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Effect of a microbial inoculant on fermentation characteristics, aerobic stability, intake, and digestibility of corn silage by rams¤

Efecto de un inóculo microbiano sobre las características fermentativas, estabilidad aeróbica, consumo y digestibilidad de ensilaje de maíz por carneros

Efeito da adição de um inoculo microbiano sobre as características de fermentação, estabilidade aeróbica, digestibilidade e consumo de silagem de milho em ovelhas

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Summary

Background: ensiling of whole-plant corn in tropical environments generally results in poor fermentation, and silages are susceptible to aerobic deterioration resulting in low acceptability by the animals. **Objective:** to determine the effect of a microbial additive on ensiling characteristics, aerobic stability, intake and digestibility by rams of diets containing corn silage. **Methods:** whole-plant corn (*Zea mays* L. vr. Mayorbella; 38.26% of DM; 3.8-5.0 cm length) was ensiled with or without a microbial starter in 208 L-capacity plastic bags. Triplicate samples of vegetative material were analyzed for pH and fermentation products after 0, 7, 13, 28, and 45 d. Aerobic stability of the fermented forage was determined by measuring increases in pH and temperature after 72 and 96 h, respectively. The DM, CP and NDF intake and digestibility of diets containing the silages were determined using ten crossbred rams. Diets consisted of 50% grass hay and 50% of either inoculated or control silage fed separately. **Results:** corn fermented with the additive had lower ($p<0.05$) pH (3.6 vs. 3.9) and higher ($p<0.05$) lactic acid content than untreated silage (1.70 vs. 1.29). Inoculation delayed the aerobic deterioration of the silage as evidenced by lower ($p<0.05$) pH and temperature. Intake of DM, CP, and NDF as a proportion of total dietary DM, CP, and NDF consumption increased ($p<0.05$), as did CP digestibility ($p<0.05$) by 6.20% units, in rams fed hay- treated silage-SBM diets. **Conclusions:** ensiling tropical corn with the microbial additive enhanced fermentation, delayed aerobic deterioration, and improved dietary CP digestibility by rams.

Keywords: *additive, ensiling, forage, nutritive value.*

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Resumen

Antecedentes: el ensilaje de la planta entera de maíz en ambientes tropicales generalmente resulta en fermentaciones pobres y los ensilajes son susceptibles al deterioro aeróbico y a la baja aceptabilidad por parte de los animales. **Objetivo:** determinar el efecto de un aditivo microbiano sobre las características fermentativas, la estabilidad aeróbica y el consumo y la digestibilidad por carneros alimentados con dietas que contenían ensilaje de maíz. **Métodos:** plantas enteras de maíz (*Zea mays* L. vr. Mayorbella; 38,26% de MS; con 3,8 a 5,0 cm de largo) fueron ensilados con o sin la adición del iniciador microbiano en bolsas plásticas con capacidad de 208 L. Después de 0, 7, 13, 28 y 45 d se analizaron en triplicado muestras del material vegetativo por tratamiento para determinar el pH y productos de fermentación. La estabilidad aeróbica del forraje fermentado se determinó utilizando como criterios incrementos en el pH y la temperatura después de 72 y 96 h, respectivamente. El consumo y la digestibilidad de MS, proteína bruta (PB) y fibra detergente neutra (FDN) de las dietas con el ensilaje se determinó usando diez carneros alimentados con dietas que consistieron de 50% de heno de gramínea y 50% del ensilaje con o sin inóculo y ofrecidos por separado. **Resultados:** el pH del forraje fermentado con el aditivo microbiano fue menor ($p<0,05$; 3,6 vs. 3,9) y el contenido de ácido láctico mayor ($p<0,05$; 1.70 vs. 1.29) que en el ensilaje no tratado. La inoculación atrasó el deterioro aeróbico del ensilaje resultante evidenciado por el menor ($p<0,05$) pH y temperatura. El consumo de MS, PB y FDN procedentes del ensilaje como proporción del consumo total de MS, PB y FDN y la digestibilidad de la PB fue mayor ($p<0,05$) en 6,20 unidades porcentuales, en ovinos alimentados con la dieta de heno-ensilaje-soya conteniendo el producto microbiano. **Conclusiones:** el ensilaje de maíz tropical tratado con el aditivo microbiano, mejoró las características fermentativas, atrasó el deterioro aeróbico y aumentó la digestibilidad de PB dietética por carneros.

