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Original article

Phosphate limestone, soil improver and nutrient source for *Macroptilium Atropurpureum* cv. Siratro

La caliza fosfatada, mejorador del suelo y fuente de nutrientes para *Macroptilium Atropurpureum* cv. Siratro

Yunior Pérez González[1*], Pedro José González Cañizares[1], Juan Francisco Ramírez Pedroso[2]

[1]Instituto Nacional de Ciencias Agrícolas (INCA). Carretera a Tapaste, km 31/2, San José de las Lajas, Mayabeque. Cuba. CP-32700. Gaveta Postal No. 1.

[2]Centro de Plantas Proteicas y Productos Bionaturales (CIPB) Calle 5ta Av. y 246 Complejo Barlovento, Playa. La Habana, Cuba.

ABSTRACT: The search for fertilization alternatives for forage crops based on the use of domestically produced nutrient sources is imperative to reduce the costs of animal feed production and to reduce the use of external inputs. Based on this premise, an experiment was conducted under semi-controlled conditions to determine the effect of phosphate limestone, a mineral of national origin, on soil fertility, nutritional status and yields of *Macroptilium atropurpureum* cv. Siratro, grown in a low fertility soil. Five treatments (1 t ha^{-1} lime, 50 kg ha^{-1} P_2O_5, 1 t ha^{-1} lime+ 50 kg ha^{-1} of P_2O_5, 1 t ha^{-1} phosphate lime and a control without lime and phosphorus) were evaluated in a completely randomized design. Indicators of soil fertility, biomass macronutrient concentrations, nodulation effectiveness and crop yields were evaluated. Phosphate limestone reduced soil acidity and increased its assimilable P content. Its application increased biomass N, P and Ca concentrations, the number and effectiveness of root nodules, and plant aerial and root biomass yields, with results similar to those obtained with lime and phosphoric fertilizer. It is concluded that the use of phosphate limestone constitutes an agronomically effective alternative to improve soil fertility, reduce the use of synthetic phosphoric fertilizer and increase the productivity of *Macroptilium atropurpureum* cv. Siratro.

Key words: nutritional status, soil fertility, forage legume, yield.

RESUMEN: La búsqueda de alternativas de fertilización para cultivos forrajeros a partir del uso de fuentes de nutrientes de producción nacional, constituye un imperativo para disminuir los costos de la producción de alimentos para los animales y reducir el uso de insumos externos. Basado en esta premisa, se realizó un experimento en condiciones semicontroladas, para conocer los efectos de la caliza fosfatada, un mineral de procedencia nacional, en la fertilidad del suelo, el estado nutricional y los rendimientos de *Macroptilium atropurpureum* cv. Siratro, cultivado en un suelo de baja fertilidad. Fueron evaluados cinco tratamientos (1 t ha^{-1} cal, 50 kg ha^{-1} P_2O_5, 1 t ha^{-1} cal+ 50 kg ha^{-1} de P_2O_5, 1 t ha^{-1} caliza fosfatada y un testigo sin cal y fósforo) en un diseño completamente aleatorizado. Se determinaron indicadores de la fertilidad del suelo, las concentraciones de macronutrientes en la biomasa, la efectividad de la nodulación y los rendimientos del cultivo. La caliza fosfatada redujo la acidez del suelo y aumentó su contenido de P asimilable. Su aplicación incrementó las concentraciones de N, P y Ca de la biomasa, el número y efectividad de los nódulos radicales y los rendimientos de biomasa aérea y radical de las plantas, con resultados similares a los que se obtuvieron con la cal y el fertilizante fosfórico. Se concluye que el uso de la caliza fosfatada constituye una alternativa agronómicamente efectiva, para mejorar la fertilidad del suelo, reducir el uso de fertilizante fosfórico sintético y aumentar la productividad de *Macroptilium atropurpureum* cv. Siratro.

Palabras clave: estado nutricional, fertilidad del suelo, leguminosa forrajera, rendimiento.

