

## Effect of ferulic acid esterase enzyme application on the *in vitro* digestibility and *in situ* rumen degradability of tropical grasses

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**Introduction** Tropical grasses are the primary staple diet of most of the domesticated ruminants in the tropical and subtropical regions of the world. However, dry matter (DM) digestibility (DMD) and intake levels of these C<sub>4</sub> grasses are considerably low, partly because of high lignin contents. Ferulic acid also impedes fibre digestion in such forages due to formation of cross linkages with digestible xylans. Certain esterase enzymes have been shown to cleave ferulic acid cross linkages in wheat bran but no studies have examined whether such enzymes can be used to increase the digestion of tropical grasses. Therefore, the objective of this work was to evaluate the effect of a ferulic acid esterase preparation applied at different rates on the digestibility of C<sub>4</sub> grasses.

**Materials and methods** An enzyme complex (Depol 740L, BioCatalyst UK) containing high esterase activity (32 U/ml) was sprayed on 12-week regrowths of ground (1 mm), Pensacola bahiagrass, Coastal bermudagrass, and Tifton 85 bermudagrass hays at 0, 0.5, 1, 2, 3 % DM. The samples were digested in rumen fluid within Ankom® Daisy II incubators, Ankom Technologies USA for 6, 24, and 48 hours. *In vitro* 96 h rumen fluid-pepsin DMD was measured using the two stage, rumen fluid-pepsin technique. Ground (4 mm) samples were also weighed in duplicate into porous, polyester bags and placed in the rumen of two cannulated, cows for 0, 3, 6, 9, 12, 24, 48, 72, 96, and 120 h to estimate the rate and extent of digestion. A 2 (cows) x 3 (hays) x 5 (enzyme rates) factorial arrangement of treatments was used for this study. The cows used for both parts of the study were fed Pensacola bahiagrass *ad lib.* and 900g of soybean meal. Ruminal degradation parameters were estimated using an exponential model. The results described are those for means across the three forages.

**Results and discussion** Forage neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin concentrations ranged from 814 to 889, 450 to 538 and 90 to 130 g/kg DM. The *in vitro* dry DMD of the bermudagrasses (440 g/kg) were greater than that of the bahiagrass (398 g/kg). As the enzyme application rate increased, NDF and hemicellulose concentrations decreased linearly (P=0.001) while there were linear and cubic increases in ADF concentration (P=0.033) and water-soluble carbohydrate concentration (P= 0.04), respectively. Increasing the rate of enzyme application also linearly increased the *in vitro* rumen fluid DMD of the hays incubated for 6 h (P= 0.001) or 24 h (P= 0.03). This suggests that enzyme application increased the rate of digestion of the grasses and could potentially increase their intake in cattle. Increasing the enzyme application rate also resulted in a cubic increase (P=0.001) in the 96 h *in vitro* rumen fluid-pepsin DMD of the hays. The 24 h NDF digestibility of the hays increased linearly (P=0.002) with increasing enzyme application but there were no increases in 6 or 48 h NDF digestibility. Enzyme application also resulted in a cubic increase in the wash value (P=0.001, Table 1), quartic increases in the total degradability (P=0.005) and the rate of degradation (P=0.005), and a cubic decrease in the lag phase (P= 0.03) which reflects how long it takes for the rumen microbes to begin to digest the hays.

**Table 1** Effect of esterase enzyme application on the *in situ* rumen degradability of tropical hays. (g/kg DM)

	Enzyme application rate					Mean	SEM	P value <sup>d</sup>
	0x	0.5x	1x	2x	3x			
Wash loss <sup>a</sup>	90	93	100	124	110	103	4.3	0.001 C
Slowly degradable fraction	553	559	522	539	552	555	17.6	0.070
Total degradability	643	651	623	663	661	648	15.9	0.005 Q
Degradation rate <sup>b</sup>	0.028	0.026	0.033	0.026	0.026	0.028	0.003	0.005Q
Lag phase <sup>c</sup>	6	5	7	6	4	6	1.1	0.030 C

<sup>a</sup> Wash loss or immediately soluble fraction; <sup>b</sup> Expressed per hour; <sup>c</sup> L refers to the lag phase before the start of digestion (h). <sup>d</sup> Letters represent significant (P<0.05) polynomial contrasts: C = cubic effect, Q = quartic effect.

**Conclusions** Application of the esterase enzyme increased the *in vitro* DMD and NDF digestibility of the hays and also increased the rate and extent of degradation.