Palabras clave: *aditivos, ensilaje, forraje, silage, valor nutritivo.*

Resumo

Antecedentes: a silagem da planta inteira de milho cultivado em ambientes tropicais geralmente resulta em fermentações pobres nutricionalmente e silagens susceptíveis à deterioração aeróbia, com uma baixa consequente da aceitabilidade pelos animais. **Objetivos:** determinar o efeito de um aditivo microbiano sobre as características fermentativas e a estabilidade aeróbia e o consumo e a digestibilidade por carneiros alimentados com dietas incluindo silagem de milho tropical. **Métodos:** plantas inteiras de milho tropical (*Zea mays* L. vr Mayorbella; 38,26% MS; 3,8-5,0 cm de comprimento), e se fez silagem com e sem adição do fermento microbiano em sacos plásticos com capacidade de 208 L. Após de 0, 7, 13, 28 e 45 d analisaram-se por triplicado amostras de material vegetativo por tratamento para determinar o pH e os produtos da fermentação. A estabilidade aeróbia da forragem fermentada determinou-se utilizando como critérios, incrementos no pH e na temperatura após 72 e 96 h, respectivamente. O consumo de MS, PB e a FDN das dietas que incorporavam silagem determinou-se utilizando dez carneiros alimentados com dietas que consistiram de 50% de feno de gramínea tropical e 50% de silagem de milho com e sem o inoculo e oferecido por separado. **Resultados:** o pH da forragem fermentada com o aditivo microbiano foi menor ($p<0,05$; 3,6 vs. 3,9) e o conteúdo de ácido láctico maior ($p<0,05$; 1,70 vs. 1,29) que na silagem não aditivada. A inoculação atrasou o deterioro aeróbico da silagem resultante, evidenciado pelo menor pH ($p<0,05$) e temperatura. O consumo de MS, PB e FDN procedentes da silagem como proporção do consumo total de MS, PB y FDN e a digestibilidade da PB foi maior ($p<0,05$) por 6,20 unidades porcentuais, nos carneiros alimentados com a dieta de feno-silagem-soja, contendo o produto microbiano. **Conclusões:** a silagem de milho cultivado no trópico e tratado com o aditivo microbiano melhorou as características fermentativas, atrasou o deterioro aeróbico e aumentou a digestibilidade da PB da dieta dos carneiros que se alimentaram com esta ração.

Palavras chave: *aditivos, ensilagem, forragem, valor nutricional.*

Introduction

Additives containing lactic acid-producing bacteria (LAB) have long been used in silages to improve fermentation characteristics, aerobic stability, and animal performance (Muck, 1988). The expected changes during the ensiling process with the use of microbial additives containing LAB include the dominance of these microorganisms during the fermentation process, an increase in the ratio of lactic acid to other fermentation products (i.e. acetic acid, ethanol), faster pH decline, reduction of proteolysis, and increased DM recovery (McDonald, *et al*., 1991).

The advantages of using biological inoculants obtained by suitable selection of homofermentative (i.e. *Lactobacilus plantarum*) or heterofermentaive LAB strains (i.e. *Lactobacillus buchneri*) have been reported by various researchers and experimental results show that inoculants have a beneficial effect on fermentation quality and aerobic stability of silages (Johnson *et al*., 2003; Hu *et al*., 2009; Cao *et al*., 2011; Lynch *et al*., 2012). Microbial inoculants contaning homo-fermentative LAB strains (i.e. *Lactobacillus plantarum*) improved the ensiling of corn silage by decreasing pH and shifting fermentation toward lactic acid production (Johnson *et al*., 2003; Hu *et al*., 2009; Cao *et al.,* 2011). Likewise, the use of microbial products contaning hetero-fermentative strains have been associated with delayed deterioration after silage is exposed to air (Filya, 2003). Differences have been noted in chemical composition and epiphytic microflora of forages harvested in tropical and temperate environements (Van Soest, 1994; Rodríguez, 1996). The inherent characteristics of tropical forages -such as high content of cell-wall components, low content of water soluble carbohydrates, and high populations of undesirable epiphytic microflora (i.e. yeast, coliforms)- might alter the effectiveness of microbial additives contaning LAB.

