Research Note

CELL WALL DISAPPEARANCE IN A FORAGE SORGHUM HYBRID AND JOHNSON GRASS AFTER TREATMENT WITH A COMMERCIAL MULTI-ENZYME PREPARATION[1]

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Because of seasonal rainfall in tropical climates, production of high quality conserved forage for use as feedstuff during dry periods is critical. Preservation of forage by ensiling requires adequate amounts of water soluble carbohydrates (WSC) to ensure a fermentation dominated by lactic acid-producing bacteria (LAB). However, tropical grasses are generally low in WSC (Van Soest, 1994), thereby limiting the fermentation process. In temperate climates, studies have yielded variable results from the use of commercial enzyme preparations to accelerate the fermentation by providing higher amounts of WSC produced from the hydrolysis of starch, cellulose and hemicellulose to oligosacharides and simple sugars (Jasten and Moore, 1988; Van Vuueren et al., 1989; Chen et al., 1994). Reports indicate that the efficacy of an enzyme preparation can be influenced by forage species, environmental conditions and application rates (Henderson et al., 1982; Spoeltra and Van Wikselaar, 1992). There is limited information regarding the utilization of enzyme preparations to improve silage fermentation in tropical climates. Rodríguez (1996) found that a multi-enzyme preparation applied at the recommended rate did not improve the ensiling characteristics of forage sorghum. The objective of this study was to determine the effects of this multi-enzyme preparation, applied at different rates, on the in vitro cell wall disappearance from Johnson grass and forage sorghum hybrids grown in Puerto Rico.

Johnson grass [*Sorghum halepense* (L.) Pers.] at 45 d (22.6% DM) and 110 d (43.8% DM) of regrowth and a forage sorghum hybrid [*Sorghum bicolor* (L.) Moench, hi energy hybrid, Agripro- seed, Hereford, TX] at 90 d of growth were harvested manually at the Lajas Agricultural Experiment Station, University of Puerto Rico. Samples from each forage were oven-dried at 60°C for 72 h, ground in a Udy mill (Arthur Thomas Co. Philadelphia; 1-mm screen)[6], and stored at room temperature (27 to 30°C) until analysis. For

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[6]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

cell-wall disappearance determination, triplicate samples from each forage (0.5 g) were treated with four levels of a liquid multi-enzyme preparation (Viscozyme™ 120L, Novo Nordisk Bioindustrials, Danbury, CT) containing arabinase, cellulase, ß-glucanase, hemicellulase and xylanase (Table 1). The enzyme preparation was applied on a dry weight basis at 0, 1, 4 and 8× the rate suggested by the manufacturer (0.1% of fresh material). Forage samples were incubated at 40°C for 7 d in 75-ml test tubes containing the respective enzyme application rates and sufficient sodium acetate buffer (0.1 M; pH = 4.5) to provide a total volume of 50 ml. Plant cell-wall fractions, neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (calculated as the difference between NDF and ADF), and cellulose (calculated as the difference between ADF and lignin) were determined at the beginning and end of the incubation period (Goering and Van Soest, 1970; Van Soest et al., 1991). Disappearances of plant cell-wall fractions were calculated as the differences between the initial and final proportions of each fraction in the forage residue. Analysis of variance was performed as a completely randomized design with a 2 (forage species) by 4 (enzyme application rates) factorial arrangement of treatments (Steel and Torrie, 1980) using the General Linear Model Procedure of SAS (1990). Mean separation was performed by a Bonferroni-test.

Initial structural carbohydrate contents of the forages evaluated (Table 2) were similar to values previously reported for tropical grasses (Minson and McLeod, 1970; Vicente-Chandler et al., 1983). As expected, Johnson grass harvested at 110 d of regrowth had more cell wall material than that harvested at 45 d. Forage sorghum had a lower cell wall content than Johnson grass, but higher lignin content.

Neutral detergent fiber disappearance was greater ($P < 0.01$) in Johnson grass harvested at 45 d of regrowth than in the same species harvested at 110 d of regrowth, and in forage sorghum harvested at 90 d of growth (Figure 1). For both forage species evaluated, adding the enzyme preparation at 4× the recommended rate increased NDF disappearance, but at the recommended application rate NDF disappearance was similar to that of the untreated forage (Figure 2). Increasing the enzyme preparation to 8× the recommended application rate provided greater ($P < 0.01$) NDF disappearance than 0, 1 and 4× the suggested rate. This result differs from previously reported experiments eval-

TABLE 1.—*Description of the commercial multi-enzyme preparation evaluated.*

Trade Name	Viscozyme™ 120L
Supplier	Novo Nordisk Bio-Industrials, Danbury, CT
Source	*Aspergillus niger*
Active Enzymes	arabinase
	cellulase
	ß-glucanase
	hemicellulase
	xylanase
Declared Activity	120 FBG/ml[1]
Recommended Application Rate	0.1% of fresh material
Optimum pH conditions	3.3 to 5.5
Optimum temperature conditions	40 to 50°C
g protein/100 ml enzyme	

[1] FBG = Fungal beta-glucanase; where 1 FBG is the amount of enzyme which under standard conditions liberates glucose or other reducing carbohydrate with a reducing power corresponding to 1 µmol glucose per minute.

