

## PF 27. FERMENTATION CHARACTERISTICS OF FORAGE SORGHUM ENSILED WITH COMMERCIAL ENZYME PREPARATIONS

A. A. Rodríguez<sup>1</sup>, S. R. Rust<sup>1</sup>, M. L. Schlegel<sup>1</sup>, E. O. Riquelme<sup>2</sup>, M. T. Yokoyama<sup>1</sup>, and R. J. Burnett<sup>1</sup>

<sup>1</sup>Michigan State University. <sup>2</sup>East Lansing and University of Puerto Rico, Mayaguez Campus.

### Resumen

#### Características fermentativas de sorgo forrajero ensilado con preparaciones enzimáticas comerciales

Se evaluó el efecto de la adición de preparaciones enzimáticas comerciales sobre las características fermentativas de sorgo forrajero. El forraje fue cosechado a 90 días de crecimiento (20.04 % MS) y fue picado en pedazos de 2.5 cm en la Finca Experimental Agrícola localizada en Michigan State University en East Lansing. Antes de su ensilado, el forraje picado se trató con preparaciones de enzimas que se aplicaron a razón de 1 ó 2 veces la dosis recomendada por el distribuidor. Los tratamientos experimentales incluyeron: sin aditivo (testigo), Viscozyme (0.1 % de forraje fresco), Ecogram (0.08 y 0.16 mL/kg de forraje fresco) y Cellulase G (0.25 y 0.50 mL/kg de forraje fresco). Se abrieron tres silos por tratamiento después de tres períodos de fermentación (0, 40 y 100 días) y el ensilaje resultante fue analizado para determinar pH, productos de fermentación y contenido de carbohidratos solubles y estructurales. El pH del ensilado de sorgo forrajero fue similar en todos los tratamientos evaluados. El contenido de ácido acético y etanol difirió entre las preparaciones enzimáticas evaluadas y entre días de fermentación. Sin embargo, no se observaron cambios consistentes debido a ningún tratamiento. El contenido de glucosa fue mayor en sorgo ensilado con 1 ó 2 veces la dosis recomendada de Cellulase G al compararse con ensilajes sin aditivo, pero el contenido de xilosa fue menor. Ningún tratamiento experimental afectó el contenido de carbohidratos estructurales. En resumen, la adición de preparaciones enzimáticas comerciales no tuvo un efecto consistente sobre las características fermentativas de sorgo forrajero.

**Palabras claves:** Enzimas, sorgo forrajero, ensilaje.

**Key words:** Enzymes, forage sorghum, silage.

### Introduction

Variable response to enzyme addition to enhance the fermentation of plant material has been shown (Kung Jr. *et al.*, 1991; Selmer-Olsen, 1994; Sheperd *et al.*, 1995). Enzyme preparations differ in declared activity, percentage of individual enzymes, source, and recommended application rates. Previous results (Rodríguez *et al.*, 1994) have indicated that an enzyme preparation containing 5 different enzymes and applied at the recommended rate (0.1 % of fresh material) did not improved the fermentation characteristics of forage sorghum. Other experiments (Rodríguez *et al.*, 1996) also showed that under controlled conditions (e.g. pH, temperature), commercial enzyme preparations applied at rates greater than recommended did not improve NDF disappearance of forage sorghum, and differ in their ability to degrade the NDF fraction of forage sorghum. The objective of this experiment was to evaluate the effect of three commercial enzyme preparations on the fermentation characteristics and carbohydrate content of forage sorghum.

### Materials and methods

Forage sorghum (Hi Energy Hybrid, Agri-pro Seed, Hereford, TX) was harvested at 90 days of growth (20.04 % DM) at Michigan State University, East Lansing and chopped mechanically into 2.5 cm pieces. Chopped forage, prior to ensiling, was treated with three commercial enzyme preparations assigned to one of six treatments; no additive (control, T1), Viscozyme, 0.1% of fresh material, T2), Ecogram (0.08 mL/kg of fresh material, T3; and 0.16 mL/kg of fresh material, T4), and Cellulase G (0.25 mL/kg of fresh material, T5; and 0.50 mL/kg of fresh material, T6). Treatments were applied to weighed portions (1.6 kg) of forage, manually mixed, and packed into PVC laboratory silos fitted with release valves to provide gas escape. Laboratory silos were maintained at room temperature (27-30 °C) until opened. Triplicate samples from each treatment at each ensiling period were analyzed for pH, fermentation end-products (acetic and lactic acids, and ethanol), and water soluble (glucose, fructose, galactose, xylose and arabinose) and structural (NDF, ADF, cellulose, and hemicellulose) carbohydrate content. For pH determination, 50 g of forage from each silo at each sampling day were placed into 450 mL of distilled water (w/v) and homogenized for 5 min with a stomacher apparatus. Homegenates were strained through

