of the product (Woolford, 1984). The YM population did not change during the entire aerobic exposure period for both fermentation lengths of CS. However, lactic acid content was greater (1.7 g/kg DM *vs.* 1.2 g/kg DM) for CS fermented 65 d *vs.* 29 d. The CS was stable to aerobic conditions during d 3, regardless of fermentation length according to the pH, TB and YM populations. The YM populations reached 7.2 and 4.2 cfu/g for PS and CS, respectively, which indicates that YM were the main microorganisms associated with aerobic deterioration.

Differences in TB population coincided with changes in pH and YM population of PS exposed to air. The rapid pH increase of fermented pineapple by-products over a short period of time was associated with proliferation of opportunistic bacteria, which are favored when acidity of the substrate decreases. However, no significant changes in TB population were observed between the two fermentation lengths for CS. Overall, fermented by-products deteriorate after aerobic exposure for 1 d -based on TB, YM (for PS), and temperature. However, the extended fermentation period (65 d) somewhat delayed the deterioration process, especially in pineapple residues.

In addition, further research is needed to evaluate whether adjusting the DM content of fruit by-products or using additives can improve fermentation and aerobic stability of the resulting silage. The effect of feeding PS and CS on productive performance of ruminants also awaits research attention.

Finally, despite the high moisture content of pineapple and citrus by-products, silage production is a viable and potentially beneficial alternative for their preservation, as indicated by the fermentation products and the chemical and microbiological composition of fresh and ensiled materials. The present results indicate that 20% substitution of TGH with PS or CS did not affect DM and CP intake or digestibility. It was also demonstrated that fermented pineapple and citrus residues were susceptible to aerobic deterioration, especially resulting from the short fermentation period (29 d).

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