

Phosphorus fertilization on biomass and bromatological composition of forage grasses in calcareous soils¹

Adubação fosfatada na biomassa e composição bromatológica de gramíneas forrageiras em solos calcários

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HIGHLIGHTS:

Forage grasses require high rates of P_2O_5 (449.4 to 554.9 kg ha⁻¹) in calcareous soils to achieve high dry matter production. The forage grasses produced greater biomass on the Vista Hermosa soil across all cuttings. The neutral detergent fiber and acid detergent fiber contents did not respond to phosphorus fertilization.

ABSTRACT: Calcareous soils reduce phosphorus availability, affecting pasture growth and quality. The purpose of this study was to evaluate the biomass and bromatological content response of three forage grasses to increases in phosphorus fertilization in two calcareous soils in the department of Córdoba, Colombia. A full factorial $2 \times 3 \times 6$ experimental design was used, involving two calcareous soils (Vista Hermosa and Carolina), three forage grass cultivars (King Grass, Cuba OM-22, and Purple Elephant), and six phosphorus rates (0, 80, 150, 250, 400, and 650 kg ha⁻¹ P₂O₅). The response variables included forage dry matter, evaluated over three cuts, and bromatological parameters (neutral detergent fiber, acid detergent fiber, protein, and fat). The grasses developed greater dry matter on the Vista Hermosa soils across all three cuttings, with a 34.43% mean difference compared to Carolina. The calcareous soils showed a response to the application of phosphorus at high rates (above 400 kg ha⁻¹). The pasture neutral detergent fiber and acid detergent fiber in the Vista Hermosa soil were 4.10 and 7.74% lower than in the Carolina soil. In conclusion, 449.4 to 554.9 kg ha⁻¹ P₂O₅ are required to achieve high biomass yields in both forage grasses, and the Vista Hermosa soils were the most favorable in terms of biomass gain and low fiber content.

Key words: pastures, phosphorus rates, dry matter, cuttings, fiber

RESUMO: Os solos calcários reduzem a disponibilidade de fósforo, afetando o crescimento e a qualidade das pastagens. Esta pesquisa teve como objetivo avaliar a resposta da biomassa e da composição bromatológica de três gramíneas forrageiras ao aumento da dose de fósforo em dois solos calcários no departamento de Córdoba, Colômbia. Foi utilizado um delineamento experimental fatorial completo $2 \times 3 \times 6$, referentes à dois solos calcários (Vista Hermosa e Carolina), três cultivares de gramíneas forrageiras (Capim Elefante Roxo, King Grass e Cuba OM-22) e seis doses de fósforo (0, 80, 150, 250, 400 e 650 kg ha⁻¹ de P₂O₅). As variáveis de resposta incluíram matéria seca da forragem, avaliada em três cortes, e parâmetros bromatológicos (fibra em detergente neutro, fibra em detergente ácido, proteína e gordura). As gramíneas apresentaram maior produção de massa seca nos solos de Vista Hermosa em todos os três cortes, com uma diferença média de 34,43% em relação a Carolina. Os solos calcários mostraram resposta à adição de fósforo em altas doses (acima de 400 kg ha⁻¹). A fibra em detergente neutro e a fibra em detergente ácido das pastagens no solo de Vista Hermosa foram 4,10 e 7,74% menores do que no solo de Carolina. Em conclusão, são necessários de 449,4 a 554,9 kg ha⁻¹ de P₂O₅ para alcançar altos rendimentos de biomassa em ambas as gramíneas forrageiras, sendo os solos de Vista Hermosa os mais favoráveis em termos de ganho de biomassa e baixos teores de fibra.

Palavras-chave: forrageiras, doses de fósforo, matéria seca, cortes, fibra

INTRODUCTION

Calcareous soils are widely distributed in arid and semi-arid regions and are characterized by high amounts of calcium carbonate - CaCO_3 (Taalab et al., 2019), as well as low contents of organic matter, nitrogen (N), micronutrients, and low availability of phosphorus (P), which is reduced to multiple insoluble forms after reacting with calcium (FAO, 2021). The deficiency of this element is one of the main chemical limitations in calcareous soils (Kumar & Kumari, 2025). According to El-Saied et al. (2016), calcareous soils are alkaline due to the presence of CaCO_3 , which dominates the chemical activity and its dissolution increases the concentration of bicarbonate - HCO_3^- in solution, and its reaction products are $\text{CaCO}_3 + \text{H}_2\text{O} \leftrightarrow \text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^-$, which neutralize the soil in the pH 7.5 to 8.5 range (Taalab et al., 2019); these reactions can precipitate phosphorus into insoluble forms, reducing its availability to plants and promoting the occurrence of nutrient deficiencies in crops.

The forage grasses in Colombian livestock have been very well received, as they are a source of food for dual-purpose cattle and among which grasses King Grass (hybrid between *Pennisetum purpureum* × *Pennisetum typhoides*), Purple Elephant (*Pennisetum purpureum* Schumach), and Cuba OM-22 (hybrid between *Pennisetum purpureum* × *Pennisetum glaucum*) are used, which are replacing traditional grazing forage systems on calcareous soils (Ros et al., 2020). According to Maldonado-Peralta et al. (2019), Botero-Londoño et al. (2021), and Ramos et al. (2022), in these grasses, the following characteristics stand out: high yields of green matter (around 50 t ha⁻¹ per cutting), dry matter (between 7 and 12 t ha⁻¹ per year), and high protein contents (between 6 and 12% on a dry matter basis).

The forage grasses established in the calcareous soils of the department of Córdoba-Colombia present nutritional deficiencies of P, which is one of the most macronutrients that crops need in sufficient quantity to ensure their growth (Chtouki et al. 2024). According to El-Saied et al. (2016), in calcareous soils, when phosphorus-containing fertilizers are added, soluble P found in the soil reacts with calcium (Ca^{2+}) and CaCO_3 ions, forming low-solubility calcium phosphates.

Phosphorus deficiency negatively influences nutrient acquisition, biomass yield, and forage quality (Ros et al., 2018; Faria et al., 2023). According to Botero-Londoño et al. (2021), this nutrient availability is essential to increase the biomass and quality of these forage grasses. Phosphorus is the second most important essential macronutrient for plants, playing a vital role in the synthesis of adenosine triphosphate (ATP), the organic molecule that fuels all plant biological functions. In addition to acting as an intermediary in signal transduction processes, it is an integral part of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids, and sugar phosphates (Chaitra et al., 2024). In pastures, phosphorus is a key element for plant nutrition. According to McKenzie (2015), these cultivars extract approximately 2 kg of phosphorus from the soil per ton of dry matter.

An effective strategy to guarantee optimal P availability in these soils is phosphate fertilization, with concentrated sources, in bands and high amounts, maintaining the balance with other nutrients (Taalab et al., 2019); and the physiological response of

some grasses to phosphorus availability (Oliveira et al. 2018). Due to the few studies regarding the application of P and the forage response in the calcareous soils of Córdoba, the purpose of present study was to determine forage grasses' biomass and bromatological content response to increases in phosphorus in the form of P_2O_5 in two calcareous soils.

MATERIAL AND METHODS

The experiment was carried out in 2018-2019, in the greenhouses of the Faculty of Agricultural Sciences of the University of Córdoba, Córdoba, Colombia, located at the geographical coordinates 8° 48' N and 75° 52' W, and at an altitude of 18 masl. The climate in this area has annual mean temperature of 27.4 °C, 84% relative air humidity, 1,346.1 mm rainfall, and 2,108.2 hours of sunshine, conditions that classify this area as a tropical dry forest according to the Holdridge life zone classification system (Palencia et al., 2006).

Calcareous soil was collected from two locations in the department of Córdoba, a volume of 800 kg at each site at a 0 – 20 cm depth. The first locality corresponded to the Vista Hermosa soil, located in the municipality of Montería (8° 48' 27.5" N and 75° 56' 26.1" W), and the second to the Carolina soil, located in the municipality of Planeta Rica (8° 49' 27.4" N and 75° 62' 61.0" W), as shown in Figure 1.

