Revista Brasileira de Ciência do Solo

Division - Soil Use and Management | Commission - Soil Fertility and Plant Nutrition

Evaluation of the nutritional state and proposal of sufficiency ranges for *Megathyrsus maximus* 'BRS Tamani' using the compositional nutrient diagnosis and boundary line methods

Antonia Marta Sousa de Mesquita[(1)], Danilo Eduardo Rozane[(2)], Roberto Cláudio Fernandes Franco Pompeu[(3)], Magno José Duarte Cândido[(4)], Marcos Neves Lopes[(5)], William Natale[(6)], Mariana Passos da Conceição[(2)], Juliana Domingues Lima[(2)], Rita de Kássia Oliveira Tavares[(1)]* and Henrique Antunes de Souza[(7)]

[(1)] Universidade Estadual do Vale do Acaraú, Centro de Ciências Agrícolas e Biológicas, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, Sobral, Ceará, Brasil.
[(2)] Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Agronomia de Registro, Registro, São Paulo, Brasil.
[(3)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Caprinos e Ovinos, Sobral, Ceará, Brasil.
[(4)] Universidade Federal do Ceará, Departamento de Zootecnia, Fortaleza, Ceará, Brasil.
[(5)] Instituto Federal de Educação, Ciência e Tecnologia do Ceará, *Campus* Crateús, Ceará, Brasil.
[(6)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroindústria Tropical, Fortaleza, Ceará, Brasil.
[(7)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Meio-Norte, Teresina, Piauí, Brasil.

*** Corresponding author:**
E-mail: kassiaoliveira120@gmail.com

Received: August 19, 2025

Approved: December 12, 2025

How to cite: Mesquita AMS, Rozane DE, Pompeu RCFF, Cândido MJD, Lopes MN, Natale W, Conceição MP, Lima JD, Tavares RKO, Souza HA. Evaluation of the nutritional state and proposal of sufficiency ranges for *Megathyrsus maximus* 'BRS Tamani' using the compositional nutrient diagnosis and boundary line methods. Rev Bras Cienc Solo. 2026;50:e0250152.
https://doi.org/10.36783/18069657rbcs20250152

Editors: José Miguel Reichert and Adelson Paulo de Araújo.

ABSTRACT: Brazil has the largest commercial cattle herd in the world, with 197.2 million head in 2023, representing approximately 12 % of the global herd. Cattle raising in Brazil is primarily carried out in pasture grazing systems. In this context, the adoption of well-adapted, high-yielding, and nutritious forage crops is essential for the sustainability of cattle raising, cost reduction, and increased yield efficiency. The cultivar 'BRS Tamani', a *Megathysus maximus* hybrid, stands out for its short plant height, high nutritional value, and resistance to pasture spittlebug and is recommended for well-drained soils of medium to high fertility, typical of the Brazilian Cerrado region. Despite its high potential, information is lacking regarding nutritional standards for the cultivar, which hinders precise fertilization recommendations. This study aimed to establish nutritional sufficiency ranges for BRS Tamani using the Compositional Nutrient Diagnosis (CND) and boundary line methods, aiming to improve nutrient management and promote yield gains. A database was used to compile information from 123 experimental units of BRS Tamani during the period from July to December 2017. Leaf samples were collected at various growth cycles / cuttings of the forage crop, and chemical analyses were performed to determine contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). The CND method was used to calculate nutritional levels and identify nutrient imbalances. The boundary line method was applied to establish relationships between yield and the levels of each nutrient. The following critical levels were proposed by the CND: N = 20 g kg^{-1}; P = 2.2 g kg^{-1}; K = 20 g kg^{-1}; Ca = 2.3 g kg^{-1}; Mg = 2.8 g kg^{-1}; and S = 1.4 g kg^{-1}. The values obtained by the boundary line method were as follows: N = 23 g kg^{-1}; P = 2.3 g kg^{-1}; K = 20 g kg^{-1}; Ca = 2.6 g kg^{-1}; Mg = 2.8 g kg^{-1}; and S = 1.3 g kg^{-1}.

Keywords: nutritional diagnosis, *Megathyrsus maximus*, pasture.

INTRODUCTION

Brazil, with the largest commercial cattle herd in the world, plays a crucial role in global meat production, accounting for approximately 12 % of the global cattle herd and consolidating its position as the second-largest producer and leading exporter of beef (ABIEC, 2023). In 2023, Brazil exported 2.29 million tons of beef to 157 countries, underscoring the sector importance to the Brazilian economy. The cattle herd, at 197.2 million head in 2023, is predominantly characterized by pasture-based grazing (ABIEC, 2023).

Within this context, the search for better-adapted, higher-yielding, and high-quality forage crops is a foundational support for the sustainability of Brazilian cattle raising. To diversify the use of forage crops in pastures and intensify Brazilian livestock production, Embrapa (Empresa Brasileira de Pesquisa Agropecuária), in partnership with Unipasto (Forage Breeding Research Association), released the cultivar 'BRS Tamani', a *P. maximum* hybrid that meets the demand for a forage crop with short plant height, simplified management, and resistance to pasture spittlebug (Jank and Santos, 2021).

The BRS Tamani stands out for its higher nutrient value compared to the cultivar 'Massai' and is recommended for well-drained soils of medium to high fertility in all the states of the Cerrado biome under annual rainfall greater than 800 mm and up to six months of dry season (Jank and Santos, 2021).

Thus, this cultivar can be used in different production systems and growing environments, such as in integrated crop-livestock-forest systems or in intercropping with corn, with diverse purposes, such as generating crop residue (cover crop) for a no-tillage system or as pasture (Silva et al., 2020; 2024) in grain production environments in the Brazilian Cerrado. In addition, BRS Tamani has been considered an excellent option as a forage crop for establishing pastures in the Brazilian semi-arid region (Fontinele et al., 2022).

