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Optimal quadrat area and sample size to estimate the forage mass of stargrass

Área da moldura e tamanho de amostras para estimar a massa de forragem de capim-estrela

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Abstract

The objective of this study was to evaluate the sample size and area of the quadrats necessary to accurately estimate the forage mass (FM) of a fenced pasture of stargrass (*Cynodon nlemfuensis* cv. Florico) during the winter. Five metal quadrats were used: a 0.09 m² square (0.30 m side), a 0.25 m² square (0.50 m side), a 0.25 m² circle (0.28 m diameter), a 0.5 m² rectangle (0.5 x 1.0 m), and a 1 m² square (1.0 m side), each with eight replicates. The size and shape of the quadrats were determined based on cumulative variances to identify combinations that minimized the coefficient of variation (CV). The minimum sample size required to estimate the FM, morphological components and height was established by the CV maximum curvature method. The 0.25 m² square quadrat (0.5 m side) presented the lowest cumulative CV in estimating the FM and the dry mass of dead material. However, for the estimation of the leaf and stem dry mass, the 1.00 m² square quadrat (1.00 m side) presented the lowest CV. Using the 0.25 m² square quadrat, a minimum number of six samples were required for the FM estimation, and eight samples were required for estimating the mean height of the stargrass pasture. Therefore, at least eight samples are recommended to obtain accurate results for the estimation of both variables.

Key words: Cumulative coefficient of variation. *Cynodon nlemfuensis*. Height. Morphological composition.

Resumo

O objetivo desse estudo foi avaliar o tamanho e a área da moldura necessários para estimar a massa de forragem (MF) de um pasto vedado de capim-estrela (*Cynodon nlemfuensis* cv. Florico) durante o inverno. Foram utilizadas cinco molduras metálicas: quadrado de 0,09 m² (0,30 m de lado), quadrado de 0,25 m² (0,50 m de lado), circular de 0,25 m² (0,28 m de raio), retangular de 0,5 m² (0,5 x 1,0 m) e quadrado de 1 m² (1,0 m de lado), cada uma com oito repetições. A dimensão e o formato da moldura foram determinados com base nas variâncias acumuladas para escolha da combinação com menor coeficiente de variação (CV). O número mínimo de amostras necessárias para estimativa da MF, dos componentes morfológicos e da altura foi estabelecido pelo método da máxima curvatura do CV. A moldura de formato quadrado e 0,25 m² de área (0,5 m de lado) apresentou menor CV acumulado

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para estimar a MF e a massa seca de material morto. No entanto, para estimativa da massa seca de folha e de colmo, a moldura de formato quadrado e área de 1,00 m² (1,00 m de lado) apresentou menor CV. Com o uso da moldura de área 0,25 m² de formato quadrado, foi estabelecido o número mínimo de seis amostras necessárias para estimativa da MF e oito amostras para estimativa da altura média do pasto de capim-estrela. Portanto, indicam-se no mínimo oito amostras para obter resultados acurados da estimativa de ambas variáveis.

Palavras-chave: Altura. Coeficiente de variação acumulado. Composição morfológica. *Cynodon nlemfuensis*.

Introduction

The use of *Cynodon* forage grasses in intensive production dairy cattle pasture systems has increased in recent years and has shown a high capacity to reduce costs and increase profits (CARVALHO et al., 2013). *Cynodon* pastures display a high accumulation of forage, a good leaf/stem ratio, and excellent nutritive value (OLIVEIRA et al., 2011). In addition, these plants present great adaptability to different environments and to diverse soil and climate conditions, have a high resistance to management (LIMA; VILELA, 2005) and can be used for both grazing and silage production (PEDREIRA, 2010).

According to Nascimento and Renvoize (2001), *Cynodon nlemfuensis* originates in eastern and central West Africa and is cultivated in tropical and subtropical countries around the world. This species has several cultivars, but the most common is “Puerto Rico stargrass”, also known as the Florico cultivar, which is described as a perennial stoloniferous grass that reaches 30 to 70 cm in height (PEDREIRA, 2010).

