Research Paper

Evaluation and reparametrization of mathematical models for prediction of the leaf area of *Megathyrsus maximus* **cv. BRS Zuri**

Evaluación y reparametrización de modelos matemáticos para la predicción del área foliar de Megathyrsus maximus cv. BRS Zuri

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Abstract

The aim of this study was to verify the precision and accuracy of 5 models for leaf area prediction using length and width of leaf blades of *Megathyrsus maximus* cv. BRS Zuri and to reparametrize models. Data for the predictor variables, length (L) and width (W) of leaf blades of BRS Zuri grass tillers, were collected in May 2018 in the experimental area of Embrapa Gado de Corte, Mato Grosso do Sul, Brazil. The predictor variables had high correlation values (P<0.001). In the analysis of adequacy of the models, the first-degree models that use leaf blade length (Model A), leaf width × leaf length (Model B) and linear multiple regression (Model C) promoted estimated values similar to the leaf area values observed (P>0.05), with high values for determination coefficient (>80%) and correlation concordance coefficient (>90%). Among the 5 models evaluated, the linear multiple regression (Model C: $\beta 0 = -5.97$, $\beta 1 = 0.489$, $\beta 2 = 1.11$ and $\beta 3 = 0.351$; R² = 89.64; P<0.001) and as predictor variables, width, length and length × width of the leaf blade, are the most adequate to generate precise and exact estimates of the leaf area of BRS Zuri grass.

Keywords: Leaf length, leaf width, non-destructive assessment, regression, research methods, tropical pasture.

Resumen

El objetivo de este estudio fue verificar la precisión y exactitud de 5 modelos para la predicción del área foliar, utilizando el largo y ancho de las láminas foliares de *Megathyrsus maximus* cv. BRS Zuri, y para reparametrizar los modelos usados. Los datos de las variables predictivas fueron largo (L) y ancho (W) de las hojas de brotes de cv. BRS Zuri los cuales fueron recolectados en mayo de 2018 en el área experimental de Embrapa Gado de Corte, Mato Grosso do Sul, Brasil. Las variables predictivas mostraron valores altos de correlación (P<0.001). En el análisis de adecuación de los modelos, aquellos de primer grado que utilizan el largo de la hoja (Modelo A), el ancho × largo de la hoja (Modelo B) y la regresión lineal múltiple (Modelo C) llevaron a valores estimados similares a los valores de área foliar observados (P>0.05), con valores altos para el coeficiente de determinación (>80%) y el coeficiente de correlación de concordancia (>90%). Entre los 5 modelos evaluados, la regresión lineal múltiple (Modelo C: $\beta 0 = -5.97$, $\beta 1 = 0.489$, $\beta 2 = 1.11$ y $\beta 3 = 0.351$; R² = 89.64; P <0.001) y, como variables predictivas el ancho, el largo y el producto ancho × largo de las hojas, representan el método más adecuado para generar estimativos precisos y exactos del área foliar del pasto *M. maximus* cv. BRS Zuri.

Palabras clave: Ancho foliar, largo foliar, mediciones no destructivas, métodos de investigación, pastos tropicales, regresión.

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Introduction

Studies involving the leaf areas of tillers are necessary to understand the processes of organogenesis, tissue expansion and photosynthesis of the forage canopy (Gastal and Lemaire 2015). To estimate leaf area of the tiller directly, it is necessary to measure, via area integrator or by other means of image digitalization, all leaves on the tiller. However, owing to the scarcity of labor or financial resources for the acquisition of customized equipment, it is necessary that researchers develop and/or use alternative and efficient techniques.

Non-destructive methods for measuring leaf area using regression equations are already used in several crops of agronomic interest, e.g. *Theobroma cacao*, *Arachis pintoi*, *Stylosanthes* spp., *Calopogonium mucunoides*, *Neonotonia wightii* and *Coffea canefora* (Santos et al. 2014; Homem et al. 2017; Espindula et al. 2018). In forage grasses, few published studies have evaluated and validated models to make accurate leaf area estimates (Toebe et al. 2019; Bezerra et al. 2020; Fernandes et al. 2020a).