Optimizing nutritional management is crucial to maximizing the yield potential of BRS Tamani. Leaf analysis, which is widely used, enables identification of nutrient deficiencies or excesses, making it possible to implement more efficient management and fertilization practices (Wadt et al., 2013).

In this context, Compositional Nutrient Diagnosis (CND), proposed by Parent and Dafir (1992), emerges as an advanced tool for interpreting plant tissue analysis. The CND method considers nutrient balance, and detects imbalances that may not be identified by traditional methods. It provides a correction factor for non-measured dry matter components (R factor), as well as generates variables (Zi) that allow multivariate analyses (McCray et al., 2010). In addition, the boundary line method has been used as an alternative approach for analyzing databases (from soil or plant samples) to generate sufficiency ranges for leaf diagnosis or the evaluation of soil fertility classes. It considers the upper part of the point cloud, which represents the relationship between the property of interest and the yield of the crop under study (Lima Neto et al., 2020a; Souza et al., 2024). Both methods have been considered effective for proposing sufficiency ranges to assess the nutritional status of crops (Ali, 2023).

This study aimed to establish nutritional standards for BRS Tamani forage using the CND and the boundary line methods, to improve fertilization recommendations and optimize crop nutrient management. The results may contribute to the sustainability and productivity of Brazilian cattle production by providing accurate, reliable information for the nutritional management of BRS Tamani.

MATERIALS AND METHODS

Database location and information

The database comprised results from studies conducted by the Forage Crop Teaching and Research Group at the Federal University of Ceará, in Fortaleza, Ceará, Brazil (30°43’02” S and 38°32’35” W), from July to December 2017. The region under study is classified as Aw tropical rainy according to the Köppen classification system, with a mean annual rainfall of 1,615 mm (Carvalho, 2013), and the soil in the area is classified as *Argissolo Amarelo eutrófico típico* (Santos et al., 2018).

The experimental area was managed under a low-pressure fixed sprinkler irrigation system (operating pressure <2.0 kg cm^{-2}) for 40 min in the morning (6:00 h), applying a net irrigation depth of 6.8 mm day^{-1}. The cultivar *Megathysus maximus* ‘BRS Tamani’ was sown in April 2017, and a uniformity cut was performed in August. The samples that composed the present database were collected during the second half of that year at different cutting times (after the uniformity cut).

A database with information from 123 experimental units was used. This information consisted of leaf macronutrient results for BRS Tamani, collected over four growth cycles, or cuttings of approximately 30 days each, after the uniformity cut. For leaf sampling, plants/tillers with recently expanded leaves were randomly sampled from experimental plots (four to five subsamples to form a composite sample), always taking care to avoid plot borders and conspicuous signs of soil/vegetation differences. The leaves that were sampled at the end of the vegetative period were those most recently expanded and with the presence of the ligule, so as to sample the whole leaf (leaf blade + ligule) during the active growth stage.

The leaves were washed with a 1 mL L^{-1} neutral detergent solution and then with running water, immersed in a 30 mL L^{-1} HCl solution for 15 seconds, and finally, rinsed with distilled water. After that, the leaves were placed in paper bags in a forced-air circulation laboratory oven at 65 ± 5 °C until reaching constant weight. Then the samples were ground in a Wiley mill (with a 2 mm screen). Evaluation of nutrient concentration followed the method of Bataglia et al. (1983). Nitrogen (N) content was determined using sulfuric acid digestion, followed by Kjeldahl distillation. For phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), nitroperchloric acid digestion was performed. Potassium, Ca, and Mg concentrations were obtained by atomic absorption spectrophotometry. Phosphorus and S were measured by colorimetry, using the method of Murphy and Riley (1962) in a spectrophotometer.

Compositional nutrient diagnosis

The CND standards were established using the database that integrated information on leaf nutrient concentrations and total forage dry matter (TFDM) production (kg ha^{-1}) from BRS Tamani. Data analysis showed considerable variation in the yield of the cultivar, with a minimum value of 251.20 kg ha^{-1} and maximum value of 4,083.18 kg ha^{-1}. Mean yield was 1,752.38 kg ha^{-1}, with a standard deviation of 652.4 kg ha^{-1}, indicating the variability of the results.

The analysis of leaf tissue composition assumes that it can be considered a closed system, in which the nutrients represent the quantified components, whereas the elements not determined in the dry matter constitute the non-quantified components, expressed by the term *Rd* (Parent and Dafir, 1992). Based on this concept, the relationship among the leaf tissue components can be modeled as a simplex (S^d), derived from the ratios of d+1 (Equation 1):

$$S^d = \begin{Bmatrix} N > 0; P > 0; K > 0; Ca > 0; [\dots] Fe > 0; Mn > 0; Zn > 0 \\ N + P + K + Ca + [\dots] + Fe + M + Zn + R_d = 100 \end{Bmatrix} \quad \text{Eq. 1}$$

in which: *100* is the total dry matter value (%); *N, P, K*, ... are the proportions of these nutrients in the dry matter (%); and *Rd* corresponds to the residue or non-measurable concentrations in the dry matter (%), calculated in the following manner (Equation 2):

$$R_d = 100 - \sum_{i=1}^{d} Xi \qquad \text{Eq. 2}$$

in which: R_d is the residue or concentration of non-measurable nutrients in the dry matter (mg kg^{-1}); *Xi* is the concentration of each nutrient in the leaf tissue (mg kg^{-1}); and *d* is the number of nutrients evaluated.

To eliminate scale dependence, the nutrient proportions were transformed into scale-invariant variables through division by the geometric mean (G) of the d+1 components (Equation 3). Equation 3 reflects the dependent nature of the compositional data, where the variation in one component affects the relative values of the others (Aitchison, 1982).

$$G = \left(\prod_{i=1}^{d} Xi\right)^{\frac{1}{(d+1)}} = (N \times P \times K \times [\dots] \times Rd)^{\frac{1}{(d+1)}} \qquad \text{Eq. 3}$$

in which: *G* is the geometric mean of the dry matter constituents (g kg^{-1}); R_d is the residue or concentration of non-measurable nutrients in the dry matter (mg kg^{-1}); *Xi* is the concentration of each nutrient in the leaf tissue (g kg^{-1}); and *d* is the number of nutrients evaluated.