The soil samples were sent to the Soil and Water Laboratory of the University of Córdoba, where a drying, grinding, and sieving process (4 mm) was carried out and, later, a 1 kg subsample was collected for chemical analysis as for fertility. These results showed that the soil from Vista Hermosa exhibited an alkaline chemical reaction (Imas, 2000), with a 7.6 pH, while the soil from Carolina had a 7.36 pH. Regarding the sum of bases (SB), both soils showed high values, with 37.6 and 73.8 $\text{cmol}_c \text{kg}^{-1}$, marking the first significant difference between the two. Similarly, calcium levels were also high in both soils, with 30.6 and 62.4 $\text{cmol}_c \text{kg}^{-1}$ for the respective locations, again highlighting a marked difference between them (Table 1). These soils were classified as Calciaquert (Soil Survey Staff, 2022).

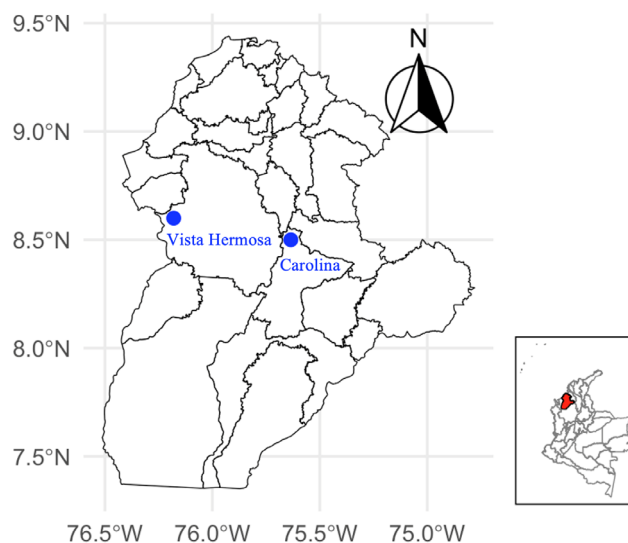


Figure 1. Geographical location of the calcareous soils Vista Hermosa and Carolina in Córdoba, Colombia

Table 1. Chemical characterization of calcareous soils Vista Hermosa and Carolina

Soil	pH 1:1	OM (%)	S (mg kg ⁻¹)	P (mg kg ⁻¹)	Ca	Mg	K (cmol _c kg ⁻¹)	Na	SB	Cu	Fe (mg kg ⁻¹)	Zn	Mn
Vista Hermosa	7.7	2.15	17.6	19.7	30.6	5.6	1.15	0.2	37.6	0.3	0.4	0.3	6.4
Carolina	7.3	1.88	24.8	18.5	62.4	10.4	0.69	0.3	73.8	0.1	3.4	0.1	1.0

pH - Soil-H₂O ratio 1:1; OM - Walkley-Black; S - Monobasic calcium phosphate 0.008 mol L⁻¹; P - Bray-II modified; Ca, Mg, K and Na - Ammonium acetate 1.0 mol L⁻¹ pH 7.0; SB - Sum of bases; Cu, Fe, Zn and Mn - Mehlich-1

This supports their classification as calcareous soils due to the high calcium content, which was confirmed in the field by the strong effervescence observed upon the application of diluted nitric acid, used as a qualitative indicator (Loeppert & Suárez, 1996). Regarding phosphorus, the levels found were intermediate, with 19.7 and 18.5 mg kg⁻¹, showing no significant differences between the soils. Finally, the concentrations of micronutrients Cu, Fe, Zn, Mn, and B were deficient in both soils, with values of 0.3, 0.4, 0.3, 6.4, and 0.15 and 0.1, 3.4, 0.1, 1.0, and 0.13 mg kg⁻¹, respectively, in Vista Hermosa and Carolina soils. Based on these characteristics, FAO (2021) primarily classifies them within the reference soil group of Calcisols and related calcic subgroups of other soils (Table 1).

A completely randomized design with a complete 2 × 3 × 6 factorial arrangement and four repetitions was used, where the first factor corresponded to the calcareous soil type (Vista Hermosa and Carolina); the second factor, to the forage grasses: King Grass (KG), Purple Elephant (PE), and Cuba OM-22 (COM-22); and the third factor, to the rates of phosphorus in the form of P₂O₅, equivalent to 80, 150, 250, 400, and 650 kg ha⁻¹ and a control without fertilization (Figure 2). The application rates were selected due to the high fixation and/or precipitation of phosphorus as calcium phosphate in these soils. The fertilizer used was diammonium phosphate (DAP), containing 46% phosphorus in the form of P₂O₅.

The experimental units were constituted by transparent plastic bags with 8 kg of calcareous soil, on which the phosphorus respective rates were applied 15 days before sowing (pre-sowing). At planting, three cuttings or asexual seeds were initially placed per bag. After 15 days, thinning was carried out

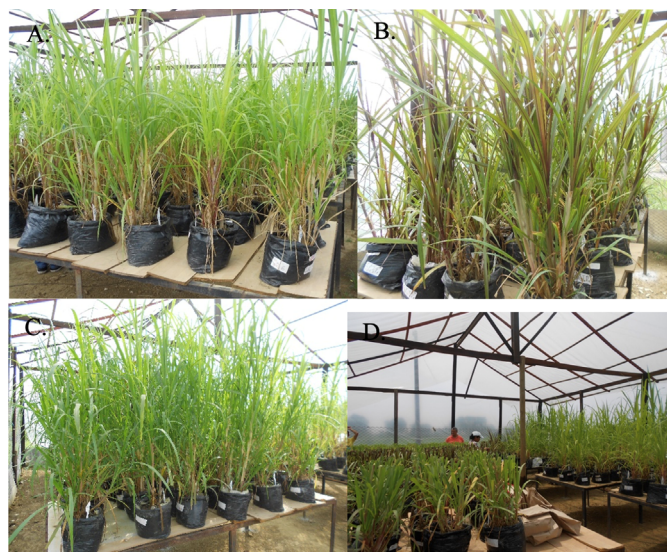


Figure 2. Phosphorus fertilization experiment on three forage grasses grown in two calcareous soils: King Grass (A), Purple Elephant Grass (B), Cuba OM-22 (C), and harvesting of the forage grasses (D)

to retain the two most vigorous plants. Subsequently, nitrogen fertilization was applied using a mixture of 200 kg ha⁻¹ urea and 100 kg ha⁻¹ ammonium sulfate [(NH₄)₂SO₄], as well as potassium fertilization with 150 kg ha⁻¹ potassium chloride (KCl). The per-hectare rates of nitrogen, phosphorus, and potassium sources, calculated for a 20 cm soil depth, were adjusted and applied to the bags in proportion to the soil weight they contained. The soil in the experimental unit bags was maintained at field capacity through manual irrigation, and the forage grasses were managed following appropriate agronomic practices for weed, pest, and disease control (Figure 2).

The response variables to evaluate the effect of phosphorus application on calcareous soils and forage grasses were forage dry mass and bromatological content. The forage dry mass was quantified through three cuttings performed at a height of 20 cm above ground level, carried out at 75, 105, and 135 days after sowing (DAS). The plant material was dried in a forced-air oven at 70 °C for 72 hours and then ground in an IKA MSF-1 micro-mill with a 1 mm mesh.

The forage bromatological content was determined on a 500 g sample of leaf dry mass from the third cutting (135 DAS). The bromatological parameters (%) were the following: neutral detergent fiber (NDF) and acid detergent fiber (ADF) by the Ankom method (Van Soest, 1967); fat (Soxhlex with immersion), and for crude protein (%N × 6.25) nitrogen was quantified by the Kjeldahl method (IGAC, 2026).