TABLE 2.—*Composition of initial plant cell wall fractions of Johnson grass and forage sorghum.*

	Forage species					
	Johnson–45 d		Johnson–110 d		Sorghum–90 d	
Cell-wall fraction	Mean	SD[1]	Mean	SD	Mean	SD
Neutral Detergent Fiber	67.38	1.35	69.87	0.73	64.00	1.34
Acid Detergent Fiber	36.05	0.09	38.53	1.09	35.18	1.10
Hemicellulose	31.33	1.26	31.34	1.75	28.82	0.24
Cellulose	32.87	0.26	33.78	1.06	29.36	0.80
Lignin	3.17	0.16	4.74	0.22	5.81	0.42

[1]Standard deviation.

uating forage sorghum in temperate climates (Rodríguez et al., 1995), where increased application rates of the same enzyme did not improve NDF disappearance from forage sorghum harvested at 90 d of growth. In the latter study, NDF disappearance was similar for application rates up to 8 times the manufacturer's recommendation. The suggested application rate for this enzyme preparation appears to be inappropriate for forages harvested in tropical climates.

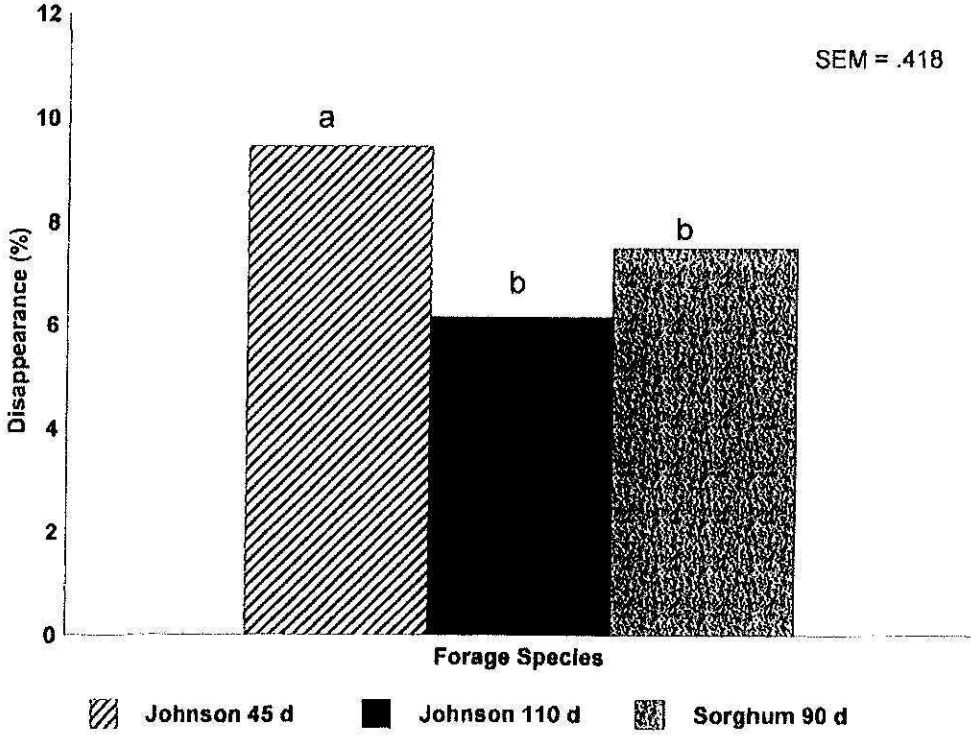

FIGURE 1. Neutral Detergent Fiber disappearance from Johnson grass and forage sorghum after treatment with a commercial enzyme mixture (Means with different letters differ, $P < 0.01$).