Megathyrsus maximus cv. BRS Zuri has high phenotypic plasticity, is adapted to cultivation in association with other grass species (Barbosa et al. 2018) or in monoculture (Veras et al. 2020) and is highly productive under moderate doses of nitrogen (Gomide et al. 2019). Understanding of the interrelation of this grass with the environment must be investigated due to its high forage potential (Freitas et al. 2018; Braga et al. 2019; Silva et al. 2020).

Length and width of leaf blades are frequently measured in experimental tests in forage and pasture studies due to the ease of obtaining these data in the field, so the hypothesis to be tested is that: length and width of leaf blades can be used as predictor variables in regression models to accurately estimate leaf area of BRS Zuri grass.

The aim of this study was to verify the precision and accuracy of models for estimating leaf area using length and width of leaf blades of *Megathyrsus maximus* cv. BRS Zuri as predictor variables and to reparametrize existing models.

Materials and Methods

Experimental area information

Data for the predictor variables, length (cm) and width (cm) of leaf blades of BRS Zuri grass tillers, were

collected in May 2018 in the experimental area of Embrapa Gado de Corte, Campo Grande, Mato Grosso do Sul, Brazil (20°27' S, 54°37' W; 530 masl).

According to the Köppen classification the climate of the region is of the tropical rainy savanna type, subtype Aw, characterized by a well-defined dry period during the coldest months of the year and a rainy period during the hottest months. The temperature and precipitation data during the experimental period, recorded by the meteorological station (A702 - INMET) located in the municipality of Campo Grande, revealed a good distribution of rainfall during the period of data collection (<u>Barbosa et al. 2018</u>).

The soil in the experimental area is classified as Red Dystrophic Latosol, characterized by clayey texture. Information related to establishment, soil chemical composition, fertilizer and pasture management and experimental period was presented by Barbosa et al. (2018).

Pasture was sown in February 2017 in an area of 0.75 ha, divided into 3 paddocks of 0.25 ha, managed in an intermittent grazing system, with a pre-grazing height of 80 cm and moderate grazing intensity (50%). Grazing was carried out by Caracu cows from the Embrapa herd.

Procedures used to measure leaf area

To estimate leaf area of the BRS Zuri grass pastures, 100 expanding or fully expanded green leaf blades were collected during the pre-grazing period. Length and width of all leaf blades were measured, with width measured at the median portion of each leaf blade; measurements were taken with a rule graduated in centimeters (Silva et al. 2013; Diavão et al. 2017; Fernandes et al. 2020b). All leaf blades were processed in the area integrator model LICOR3000 to measure the observed leaf area (Sbrissia and Da Silva 2008; Sbrissia et al. 2018). In the leaf area integrator, the leaf blade is inserted, and after the scanner process, the entire surface area of the object inserted in the equipment is calculated.

Models used for leaf area estimates

Based on reports by Diavão et al. (2017), Bezerra et al. (2020) and Fernandes et al. (2020a), it was possible to verify that there is no standard model for measuring leaf area in forage grasses. We tested 5 models for estimating leaf area based on measures of leaf length and leaf width:

Model A: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \epsilon i$; Model B: PLAi (cm²) = $\beta 0 + \beta 1 \times (L \times W) + \epsilon i$; Model C: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \beta 2 \times W + \beta 3 \times (L \times W) + \epsilon i$: Model D: PLAi (cm²) = $\beta 0 \times (L)^{\beta 1} + \epsilon i$; Model E: PLAi (cm²) = $\beta 0 \times (L \times W)^{\beta 1} + \epsilon i$; where: PLAi (cm²) = predicted leaf area; L (cm) = leaf blade length; W (cm) = leaf blade length; W (cm²) = product of length and width of leaf blade; $\beta 0$ = equation intercept; $\beta 1, \beta 2$ and $\beta 3$ = slope of the equation; and ϵi = error associated with that observed in the response variable.

Statistical analysis

The independent variables were correlated ('L', 'W' and 'L \times W') with observed leaf area. The correlation matrix was obtained using the corrgram package (Wright 2018). Descriptive analysis (mean, maximum, minimum and standard deviation), parameter estimates and correlation matrix were performed using software R version 4.0.