eight layers of cheesecloth and pH analyzed with a pH meter fitted with a combination electrode. Fermentation end-products and water soluble carbohydrates were determined by ion exchange exclusion HPLC analysis following the general procedure of Canale *et al.* (1984). Structural carbohydrate content; NDF, ADF, hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between ADF and lignin); were determined by the Van Soest method (Van Soest *et al.*, 1991). Data was analyzed as a completely randomized design with a 6 (enzyme preparations) by 3 (ensiling periods) factorial arrangement of treatments using the Linear Model Procedure of SAS (1990). Bonferroni t- test was used for mean separation.

## Results and discussion

Significant interactions between enzyme treatments by day of ensiling for pH or fermentation end-products were not observed (table 1). Over the entire ensiling period, pH tended to be higher ( $P < .09$ ) in forage sorghum treated with Viscozyme (T2) in comparison to forage treated with the other enzyme treatments, but was similar to control silage. Forage sorghum treated with Ecogram (T3 and T4) or Cellulase G (T5 and T6) at 1 or 2 X the suggested application rate had lower ( $P < .05$ ) acetic acid content than control silage or silage treated with Viscozyme (T2). However, ethanol content was higher ( $P < .05$ ) in silages containing Viscozyme and the 2 X application rate of Cellulase G as compared to the other enzyme treatments. For all enzyme treatments, lactic acid content was similar over the entire ensiling period.

**Table 1. Effect of enzyme treatment and day of ensiling on pH and fermentation end-products of forage sorghum silage.**

Item	Day of Ensiling	Treatment						SEM <sup>a</sup>	Probability		
		1	2	3	4	5	6		E <sup>b</sup>	D <sup>c</sup>	E*D <sup>d</sup>
pH	0	5.23	5.36	5.23	5.30	5.16	5.23	.043	.094	.001	.127
	40	3.53	3.56	3.53	3.53	3.55	3.56				
	100	3.68	3.66	3.55	3.48	3.58	3.56				
Acetic acid <sup>e</sup>	0	0.25	0.21	0.13	0.10	0.19	0.16	.101	.047	.001	.300
	40	1.60	1.68	1.60	1.58	1.59	1.51				
	100	1.68	1.77	1.41	1.33	1.21	1.52				
Lactic acid <sup>e</sup>	0	0.77	0.60	0.55	0.52	0.70	0.53	.632	.126	.001	.257
	40	8.26	7.84	6.70	9.54	8.33	6.99				
	100	6.28	6.00	7.82	7.59	7.35	5.80				
Ethanol <sup>e</sup>	0	0.13	0.23	0.06	0.43	0.06	0.07	.166	.001	.001	.963
	40	0.73	1.20	0.77	1.06	0.56	0.84				
	100	0.61	0.98	0.57	0.96	0.69	0.60				

<sup>a</sup> Standard error of the mean. <sup>b</sup> Effect of enzyme. <sup>c</sup> Effect of day of ensiling. <sup>d</sup> Interaction of enzyme by day of ensiling. <sup>e</sup> g/100 g DM.

Glucose content was higher ( $P < .05$ ) after 40 days post-ensiling in silages containing 1 or 2 X the recommended application rate of Ecogram and Cellulase G as compared to control or Viscozyme treatments (table 2). After 100 days post-ensiling, silages containing Ecogram and Cellulase G had greater ( $P < .05$ ) glucose content than other enzyme treatments. In contrast to glucose content, xylose concentrations were higher ( $P < .05$ ) after 40 and 100 days post-ensiling in silages without enzyme additive or forage treated with Viscozyme. Over the entire ensiling period, concentrations of fructose, galactose and arabinose were similar regardless of enzyme treatment. After 100 days post-ensiling, there was a tendency ( $P < .09$ ) for control silage to have greater ADF content than the other silages. Neutral detergent fiber, hemicellulose and cellulose content were not different for the various treatments. However, for all cell-wall fractions, a small numerical decrease was observed in silages containing enzyme mixtures as compared to control silage.