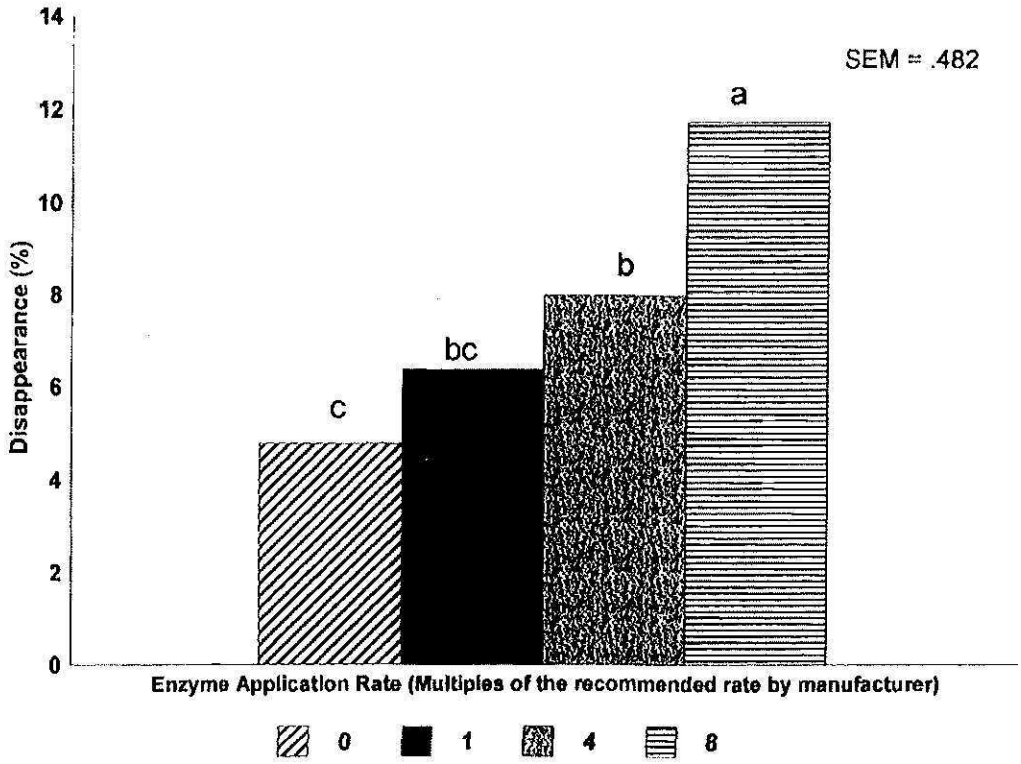

FIGURE 2. Enzyme application rate on Neutral Detergent Fiber disappearance from Johnson grass and forage sorghum (Means with different letters differ, $P < 0.01$).

Acid detergent fiber disappearance from Johnson grass harvested at 45 d of regrowth and treated with the enzyme preparation at 1 and 4× the suggested rate was greater ($P < 0.01$) than that of untreated samples, but less than that with 8× the recommended application rate (Figure 3). None of the enzyme application rates increased ADF disappearance from Johnson grass harvested at 110 d of regrowth as compared to that of the untreated forage. In forage sorghum, ADF disappearance increased as application rate of the enzyme preparation increased. The lower response to the enzyme preparation observed in the ligno-cellulolytic fraction of Johnson grass harvested at 110 d of regrowth may be explained by a greater thickness of the cell wall than in more immature forages (Van Soest, 1994). As plants mature, changes occur in the degree of polymerization within and between glucose polymers within the cell wall. Crystallinity of the cellulose fraction and physical incrustation of cellulose with lignin both increase. These factors collectively render the plant material less susceptible to enzyme degradation.

Hemicellulose disappearance in Johnson grass harvested at 45 d of regrowth was increased only when enzyme application was 8× the recommended rate (Figure 4). In Johnson grass harvested at 110 d of regrowth, hemicellulose disappearance increased stepwise as enzyme application rate increased. In sorghum forage, application rates of 1 and 4× the recommended rate resulted in greater hemicellulose disappearance than in untreated forage, but lower than with 8×. Hemicellulose disappearance was observed in the control samples of both forage species evaluated. This disappearance must have resulted from either microbial activity or solubilization of the hemicellulose. The hemicellulose of immature forages seems to be more soluble, but less susceptible to enzyme attack. This concept is supported by the observation of increased hemicellulose disappearance in the most immature forage only at a high enzyme level.