The estimation of forage dry mass (FM) in pasture is of fundamental importance, both for scientific research and rational exploration in commercially managed areas (CÓSER et al., 1998). To establish an optimal grazing use and management program, it is necessary to define the quantitative and qualitative criteria of the vegetation, that is, to estimate FM in a simple and precise way so that the appropriate stocking rate can be calculated for a pre-established forage supply, thus allowing for inferences regarding the observed variations in animal performance (ESTRADA et al., 1991;

PACIULLO et al., 2004), with the ultimate goal of making the system sustainable and productive. Thus, identifying the best quadrats and sample size is important to reduce sampling errors of the FM and morphological components (leaf, stem and dead material), as the size and shape of the quadrat (plots, subplots or samples) can interfere with the accuracy of the method evaluation (FRAME, 1981).

The use of indirect methods is a practical and quick way to estimate FM, although they should be validated with direct methods. An example of an indirect method is the use of pasture height. Abramides et al. (1982) found that using the mean uncompressed pasture height is a viable technique to estimate FM, especially in grasses displaying a prostrate growth habit. However, when pasture displays free growth, the use of height is not precise enough to determine the FM, and thus, only an approximation is attained (CAUDURO et al., 2006).

Similar to the shape and size of the quadrat, the number of samples or repetitions can also interfere with precision and accuracy. The optimal sample size is associated with the area (shape and dimension) of the quadrat (FRAME, 1981). The sample size required to obtain a reliable estimate depends on the homogeneity of the pasture structure; the greater the heterogeneity of the area is, the greater the number of samples required for a reliable estimation. One method to obtain the optimal sample size is to establish the minimum number of samples to be collected by obtaining the curve of the cumulative coefficients of variation as a function of the number of samples (LOPES et al., 2000), which is visualized graphically by the method of maximum curvature of the coefficient of variation (CV) described by

Federer (1955).

Mannetje (2000) noted that FM sampling should be preceded by a cost/benefit analysis that considers labor, precision in assessments, and the need for manpower and resources. There is usually a large variation in FM in pasture, and the literature does not provide a recommended sample size for different forage plants. To calculate the sample size required for a given precision (SHAW et al., 1987), a preliminary study is recommended to generate an estimate of the variation between treatments and sampling in treatments.

In a study performed by Carvalho et al. (2010) to determine an appropriate quadrat area (size and shape) to estimate the FM of *Brachiaria humidicola* pastures, the coefficients of variation ranged from 11.17% to 28.45%. Thus, the determination of the size and shape of the quadrat is of great relevance because it allows selection of the area (shape and dimension) that minimizes variability. Defining criteria for pasture sampling is one of the main problems encountered by researchers attempting to reduce experimental errors from plot heterogeneity (GUZMAN et al., 1992).

The objective of this study was to determine the area of the quadrat (shape and size) and the minimum sample size for estimating FM and the uncompressed height of Puerto Rico stargrass (*Cynodon nlemfuensis* cv. Florico) pastures.

Materials and Methods

The study was conducted during the winter of 2010 at the Pesagro-Rio experimental station, located in the municipality of Seropédica, state of Rio de Janeiro, Brazil at a latitude of 22°45'S, a longitude of 43°41'W, and an altitude of 33 m.

The climate of the region is type Aw according to the Köppen classification. The mean annual rainfall is 1,300 mm, and the summers are hot and rainy. The dry season occurs from April to September, and the rainy season occurs from October to March.

The soil of the experimental area was classified as yellow, dystrophic Argisol, with a moderate A horizon and a sandy/medium texture. This climate is designated as a tropical deciduous forest with good drainage.

