OBSERVATION OF BUNDLE SHEAT DIGESTION OF *Panicum maximum* Jacq.

B. Lempp[1], V.B.P. Euclides[2], M. da. G. Morais[1], and D.M. Victor[1]

[1]Universidade Federal de Mato Grosso do Sul, C. P. 533, Dourados, MS, 79804-970, Brasil

[2] Embrapa Gado de Corte, Caixa Postal 154, Campo Grande, MS, 79002-970, Brasil

Abstract

Cross-sections of available forage and extrusa fractions of leaf blades of Tanzânia-1, Mombaça and Massai grasses were evaluated for anatomic structure and observations of residue of *in vitro* digestibility. The observations showed greater frequency of scherenchyma girders in leaf blades of cv Massai, compared with order grasses. Tanzânia-1 and Mombaça residues were more susceptible to digestion, while Massai presented more frequently scherenchyma girders associated with less digestible bundle sheath.

Keywords: Massai, microscope, Mombaça, Tanzânia-1.

Introduction

Observations of leaf blades digestion residue revealed variation in the ease with which tissues are digested by rumen microbes, *i.e.* mesophyll is rapidly digested whereas parenchyma bundle sheath is very slow. The chemical nature of bundle sheath, however is not well known. The walls of bundle sheath cells in C_4 grasses are different from those of the mesophyll cells. They show secondary thickening, are about five times the thickness of mesophyll walls (Wilson, 1990), and are weakly lignified as judged from safranin O staining (Lempp, 1997). The C_4 grasses contain a high proportion of bundle sheath, with are rich in

protein and starch and hence are a significant source of easily digestible constituents. For *Panicum maximum* this proportion reached 29.40 % (Lempp, 1997). The apparent lignification, however, does not prevent digestion but may render it incomplete and always slow, taking 48 to 72 h or longer (Akin *et al.*, 1983). These cells may influence dry matter digestibility due to the characteristics of their walls which determine the availability of their polysaccharides to rumen microbes. Our objective was to evaluate the bundle sheath cells digestibility by observation of leaf blades digestion residue and beef cattle feces.

Material and Methods

This research was performed at Embrapa Beef Cattle Research Center (Campo Grande, MS, Brazil). The experiment was conducted in *Panicum maximum* cvs Massai, Mombaça, and Tanzânia-1 plots. Samples were collected from three oesophagally-fistulated steers in December of 1998. The extrusa of each animal was collected in four consecutive days, homogenized, and leaf blade fragments were manually separated. Additionally, excrements of four steers were collected and observed under a light-microscope. Fragments of leaf blades from extrusa *in natura* (1-2 g) were placed in nylon bags (40 μm pores – 12 cm^2) and incubated (Tilley and Terry, 1963). After six, 12, 24, 48, and, 72 h were fixed in formalin-acetic acid-alcohol (FAA). Ten microscope slides with the incubation residue of each sample/time was observed in a light-microscope, and photographed. Samples of leaf blade fragments consisted of a one centimeter section from the median portion of the penultimate expanded leaf blade. Each sample was preserved in FAA, processed according to Daykin and Hussel (1985). Transverse sections of 10 μm were safranin O (red) stained for observations at the light microscope.Bundle sheath cells were retained by a 248 μm nylon mesh (modified from Grabber and Jung, 1991). Cell wall analyses were then performed.

Results and Discussion

Observations under the light-microscope of digestion residues indicated negligible differences among cultivars after six, 12, and 24 h of incubation. The slight differences in relation to bundle sheath cell disappearance at those times could be a result of slow digestibility of the cells compared to mesophyll cells. Mesophyll cells almost disappeared after the 24 h incubation period. The thickness of bundle sheath cell walls may explain the slow digestion. Akin and Burdick (1975) studying the bundle sheath cells of *Cynodon dactylon* observed a laminated cell wall structure of 0.5 to 1.0 μm in width, and a possible lignification of the cell wall. In our study the quadruple-stain triarch technique of the tissues revealed a positive reaction to the safranin O in the bundle sheath cells for the three cultivars studied, indicating the presence of phenolic compounds. Differences among cultivars were more evident after the 48 h incubation period. Greater digestion of bundle sheath cells of cvs. Tanzania-1 and Mombaça were observed compared to cv. Massai (Figure 1). In the cv. Massai residue, a frequent detachment of the bundle sheath cells after 48 and 72 h of incubation could be observed, as well as few cell wall residues and structured leaf blade fragments. Microscopic observations of leaf blades from available forage revealed greater frequency of sclerenchyma *girder* (arrangement of epidermal cells and bundle sheath cells through sclerenchyma cells) for the Massai in relation to the other two cultivars. This specific anatomical arrangement interferes with the middle lamella dissolution, consequently leading to longer retention time of the particles in the rumen (Wilson *et al*., 1989). Microscopic observations of excrements also confirmed the low digestibility of the Massai bundle sheath cells detected by the digestion residue observations. A high frequency of detached cells as well as adhered to the vascular tissue were noticed in the excrements (Figures 2A and 2B). The Massai digestion residue at the 72 h was very similar to the excrement residues. Variations in the chemical cell wall composition might explain the lower digestibility of