**Table 2. Effect of enzyme treatment and day of ensiling on carbohydrate content of forage sorghum silage.**

Carbohydrate	Day of Ensiling	Treatment						Probability			
		1	2	3	4	5	6	SEM <sup>a</sup>	E <sup>b</sup>	D <sup>c</sup>	E*D <sup>d</sup>
<u>Water Soluble</u>											
Glucose <sup>e</sup>	0	6.62	6.64	6.27	6.50	6.22	6.54	.386	.011	.001	.029
	40	1.66 <sup>f</sup>	1.78 <sup>f</sup>	2.42 <sup>g</sup>	2.85 <sup>g</sup>	2.43 <sup>f</sup>	2.68 <sup>f</sup>				
	100	0.94 <sup>f</sup>	1.06 <sup>f</sup>	1.94 <sup>g</sup>	1.91 <sup>g</sup>	2.91 <sup>g</sup>	2.94 <sup>g</sup>				
Fructose <sup>e</sup>	0	6.44	6.33	6.67	6.17	6.86	6.12	.237	.597	.001	.833
	40	0.29	0.19	0.34	0.16	0.34	0.46				
	100	0.54	0.44	0.43	0.30	0.43	0.58				
Galactose <sup>e</sup>	0	0.15	0.19	0.11	0.16	0.12	0.22	.045	.340	.006	.483
	40	0.23	0.28	0.28	0.12	0.26	0.26				
	100	0.24	0.25	0.18	0.25	0.24	0.27				
Xylose <sup>e</sup>	0	0.07	0.05	0.06	0.05	0.06	0.07	.071	.001	.001	.033
	40	0.37 <sup>h</sup>	0.32 <sup>h</sup>	0.18 <sup>fg</sup>	0.08 <sup>f</sup>	0.20 <sup>fg</sup>	0.24 <sup>gh</sup>				
	100	0.68 <sup>h</sup>	0.63 <sup>h</sup>	0.34 <sup>g</sup>	0.18 <sup>f</sup>	0.45 <sup>g</sup>	0.32 <sup>g</sup>				
Arabinose <sup>e</sup>	0	0.16	0.18	0.09	0.15	0.13	0.14	.062	.450	.009	.370
	40	0.09	0.09	0.07	0.06	0.07	0.03				
	100	0.13	0.14	0.10	0.11	0.10	0.13				
<u>Structural</u>											
NDF (%)	0	61.64	63.04	60.62	60.54	60.03	61.86	1.034	.678	.001	.270
	40	58.08	56.10	57.44	58.81	58.17	57.34				
	100	59.53	56.33	58.12	57.42	57.17	56.81				
ADF (%)	0	37.31	38.77	36.16	35.41	35.79	34.78	1.200	.087	.129	.321
	40	35.79	34.40	35.48	35.08	35.07	33.55				
	100	36.12	33.56	34.85	34.80	35.33	33.13				
Hemicellulose (%)	0	24.32	24.26	24.36	24.12	24.57	25.75	1.379	.716	.003	.785
	40	22.28	21.69	21.96	23.03	23.10	24.79				
	100	23.41	22.76	22.59	22.61	21.84	23.88				
Cellulose (%)	0	32.50	34.33	31.68	31.99	31.20	31.66	.907	.338	.001	.491
	40	31.71	30.63	30.67	32.20	30.29	30.85				
	100	31.85	29.92	29.85	29.63	30.14	29.83				

<sup>a</sup> Standard error of the mean. <sup>b</sup> Effect of enzyme. <sup>c</sup> Effect of day of ensiling. <sup>d</sup> Interaction of enzyme by day of ensiling. <sup>e</sup> g/100 g DM. <sup>fg</sup> Means with unlike superscripts in the same row within an item heading differ (P < .05).

Results from this experiment indicate that forage sorghum treated with Ecogram or Cellulase G increased the residual glucose content after 100 days of fermentation. However, neither enzyme preparation increased the acidity or lactic acid content of the resulting silage, and a decrease in xylose content was observed. Application of the enzyme mixture did not significantly decrease cell-wall components of forage sorghum silage. Even though the higher residual content due to enzyme addition may improve digestibility of the resulting silage, it may lead to more aerobic deterioration (Spoeltra and Van Wikselaar, 1992). Therefore more research is needed to evaluate the effects of different enzyme preparations on the fermentation characteristics of silages.

### Conclusions

Addition of enzyme preparations did not consistently affect pH, fermentation end-products or structural carbohydrate content of forage sorghum silage. Commercial enzymes differ in their ability to increase the residual soluble carbohydrates in forage sorghum silage. More research is needed (e.g. feeding trials) to justify the use of enzyme preparations as a silage additive.

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