The criteria for assessing the adequacy of the models were: determination coefficient (R²); F test, for the identity of the parameters ($\beta 0 = 0$ and $\beta 1 = 1$) of the regression of the data predicted by the observed ones; the correlation concordance coefficient (CCC); the square root of the mean square of the prediction error (RMSEP); and the decomposition of the mean square of the prediction error (MSEP) into mean bias, systematic bias and random error (Tedeschi 2006), using the Model Evaluation System version 3.1.16. For the chosen models, reparametrization estimates ($\beta 0$, $\beta 1$, $\beta 2$ and $\beta 3$) were performed.

Results

Correlation between observed leaf area and predictor variables

The predictor variables had high correlation values (P<0.001). The 95% confidence interval reveals that the observed leaf area values are highly correlated with leaf blade width (P<0.001), leaf blade length (P<0.001) and the product of leaf blade length and width (P = 0.009) (Figure 1).

Model evaluation

In the analysis of adequacy of the models, the first-degree models that use leaf blade length (Model A), leaf width × length (Model B) and multiple regression (Model C) promoted estimated values similar to the leaf area values observed (P>0.05), with high values for determination coefficient (>80%) and correlation concordance coefficient (>90%). For these models, the RMSEP decomposition allowed us to observe that 99 and 100% of the error is of random origin. Leaf area predictions using the 'D' and 'E' models were underestimates ($\beta 0 \neq 0$ and $\beta 1 \neq 1$) in relation to the observed leaf area values (Table 1 and Figure 2).

Model reparametrization

After the model validation analysis, it was possible to measure the parameters for models A, B and C (Table 2).



Figure 1. Correlation matrix between variables used to estimate leaf area and observed leaf area of *Megathyrsus maximus* cv. BRS Zuri. $OLA = observed leaf area; W (cm) = leaf blade width; L (cm) = leaf blade length; L \times W (cm²) = product of leaf blade length and width.$

Item	Observed	Model ¹					
	_	А	В	С	D	Е	
Mean (cm ²)	41.67	40.70	40.66	40.62	39.75	41.10	
Standard deviation	24.93	23.24	23.62	23.61	28.43	25.95	
Maximum (cm ²)	91.44	88.08	87.80	88.13	120.09	94.69	
Minimum (cm ²)	0.15	-11.57	1.71	-3.90	0.06	0.15	
P value	-	0.996	0.998	0.999	< 0.001	0.020	
R ² (%)	-	86.20	88.80	89.64	84.60	88.60	
CCC (%)	-	92.60	94.10	94.60	91.20	94.00	
RMSEP	-	86.02	69.62	63.69	125.07	76.70	
MB (%)	-	0.007	0.002	0.000	0.605	0.300	
SB (%)	-	0.000	0.001	0.000	23.01	7.30	
RE (%)	-	99.99	99.99	100.00	76.38	92.39	

Table 1. Evaluation of the adequacy of models for predicting leaf area of Megathyrsus maximus cv. BRS Zuri.

¹Model A: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \epsilon i$; Model B: PLAi (cm²) = $\beta 0 + \beta 1 \times (L \times W) + \epsilon i$; Model C: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \beta 2 \times W + \beta 3 \times (L \times W) + \epsilon i$; Model D: PLAi (cm²) = $\beta 0 \times (L)^{\beta 1} + \epsilon i$; Model E: PLAi (cm²) = $\beta 0 \times (L \times W)^{\beta 1} + \epsilon i$. PLAi (cm²) = predicted leaf area; $\beta 0$ = intercept of the equation; $\beta 1$, $\beta 2$, $\beta 3$ = parameter slopes; ϵi = error associated with that observed in the response variable. W (cm) = leaf blade width; L (cm) = leaf blade length; L \times W (cm²) = product of leaf blade length and width. R² = adjusted coefficient of determination; P value = probability, associated with the F test for the identity of the parameters, of the regression of the data observed by the predicted ones; CCC = correlation concordance coefficient; RMSEP = square root of the mean square of the prediction error; MB = mean bias; SB = systematic bias; RE = random error. The values in bold indicate the models that present the best fit, as well as the similarity of the estimated values with those observed.