To analyze the multivariate relationships among nutrients, the natural logarithm of the ratio of each nutrient concentration to the geometric mean of the nutritional composition in leaf sampling was used. This procedure, known as the centered log-ratio (clr) transformation, allows expression of each simplex component in relation to the others (Equation 4).

$$clr_{nutrient} = V_N = \ln\frac{N}{G};\ V_P = \ln\frac{P}{G};\ V_K = \ln\frac{K}{G}; [\dots]; V_{Rd} = \ln\frac{R_d}{G} \qquad \text{Eq. 4}$$

in which: $clr_{nutrient}$ is the centered log-ratio of the nutrients; V_N, V_P, V_K, *[...]*, V_{Rd} are the centered log-ratio transformation (*clr*) values for N, P, K, ..., R_d (mg kg^{-1}); and *G* is the geometric mean of the dry matter constituents (g kg^{-1}).

Logarithmic transformation of the values was carried out to correct data dispersion using equation 5.

$$V_N + V_P + V_K + V_{Ca} + V_{Mg} + [\dots] + V_{Rd} = 0 \qquad \text{Eq. 5}$$

in which: V_N, V_P, V_K, *[...]*, V_{Rd} are the centered log-ratio values of the nutrient variables.

The Mahalanobis distance (Parent et al., 2009) was applied to detect and remove outliers before classification of the CND indices (Equation 6). Nutrient limitation order was established based on the arithmetic mean of the concentrations observed in the low-yielding population.

$$D^2 = \sum_{i=1}^{d} (clr_i - clr_i{}^{*})^T COV^{-1} (clr_i - clr_i{}^{*}) \qquad \text{Eq. 6}$$

in which: clr_i is the sample to be compared; clr_i* is the mean of the reference population (high yield); COV^{-1} is the inverse covariance matrix of the reference population; and *T* means that the matrix must be transposed.

Samples were categorized as high- or low-yielding according to the methodology proposed by Khiari et al. (2001). Compositional nutrient diagnosis standards were established using means and standard deviations, derived from the centered log-ratio transformed nutrient relationships (V_X) of high-yield specimens; in other words, V_N*, V_P^*, V_K^*, ... V_R^* and SD_N^*, SD_P^*, SD_K^*, ... SD_R^*, respectively. The CND indices for the elements were then calculated using equation 7:

$$I_N = \frac{(V_N - V_N{}^*)}{\sigma_N};\ I_P = \frac{(V_P - V_P{}^*)}{\sigma_P};\ I_K = \frac{(V_K - V_K{}^*)}{\sigma_K}; [\ldots];\ I_{Rd} = \frac{(V_{Rd} - V_{Rd}{}^*)}{\sigma_{Rd}} \qquad \text{Eq. 7}$$

in which: I_N, I_P, I_K, *[...]*, I_{Rd} are the nutrient balance indices; V_N, V_P, V_K, *[...]*, V_{Rd} are the centered log-ratio values of the nutrient variables; V_N*, V_P*, V_K*, *[...]*, V_{Rd}* are the means of the reference population; and σ_N, σ_P, σ_K, *[...]*, σ_{Rd} are the standard deviations of the variables in the reference population (high yield).

The overall nutritional imbalance of the sample was evaluated through calculation of the CND-r² index, which consists of the sum of squares of the nutrient balance indices, as shown in equation 8.

$$CND{-}r^2 = I_N{}^* + I_P{}^* + I_K{}^* + \cdots + I_R{}^* \qquad \text{Eq. 8}$$

Based on the correlation between the leaf nutrient concentrations and their respective CND indices, specific equations were developed for each nutrient. The critical level of each nutrient was determined by setting the nutrient index to zero, which indicates the equilibrium point. The sufficiency ranges were established based on the critical level, with upper and lower limits calculated by adding and subtracting 2/3 of the standard deviation of the nutrient concentrations in the general population (Kurihara et al., 2013; Souza et al., 2013, 2015; Santos and Rozane, 2017; Rozane et al., 2020; Lima Neto et al., 2020b; Rodrigues et al., 2022). The analyses cited were carried out using R software, with the package described in Van Den Boogaart et al. (2013).

Boundary line

The boundary line calculations were performed according to Blanco-Macías et al. (2009), Lafond (2009, 2013), Quesnel et al. (2006), and Bhat and Sujatha (2013). Before beginning the calculations, possible outliers were identified and excluded using a box plot, always considering normality (Shapiro-Wilk) for crop yield.

The first step was to plot soil nutrient content data (x-axis) against relative yield (y-axis). The second step was to select points located at the upper boundary of the scatterplot. In this case, the maximum and minimum contents values were identified, the difference between them was determined, and this difference was divided by "n" classes. Then, the highest point (concentration) within each interval was selected (Blanco-Macías et al., 2009).

Given that the study used 123 samples, a maximum of 15 classes was employed, as the model was developed with less than 25 % of the observations. This was to limit the selection of points to the upper boundary of the scatterplot and to maximize the probability of developing statistically significant models by increasing the number of observations (Vizcayno-Soto and Côté, 2004). The number of classes used was considered the square root of the number of samples, after outliers were excluded. Some authors suggest that the number of classes be greater than 10 or even up to 20 (Vizcayno-Soto and Côté, 2004; Blanco-Macías et al., 2009; Bhat and Sujatha, 2013).

