Microhistological Estimation of Leaf Blade Percentage in Diets from Monoespecific Pastures

P. Sierra
Universidad Nacional de Mar del Plata, Argentina

M. S. Cid
Universidad Nacional de Mar del Plata, Argentina

M. A. Brizuela
Universidad Nacional de Mar del Plata, Argentina

C. M. Ferri
Universidad Nacional de La Pampa, Argentina

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MICROHISTOLOGICAL ESTIMATION OF LEAF BLADE PERCENTAGE IN DIETS FROM MONOESPECIFIC PASTURES

P. Sierra[1], M.S. Cid[2,3], M.A. Brizuela[2,4] and C. Ferri[5]

[1] Fac. Cs. Ex. y Nat., and 2Fac. Cs. Agrarias (FCA),Universidad Nacional de Mar del Plata (UNMdP); rtbrizue@criba.edu.ar, cc 276 (7620) Balcarce, Bs.As., Argentina

[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

[4] Comisión Investigaciones Científicas Pcia. Buenos Aires (CICBsAs)

[5] Fac. Agronomía, UNLPam

Abstract

Although a decrease in the leaf-stem ratio affects the nutritive value of pastures, herbivores can reinforce selection for leaf blades to maintain the quality of their diets. This study evaluated whether the percentage of fragments with furrows in blades could be used to estimate the relative intake of this part of the leaves by herbivores grazing monoespecific pastures. It was worked with vegetation of kleingrass (*Panicum coloratum* L.) from paddocks with three deferment periods. Blade samples, and different plant part mixtures hand compounded were *in vitro* digested. The digestion residues were microhistological analyzed determining the number of fragment with furrows (#FWF), and the total number of fragments (T#F). The blade percentages in mixtures was computed as: Estimated $\%Blade_{mixtures} = (((\#FWF_{mixtures} * 100 / \%FWF_{blades}) / T\#F_{mixtures}) * 100$. The %FWF in blade samples (19$\pm$ 1.5%) was not affected (P>0.05) by changes in plant maturity determined by the length of the deferment period. The relationship between the actual blade percentages (y), and those determined by microanalysis (x) in mixtures was 1:1. This suggests that the microanalysis of

feces or digestive contents could be used to estimate the percentages of blades in the diet of herbivores grazing monoespecific pastures.

Keywords: Blade percentage quantification, diets, microanalysis, *Panicum coloratum*

Introduction

Grass canopy architecture affects ingestive behavior of grazing livestock (Chacon y Stobbs 1976, L´Huillier *et al*. 1984, Burlison *et al.* 1991), being the leaf-stem ratio one of the most important factors that determinate leaf blade selection and forage intake.

Botanical composition of herbivore diets can be quantified by microhistological analysis of feces or gastrointestinal contents. However, the utility of this technique to predict the relative intake of leaf blades has received little consideration (Cid and Brizuela 1990). In most grasses, only the adaxial leaf blade has furrows in whose bases are the bulliform cells (Metcalfe 1960). Thus, the microhistological quantification of fragments with furrows could be used to estimate the percentage of blade of a given grass species in herbivore diets. Kleingrass (*Panicum coloratum* L.), is a warm season grass recently introduced in the Semi-arid Pampean Region, whose deferred utilization could provide nutritious forage for winter grazing systems (Stritzler *et al.* 1996). This study evaluated whether the percentage of fragments with furrows in leaf blades allows to estimate the relative contribution of this part of the leaves to digested samples which simulate diets of herbivore grazing monospecific pastures.

Material and Methods

The study was conducted with vegetation samples coming from kleingrass (*Panicum coloratum* L.) paddocks with different canopy structure determined by three deferment

periods in Santa Rosa (36°46'S; 64°16'W), La Pampa province, Argentina. Deferments periods began in mid December of 1997 (T1), and early January (T2) and February (T3) of 1998. Each treatment was replicated twice. In mid May, when early frosts stopped pasture growths, it was harvested plant biomass from each treatment by clipping three 2 m^2 frames to ground level in each paddock. Latter, the different plant parts (blades, sheaths, culms and inflorescences) were sorted, dried (24h, 60^oC), and ground in a Willey mill with a 1 mm sieve screen.