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*Author for correspondence. pyunior617@gmail.com

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INTRODUCTION

Livestock production in Cuba is primarily based on the use of pastures and forages as the main sources of animal feed, which necessitates the generation of high volumes of biomass with sufficient quality to meet their nutritional requirements (1). Pasture quality can be enhanced through the inclusion of legumes in grazing systems. These legumes not only provide a more balanced diet in terms of energy and protein particularly during the dry season but also serve as natural sources of nitrogen for the soil (2). The contribution of forage legumes to pasture systems is well recognized due to their ability to fix atmospheric nitrogen through symbiosis between the plants and bacteria of the genus *Rhizobium* and others, resulting in increased protein content in the animals' diet (3). Among forage legumes, the species *Macroptilium atropurpureum* cv. Siratro is highly accepted by ruminants due to its excellent nutritional quality (4). Its use in association with grasses, either as part of the herbaceous component of silvopastoral systems or as a protein bank, improves the dietary quality of livestock and, consequently, their productive indicators. Although Siratro is cultivated across a wide range of soil types, high acidity and low fertility have been shown to limit its growth and development. Under acidic soil conditions, its forage value decreases significantly. Additionally, low phosphorus content in the soil restricts root and shoot growth and negatively affects the formation and effectiveness of nodules where biological nitrogen fixation occurs (5). In such cases, it is necessary to correct these deficiencies to allow the crop to express its productive potential (4). Liming is the most commonly used agricultural practice to correct soil acidity and, consequently, increase crop productivity (6). In the case of Siratro, liming has been shown to enhance both its productivity and nutritional value (7). Phosphorus fertilization has also proven effective in improving yield and persistence of forage legumes, whether in association with other crops or in protein banks, particularly in soils with severe phosphorus deficiency (8). However, both the application of soil amendments and synthetic fertilizers are costly agricultural activities, especially in the case of fertilizers, which must be imported and are subject to rising prices in the international market. Therefore, the search for local nutrient sources as an alternative to enhance forage crop productivity while reducing reliance on external inputs represents a challenge for sustainable livestock feed production (9). Based on these premises, the present study was conducted to evaluate the effects of phosphate limestone, a nationally sourced mineral, on soil fertility, nutritional status, and yield of *Macroptilium atropurpureum* cv. Siratro cultivated under semi-controlled conditions in acidic, low-fertility soil.

MATERIALS AND METHODS

The experiment was conducted under semi-controlled conditions in the greenhouse area of the National Institute of Agricultural Sciences (INCA), located in San José de las Lajas municipality, Mayabeque province. Five treatments were evaluated, consisting of the application of 1 t ha^{-1} of lime, 50 kg ha^{-1} of P_2O_5, 1 t ha^{-1} of lime + 50 kg ha^{-1} of P_2O_5, 1 t ha^{-1} of phosphate limestone, and a control without amendments or phosphorus fertilizer, arranged in a completely randomized design with six replications.

Plastic pots with a capacity of 3.5 L, previously perforated at the bottom to facilitate drainage, were used. Each pot was filled with 3 kg of soil collected from the Experimental Station of Pastures and Forages in Cascajal, located at 22° 39′ N latitude and 80° 24′ W longitude, in the municipality of Santo Domingo, Villa Clara province. The soil was classified as Petroferric Ferruginous Nodular Gleysol (9,10), and its main chemical characteristics at a depth of 0-20 cm are presented in Table 1.

The soil exhibited high acidity, characterized by a strongly acidic pH, elevated levels of exchangeable acidity ($H^+ + Al^{3+}$), and a very low percentage of base saturation (V), as well as low organic matter content and very low levels of available phosphorus and exchangeable cations (11).

Procedure for pot filling and treatment application

For pot filling, soil was collected from a depth of 0-20 cm and sieved through a 5 mm mesh. In treatments involving lime and phosphate limestone, both materials were applied at a rate of 1.5 g per pot, equivalent to 1 t ha^{-1}. In treatments with phosphorus fertilizer, 75 mg of P_2O_5 per pot was added, equivalent to 50 kg ha^{-1} of P_2O_5, using triple superphosphate as the carrier. All treatments received basal potassium fertilization at a rate of 150 mg of K_2O per pot, equivalent to 100 kg ha^{-1}, and using potassium chloride as the carrier.