More information is needed on the possible beneficial effects of inoculants containing mixtures of homo and hetero-fermentaive LAB on ensiling characteristics, aerobic stability and performance of animals fed tropical corn silage. Previous experiments showed that a microbial inoculant containing homo and hetero-fermentative LAB improved fermentation characteristics and aerobic stability of tropical corn ensiled under laboratory conditions (Rodriguez *et al.,* 2009). Tropical corn treated with the inoculant and fermented during 45 d yielded silage with lower ($p<0.05$) pH and higher ($p<0.05$) lactic acid content than untreated silage. Furthermore, microbial addition lowered ($p<0.05$) the NH_3-N/total-N ratio in inoculated silage compared with the control. However, more studies on the effect of corn grown in tropical environments and treated with microbial inoculants need to be conducted. Therefore, this study was aimed to determine the fermentation characteristics, aerobic stability, DM and nutrient intake and digestibility of tropical corn ensiled with a microbial additive containing a blend of homo-fermentative and hetero-fermentative LAB strains by rams.

Materials and methods

Ethical considerations

This study was approved by the Ethics Committee for the Use of Animals of the Agricultural Experimental Station, University of Puerto Rico (August 17, 2011, Project Z-223).

Silage preparation

Tropical corn (*Zea mays* L. vr. Mayorbella) was harvested under farm conditions at 38.2% DM and chopped to 3.8 to 5.0 cm pieces at the University of Puerto Rico (Agricultural Experimental Station), located in the southwestern region of the island (18°01’51.02” N – 67°04’27.05”). Chopped whole-plant corn was ensiled with or without the addition of the microbial starter contaning the LAB strains *Enterococcus faecium, Lactobacillus brevis, and Lactobacillus plantarum*, diluted in distilled water at 1:25 proportions, and applied to weighed portions of forage at the rate recommended by the manufacturer (1×10^{10}cfu/g). Forage was ensiled in 208 L plastic bags (Husky 45-Gallon Heavy Duty Trash Bags, Gran Prairie, TX, USA) sealed with a vacuum pump (Ridgid, Model # WD1451, 14-gal. 6-Peak High Performance Wet/Dry Vacuum, Ridge Tool Company, Elyria, Ohio, USA) and maintained at room temperature (26-28 °C).

Chemical analyses and fermentation parameters

Triplicate samples of fresh forage from each experimental treatment were obtained before ensiling and analyzed to determine initial pH and chemical composition (DM; organic matter, OM; ash, CP, NDF, with α-amylase but not corrected for ash, ADF; and hemicelulose (Ankom Technology Corporation, Macedon, New York, USA) using standard procedures (AOAC, 1990; Van Soest *et al.,* 1991). To determine progressive changes in pH and fermentation products during the ensiling process, triplicate samples of fermented vegetative material treated or not with the microbial inoculant were analyzed after five fermentation periods (0, 7, 13, 28, and 45 d). Samples were prepared using the composite of six core sub-samples from the center of each plastic-bag silo, corresponding to each treatment and fermentation period, using a forage sampler (Penn State Sampler; 3.5 cm diameter and 40 cm length; Nasco, Fort Atkinson, WI). To determine pH, 50 g of silage from each plastic-bag silo at each sampling day were placed into 450 ml of distilled water (w/v) and homogenized for 5 min with a stomacher apparatus (Tekman 3500, Tekman, Cincinnati, OH, USA). Homogenates were strained through eight layers of cheesecloth and analyzed for pH. Homogenates from each treatment and sampling day were analyzed in a commercial laboratory (Dairy One Forage Lab, Ithaca, NY) to determine the fermentation products; lactic, acetic, butyric, and propionic acids using an ion-exchange high performance liquid chromatography (Biorad aminex HPX-87H), and the NH_3-N/Total-N ratio was determined using an oxidation method (Strickland and Parson, 1972)