An aliquot of each blade sample was used to estimate by microanalysis the percentage of fragments with furrows ($\%FWF_{blades}$) in this part of the plant. At the same time, an operator who was not involved in the development of the study artificially prepared 12 mixtures containing different known amounts of blades, sheaths, culms and inflorescences. To simulate the effect of animal digestion both, blade samples and mixtures, were *in vitro* digested by the Tilley and Terry (1963) procedure, and the digestion residues were prepared for microhistological analysis according to Sparks and Malechek (1968). Finally, it was registered the number of fragment with furrows (#FWF), and the total number of fragments (T#F) in each blade samples and mixtures, by analyzing 20 fields of 5 slides at 100x power magnification, and computed the percentage of fragments with furrows (%FWF).

The estimated blade percentage in mixtures (Estimated $\%B_{mixtures}$) was computed as:

$$\text{Estimated } \%B_{mixtures} = \frac{(\#FWF_{mixtures} * 100 / \%FWF_{blades})}{T\#F_{mixtures}} * 100$$

Where the numerator estimates the number of leaf fragments in the mixtures.

The effect of plant maturity on the %FWF in blade samples was evaluated by ANOVA in a completely randomized design. We used simple correlation and linear regression to explore the relationships between the estimated blade percentage in mixtures (x), and the

actual one (y), evaluating by *t*-tests if a=0 and b=1, that is if the percentages of blades in mixtures could be predicted by microanalysis.

Results

The blade adaxial surface of kleingrass has deep furrows close to each other with large bulliform cells at their bases (Figure 1). This allows an easy identification of the fragments with furrows of this species, in blade samples as well as in mixtures of different plant parts.

The percentage of fragments with furrows in blade digested samples (19±1.5%) was not affected (P>0.05) by the plant maturity determined by the length of the deferment, and the relationship between the actual blade percentages (y), and those determined by microanalysis (x), was 1:1 (Figure 2).

Discussion

In kleingrass, the microhistological quantification of the percentages of fragments with furrows allows to estimate the percentages of leaf blades in digested mixtures of different plant parts. The specific identification of the fragments by microanalysis is made according to leaf epidermal features, because the epidermis is the only vegetal tissue that resists digestion and has taxonomic value. Although in grasses the epidermal features of the leaves (Metcalfe 1960, Cid and Brizuela 1990) change according to their insertion level, the proportion of fragments with furrows in kleingrass was similar in blades coming from paddocks with different deferment periods. This indicates that, at least in this species, this character is not affected by leaf maturity.

In leaf blade samples it can be expected that 50% of the epidermal fragments belongs to each blade surface. In the kleingrass digested blade samples the percentage of fragments

with furrows was almost 20%. Thus, blade fragmentation and digestion determined that only the 40% of the adaxial surface fragments had furrows. Because in cool season grasses the distance between adaxial furrows is higher than in the warm season species (Norton 1982), in those species the percentage of fragments with furrows would probably be lower.

These results indicated that the microanalysis of feces or digestive contents could be used to estimate the percentages of blades in the diet of herbivores grazing monospecific pastures. Whether this procedure can be used to estimate the blade percentages of different species in gramineous multispecific diets will depend on the anatomical structure and resistance to digestion of the involved species, and on the possibility to differentiate the fragments with furrows coming from each one of them.