This approach led to the selection of samples in adjacent intervals with large yield differences, suggesting that the lower-yielding samples were not under optimal conditions for that level of nutrition (Vizcayno-Soto and Côté, 2004; Quesnel et al., 2006; Bhat and Sujatha, 2013). Thus, classes were excluded, considering the creation of a future concave quadratic model, that is, with an ascending curve up to the maximum point and descending after that. Classes were excluded using the procedure of Vizcayno-Soto and Côté (2004) and Quesnel et al. (2006), according to equations 9, 10, 11 and 12.

$$RY > RY_{-1}\ and\ RY_{+1} \qquad \text{Eq. 9}$$

$$RY < RY_{-1} and\ RY_{+1} \qquad \text{Eq. 10}$$

$$RY < (RY_{-1}\ and\ RY_{+1})/2 \qquad \text{Eq. 11}$$

$$\frac{RY}{\left[\frac{RY_{-1} + RY_{+1}}{2}\right]} < 90\ \% \qquad \text{Eq. 12}$$

in which: RY, RY_{-1}, and RY_{+1} are the relative yields and the adjacent lower and upper points, respectively.

If these criteria were met, the sample from the class in question was excluded (Vizcayno-Soto and Côté, 2004; Quesnel et al., 2006). In addition, the yield of the sample selected from the first class could not be higher than that of the second class. Otherwise, the first class could be discarded and the procedure initiated with the second class. Analogous reasoning was performed for the last class selected, which could be discarded if the relative yield was higher than that of the preceding class. After exclusion of possible classes with values that do not meet the assumptions described, a second-degree polynomial function was generated. The optimal concentration, or critical level (CL), was determined by solving the first derivative of the quadratic regression equation (Equation 13).

$$C = \frac{-b}{2a} \qquad \text{Eq. 13}$$

in which: *a* and *b* are the coefficients of the quadratic equation.

Thus, a sufficiency range was generated, corresponding to 95 % of the maximum yield, with subsequent substitution into equation 14 (Quesnel et al., 2006; Blanco-Macías et al., 2009).

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \qquad \text{Eq. 14}$$

in which: *a*, *b*, and *c* are coefficients of the quadratic equation, and *x* is the corresponding value of the nutrient concentration in the soil used to generate fertility classes.

With the critical levels and sufficiency ranges determined, reasoning analogous to that used for the results from the CND was applied. The values were compared with the indices proposed for forage crops by Quaggio et al. (2022). Electronic spreadsheets were used to carry out these analyses.

RESULTS

Leaf contents

The descriptive statistics of the data under analysis are shown in table 1, which contains the nutrient composition and the yield of 123 experimental units of the BRS Tamani forage cultivar. The application of Mahalanobis distance (Parent et al., 2009) enabled the identification and removal of 13 outliers, resulting in a final database with 110 experimental units, with yields ranging from 251.2 to 3084.1 kg ha^{-1}, a mean of 1672.0 kg ha^{-1}, and a standard deviation of 567.3 kg ha^{-1} (Table 1). The evaluation of leaf nutrient composition showed that, except for calcium, with a mean content lower than the recommended range, the mean contents of the nutrients were in accordance with the standards established by Quaggio et al. (2022).

Table 1. Minimum, maximum, mean, standard deviation (SD), and coefficient of variation (CV) of the leaf nutrient contents and yield obtained in the pasture of the cultivar BRS Tamani (n = 110)

Variable	N	P	K	Ca	Mg	S	Yield
	g kg^{-1}						kg ha^{-1}
Minimum	11.20	1.49	11.00	1.60	2.10	0.90	251.2
Maximum	41.30	3.40	26.40	3.80	3.95	2.70	3084.1
Mean	19.47	2.30	18.12	2.60	2.87	1.43	1672.0
SD	5.33	0.41	3.17	0.44	0.41	0.36	567.3
CV (%)	0.27	0.18	0.17	0.17	0.14	0.25	0.3

Pearson correlation was performed to assess the interactions between leaf nutrient concentrations and total forage dry matter (Table 2). Among the 21 correlations, seven were significant, which were classified as moderate positive correlations for TFDM-K, N-Mg, and N-S; a moderate negative correlation only for Ca-K; weak positive correlations for N-K and Ca-Mg; and a weak negative correlation for TFDM-Ca, according to the classification system of Dancey and Reidy (2006), in which the intensity of the correlation coefficient magnitudes are classified into ranges, from 0.10 to 0.30 for weak correlation, 0.40 to 0.60 for moderate correlation, and 0.70 to 1.0 for strong correlation.

Compositional nutrient diagnosis

The population was divided into high- and low-yield subpopulations, according to the methodology proposed by Khiari et al. (2001). The inflection point of the cumulative function, corresponding to 1658.96 kg ha^{-1}, was used as the criterion for segregation of the subpopulations. Of the 110 plots evaluated, 62 (56.4 %) had total forage dry matter production above 1658.96 kg ha^{-1}, constituting the high-yield (reference) subpopulation, while the 48 remaining plots formed the low-yield subpopulation (Figure 1).

The high-yield population showed variability ranging from 1689.7 to 3084.1 kg ha^{-1}, with a mean of 2081.9 kg ha^{-1} and standard deviation of 323.2 kg ha^{-1}. The low-yield population, in turn, exhibited yield ranging from 251.2 to 1611.0 kg ha^{-1}, with a mean of 1142.4 kg ha^{-1} and standard deviation of 320.5 kg ha^{-1} (Table 3). Regarding nutritional composition, with the exception of potassium in the low-yield population, which had concentrations below recommended levels, the other nutrients in both populations were within the limits established by Quaggio et al. (2022) for forage crops in the state of São Paulo (Table 3).

After separation into high- and low-yield populations, new correlation matrices were calculated for leaf nutrient content and yield (Table 4). In the high-yield population, two significant correlations were observed; in the low-yield population, four were observed. In the high-yield population, TFDM-N and Ca-Mg showed a moderate positive correlation, whereas in the low-yield population, moderate correlations among nutrients were observed for TFDM-N, N-Mg, N-S, and K-Ca (Table 4).