Aerobic stability

Aerobic stability of the fermented forage was determined by increments in pH and temperature as criteria to assess silage deterioration (Rodríguez, 1996). Triplicate plastic-bags (silos) from each treatment after 45 d fermentation were exposed to air for 3 d. Samples from each treatment were collected for pH analyses after 0, 24, and 72 h of aerobic exposure. Temperature was monitored after 0, 2, 4, 8, 14, 20, 26, 72, 90, and 96 h of aerobic exposure with a thermometer (Sudbury Compost Thermometer, Baled Hay Compost, NASCO, Fort Atkinson, WI, USA) embedded in the surface of the exposed silage.

Nutrient intake and digestibility

Ten cross-bred rams of similar age (12 months old) and weight (BW = 24.3 ± 4.3 Kg) were used to determine the effects of the inoculant treatment of tropical corn silage on DM, CP and NDF intake and digestibility. Rams were dewormed (ivermectin; 1 mL/50 Kg BW) on day -21 before the start of the trial and randomly placed in metabolic stalls, where they had free access to diets and water throughout the study. Animals were allowed a 7-d adaptation period to the facilities while being fed TGH (mixtures of *Panicum maximum*, *Digitaria decumbens* and *Sorghum halapense*) *ad libitum*. After the adaptation period, five animals per treatment were assigned to the corn silage-based diets, treated or untreated with the microbial additive, supplemented with 50g/d (dry matter basis; DMB) of soybean-meal (SBM) and free choice mineral blocks. Diets consisting of separated offerings of 50% TGH and 50% inoculated or control silage were fed daily at 3% body weight (DMB). The experimental protocol consisted of two periods, each with a 12 d adaptation to the diets, and a 7 d collection phase in which daily feed offered, orts, and feces output were recorded to determine DM and nutrient digestibility. Forage samples and individual fecal samples, obtained by weighing a daily 10% aliquot, were composited and stored (-20 °C) until analyzed for crude protein according to the AOAC (1990) and NDF contents (Van Soest *et al*., 1991). Rams were weighted at the beginning and end of each experimental period to calculate diet consumption in relation to body weight. The ratios of DM and nutrient intake from each dietary component (TGH and treated or untreated silage) as a proportion (%) of total dietary intake and amount offered were also calculated.

Experimental design and statistical analyses

Data on the silage fermentation process were analyzed as a completely randomized design with a 2 (microbial additive or not) by 5 (ensiling period, 0, 7, 13, 28, and 45 d) factorial arrangement of treatments, using the General Linear Model subroutine of SAS® (1990) statistical software version 6.12 (SAS Inst. Inc, Cary, NC, USA). The Tukey-t test was used for means separation. Aerobic stability was analyzed as a completely randomized design using the PROC MIXED procedure of SAS statistical software with a 2 (inoculants treatment) by 3 (aerobic exposure time, 0, 24, and 72 h) factorial arrangement of treatments for pH. Temperature was analyzed as a 2 (inoculants treatment) by 10 (aerobic exposure time, 0, 2, 4, 8, 14, 20, 26, 72, 90, and 96 h) factorial arrangement using the above mentioned statistical procedures. A cross-over design with two treatments and five replicates was utilized to analyze the intake and digestibility data. For each variable determined (i.e. DM, CP, NDF intake, and digestibility) the model included, within each experimental period, data from each ram as repetitive measurements during the seven days of data collection.

Results

Vegetative material

The initial chemical composition of the vegetative material utilized in this experiment was similar between treatments and represents the typical values for corn harvested in tropical climates (Table 1)

Table 1. Initial chemical composition of whole plant tropical corn treated or not with a microbial inoculant prior to ensiling.