The experimental area consisted of an approximately 0.1 ha homogeneous Puerto Rico stargrass (*Cynodon nlemfuensis* cv. Florico) pasture containing flowering plants with a mean uncompressed height of 17 cm. The pasture was fenced for a month before assessment.

The experiment was conducted using a completely randomized experimental design with five quadrants and eight replications. The quadrants consisted of five metal structures with different areas and shapes (a 0.09 m² square, a 0.25 m² square, a 0.25 m² circle, a 0.5 m² rectangle, and a 1 m² square).

Two evaluations were carried out. In the first evaluation (09/01/2010), the optimal area (shape and size) of the quadrat was determined, and in the second evaluation (09/08/2010), the minimum sample size for estimating the FM and the mean uncompressed height of the star grass pasture was determined.

In the first evaluation, the five metallic structures described were tested, and systematized sampling was performed every eight meters between samples. The fodder contained inside the quadrats was cut close to the ground and immediately wrapped in labeled plastic bags. These samples were weighed and sub-sampled into parts; they were fractionated into leaves (leaf blades), stems (stem + sheath) and dead material. The subsamples were weighed and dried in a forced air oven at 65°C for 72 hours to determine their dry matter values and dry masses (total, leaf, stem and dead material values). Thus, the optimal size and shape of the quadrat (to accurately measure the FM) was determined based on the cumulative variances of the tested quadrats, and the chosen combination provided the lowest CV (a 0.25 m² square with 0.5 m sides).

In the first evaluation (09/01/2010), the mean uncompressed grass height was evaluated at 40 points, using a ruler graduated in millimeters, between the curvature of the highest leaf at the sampling point and the soil level (FRAME, 1981). Sampling was carried out in a systematized manner every four meters.

Based on the results obtained in the first evaluation (09/01/2010), the second evaluation was performed on 09/08/2010, and 40 samples were collected from the square quadrat with an area of 0.25 m². Sampling was performed in a systematized way every four meters between samples. The samples were processed as described in the first evaluation. Three height measurements were performed in each sample unit using a ruler graduated in millimeters.

The minimum sample sizes required for estimating FM, the dry masses of leaf, stem, and dead material, and mean uncompressed pasture height were assessed graphically by the CV% maximum curvature method (FEDERER, 1955). According to Lopes et al. (2000), after obtaining the curve of the cumulative CV as a function of the sample size, a line is drawn from the point of origin

of the curve to its final point. Next, another line is drawn parallel to the first and tangent to the curve. From the point of tangency, a line perpendicular to the (x) axis is drawn, where the intercept point indicates the minimum sample size required for accurate sampling.

The data were initially tested for the basic assumptions of the analysis of variance (normality of the experimental errors, homogeneity of the variances and additivity of the model) and were then analyzed using the PROC GLM procedure of the statistical package SAS® (Statistical Analysis System), version 8.2 for Windows. The means of the treatments were compared by the Tukey test at 5% probability. To determine correlations, the SAS® PROC REG was used.

Results and Discussion

Forage dry mass (FM), leaf dry mass (LDM), stem dry mass (SDM) and dead material dry mass (DMDM) did not vary ($p>0.05$) with treatment (Table 1), indicating that the criterion adopted for the quadrat shape and size should be the lowest CV value (GUZMAN et al., 1992).

Table 1. Estimates of the coefficient of variation for forage dry mass (FM), leaf dry mass (LDM), stem dry mass (SDM) and dead matter dry mass (DMDM) of Puerto Rico stargrass (*Cynodon nlemfuensis*) as a function of quadrat shape and area.

Quadrat area	Coefficient of Variation (%)			
	FM	LDM	SDM	DMDM
0.09 s	39	72	56	66
0.25 s	26	32	42	21
0.25 c	28	46	39	52
0.50 r	39	50	46	45
1.00 s	27	25	39	35
(kg ha ⁻¹ dry mass)				
0.09 s	2413	555	877	1151
0.25 s	2108	469	997	863
0.25 c	2428	475	926	1361
0.50 r	1570	456	721	629
1.00 s	2150	437	817	1186

s = square; r = rectangle; c = circle.