Table 2. Pearson correlation matrix between the leaf nutrient concentrations and yield in pastures of the cultivar BRS Tamani (n = 110)

Properties	N	P	K	Ca	Mg	S
TFDM	0.20	-0.08	0.42*	-0.36*	0.03	0.00
N		0.25	0.27*	-0.02	0.42*	0.48*
P			-0.03	0.02	0.19	0.15
K				-0.43*	0.09	0.24
Ca					0.27*	0.00
Mg						0.26

*: Significant at $p<0.05$ by the t-test; TFDM: total forage dry matter.

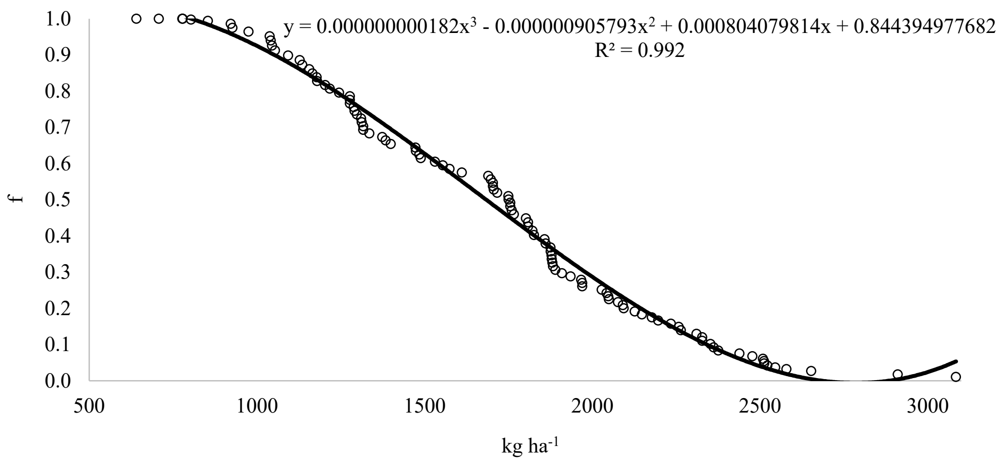

Figure 1. Cumulative function of the data on total forage dry matter (kg ha^{-1}) in pasture of the cultivar BRS Tamani (n = 110).

Table 3. Minimum, maximum, mean, standard deviation (SD), and coefficient of variation (CV) of the results of leaf nutrient contents and yield obtained in the pasture of the cultivar BRS Tamani for the high- and low-yield populations

Variable	N	P	K	Ca	Mg	S	Yield
	g kg^{-1}						kg ha^{-1}
High yield (n = 62)							
Minimum	14.0	1.5	14.3	1.6	2.2	0.9	1689.7
Maximum	30.8	3.4	26.4	3.7	3.7	2.4	3084.1
Mean	20.8	2.3	19.6	2.4	2.9	1.5	2081.9
SD	4.0	0.4	2.8	0.4	0.4	0.3	323.2
CV (%)	0.19	0.18	0.14	0.17	0.13	0.22	0.2
Low yield (n = 48)							
Minimum	11.2	1.6	11.0	2.2	2.1	0.9	251.2
Maximum	41.3	3.3	22.7	3.8	4.0	2.7	1611.0
Mean	17.8	2.3	16.2	2.8	2.8	1.4	1142.4
SD	6.3	0.4	2.6	0.4	0.4	0.4	320.5
CV (%)	0.35	0.18	0.16	0.14	0.16	0.29	0.3

Table 4. Pearson correlation matrix between the leaf nutrient contents and yield in high- and low-yield pastures

Attributes	N	P	K	Ca	Mg	S
High yield (n = 62)						
TFDM	0.49*	-0.09	-0.07	0.17	-0.11	0.06
N		0.18	0.19	0.16	0.27	0.29
P			0.06	0.00	0.13	0.17
K				-0.14	-0.16	0.25
Ca					0.41*	-0.05
Mg						0.21
Low yield (n = 48)						
TFDM	-0.51*	-0.14	0.08	-0.23	-0.27	-0.32
N		0.36	0.12	0.10	0.49*	0.61*
P			-0.14	0.01	0.27	0.15
K				-0.44*	0.19	0.21
Ca					0.37	0.14
Mg						0.29

*: Significant at $p<0.05$, by the t-test; TFDM: total forage dry matter.

Analysis of the high-yield population revealed a positive correlation between the nutritional imbalance index (CND-r^2) and Mahalanobis distance (D^2) (Figure 2), that is, the higher the D^2 values, the greater the nutritional imbalance of the plants, with a coefficient of determination (R^2) of 0.55.

The standards of the CND indices for each nutrient were established based on the mean leaf nutrient contents and the standard deviation of the high-yield population (Table 5). With the CND index standards thus established, the mean nutrient contents in the range considered suitable for BRS Tamani were compared with those proposed in the manual of liming and fertilization recommendations for the state of São Paulo (Quaggio et al., 2022) (Figure 3). The nutritional imbalance result (CND-r^2) was 27.51. According to the standards established by the method, the contents recommended by the state manual were underestimated for N, P, and S and overestimated for Ca.

The relationship between the CND-r^2 index across all plots and yield values (Table 6) was described by a linear equation with a coefficient of determination (R^2) of 25 % ($R^2 = 0.25$). The mathematical models for regression analysis between the CND-r^2 index of the nutrient and its respective leaf content, with the exception of Mg ($R^2 = 0.61$), exhibited coefficients of determination greater than 70 % ($R^2 \geq 0.70$), reaching 90 % for N ($R^2 = 0.90$).