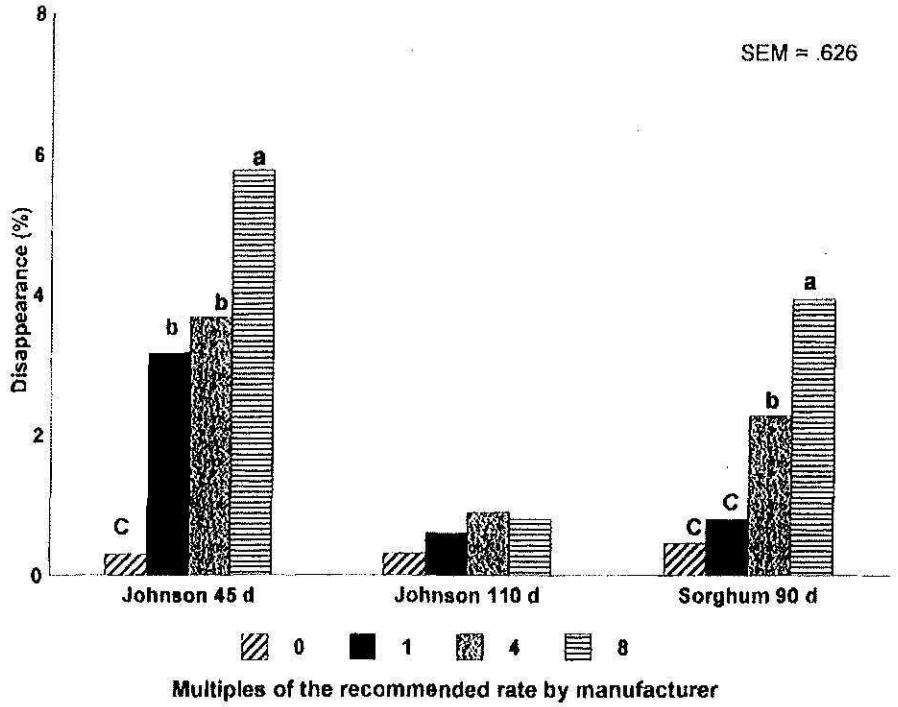

FIGURE 3. Interaction between enzyme application rate and forage species, on Acid Detergent Fiber disappearance from Johnson grass and forage sorghum (Means with different letters differ, $P < 0.01$).

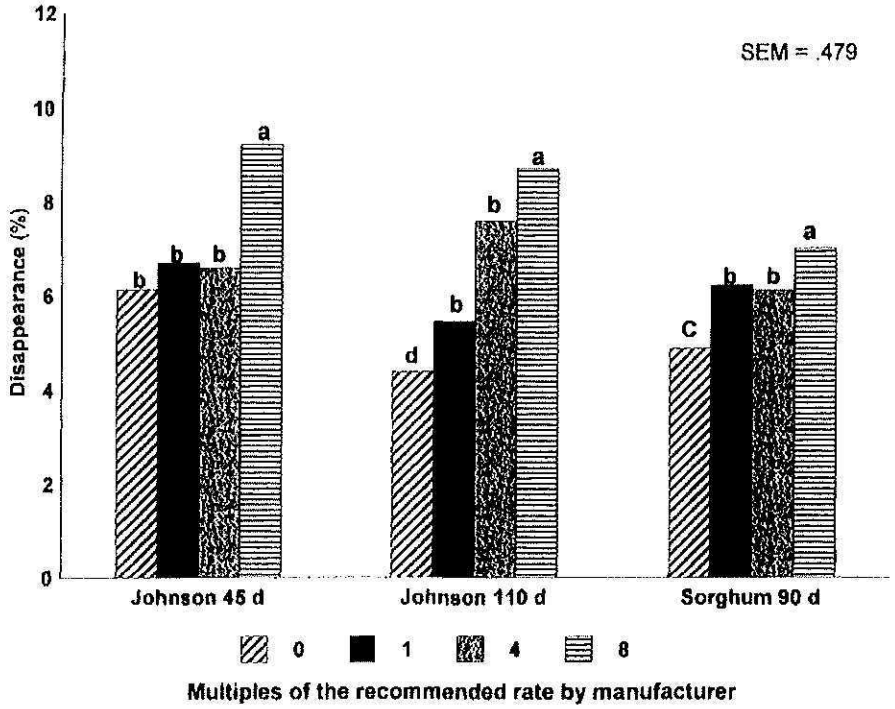

FIGURE 4. Interaction between enzyme application rate and forage species on hemicellulose disappearance from Johnson grass and forage sorghum (Means with different letters differ, $P < 0.05$).

Cellulose disappearance from Johnson grass harvested at 45 d of regrowth and forage sorghum followed a similar pattern for ADF disappearance when treated with the multi-enzyme preparations (Figure 5). With both forages, highest cellulose disappearance values were obtained when the enzyme preparation was applied at 8× the recommended rate. With Johnson grass harvested at 110 d, cellulose disappearance was improved only when the enzyme complex was applied at 8× the recommended rate, and this disappearance occurred at a lower order of magnitude than that observed with the immature forage.