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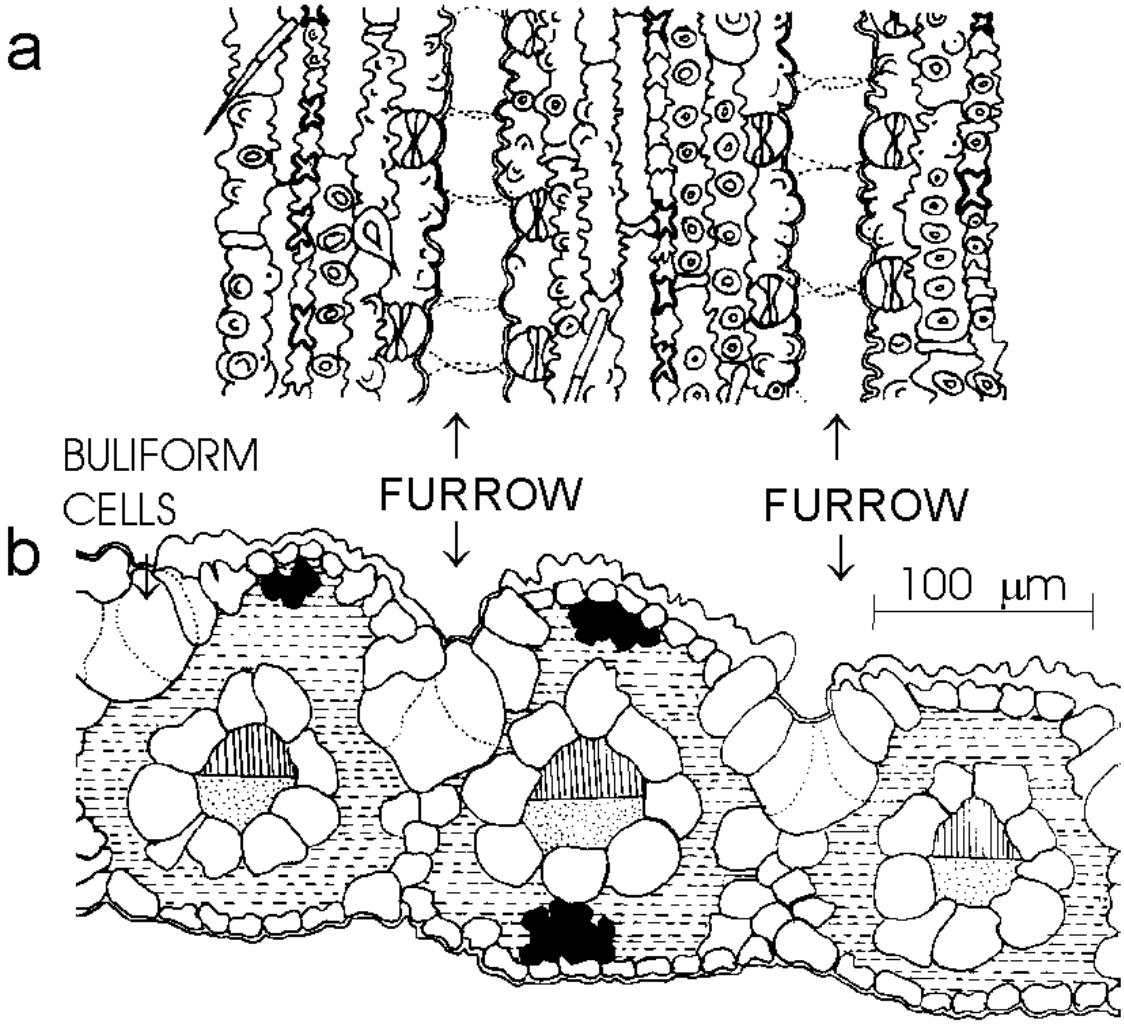

Figure 1 - Superficial view of the adaxial surface (a), and transverse section (b) of *Panicum coloratum* L. leaf blades. The bulliform cells in the bases of the furrows have larger diameters in their internal section (see b), and for this reason in superficial view their size and shape change when the microscope focus is displaced (see a).

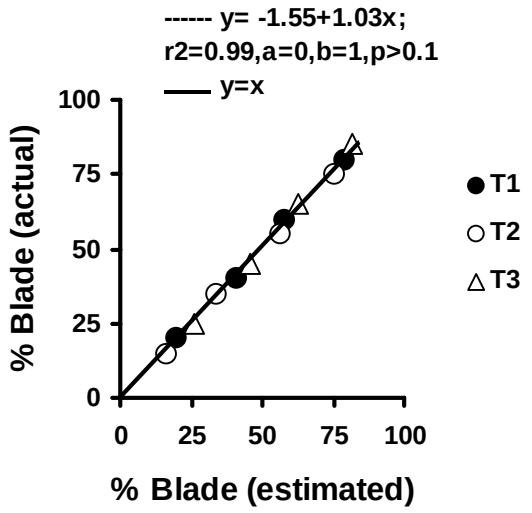

Figure 2 - Relationship between actual (y) and estimated (x) percentages of blades in digested mixtures of *Panicum coloratum* L. plant parts (blades, sheaths, culms and inflorescences). Mixtures were compounded with vegetation coming from different deferment period: December (T1), early January (T2) and February (T3).