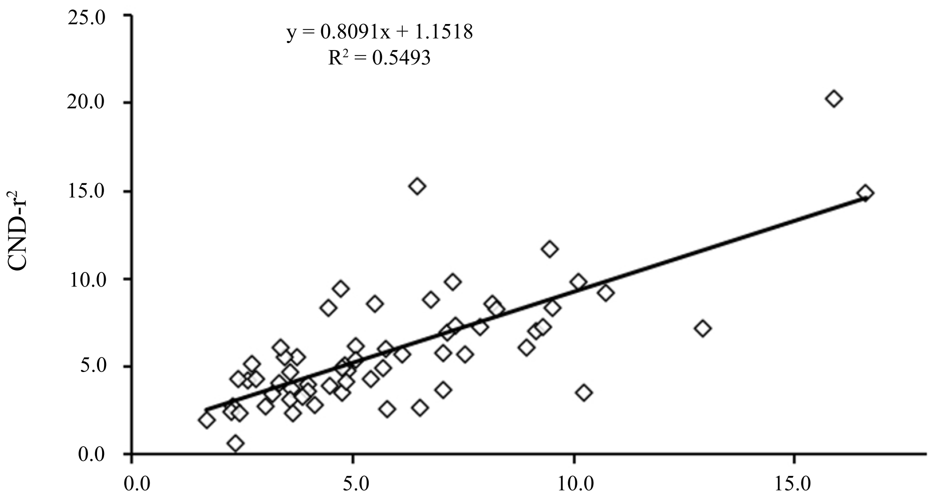

Figure 2. Relationship between the nutritional imbalance index (CND-r^2) and Mahalanobis distance (D^2) in the high-yield population (n = 64), considering forage dry matter per hectare as the yield benchmark.

Table 5. Compositional nutrient diagnosis (CND) standards of the reference population (n = 62), considering total forage dry matter

	V_N	V_P	V_K	V_{Ca}	V_{Mg}	V_S	
Mean	0.07	-2.13	0.02	-2.07	-1.88	-2.59	
Standard Deviation	0.16	0.16	0.14	0.16	0.11	0.18	
	IN	IP	IK	ICa	IMg	IS	CND-r^2
Upper limit	1.64	2.04	2.64	2.57	2.11	2.13	20.28
Lower limit	-2.92	-2.46	-2.37	-2.04	-2.12	-1.86	0.64
Mean deviation	0.81	0.83	0.76	0.79	0.83	0.82	2.48
Skewness	-0.71	-0.04	-0.19	-0.14	-0.10	0.04	1.81

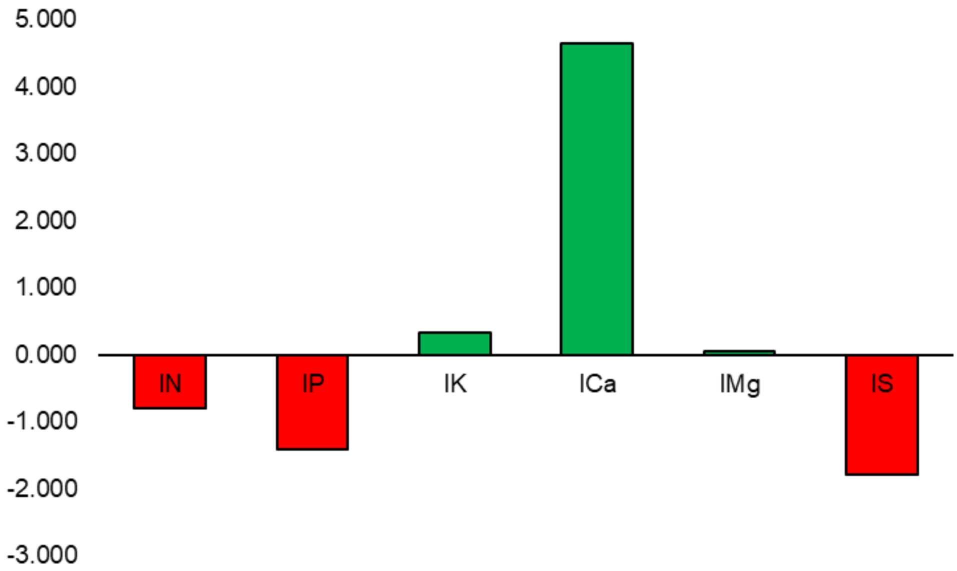

Figure 3. Comparison between the mean contents recommended for the forage cultivar BRS Tamani (Quaggio et al., 2022) and the nutritional imbalance index (CND-r^2) of the high-yield population, considering total forage dry matter as the yield benchmark.

Table 6. Statistical models used to determine critical nutrient levels based on compositional nutrient diagnosis (CND) indices, considering the total forage dry matter of the cultivar BRS Tamani as the yield benchmark

Nutrient	Equation	R^2	Critical level
			g kg^{-1}
N	IN = $-0.0053x^2 + 0.4534x - 7.064$	0.90	20.49
P	IP = $-0.8003x^2 + 5.9163x - 9.0856$	0.74	2.18
K	IK = $0.3272x - 6.3967$	0.74	19.55
Ca	ICa = $-0.5476x^2 + 5.1757x - 9.0927$	0.76	2.33
Mg	IMg = $-0.9841x^2 + 7.6828x - 13.802$	0.61	2.80
S	IS = $-1.1163x^2 + 6.2384x - 6.5336$	0.89	1.40
Yield	Iprod = $-0.0051x + 16.534$	0.25	

Boundary line

Regarding the ranges generated by the boundary line method, the number of classes used for the macronutrients, after exclusions, was 5 for N (Figure 4a), 5 for P (Figure 4b), 5 for K (Figure 4c), 6 for Ca (Figure 4d), 5 for Mg (Figure 4e), and 5 for S (Figure 4f), considering a total of 11 initial classes. The coefficients of determination (R^2) ranged from 0.69 to 0.97.

Sufficiency ranges

The sufficiency ranges proposed by the CND method for BRS Tamani were compared with those recommended by Quaggio et al. (2022), where, generally, a reduction in the amplitude of the suitable ranges was observed. However, the ranges proposed by the boundary line method were close to those suggested by the CND, and the critical levels proposed by the two methods were also similar (Table 7).