Data was analyzed using a three-way analysis of variance. For post hoc comparisons, Tukey's HSD test ($p \leq 0.05$) was applied to evaluate differences between the levels of the independent factors: type of calcareous soil and forage grass species. Regarding the interaction between calcareous soil and forage grass, specific contrasts were performed using Student's t-test ($p \leq 0.05$) to compare the means. Meanwhile, the interaction between calcareous soil and phosphorus rate, as well as forage grasses and phosphorus rate, were analyzed using regression analysis ($p \leq 0.05$). In the third cutting, a principal component analysis (PCA) was carried out for the forage dry matter and bromatological content on the combinations of forage grasses and phosphorus rates for each calcareous soil. The data was processed and graphed with the statistical software R, version 4.4.2 (R Core Team, 2024).

In addition, once the mathematical models for the interaction calcareous soils-phosphorus rate and forage grasses-phosphorus rate were obtained, and the response variables were adjusted to decreasing quadratic equations, the model was derived to obtain the optimal rate and the critical level of phosphorus (kg ha⁻¹), which is equivalent to 100 and 90% of the maximum biomass production, which were obtained with the application of phosphorus (Corréa et al., 2008).

RESULTS AND DISCUSSION

Forage dry matter was statistically influenced by the type of calcareous soil ($p \leq 0.001^{***}$), the forage grass cultivar ($p \leq 0.05^*$), and the phosphorus rates ($p \leq 0.001^{***}$) across all cuttings. Significant interaction effects were also observed between forage grass type and calcareous soil ($p \leq 0.001^{***}$), as well as between calcareous soil and phosphorus rates ($p \leq 0.001^{***}$) in the third cutting. In the first cutting, a significant interaction was also observed between forage grass type and phosphorus rates ($p \leq 0.001^{***}$) (Table 2).

Figure 3 shows that the forage grasses achieved higher dry matter production in the Vista Hermosa (VH) soil across all three cuttings. In the first cutting (Figure 3A), grasses established in this soil produced 24.17 g of dry matter, representing a 70.57% increase compared to the Carolina soil (14.17 g). In the second cutting (Figure 3B), 44.6 g were recorded in the Vista Hermosa soil, exceeding the values obtained in the Carolina soil (36.4 g) by 22.53%. Finally, in the third cutting (Figure 3C), production in the Vista Hermosa soil reached 160 g, corresponding to a 10.35% increase relative to the Carolina soil, which yielded 145 g.

The difference in dry matter accumulation in the grasses could be related to the chemical characteristics of the soils. The Vista Hermosa soil showed a lower calcium content ($30.6 \text{ cmol}_c \text{ kg}^{-1}$), although with a 7.8 pH, indicating a more advanced soil development compared to the Carolina soil

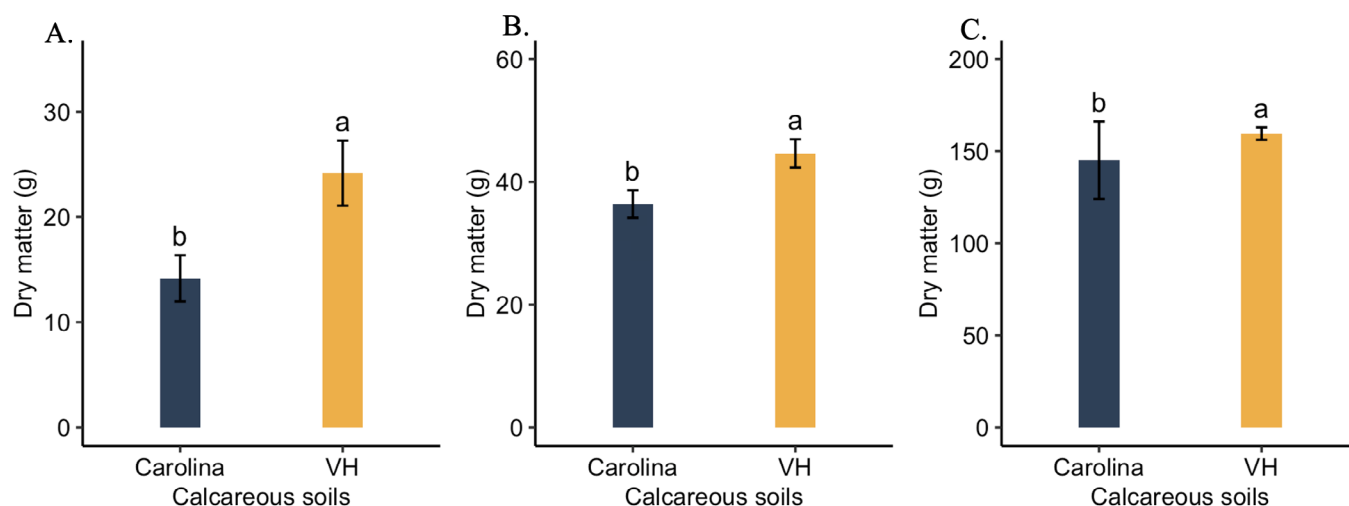
($62.4 \text{ cmol}_c \text{ kg}^{-1}$) (Table 1). These values are characteristic of Calcisol-type soils, which may have contributed to reduced phosphorus precipitation due to the lower calcium concentration, thereby increasing its availability to plants (Cambrollé et al., 2015; Taalab et al., 2019; FAO, 2021). Additionally, the high organic matter content (2.15%) (Table 1) may have contributed to the generation of organic acids during the mineralization process, promoting phosphorus solubilization and enhancing its availability for plant uptake (Padghan et al., 2024).

The forage grass dry matter response varied across the three cuttings (Figure 4). In the first cutting (Figure 4A), the dry matter yield of the Cuba OM-22 and King Grass cultivars did not show significant differences between them, with a mean of 20.63 g; however, both cultivars outperformed the Purple Elephant grass by 27.03%, regardless of the calcareous soil type and phosphorus rates. In the second cutting (Figure 4B), significant differences in dry matter yield were observed between Cuba OM-22, which recorded 42.2 g, and Purple Elephant, with 38.6 g; King Grass showed an intermediate value of 40.7 g, with no significant differences compared to the other two cultivars. In the third cutting (Figure 4C), no statistical differences were observed between the COM-22 grass (151 g per plant) and Purple Elephant (163 g per plant), which had a mean of 157 g per plant. This value exceeded the King Grass dry matter yield (143 g per plant) by 9.79%. These results suggest that the Cuba OM-22 grass exhibited greater stability in dry

Table 2. F ratio and p-value of the analysis of variance for the forage grass (G) dry matter in calcareous soils (S), with the addition of phosphorus rates (P) in three cutting periods