Component[1] (g/Kg)	Control	Inoculated
Dry matter	396.8	387.6
Organic matter[2]	925.5	920.5
Ash	75.5	79.5
Crude protein[2]	80.2	87.6
Neutral detergent fiber[2]	629.4	610.5
Acid detergent fiber[2]	326.7	330.1
Hemicelulose[2]	302.7	280.4

[1]Means of three replicates
[2]Dry matter basis

Fermentation characteristics

Data encompassing the entire fermentation process show that tropical corn fermented with the microbial inoculant had lower ($p<0.001$) pH and higher ($p<0.001$) lactic acid content than untreated silage (Table 2).

Conversely, propionic acid content was higher ($p<0.05$) in the control compared with the treated silage. However, concentration of this acid was not sufficient to play a major role in the fermentation process. No significant responses ($p>0.05$) to treatments on acetic and butyric acid contents and NH_3-N/Total-N ratio were observed.

Values for pH and fermentation products over the course of the 45 d fermentation process of forage treated or not with the microbial inoculant are presented in Table 3. Acidity, measured by lower pH, was consistently lower ($p<0.05$) in corn fermented with the microbial product relative to the untreated vegetative material. As expected, in both treatments lactic acid was the major fermentation

Table 2. Fermentation characteristics of tropical corn silage fermented with or without a microbial inoculant.

Component	Experimental treatment[2]		SEM[1]	P-value
	Control	Inoculated		
pH	4.91[a]	4.58[b]	0.02	0.001
Fermentation product (g/Kg)				
Lactic acid	1.29[b]	1.70[a]	0.22	0.001
Acetic acid	0.82	0.79	0.04	0.640
Propionic acid	0.05[a]	0.01[b]	0.01	0.038
Butyric acid	0.10	0.04	0.01	0.220
NH_3-N/N-Total, %	1.76	1.31	3.56	0.466

[1] Standard error of the mean.
[2] Means with different letters within a row indicate significant differences ($p<0.05$).

product associated with the ensiling process. There was no significant treatment by day of fermentation interaction on lactic acid content. Inoculating corn at ensiling with the commercial product did not influence the concentration of the other fermentation products quantified over the ensiling period (i.e. organic acids and NH_3-N). A tendency ($p<0.06$) toward higher acetic acid concentrations observed in the control silage in the initial stages of fermentation (from d 7 to 13), shifted to a higher value in the inoculated treatment at the last sampled day of fermentation (d 45).

Aerobic stability

Data encompassing the entire aerobic exposure period show that tropical corn fermented with the microbial inoculant had lower ($p<0.05$) pH and temperature than untreated silage (Table 4).

Corn treated at ensiling with the microbial inoculant had lower ($p<0.05$) pH than control silage after 24 h of aerobic exposure, respectively (Table 5).

A sustained rise in temperature of the inoculated silage was observed after 14 h of aerobic exposure, whereas in the control silage this occurred after only 2 h (>2 °C above the average ambient temperature of 27 °C). Temperature was numerically lower in inoculated than untreated silage (± 2 °C) after 26 h of aerobic exposure, but this difference was not sustained thereafter.

Table 3. Effects of a microbial inoculant and day of fermentation on the ensiling characteristics of tropical corn.

Component	Day of ensiling	Experimental treatment[2]		SEM[1]	P-value
		Control	Inoculated		
pH	0	6.39	6.44	0.02	0.002
	7	4.69[a]	4.32[b]		
	13	4.47[a]	4.07[b]		
	28	4.35[a]	4.00[b]		
	45	4.67[a]	4.07[b]		
Fermentation product (g/Kg)					
Lactic acid	0	0.10	0.11	0.06	0.354
	7	1.48	1.65		
	13	1.37	2.14		
	28	2.20	2.48		
	45	1.30	2.13		
Acetic acid	0	0.30	0.25	0.04	0.066
	7	0.84[z]	0.69[y]		
	13	0.94[z]	0.67[y]		
	28	1.04[z]	0.88[y]		
	45	1.01[z]	1.45[y]		
Propionic acid	0	0.00	0.00	0.04	0.230
	7	0.05	0.01		
	13	0.04	0.01		
	28	0.02	0.03		
	45	0.08	0.05		
Butyric acid	0	0.00	0.00	0.22	0.158
	7	0.02	0.01		
	13	0.03	0.02		
	28	0.07	0.01		
	45	0.15	0.03		
NH_3-N/N-Total, %	0	0.00	0.00	0.66	0.680
	7	1.33	0.00		
	13	3.46	1.67		
	28	0.96	1.45		
	45	3.08	3.44		

[1] Standard error of the mean.