The results from this experiment support the premise that forages harvested at an early stage of maturity are more susceptible to enzyme degradation than those harvested at a later stage of growth. This variability in response to the enzyme preparation among the forage species evaluated may be due to differences in concentration of individual carbohydrates, covalent bonding of phenolic acids to cell wall, or crystalline structure of the cellulose. In similar experiments conducted in temperate environments, Sheperd and Kung (1994) showed that an enzyme additive was more effective in altering the fiber content of corn silage at the earlier milk stage than at the black layer stage of maturity.

The multi-enzyme source evaluated in this experiment consisted of a preparation containing five different enzymes. Information on individual concentrations of each enzyme were not provided, and disappearance of simple sugars (arabinose, xylose) or specific sugars present in the hemicellulose polysaccharide (xylan) fraction were not measured. However, it appears that an enzyme preparation containing cellulase and hemicellulase is needed in younger forages, whereas only a hemicellulase is needed in

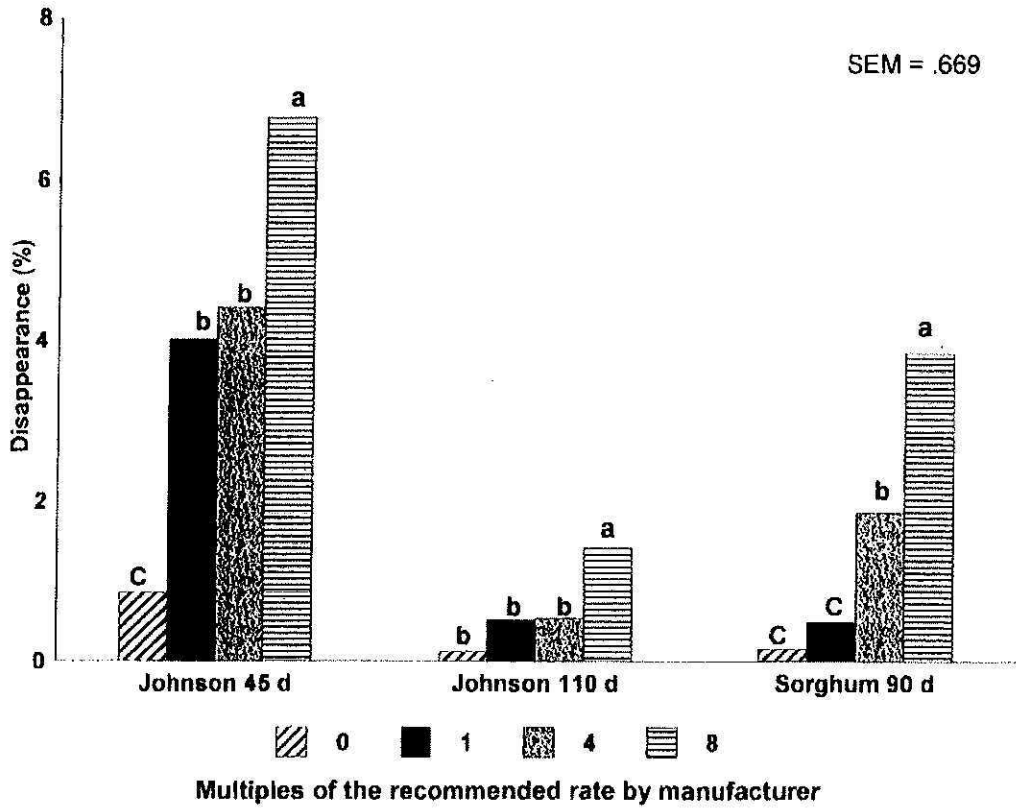

FIGURE 5. Interaction between enzyme application rate and forage species, on cellulose disappearance from Johnson grass and forage sorghum (Means with different letters differ, $P < 0.05$).

more mature forages. Similar results were observed with forages grown under temperate conditions. Hoffman et al. (1995) found that a commercial enzyme preparation reduced cell wall content in alfalfa harvested at late-bloom by reducing the hemicellulose and pectin fractions, but had little effect on the ADF and cellulose fractions.

In this experiment, cell wall disappearance differed among forage types when treated with a commercial multi-enzyme preparation. Application rates greater than those recommended by the manufacturer are needed to ensure an increase in cell wall disappearance from Johnson grass and forage sorghum harvested in a tropical climate.

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