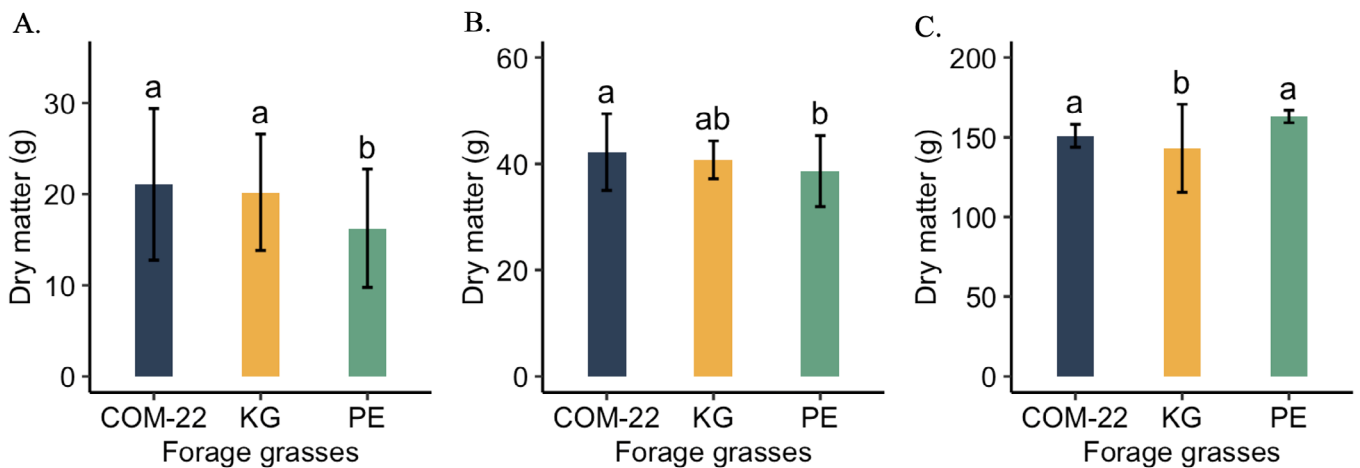
Source	DF	First cutting		Second cutting		Third cutting	
		F	p-value	F	p-value	F	p-value
Calcareous soils (S)	1	78.3	$\leq 0.001^{***}$	58.2	$\leq 0.001^{***}$	17	$\leq 0.001^{***}$
Forages grass (G)	2	6.9	0.0015^{***}	3.7	0.0274^*	10.9	$\leq 0.001^{***}$
Phosphors (P)	5	24.9	$\leq 0.001^{***}$	31.1	$\leq 0.001^{***}$	60.4	$\leq 0.001^{***}$
Interactions							
Interaction S-G	2	0.6	0.5415^{ns}	2.2	0.1153^{ns}	13.8	$\leq 0.001^{***}$
Interaction S-P	5	1.4	0.2038^{ns}	1.9	0.0885^{ns}	6.2	$\leq 0.001^{***}$
Interaction G-P	10	2.9	0.00278^{**}	0.5	0.8451^{ns}	0.9	0.53^{ns}
Interaction S-G-P	10	1.1	0.3408^{ns}	0.5	0.8676^{ns}	0.6	0.75^{ns}
CV (%)		36.36		16.00		13.82	

DF - Degrees of freedom; F - F ratio; * - Significant at $p \leq 0.05$; ** - Significant at $p \leq 0.01$; *** - Significant at $p \leq 0.001$



The bars represent the mean \pm standard deviation ($n = 4$); Same letters indicate that there were no statistical differences between the calcareous soils according to Tukey's HSD test ($p \leq 0.05$)

Figure 3. Dry matter production of forage grasses on calcareous soils during the first (A), second (B), and third (C) cutting



The bars represent the mean \pm standard deviation (n = 4); Same letters indicate that there were no significant differences between forage grasses according to Tukey's HSD test (p \leq 0.05)

Figure 4. Dry matter of forage grasses per plant during the first (A), second (B), and third (C) cutting

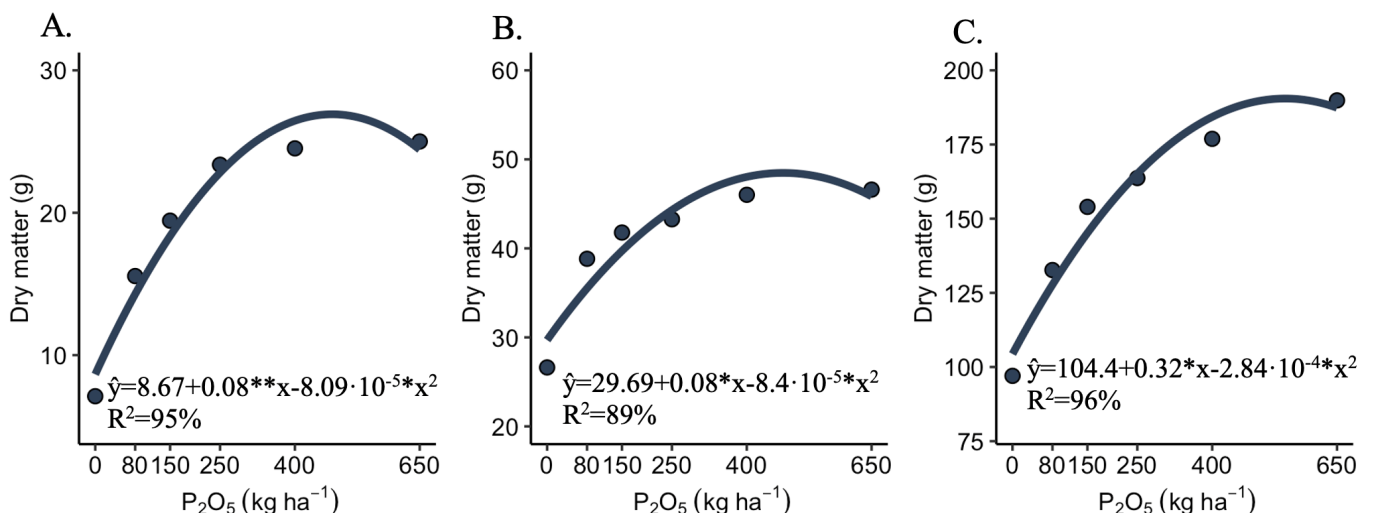
matter yield, as it maintained high biomass values in all three harvests, unlike the other grasses.

This response depends on the type of grass, its genetic constitution, the environment, the type of soil, and management practices, such as fertilization (Mitchell et al., 2019). Maldonado-Peralta et al. (2019) point out that the hybrid Cuba OM-22, which is of the genus *Pennisetum*, is characterized by its high yield, with 220 g of dry matter between 30 and 90 days. King Grass, for its part, has a remarkable ability to adapt to various soil conditions (Cardona et al., 2016) and can produce, on average, 340 g of dry matter per plant at 30 days after cutting and 392 g per plant at 90 days (Botero-Londoño et al., 2021). Finally, according to Vivas-Quila et al. (2019), Purple Elephant grass can reach an average dry matter yield of 230 g per plant of dry matter.

On the other hand, the plant roots can increase the P availability, by acidification of the rhizosphere, exudation of organic acids (Sun et al., 2019). However, according to Mitchell et al. (2019) the response of pastures depends on the genotype-environment interaction and management practices, such as fertilization. Additionally, Almeida et al. (2020), when evaluating the exudation of organic acids –

OA (citrate, isocitrate, and malate) in ruzigrass (*Urochloa ruziziensis*), palisade grass (*Urochloa brizantha*), and Guinea grass (*Megathyrsus maximus*) under different phosphorus rates, found that the concentration of these compounds in the root surface increased under phosphorus deficiency conditions. This effect was more pronounced in grasses of the *Urochloa* genus, particularly in palisade grass, which showed the highest total OA concentration in the rhizosphere, attributed to greater production of oxalate and isocitrate, a result that may be associated with the genetic variability, environment, soil and crop management, which lead to differences in their yield effects (Bocianowski et al., 2021).

The forage grass dry matter response showed a similar pattern across the three cuttings, reaching its maximum value at high phosphorus rates, after which a decline in this parameter was observed (Figure 5). In the first cutting (Figure 5A), the optimal phosphorus rate was estimated to be 449.44 kg ha⁻¹ of P₂O₅, which would result in a mean dry matter yield of 28,47 g per plant in the forage grasses. The critical level, equivalent to 90% of the optimal rate, corresponded to 404.5 kg ha⁻¹ of P₂O₅; below this value, the pastures would be in a deficiency phase. In the second cutting, the optimal



* - Indicate regression models with coefficients significant at the p \leq 0.05; ** - Indicate regression models with coefficients significant at the p \leq 0.01

Figure 5. Forage grass dry matter per plant response to the application of different phosphorus rates during the first (A), second (B), and third (C) cutting

phosphorus rate was 461 kg ha⁻¹ of P₂O₅, resulting in a mean dry matter yield of 48.59 g per plant, with a 414 kg ha⁻¹ of P₂O₅ critical level (Figure 5B). Finally, in the third cutting, the optimal phosphorus rate was 554.9 kg ha⁻¹ of P₂O₅, and at this rate, biomass production reached 191.8 g per plant; the critical level was 499.4 kg ha⁻¹ of P₂O₅ (Figure 5C).