[2] Significant differences ($p<0.05$) are indicated within the same row with different superscript letters ([a, b]). Tendencies p ($p<0.51$ - 0.10) are indicated within the same row with different superscript letters ([z, y]).

Intake and nutrient digestibility

Initial DM, CP, and cell-wall content of TGH (mixture of *Panicum maximum* Jacq., *Digitaria decumbens*, *Cenchrus ciliaris*, and *Sorghum halapense*) and whole plant corn silage, treated or not with the microbial inoculant, utilized in the feeding trial are reported in Table 6, as were the DM, CP, and NDF values of the soybean-meal used as nitrogen supplement.

Table 4. pH and temperature of tropical corn treated or not with a microbial inoculant at ensiling after three days of aerobic exposure.

Component	Experimental treatment[2]		SEM[1]	P-value
	Control	Inoculated		
pH	6.64[a]	5.33[b]	0.34	0.001
Temperature (°C)	34.13[a]	32.05[b]	4.35	0.001

[1] Standard error of the mean.

[2] Means with different superscripts letters ([a, b]) within the same row indicate significant difference ($p<0.05$).

Table 5. Aerobic stability of tropical corn treated or not with a microbial inoculant at ensiling upon exposure to air.

Component	Aerobic exposure (h)	Experimental treatment[2]		AT+2[3]	SEM[1]	P-value
		Control	Inoculated			
pH	0	5.27[a]	4.11[b]	---	0.358	0.001
	24	6.88[a]	4.33[b]	---		
	72	7.80	7.55	---		
Temperature (°C)	0	27.25	27.25	29.85	4.38	0.486
	2	30.00[4]	27.52	29.15		
	4	29.87	26.82	28.90		
	8	31.92	28.72	29.10		
	14	33.60	29.70[4]	29.15		
	20	35.57	32.20	29.45		
	26	35.42	32.90	28.85		
	72	37.90	36.67	29.10		
	90	40.82	39.60	29.85		
	96	38.90	39.15	29.30		

[1] Standard error of the mean.

[2] Means with different superscript letters ([a, b]) within the same row indicate significant difference ($p<0.05$).

[3] AT + 2 = Ambient Temperature +2 °C.

[4] Time to reach a rise in temperature over 2 °C above the ambient temperature.

Table 6. Chemical composition of tropical grass hay, whole plant corn and soybean meal (SBM) utilized in the feeding trial.

Component (g/Kg)[1]	Tropical grass hay	Control silage	Inoculated silage	SBM
Dry matter	930	386	396	905
Crude protein[2]	43	75	81	534
Neutral detergent fiber[2]	725	609	630	110

[1] Means of three replicates.

[2] Dry matter basis.

During the feeding trial, daily hay DM intake was higher ($p<0.05$) in rams fed the control diet with untreated silage than in animals fed hay and inoculated corn silage; however, silage and total DM intake were similar (Table 7). Inoculation of whole-plant corn at ensiling tended to increased the relationships silage intake/total intake by 4.84 percentage units. Conversely, animals fed the treated corn silage tended to reduced TGH intake/TGH offered ratio by 12.92 percentage units and TGH DM intake/total intake ratio by 5.76 percentage units. The greater TGH consumption by rams fed 50% TGH and 50%

untreated corn silage resulted in a higher (p<0.05) CP intake from hay/total CP intake ratio, as compared with animals fed the combination grass hay-treated silage. However, inoculated whole-plant corn silage increased the relationship CP intake from silage/Total CP intake by 3.56 percentage units. Neutral detergent fiber intake from hay was higher (p<0.05) in rams fed the untreated corn than those fed material with the microbial starter (194.3 vs. 158.4 g/d). As expected, higher hay intake resulted in higher (p<0.05) cell-wall component intake. Inoculating whole-plant corn at ensiling also decreased the relationships NDF intake from hay/total NDF intake by 10.95 percentage units, and increased the ratio NDF intake from silage/total NDF intake by 10.91 percentage units.