We verified that the quadrat with an area of 0.25 m² (square with sides of 0.5 m) provided the lowest cumulative variation for the estimation of the total FM (Table 1) of the Puerto Rico stargrass pasture, indicating this quadrant to be the most suitable for the evaluation of this variable in these pastures. Although there were no statistical tests to compare cumulative variances, the number of repetitions of the evaluated area validated this result, which was also reported by Estrada et al. (1991) and Lopes et al. (2000). A similar result was found by Carvalho et al. (2010) when they verified that in non-grazed *Brachiaria humidicola* pasture, the most suitable quadrat for FM estimation was a square with an area of 0.25 m² (with sides of 0.5 m).

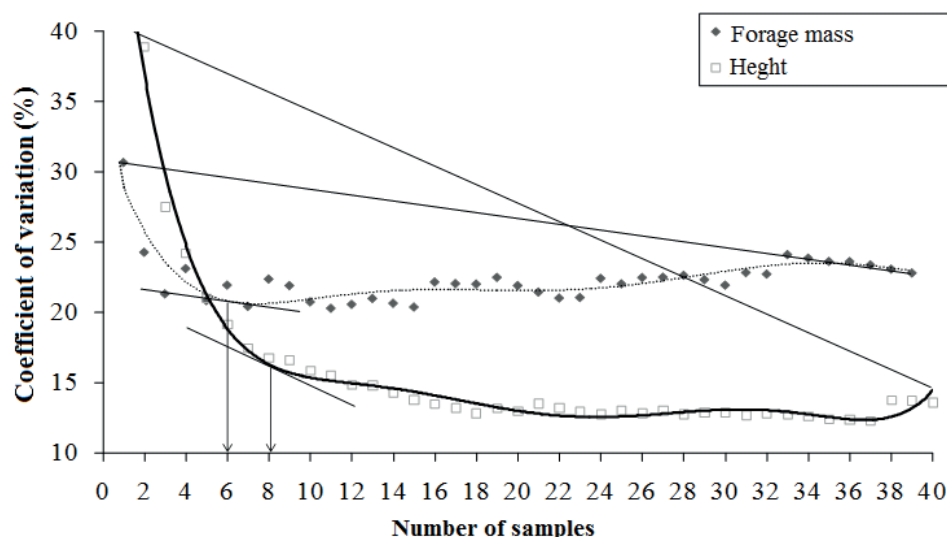
In relation to the morphological composition of the pasture, it was verified that the use of the 1.00 m² (sides of 1.0 m) quadrat provided the lowest cumulative variation in estimating the LDM and SDM (Table 1) and was more suitable for the evaluation of these variables in fenced Puerto Rico stargrass pasture. However, for the DMDM estimation, the quadrat that provided the lowest variation was the same as that most suitable for the FM (a 0.25 m² square) (Table 1).

Arruda et al. (2011) used a 0.25 m² (0.50 x 0.50 m) quadrat with ten replicates to evaluate the FM, LDM, SDM and DMDM of stargrass pasture and observed a CV of 13.63, 18.5, 23.37, and 26.17%, respectively. Therefore, variation between the morphological components, even when using the same shape and the same area, was observed.

The coefficients of variation for the estimation of the SDM, LDM and DMDM were in general higher and could be explained by losses intrinsic to the material separation process. Notably, the 1 m² square quadrat was always among the quadrats that presented the lowest CV for each evaluated parameter because, due to its greater area and, consequently, greater volume of forage collected, the existing variations in the component separation process may have been reduced. Considering that the main objective of this work was to estimate FM, the quadrat choice was based on this variable and on the operability of sample collection and processing.

For the estimation of FM, the relationship between the sample size and the cumulative CV analysis revealed a minimum number of six samples necessary to represent the variation found in the sampled area (Figure 1).