These results suggest that forage grasses (regardless of the cultivar) require high amounts of phosphorus in the form of P₂O₅ (between 449.4 and 554.9 kg ha⁻¹) to achieve high dry matter content in calcareous soils. In these soils with high CaCO₃ content, sorption and precipitation reactions increase the soil phosphorus buffering capacity, promoting its transformation into stable forms of calcium phosphate. This leads to phosphorus deficiencies and, consequently, low crop yields (Shrivastava et al., 2018; Rafiullah et al., 2020). These deficiencies caused by phosphorus immobilization must be compensated through the application of phosphorus fertilizers, which ensure greater availability to plants (Taalab et al., 2019; Somavilla et al., 2021). According to Pant & Warman (2000), it is difficult to distinguish between phosphorus adsorption and precipitation in calcareous soils due to the process of surface adsorption and precipitation, where the application of soluble P and the concentration of labile P decrease rapidly over time as a result of precipitation reactions or adsorption by CaCO₃ in calcareous soils. Ultimately, phosphorus availability directly influences dry matter production, as this nutrient is essential for increasing both biomass and forage quality (Botero-Londoño et al., 2021), given its involvement in multiple plant metabolic processes (Chaitra et al., 2024).

In the interaction between calcareous soils and forage grasses during the third cutting, we observed that in the Carolina soil, the highest dry matter production corresponded to the Purple Elephant grass, with a value of 166 g per plant, surpassing by 13.7 and 33.9% the values obtained by Cuba OM-22 (146 g per plant) and King Grass (124 g per plant), respectively (Figure 6A). In contrast, in the Vista Hermosa soil, no statistically significant differences were recorded in dry matter yield among the evaluated grasses, with a mean value

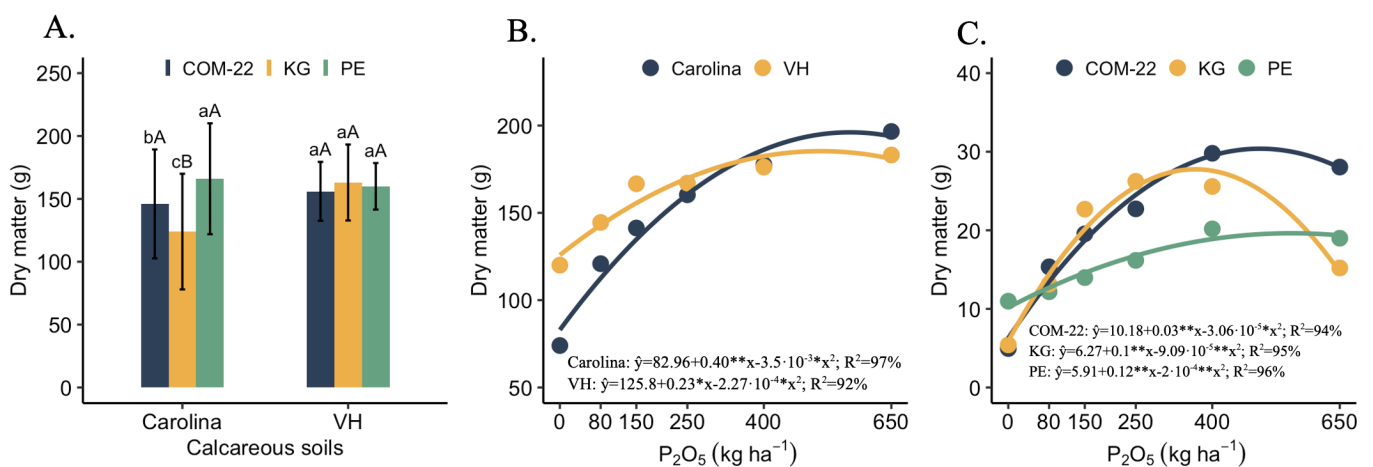
of 169 g per plant. Regarding the species specific response in both calcareous soils, only King Grass showed significant differences, with a yield of 163 g per plant in Vista Hermosa and 124 g in Carolina, representing a 31.45% difference (Figure 6A).

In the interaction between the calcareous soils of Carolina and the phosphorus rates, after modeling efficiency at 90% of the optimal rate, the highest dry mass gain in the grasses was observed with an application of 568.9 kg ha⁻¹ P₂O₅, resulting in a maximum dry matter accumulation of 196.13 g per plant (Figure 6B). Similarly, in the Vista Hermosa soil, the highest dry matter accumulation was 188.03 g per plant, corresponding to a 511.65 kg ha⁻¹ P₂O₅ rate (Figure 6B). According to Bouras et al. (2022), an increase in fresh forage yield of *Panicum antidotale* Retz was reported with the application of 90 and 108 kg ha⁻¹ of P₂O₅ in saline soils.

These results are very similar to those reported by Siri-Prieto et al. (2021), who found a dry matter yield of 189.1 g per plant in Purple Elephant grass after applying a combination of 100 kg ha⁻¹ of P₂O₅ and 100 kg ha⁻¹ of N. In other studies with Purple Elephant, Botero-Londoño et al. (2021) found a positive response to fertilization with a mixture of 340 kg ha⁻¹ (23.6% N, 12.5% P₂O₅ and 19.3% K₂O).

When analyzing the interaction between the forage grasses and the P₂O₅ rates (Figure 6C), the highest dry matter (31.55 g) was recorded with the Cuba OM-22 material from the P₂O₅ rate that is equivalent to 505.56 kg ha⁻¹, followed by King Grass, whose forage dry mass was 23.91 g with a 300 kg ha⁻¹ rate. Finally, the Purple Elephant grass registered a lower response than that described for the previous grasses, where the maximum forage dry matter was 17.53 g per plant, with a 490.19 kg ha⁻¹ rate.

These results suggest that the critical level of 90% of the maximum P₂O₅ rate, where deficiency can occur, are below 455 kg ha⁻¹ for Cuba OM-22, 290 kg ha⁻¹ for King Grass, and 441.17 kg ha⁻¹ for Purple Elephant (Figure 6C). Therefore, the technical criterion is necessary to apply P in calcareous soils (Schneider et al., 2019). In addition, these results may be directly related to genetic variability and genotype-environment interaction,



* - Indicate regression models with coefficients significant at the $p \leq 0.05$; ** - Indicate regression models with coefficients significant at the $p \leq 0.01$; The bars represent the mean \pm standard deviation ($n = 4$); Same lowercase letters indicate that no statistical differences were observed among the forage grasses within each type of calcareous soil, while the same uppercase letters indicate that there were no statistical differences for the same forage grass between the two calcareous soils, according to Student's t-test ($p \leq 0.05$)

Figure 6. Dry matter response to the interaction between calcareous soils and forage grasses in the third cutting (A), between calcareous soils and phosphorus rates in the third cutting (B), and between forage grasses and phosphorus rates in the first cutting (C)

which has been documented in corn lines (Dongdong et al., 2022), influencing root length and branching, release of P-mobilizing exudates, mycorrhizal symbiosis (Zhuhui et al., 2019), parameters that have been decisive for the acquisition of phosphorus. Similarly, Mitchell et al. (2019) indicate that the response of pastures to fertilization depends on the type of pasture and its genetic constitution, the environment, soil type, and management practices.

The protein content varied in the three factors: calcareous soils ($p \leq 0.001^{***}$), forage grasses ($p \leq 0.001^{***}$), and phosphorus rates ($p \leq 0.001^{***}$), likewise, responded to the interaction between calcareous soils and P rates ($p \leq 0.0076^{**}$), and between forage grasses and P rates ($p \leq 0.001^{***}$) (Table 3).