Table 7. Daily intake and digestibility in rams fed tropical corn silage fermented or not with a microbial inoculant and supplemented with SBM.

Component	Control	Experimental treatment[2]		P-value
		Inoculated	SEM[1]	
Dry matter intake (g/d)				
Hay	267.6^a	218.2^b	4.9	0.005
Silage	222.3	230.5	1.0	0.706
SBM	63.3	63.3		
Total	553.2	512.1	3.2	0.234
Ratio (%)				
Silage intake/silage offered	76.0	82.4	1.9	0.283
Hay intake/hay offered	91.5^z	78.5^y	2.6	0.083
Silage intake/total intake	40.1^y	45.0^z	1.0	0.076
Hay intake/total intake	48.3^a	42.6^b	0.5	0.023
Intake as % ram body weight (DMB)	2.7	2.6	1.1	0.525
Crude protein intake (g/d)				
Hay	11.5^a	9.4^b	0.8	0.001
Silage	16.8^a	18.8^b	2.0	0.001
SBM	33.8	33.8		
Total	62.2	62.0	1.1	0.114
Ratio (%)				
Cp intake silage/total Cp intake	27.0^a	30.6^b	1.1	0.001
Cp intake hay/total Cp intake	18.5^a	15.3^b	0.8	0.001
NDF Intake (g/d)				
Hay	194.2^a	158.3^b	2.4	0.001
Silage	135.0	145.2	1.4	0.245
SBM	6.9	6.9		
Total	336.2	310.5	0.9	0.119
Ratio (%)				
NDF intake silage/total NDF intake	40.1^a	51.0^b	0.8	0.001
NDF intake hay/total NDF intake	57.7^a	46.8^b	0.4	0.001
Digestibility				
DM	65.7	64.1	0.5	0.123
CP	72.2^a	78.4^b	0.4	0.002
NDF	58.4	60.0	0.9	0.967

[1] Standard error of the mean.

[2] Significant differences (p<0.05) are indicated within the same row with different superscript letters ($^{a,\ b}$). Tendencies (p<0.51 - 0.10) are indicated within the same row with different superscript letters ($^{z,\ y}$).

Discussion

The initial chemical composition of the whole-plant corn utilized in this study is in agreement with related research (Rodríguez, 1996; Prieto, 2007). Compared with native or cultivated tropical grasses typically used in livestock production systems, tropical corn is characterized by being higher in CP content and lower in cell-wall components (Arias, 1998; Sandoval, 2007; Prieto, 2007); however, it shows higher cell-wall and lower soluble cell components than the corn or sorghum harvested in temperate climates at the same stage of growth (Rodríguez, 1996; Johnson *et al*., 1997). In this experiment, lactic acid was the major end product associated with the whole plant silage tropical corn and this organic acid was higher throughout the fermentation process in the inoculated forage. However, inoculating with the commercial product did not influence the concentration of the other major fermentation products. Related research has reported a decrease in pH and an increase in lactic acid concentration in corn silages treated with additives containing LAB (Taylor and Kung, 2002; Johnson *et al*., 2003; Hu *et al*., 2009).

A summary of 17 studies on the use of corn silage inoculants demonstrated increments in lactic acid levels and a significant reduction in pH of corn silage fermented with an inoculant (Harrison *et al.,* 1996). These beneficial changes in fermentation due to inoculation are associated with the presence of homo-fermentative LAB strains in the microbial product (*Lactobacillus plantarum*). In the present study, the inoculant tested improved fermentation characteristics of whole-plant tropical corn silage as evidenced by lower pH and higher lactic acid content. A changing micro-flora during the ensiling process in which different substrates are used (i.e. lactic acid) has been reported in tropical silages, thus implying a very heterogeneous microbial population, a factor that might affect the efficacy of microbial inoculants (Rodríguez, 1996). However, the bacterial strains contained in the microbial product provide evidence of competing with undesirable microorganisms present in the chopped forage prior to ensiling and during the fermentation period and consequently improving fermentation characteristics.