DISCUSSION

The results showed that the yield of BRS Tamani is not influenced by the content of a single nutrient, but rather by nutrient interactions. Comparative analysis between the total population (Table 2) and the high- and low-yield subpopulations (Table 4) revealed distinct patterns of correlation between the leaf nutrient concentrations and the dry matter yield of BRS Tamani. In the total population, a moderately positive correlation was observed between potassium concentration and dry matter yield. In the subpopulations, moderate correlation was observed only for nitrogen, which was positive in the high-yield population and negative in the low-yield population. These results show that yield is not determined solely by the concentrations of a single nutrient, but rather by complex interactions among the nutrients, consistent with the conclusions of Marschner (2012) and Rozane et al. (2016).

The use of the Mahalanobis distance (D^2) and the nutritional imbalance index (CND-r^2), as proposed by Parent and Dafir (1992), enables the identification of nutritional imbalances even in high-yield populations. This method shows that, regardless of yield level, revising nutritional management practices is essential to optimize nutrient balance. Positive correlation between CND-r^2 and D^2, shown by a coefficient of determination (R^2) of 0.55 (Figure 2), indicates that greater nutritional imbalances are associated with larger Mahalanobis distances. This finding is consistent with the results of Rozane et al. (2020), who, in studies on grapevines, observed an R^2 of 0.42 in the reference population, reinforcing the importance of nutritional evaluation in high-yield populations.

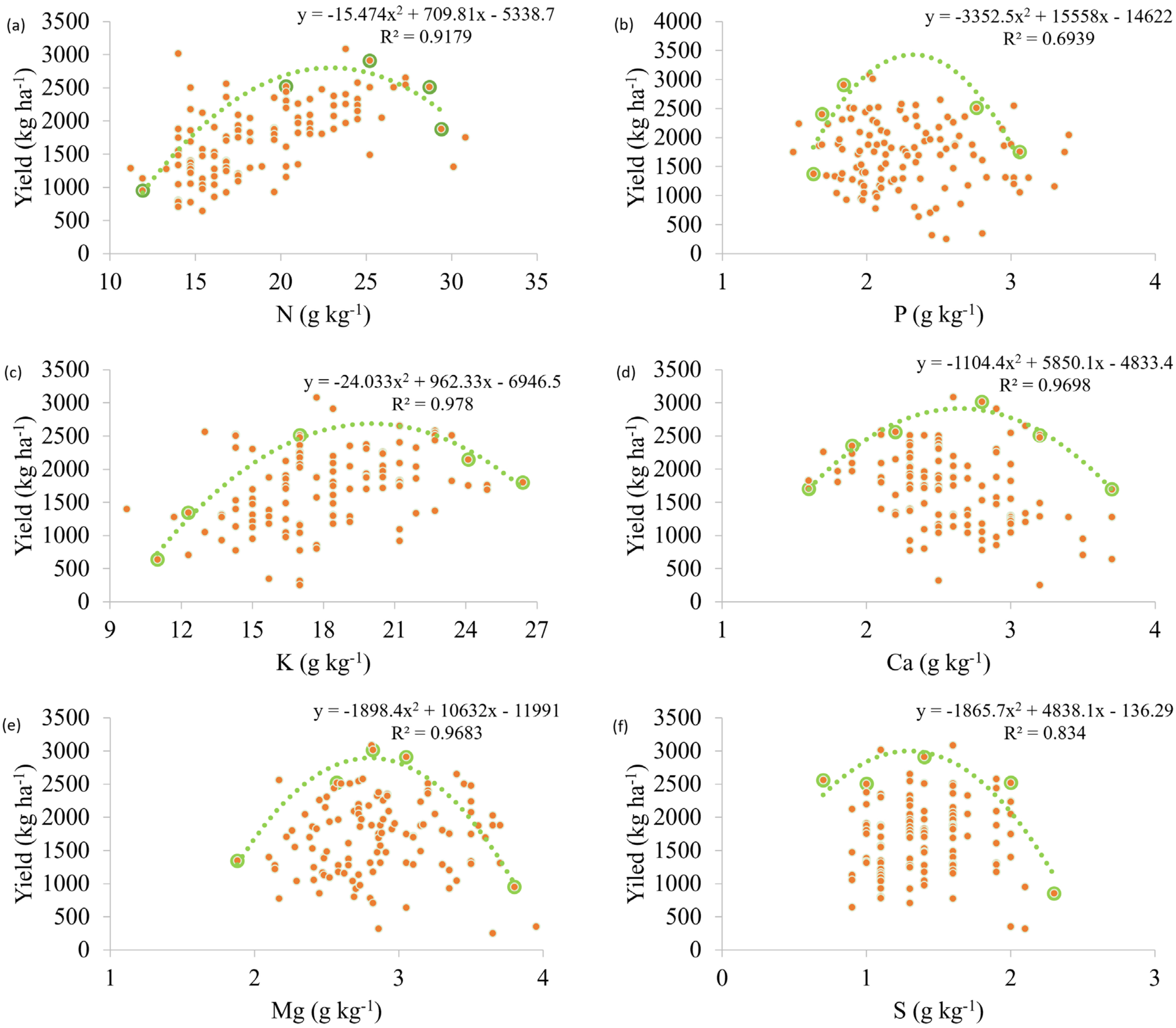

Figure 4. Relationship between the properties of leaf nitrogen (a), phosphorus (b), potassium (c), calcium (d), magnesium (e), and sulfur (f) and yield, plotted using the boundary line method for Tamani grass.