In calcareous soils, regardless of the cultivar and the phosphorus rate applied (Figure 7A), forage grasses showed a higher protein content in the Vista Hermosa soil (6.82%) compared to the Carolina soil (5.99%). This difference may be attributed to the soil characteristics (Table 1), as a lower phosphorus precipitation is likely in Vista Hermosa due to its

lower calcium concentration ($30.6 \text{ cmol}_c \text{ kg}^{-1}$). Additionally, the higher organic matter content (2.76%) could enhance mineralization, thereby increasing nitrogen availability, an essential nutrient for protein synthesis. Regarding the forage grasses, Purple Elephant (6.65%) and King Grass (6.56%) exhibited the higher protein contents, with no statistical differences between them, though both values were higher than that of Cuba OM-22 grass (6.0%) (Figure 7B).

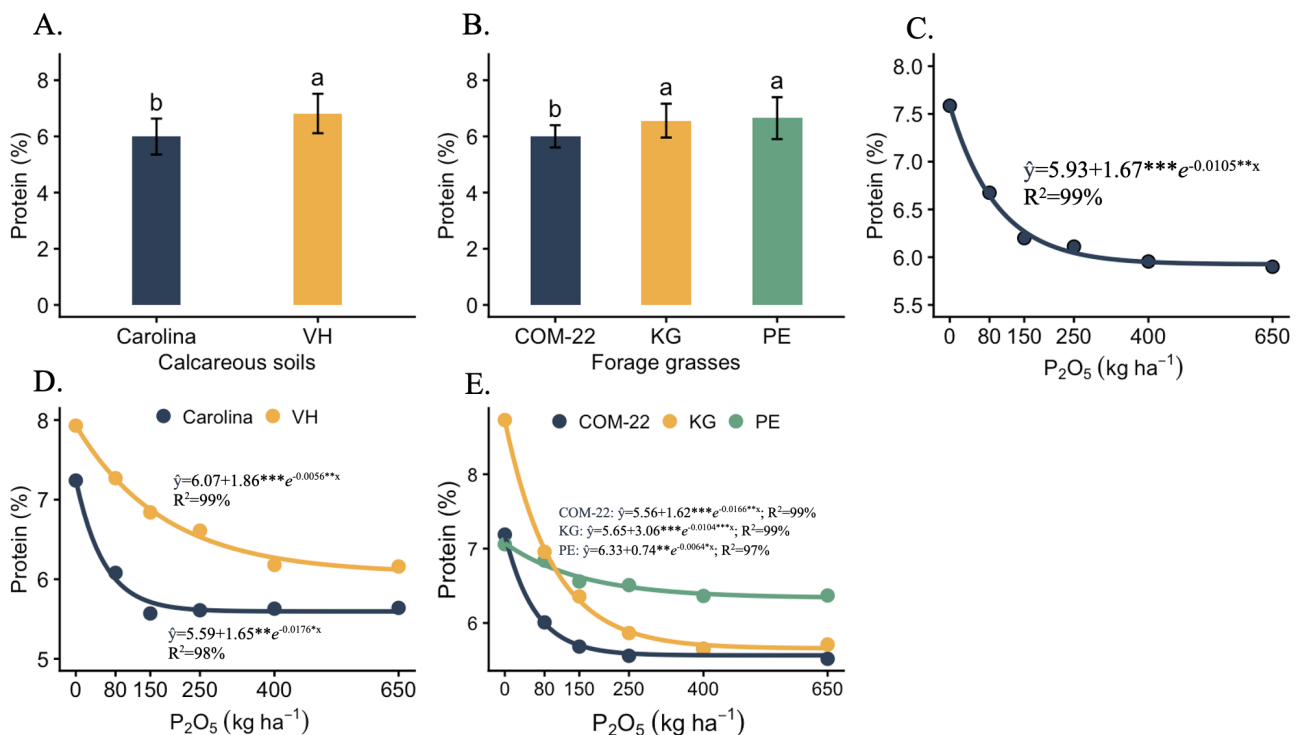
The protein content in forage grasses in response to phosphorus rates (Figure 7C), as well as the interaction between calcareous soils and phosphorus rates (Figure 7D), and between forage grasses and phosphorus rates (Figure 7E) showed similar trends. All were characterized by high protein levels at low P_2O_5 concentrations, which significantly decreased as the phosphorus rate increased, until reaching a stable level.

These results may be explained by the increase in phosphorus rates, which could promote higher dry matter production. However, a dilution effect is also likely to occur (Garay et al., 2017), which reduces foliar nitrogen content and consequently

Table 3. F ratio and p-value of the analysis of variance for the protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), and fat content of three forage grasses (G) on two calcareous soils (S) and the addition of phosphorus rates (P)

Source	DF	Protein		NDF		ADF		Fat	
		F	p-value	F	p-value	F	p-value	F	p-value
Calcareous soils (S)	1	83.5	$\leq 0.01^{***}$	6.5	0.01*	5.4	0.0217*	5.3	0.02*
Forage grass (G)	2	20.3	$\leq 0.01^{***}$	5.0	0.0077**	0.04	0.96 ^{ns}	2.2	0.11 ^{ns}
Phosphors (P)	5	33.8	$\leq 0.01^{***}$	1.5	0.19 ^{ns}	0.17	0.97 ^{ns}	4.7	$\leq 0.01^{***}$
Interaction S-G	2	2.5	0.08 ^{ns}	0.6	0.53 ^{ns}	0.23	0.80 ^{ns}	3.1	0.04*
Interaction S-P	5	3.3	0.0076***	0.9	0.47 ^{ns}	0.9	0.49 ^{ns}	1.1	0.35 ^{ns}
Interaction G-P	10	7.6	$\leq 0.01^{***}$	0.9	0.5 ^{ns}	0.55	0.85 ^{ns}	0.4	0.93 ^{ns}
Interaction S-G-P	10	1.7	0.07 ^{ns}	0.8	0.57 ^{ns}	0.54	0.86 ^{ns}	0.5	0.84 ^{ns}
CV (%)		8.43		9.98		20.78		35.72	

DF - Degrees of freedom; F - F ratio; * - Significant at $p \leq 0.05$; ** - Significant at $p \leq 0.01$; *** - Significant at $p \leq 0.001$; CV - Coefficient of variation



The bars represent the mean \pm standard deviation ($n = 4$); Same letters indicate that there were no statistical differences between the calcareous soils or forage grasses according to Tukey's HSD test ($p \leq 0.05$); * - Indicate regression models with coefficients significant at the $p \leq 0.05$; ** - Indicate regression models with coefficients significant at the $p \leq 0.01$; *** - Indicate regression models with coefficients significant at the $p \leq 0.001$

Figure 7. Forage protein content across calcareous soils (A), forage grasses (B), phosphorus rates (C), the interaction between calcareous soils and phosphorus rates (D), and the interaction between forage grasses and phosphorus rates (E)

limits protein synthesis. Moreover, in this study, uniform nitrogen (N) rates were applied, a nutrient directly related to protein production. According to Dupas et al. (2016), the crude protein content in forage can be increased by raising the nitrogen application rate, as this element contributes to amino acid formation and protein synthesis. Similarly, Ramos et al. (2022) reported an increase in foliar phosphorus content accompanied by a decrease in foliar nitrogen in pastures fertilized with phosphorus, which negatively affected protein content, given that foliar nitrogen is essential for protein accumulation. Delevatti et al. (2017) indicated that nitrogen fertilization can increase forage yield and its nutritional value in tropical regions.