Massai bundle sheath cells. Although, tropical grasses are divided into three groups: PCK, NADP-ME, and NAD-ME, the chemical nature of the cell wall has not been described. Akin *et al.* (1983) and Wilson and Hattersley (1983) reported differences in the bundle sheath cell disappearance average in *Panicum* spp. (C_4) after a 20 h incubation period *in vitro*, 23.0 % for NAD-ME and 73.6 % for PCK. Mestoma sheaths were observed in all cultivars studied. Thus, these cultivars are either NAD-ME or PCK, and differences in digestibility could be explained by the chemical composition of the cell wall. Although, a complete isolation of bundle sheath cells was not possible, similar levels of hemicellulose, lignin, NDF, and ADF were observed among the three cultivars. Greater variation was observed for cellulose levels were 28.6 %, 31.3 %, and 36.6 %, for Tanzânia-1, Mombaça, and Massai, respectively. Observations of leaf blades transverse sections, digestion residues, and excrements showed that Massai has a lower digestive weakening and bundle sheath cells digestibility. Barbosa and Euclides (1997) reported less weight gaining of beef cattle fed with Massai compared to Tanzânia-1 and Mombaça. Our findings could explain that fact. Future studies should be directed towards the digestibility of Massai bundle sheath cells, to investigate differences in the chemical nature of its cell structure, and if these differences interfere with the accessibility of rumen microorganisms to the cell wall.

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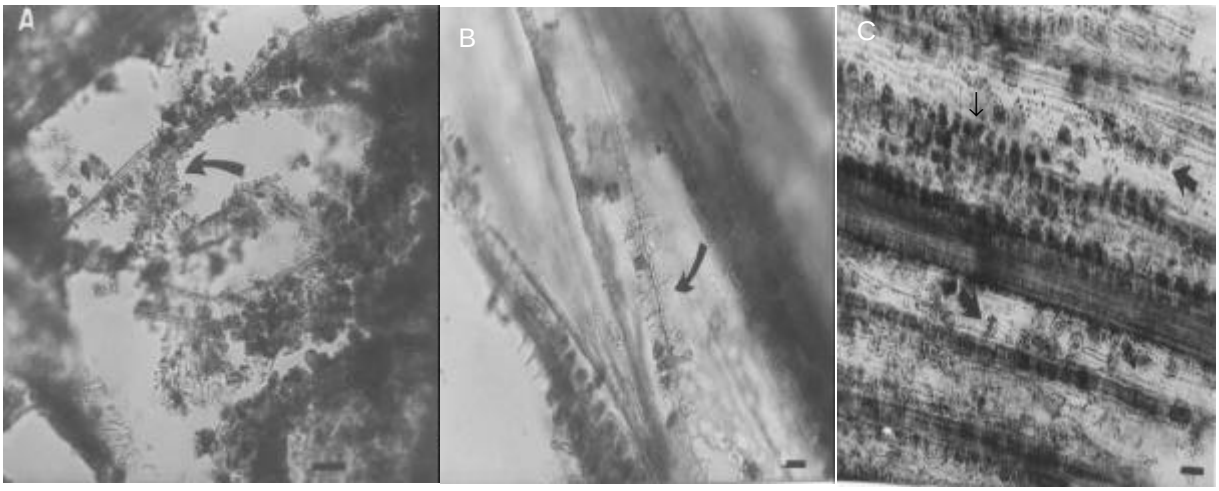

Figure 1 - Digestion residues of leaf blades extrusa after 48h h *in vitro* incubation, → cell wall residue **A.** cv Mombaça. **B.** cv. Tanzânia-1. **C.** cv. Massai, structured leaf blade fragment. (— 20 μm).

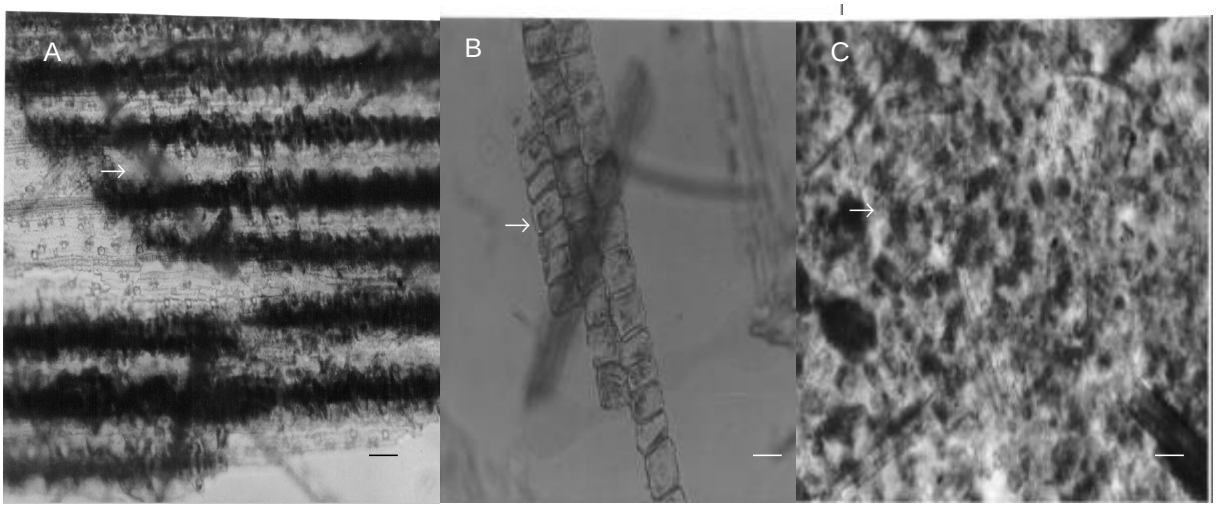

Figure 2 - A e B. Leaf blades fragments of *Panicum maximum* cv Massai in feces. **C.** Isolated bundle sheath cells of leaf blades of cv Massai. → Bundle sheath cells (— 20 μm).