Table 7. Nutrient sufficiency ranges considered adequate in forage samples determined through the CND method and the boundary line (BL) method in this study, compared to the recommendations established for forage crops (Quaggio et al., 2022)

Nutrient	Reference	Adequate range	Adequate content
N	CND - Tamani grass	17 - 24	20
	BL - Tamani grass	19 - 26	23
	Quaggio et al. (2022)	15 - 25	-
P	CND - Tamani grass	1.9 - 2.5	2.2
	BL - Tamani grass	2.1 - 2.6	2.3
	Quaggio et al. (2022)	1.0 - 3.0	-
K	CND - Tamani grass	18 - 21	20
	BL - Tamani grass	18 - 22	20
	Quaggio et al. (2022)	15 - 30	-
Ca	CND - Tamani grass	2.1 - 2.6	2.3
	BL - Tamani grass	2.3 - 3.0	2.6
	Quaggio et al. (2022)	3 - 8	-
Mg	CND - Tamani grass	2.6 - 3.1	2.8
	BL - Tamani grass	2.0 - 3.6	2.8
	Quaggio et al. (2022)	1.5 - 5.0	-
S	CND - Tamani grass	1.1 - 1.6	1.4
	BL - Tamani grass	1.0 - 1.6	1.3
	Quaggio et al. (2022)	1.0 - 1.3	-

A simulation performed between a hypothetical area using the mean nutrient concentrations recommended for the forage cultivar BRS Tamani (Quaggio et al., 2022) and the standard established by the CND method for the high-yield population (Figure 3) resulted in a CND-r^2 value of 27.5, which represents the overall nutritional imbalance index of the sample, where samples tending toward zero are considered balanced.

The coefficient of determination (R^2) obtained for the relationship between the nutritional imbalance indices (CND-r^2) and yield was 25 % (Table 6), showing that leaf nutrient contents explain a limited portion of the yield variation. However, it is essential to consider that agricultural yield is a complex phenomenon, influenced by multiple factors that act interdependently on plant growth, development, and yield, as highlighted by Tisdale et al. (1985).

Analysis of the values of the coefficients of variation obtained for Tamani grass using the boundary line method show excellent fit of the models (R^2 from 0.69 to 0.97), compared to other similar studies, with crops such as cowpea (R^2 from 0.66 to 0.94; Melo et al., 2020), *Opuntia fícus indica* (R^2 from 0.50 to 0.90; Ali, 2018), and soybean (R^2 from 0.67 to 0.89; Souza et al., 2020).

It is important to highlight that some studies indicate that the generation of sufficiency ranges using the CND and boundary line methods suggests similar and comparable values (Blanco-Macías et al., 2009), which can be confirmed in this study. In this case, the critical level value or optimal content is equal for the nutrients P, K, Mg, and S. For P and S, the difference is of only one decimal place, showing a discrepancy of less than 10 %. However, for N and Ca, the discrepancies between the optimal levels are near 15 %. Comparison with reference literature is not possible because the critical or optimal levels are not reported. However, if the critical level is considered as the lower value of the sufficiency range, the optimal concentrations proposed by the boundary line method are near those reported in the reference literature for N, Ca, Mg, and S. Nevertheless, for P, the values are greatly underestimated, and for K, greatly overestimated, regardless of the method.

The differences observed between the methods tested (CND and boundary line) and the reference literature for the proposed sufficiency ranges can be explained by the fertilization used, soil type, and soil management practices, which reinforce the need to establish regional nutritional standards. Another explanation for the differences between the proposed values and the reference literature is the need to analyze nutritional standards by forage plant genus, or even by species. The reference literature presents generalized recommendations.

Reinforcing the reasoning presented, the use of irrigation further contributes to modulate the differences observed between the present data and those reported in the reference literature, which are primarily for forage crops under dryland production systems. The use of irrigation alters the nutritional status of the plants due to greater intensification of the production system.

The results presented are a first approximation of reference values that allow qualification of plant tissue analysis results for *Megathysus maximus* BRS Tamani; that is, they enable assessment of fertilization management, enabling adjustments for the next cutting or re-entry of animals in the area. The results assist adjustments in fertilization of forage crops based on an increased technological level through the use of plant tissue analysis.

CONCLUSIONS

The critical levels proposed for assessment of leaf diagnosis in Tamani grass are N: 20 and 23 g kg^{-1}, P: 2.2 and 2.3 g kg^{-1}, K: 20 and 20 g kg^{-1}, Ca: 2.3 and 2.6 g kg^{-1}, Mg: 2.8 and 2.8 g kg^{-1}, and S: 1.4 and 1.3 g kg^{-1} by the CND (Compositional Nutrient Diagnosis) method and boundary line method, respectively. The boundary line and CND methods are efficient alternative approaches for generating sufficiency ranges for forage crops. The sufficiency ranges have a regional character and should consider the edaphoclimatic conditions of the production environment.

DATA AVAILABILITY

The data will be provided upon request.

AUTHOR CONTRIBUTIONS

Conceptualization: Antonia Marta Sousa de Mesquita (lead).

Data curation: Antonia Marta Sousa de Mesquita (equal), Danilo Eduardo Rozane (equal), Henrique Antunes de Souza (equal), Juliana Domingues Lima (equal), Magno José Duarte Cândido (equal), Mariana Passos da Conceição (equal) and William Natale (equal).

Formal analysis: Antonia Marta Sousa de Mesquita (equal), Danilo Eduardo Rozane (equal), Henrique Antunes de Souza (equal) and Magno José Duarte Cândido (equal).

Funding acquisition: Henrique Antunes de Souza (equal) and Roberto Cláudio Fernandes Franco Pompeu (equal).

Investigation: Antonia Marta Sousa de Mesquita (lead).

Methodology: Antonia Marta Sousa de Mesquita (equal), Danilo Eduardo Rozane (equal), Magno José Duarte Cândido (equal), Marcos Neves Lopes (equal) and Roberto Cláudio Fernandes Franco Pompeu (equal).

Project administration: Roberto Cláudio Fernandes Franco Pompeu (lead).

Supervision: Henrique Antunes de Souza (equal) and Roberto Cláudio Fernandes Franco Pompeu (equal).

Writing – review & editing: Rita de Kássia Oliveira Tavares (lead).

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