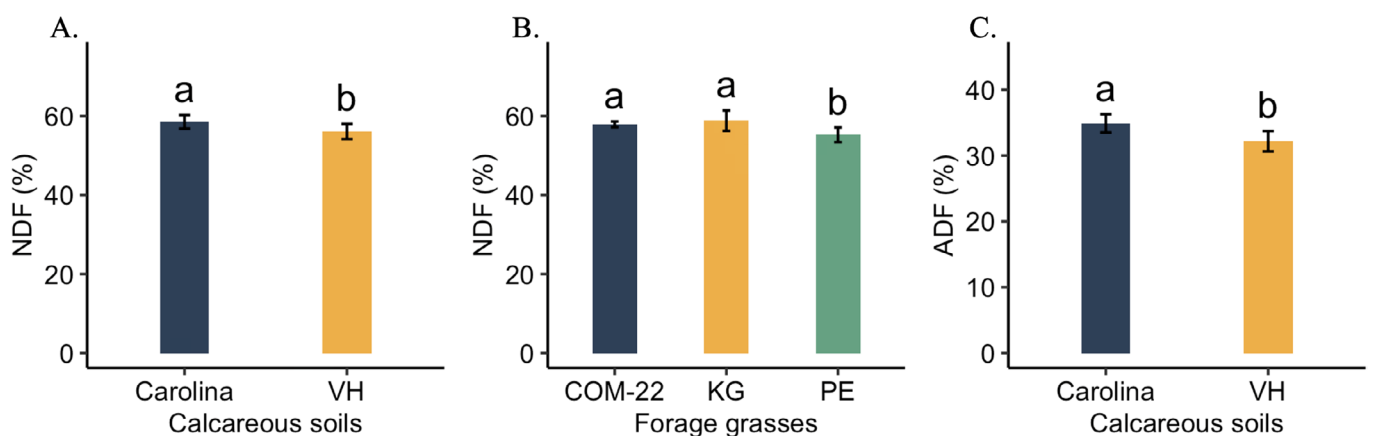
Likewise, Figure 7C showed that the protein content in forage grasses significantly decreased up to a rate of 334 kg ha⁻¹ P₂O₅, beyond which no further response was detected, stabilizing at approximately 5.93%. The protein content was higher in the Vista Hermosa soil than in the Carolina soil (Figure 7D). In the latter, a decline was recorded starting at 262 kg ha⁻¹ P₂O₅, reaching a 5.59% minimum content, while in the Vista Hermosa soil, the reduction was less pronounced, stabilizing at a higher rate of 611 kg ha⁻¹. Finally, Figure 7E showed that the phosphorus rate at which protein content stabilized varied among the forage grass species: for Cuba OM-22, it was 277 kg ha⁻¹ (5.56%); for King Grass, 443 kg ha⁻¹ (5.65%); and for Purple Elephant, 672 kg ha⁻¹ (6.33%). These results differ from those reported by Francisquini et al. (2020), who observed under field conditions that phosphorus fertilization promoted protein accumulation in *Panicum maximum*. In contrast, Botero-Londoño et al. (2021) reported, also under field conditions, a decrease in protein content in Purple Elephant grass, following the application of a 340 kg ha⁻¹ NPK mixture (23.6% N, 12.5% P₂O₅, and 19.3% K₂O).

The NDF content showed significant variation only between the calcareous soils ($p \leq 0.01$) and among the forage grass cultivars ($p = 0.0077^{**}$) (Table 3). When analyzing the mean NDF content of the three grasses established in the two soils, a higher content was observed in the Carolina soil (58.5%) compared to the Vista Hermosa soil (56.1%) (Figure 8A). These results are directly related to the Carolina soil nutritional content and fertility, which shows high levels of calcium (62.4 cmol_c kg⁻¹) and magnesium (10.4 cmol_c kg⁻¹), values that are twice as high as those recorded

in the Vista Hermosa soil. In addition, the sulfur content also contributes to soil fertility (Table 1). Therefore, technical criteria are essential for applying phosphorus in calcareous soils, aiming to increase the efficiency of phosphate fertilizers, since global P resources are non-renewable, which means that their extraction and production will become increasingly costly (Schneider et al., 2019). According to Elgersma & Søgaard (2016), there is a negative correlation between NDF and protein content, which affects the quality of pastures.

Among the forage grasses, the Purple Elephant grass recorded a lower NDF content (55.24%), and the Cuba OM-22 and King Grass grasses produced a 58.36% NDF mean (Figure 8B). However, these NDF contents are lower than those reported for Purple Elephant grass with 59.84% by Vivas-Quila et al. (2019). Therefore, it is important to highlight that the results from this research confirm the validity of describing the relationships between the characteristics of grass genotypes, calcareous soils, and the application of available P, as they have a great impact on the bromatological properties, particularly the forage cultivar NDF content. Wang et al. (2016) found approximately 56 genes associated with the synthesis of NDF and ADF, which could explain the differences in this parameter content in the forage grasses.

The acid detergent fiber content only varied statically between calcareous soils ($p = 0.0217^*$) (Table 3); the lack of ADF response of cutting grasses is consistent with the findings by Ewetola et al. (2020). In the Carolina soil, the forage grasses showed a higher acid detergent fiber (ADF) content (34.9%) compared to those grown in the Vista Hermosa soil (32.18%). This response is associated with soil fertility, as previously explained in the ADF analysis (Figure 8C). However, the ADF contents of the calcareous soils under study were lower than those reported by Vivas-Quila et al. (2019) for Purple Elephant grass, which had 42.64% in a normal soil (pH 6.05). Finally, the ADF response to phosphorus application is consistent with the findings by Shi et al. (2024), who reported no variation in this parameter in a grass species following the application of P₂O₅ in a calcareous soil (pH 8.43). In contrast, the ADF response differs from the findings by Ewetola et al. (2020), who reported an increase in this parameter in two tropical grass species after NPK fertilization in a soil close to neutrality.



The bars represent the mean \pm standard deviation ($n=4$); Same letters indicate that there were no statistical differences between the calcareous soils or forage grasses according to Tukey's HSD test ($p \leq 0.05$)

Figure 8. Neutral detergent fiber (NDF) content between calcareous soils (A) and forage grasses (B); and acid detergent fiber (ADF) content between calcareous soils (C)

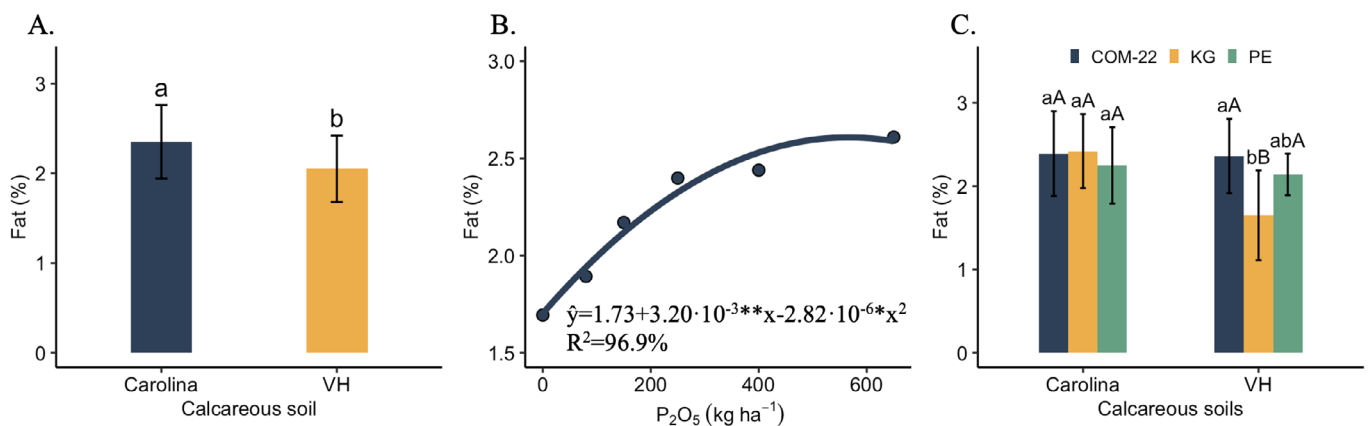
On the other hand, fat content showed significant differences between calcareous soils ($p = 0.02^*$) and among the applied phosphorus rates ($p \leq 0.001$), as well as in the interaction between calcareous soils and forage grasses ($p = 0.04^*$) (Table 3).