Aerobic stability of common tropical silages (i.e. corn, sorghum) is usually short. Previous researchers have reported that the greatest deterioration of such silage typically occurs within 1-2 d of aerobic exposure, as evidenced by changes in pH, temperature, yeast and mold populations (Rodriguez, 1996; Prieto, 2007). In this experiment, although silages were exposed to air during 96 h, the greatest changes in pH and temperature had already occurred during the first 6 to 30 h of aerobic exposure. The microbial additive evaluated in this experiment contained the hetero-fermentative lactic acid-producing bacteria strain *Lactobacillus brevis*. Such LAB strain turns lactic into acetic acid (Oude Elferink *et al.,* 2001). Acetic acid has antifungal properties and its presence has been reported to delay deterioration of silages exposed to air (Kleinschmit and Kung, 2006). The beneficial effect of hetero-fermentative strains of LAB on the aerobic stability of silages has also been observed in other studies. Taylor and Kung (2002) found that microbial inoculants containing hetero-fermentative strains decreased yeast populations in high-moisture corn silage delaying its aerobic deterioration. Driehuis and co-workers (2001) reported that inoculation with *L. buchneri* increased acetic acid production during fermentation, thus enhancing the aerobic stability of grass silage (*Lolium perenne*). In the present experiment, inoculated silage had greater acetic acid concentration than untreated vegetative material at the time of aerobic exposure (1.45 vs. 1.01%, respectively; Table 3), a factor that might have delayed its deterioration.

In this experiment a greater relative preference was observed for the fermented vegetative material when treated, rather than not treated as compared to grass hay. Factors affecting and regulating feed intake of ruminant animals are numerous and complex and span from cellular to macro-environmental levels (Forbes, 1996; Roseler *et al*., 1997; Allen, 2000). The higher observed ratio of silage DM intake/total DM intake obtained with inoculated corn silage might be associated with the organoleptic characteristics of the resulting silage (odor, taste); other pertinent differences might include the slower deterioration of silage after opening the silo.

Dry matter and NDF digestibility was similar in diets contaning TGH and silage treated or not with the microbial starter. However, CP digestibility was higher ($p<0.05$) by 6.20 percentage units in diets contaning corn silage treated with the inoculant. Numerous studies have shown that digestibility of corn silage can vary greatly due to plant genetics, environmental conditions, and handling of forage before and after harvest (Johnson *et al*, 2003; Allen, 2009). Other studies on bacterial inoculation have shown positive effects on DM and nutrient digestibility. Aksu *et al*. (2004) reported that microbial inoculants containing homo (*Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Pediococcus pentosaceus*) and heterofermentative (*Lactobacillus brevis*, *Lactobacillus buchneri*) LAB added to corn silage increased DM and NDF digestibility in sheep by 8.8 and 2.7 percentage units, respectively. In summary, the present results indicate that addition at ensiling of the microbial additive improved fermentation characteristics of tropical corn silage -as evidenced by lower pH and higher lactic acid content- and also delayed aerobic deterioration of the resulting silage. Upon comparing the two hay-silage-SBM diets, the one containing inoculated corn silage resulted in higher CP digestibility and higher proportional intakes of DM, CP, and NDF from silage but did not affect total intake of these fractions.

These results show that the inoculation at ensiling enhanced ram silage consumption by reducing or susbtituing for TGH intake. In this experiment, the higher observed ratio of silage DM intake/total DM intake obtained with the inoculated corn silage might be associated with the organoleptic characteristics of the resulting silage (odor, taste); other pertinent differences might include the fermentation products and slower deterioration of the silage after opening the silo.

Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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