Figure 2. Comparison between the observed leaf area (cm²) (x axis) and predicted (y axis) values in BRS Zuri grass. Predicted leaf area A: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \epsilon i$; Predicted leaf area B: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \epsilon i$; Predicted leaf area B: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \beta 2 \times W + \beta 3 \times (L \times W) + \epsilon i$; Predicted leaf area D: PLAi (cm²) = $\beta 0 \times (L)^{\beta 1} + \epsilon i$; Predicted leaf area E: PLAi (cm²) = $\beta 0 \times (L \times W)^{\beta 1} + \epsilon i$. PLAi (cm²) = predicted leaf area; $\beta 0$ = intercept of the equation; $\beta 1$, $\beta 2$, $\beta 3$ = parameter slope; ϵi = error associated with that observed in the response variable. W (cm) = leaf blade width; L (cm) = leaf blade length; L × W (cm²) = product of leaf blade length and width.

Model	Parameter (±standard error)					
	β0	β1	β2	β3	-	
Model A	-14.52±2.39	1.32 ± 0.053	-	-	< 0.001	
Model B	$1.44{\pm}1.63$	0.559 ± 0.020	-	-	< 0.001	
Model C	-5.97 ± 4.03	0.489±0.173	1.11±3.19	0.351±0.092	< 0.001	

Table 2. Parameters obtained in the adjusted model to make predictions for leaf area of Megathyrsus maximus cv. BRS Zuri.

Model A: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \epsilon i$; Model B: PLAi (cm²) = $\beta 0 + \beta 1 \times (L \times W) + \epsilon i$; Model C: PLAi (cm²) = $\beta 0 + \beta 1 \times L + \beta 2 \times W + \beta 3 \times (L \times W) + \epsilon i$. PLAi (cm²) = predicted leaf area; $\beta 0$ = intercept of the equation; $\beta 1$, $\beta 2$, $\beta 3$ = parameter slope; ϵi = error associated with that observed in the response variable. W (cm) = leaf blade width; L (cm) = leaf blade length; L $\times W$ (cm²) = product of leaf blade length and width. P value = probability associated with the F test for the identity of the parameters.

Discussion

For the studied equations, a high relationship between predicted and observed data for leaf area of BRS Zuri grass ($R^2 = >80\%$) was noted. However, not all models showed a good fit; the power models (D and E) using the 'L' and 'L × W' variables showed high values of systematic bias (Table 1), which indicates that the models will produce inaccurate and biased estimates (Tedeschi 2006).

This situation occurred because leaf blades of grasses generally are shaped similar to a triangle and trapezoid (<u>Sousa et al. 2015</u>), which requires a precise method or models to allow estimation of leaf area of these grasses with high reliability.

The first degree regression model that used variables 'L' and 'L × W' presented accurate estimates of leaf area of BRS Zuri grass. However, it was already consolidated in the literature that using 2 or more dimensions (e.g. 'L × W') is more appropriate as an independent variable in first degree linear models (Silva et al. 2013; Diavão et al. 2017; Toebe et al. 2019), in addition to having a high correlation with the values of the dependent variable (Figure 1). Leite et al. (2019) estimated leaf area of 2 millet genotypes with high reliability, when they used 'L × W' as a predictor variable.

Although the first degree models show considerable precision due to the low values of systematic bias, the multiple regression model using the variables 'L', 'W' and 'L × W' showed lower values of RMSEP with 100% of the error of random origin (Table 1). Therefore, we recommend using the equation 'PLAi (cm²) = -5.97 + 0.489 × L + 1.11 × W + 0.351 × (L × W) + ε i' (Table 2) to estimate leaf area of BRS Zuri grass, because it is considerably more precise and accurate than the other models presented in this research.

Conclusions

Among the 5 models evaluated, the linear multiple regression ($\beta 0 = -5.97$, $\beta 1 = 0.489$, $\beta 2 = 1.11$ and $\beta 3 = 0.351$; R² = 89.64; P<0.001) incorporating width, length and product of length and width of the leaf blade is the most

adequate for generating accurate estimates of leaf area of BRS Zuri grass. This finding supports earlier studies, which indicated a combination of length and width measurements of leaves is currently the most accurate for estimating leaf area of grasses. We encourage public and private companies to initiate further studies of evaluation, adaptation and reparametrization of leaf area prediction models for other tropical grass cultivars.

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