The forage grasses, regardless of cultivar and phosphorus rates, showed a higher fat content in the Carolina soil (2.35%) compared to the Vista Hermosa soil (2.05%) (Figure 9A). In the response of fat content in forage grasses (regardless of cultivar and type of calcareous soil) to phosphorus application, an increase in this parameter was observed as the applied rate increased, reaching a 2.64% at 567 kg ha⁻¹ P₂O₅ maximum value (Figure 9B).

When analyzing the interaction between calcareous soils and forage grasses (Figure 9C), we observed that in the Carolina soil, fat content did not show significant differences among cultivars, with a mean value of 2.35%. In contrast, in the Vista Hermosa soil, the Cuba OM-22 grass exhibited the highest fat content (2.36%), surpassing King Grass (1.65%), while Purple Elephant grass showed an intermediate value (2.14%). When comparing the grasses across both soils, King Grass accumulated a higher fat content in the Carolina soil (2.42%) compared to the Vista Hermosa soil (2.14%).

These responses may be associated with the soil's nutritional potential, characterized by high levels of calcium, magnesium, potassium, and organic matter, as well as the genotype-environment interaction, in which King Grass showed a better fat production response in both calcareous soils. These results can be explained by genetic variability, environmental conditions, and genotype-environment interaction (Whetsell et al., 2022). Likewise, Lynch (2019) explains that the genetic characteristics of each species also play an important role, as they can influence greater nutrient uptake and the formation of photosynthates that contribute to fat synthesis, as well as to forage quality and dry matter content (Carvajal-Tapia et al., 2023).

In the Carolina soil, the first principal component was associated with forage dry matter, fat content, and protein content, while the second component was primarily related to neutral detergent fiber. Together, these two components explained 72.8% of the total variability observed. Furthermore, a positive correlation was observed between dry matter and fat content, and a negative correlation between dry matter and protein content. Additionally, an inverse relationship was found between fat and protein contents (Figure 10A). These results align with the response to phosphorus rates, which promoted



The bars represent the mean \pm standard deviation (n=4); Same letters indicate that there were no statistical differences between the calcareous soils according to Tukey's HSD test ($p \leq 0.05$); * - Indicate regression models with coefficients significant at the $p \leq 0.05$; ** - Indicate regression models with coefficients significant at the $p \leq 0.01$; Same lowercase letters indicate that no statistical differences were observed among the forage grasses within each type of calcareous soil, while identical uppercase letters indicate that there were no statistical differences for the same forage grass between the two calcareous soil, according to Student's t-test ($p \leq 0.05$)

Figure 9. Fat content across calcareous soils (A), phosphorus rates (B), and the interaction between calcareous soils and forage grasses (C)

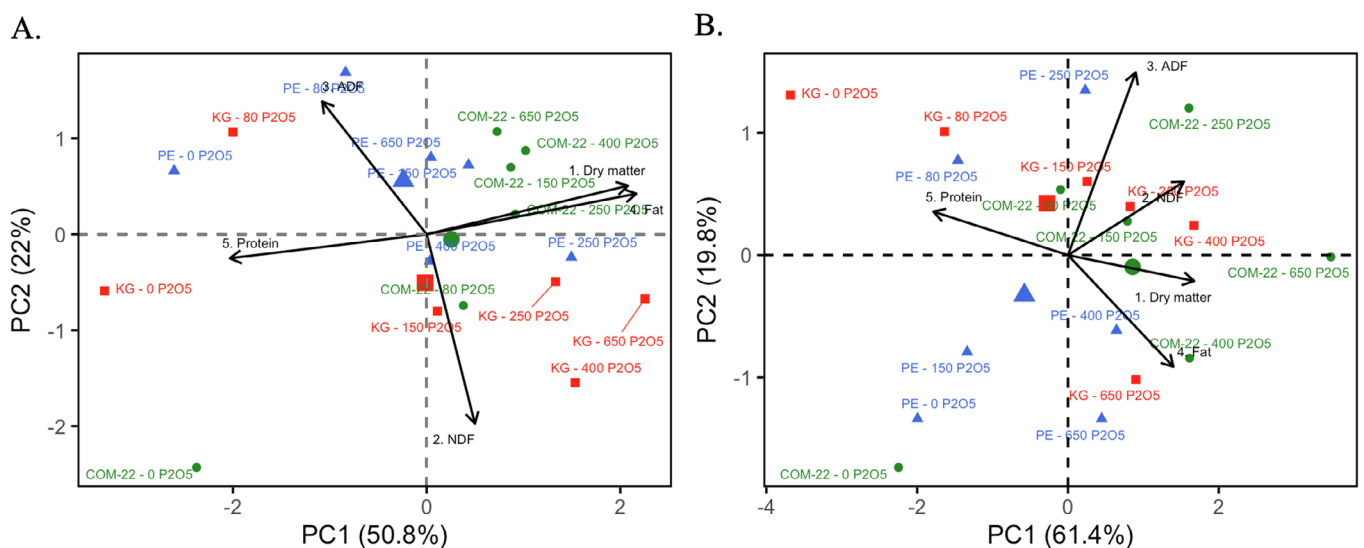


Figure 10. Principal component analysis of dry matter and bromatological parameters of the forage grasses King Grass, Purple Elephant, and Cuba OM-22 in Carolina soil (A) and Vista Hermosa soil (B), in response to phosphorus rates

increases in biomass and fat content, while protein content decreased proportionally with higher phosphorus levels. These findings are consistent with those reported by Ferreira et al. (2018) and Francisquini et al. (2020), who observed that crude protein content depends on both dry matter and the cutting age of forage grasses.

On the other hand, grasses with high protein content were characterized by having low biomass contents, as in the case of Cuba OM-22 and King Grass when phosphorus was not applied. Also, some grasses with high biomass contents presented high NDF contents and low protein contents (King Grass with 650 kg ha⁻¹ of P₂O₅). Pastures such as COM-22 and Purple Elephant grass are highlighted, since after the application of 400 kg ha⁻¹ P₂O₅, high biomass contents with moderate contents of NDF, ADF, protein, and high fat contents were observed (Figure 10A).

In the Vista Hermosa soil, the first component was explained by the dry matter, the contents of NDF, fat and protein, and the second component was explained by the ADF (81.21% of the variability). Dry matter was positively correlated with NDF and fat but negatively correlated with protein. Likewise, protein was negatively correlated with NDF and fat contents (Figure 10B). The pasture response was similar to that described in Carolina, where the Cuba OM-22 and Purple Elephant pastures once again stand out with the application of 400 kg ha⁻¹ P₂O₅, whose dry matter was the highest with moderate NDF and ADF content, and high fat content (Figure 10B).

CONCLUSIONS

1. This study found a positive response to phosphorus application in the biomass increase of the three evaluated forage grasses, with optimal phosphorus rates ranging from 449 to 555 kg ha⁻¹ P₂O₅.

2. Among the grasses evaluated in the two calcareous soils, the Vista Hermosa soil showed a higher mean dry matter yield compared to the Carolina soil. Additionally, across both soils, Cuba OM-22 and King Grass showed the higher dry matter accumulation.

3. The protein content of cutting grasses decreases with increasing phosphorus rates, and Cuba OM-22 and King Grass are the most affected, starting at a 277 kg ha⁻¹ P₂O₅ rate.

4. Phosphorus fertilization in both soils did not affect the acid detergent fiber or neutral detergent fiber contents but did influence the fat and protein contents.

Contribution of authors: Combatt-Caballero, E. M., and Paternina-Durango, E. M., participated in the research design, supervision of the work, literature review, interpretation and discussion of results, writing, revision, and preparation of the manuscript. Pérez-Polo, D. J., participated in the statistical data analysis and figure design, interpretation and discussion of results, writing, revision, and preparation of the manuscript.

Data availability statement: The authors state that the data supporting the findings of this article are available upon request from the corresponding author: Pérez-Polo, D. J. (dairoperezp@correo.unicordoba.edu.co).

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