Figure 1. Relationship between the coefficient of variation and the sample size in estimating the forage mass and height of Puerto Rico stargrass (*Cynodon nlemfuensis* cv. Florico) pasture.



Note that the optimal sample size was located at the point at which the increase in CV was equal to or less than the increase in the sample size. According to Penati et al. (2005), it is important to determine the amount of samples for which the value of the CV presents the lowest variation, that is, when the CV becomes constant regardless of the increase in the number of samples. In this sense, Carvalho et al. (2010) used a similar methodology to determine a minimum sample size of eight for the estimation of

FM in *Brachiaria humidicola* pastures.

However, for the morphological components (LDM, SDM and DMDM) of the pasture, the relationship between the number of samples and the cumulative CV did not behave similarly to the FM analysis, as the CV increased as the number of samples increased (Table 2), and it was not possible to establish a minimum sample size for these variables.

Table 2. Variation found in the estimation of each component in the 2nd and 40th samples.

Component	CV (%) of the 2nd Sample	CV (%) of the 40th Sample
Leaf	7.4	39.1
Stem	3.5	35.6
Dead matter	42.2	55.2
Total mass	30.7	22.8

Based on the relationship between the number of samples and CV, eight samples was the minimum sample size required to represent the variation of the uncompressed height of the sampled area (Figure 1). Lopes et al. (2000) verified that the minimum sample size for elephant grass under grazing conditions was 15.

Based on these results, a minimum of eight samples for the estimation of both variables (FM and uncompressed height) offers more accuracy.

Low correlations were observed between the height and FM ($r = 0.25$), LDM ($r = 0.12$), SDM ($r = 0.13$) and DMDM ($r = 0.05$). Therefore, the use of height to estimate the DM of forage and morphological components is not recommended under the conditions of the present study (fenced pasture and grass reproductive stage). However, Costa et al. (2009) verified a positive linear relationship with a high coefficient of correlation ($r = 0.94$) between height and FM in a pasture of *Brachiaria decumbens* that was in a vegetative condition and in full growth (during spring and summer).

The percentages of leaves, stems and dead material of the Puerto Rico stargrass in September were 16%, 32% and 42% respectively. According to Fagundes et al. (1999), *Cynodon* spp. pastures contain approximately 60% living material and 40% dead material, and for lenient or lighter grazing intensities, these values are closer to 50%.

In the second evaluation, we observed that the leaf/stem ratio was 0.60. This low leaf-to-stem ratio is possibly due to the stargrass presenting more stems and stolons, especially during the reproductive phase, or due to a decrease in soil water availability, which reduces the growth of new leaves, (BONFIM-SILVA et al., 2011), especially during the winter. Rodrigues et al. (2006) evaluated five *Cynodon* cultivars and observed that after 28 days of growth, Tifton-85 had the highest leaf/stem ratio (0.96), differing from the Florico and Florona cultivars, which had the lowest leaf/stem ratios (0.57 and 0.60, respectively) and were similar to those of Tifton-68 and Florakirk (0.78 and 0.75, respectively).

The area sampled in relation to the total area was 0.2%. However, to obtain 5% of the total sampled area, as indicated by Mannetje (2000), 200 samples would be required. However, the CV stabilized for both the FM and height when six and eight samples, respectively, were analyzed. According to Mannetje (1987), the proportion of the sampled area can vary from 10% to 100% in reduced pasture areas (cut-plot experiments) or less than 5% when evaluating extensive pasture areas.

Conclusions

To estimate the forage DM and height of Puerto Rico stargrass (*Cynodon nlemfuensis* cv. Florico) during the winter, we recommend using a 0.25 m² square quadrat with the collection of at least eight samples.

We do not recommend using height to estimate the forage DM and morphological components for Puerto Rico stargrass pastures during the flowering phenological stage.

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