The lime used in the experiment was sourced from the Tapaste deposit in San José de las Lajas, Mayabeque, and contained 95 % $CaCO_3$. The phosphate limestone was obtained from the Loma de Candela deposit in Güines municipality, also in Mayabeque province, and contained 65 % $CaCO_3$ and 11.5 % P_2O_5. Both deposits belong to the Basic Enterprise Unit (UEB) “Roberto Coco Peredo” of the Western Geomining Company. Prior to application, both materials were sieved through a 0.25 mm mesh.

Table 1. Chemical characteristics of the soil used in the experiment (depth: 0-20 cm)

pH H_2O	OM (%)	P (mg kg^{-1})	Exchangeable bases Ca^{2+}	Mg^{2+}	Na^+	K^+	CEC	$H^+ + Al^{3+}$	V (%)
			(cmol$_c$ kg^{-1})						
4.8	2.52	5.5	3.32	1.12	0.05	0.1	4.59	4.33	51
(0.2)	(0.17)	(0.6)	(0.3)	(0.1)	(0.01)	(0.02)	(0.31)	(0.33)	

OM: organic matter; CEC: Cation Exchange Capacity; $H^+ + Al^{3+}$: exchangeable acidity; V: base saturation. Values in parentheses indicate the confidence interval range of the means (α = 0.05)

Both liming materials and mineral fertilizers were thoroughly mixed with the soil at the time of pot filling. Subsequently, pots were watered every three days to maintain soil moisture at 80 % of field capacity and ensure interaction between the amendments and the soil. Irrigation was maintained throughout the duration of the experiment.

Seed sowing and inoculation

Sowing was performed 15 days after the application of liming materials and mineral fertilizers. Five seeds of Siratro (*Macroptilium atropurpureum* cv. Siratro), with 80 % germination, were placed in a 0.5 cm deep hole at the center of each pot and covered with the same soil. In all treatments, seeds were sprayed at sowing with a liquid inoculant produced at INCA, containing a Rhizobium isolate with a concentration of 10^9 CFU mL^{-1}, previously selected for its high efficiency in promoting Siratro growth (12). A 50 cm tall wooden stake was placed in each pot to support vertical plant growth, given the species' prostrate growth habit.

Sampling and measurements

Two harvests were conducted: the first at 40 days after sowing, cutting at 5 cm above the soil surface, and the second at soil level, 35 days after the first. In both cases, the fresh aerial biomass was weighed using a precision balance (0.01 g), and samples were dried in a forced-air oven at 70 °C for 72 hours to determine dry matter (DM) percentage, DM yield, and concentrations of N, P, K, and Ca in the biomass, following standard procedures of the Soil and Plant Analysis Laboratory at INCA (13).

In the second harvest, root fresh biomass was weighed, and DM yield was determined using the same procedure. Before drying, nodules on carefully washed roots were counted in each pot, and their internal coloration was qualitatively assessed. Ten nodules per pot were randomly selected, and effectiveness was determined by observing their internal color via transverse section. Nodules were considered effective if they exhibited a red to pink coloration, indicating the presence of leghemoglobin (14).

From the area where soil was collected for pot filling, ten soil samples were taken at a depth of 0-20 cm using the zigzag method for initial chemical characterization. After the final harvest, one soil sample was taken from each pot. Analytical determinations included pH in H_2O (potentiometry, soil-to-water ratio 1:2.5), organic matter content (Walkley and Black), available P (extraction with 0.5 mol L^{-1} H_2SO_4 and colorimetric determination), exchangeable bases (extraction with 1 mol L^{-1} NH_4Ac at pH 7, titration with EDTA for Ca and Mg, and flame photometry for Na and K), base exchange capacity (BEC) calculated as the sum of exchangeable bases, exchangeable acidity ($H^+ + Al^{3+}$) extracted with 1 mol L^{-1} KCl and titrated, and base saturation percentage (V) calculated as: BEC/[BEC + ($H^+ + Al^{3+}$)] × 100. All analyses followed the standard procedures of the Soil and Plant Laboratory at INCA (13).

Statistical processing

Data were analyzed after verifying normality and homogeneity of variances, using one-way analysis of variance according to the experimental design. When significant differences among treatments were found, Tukey's test was applied ($P<0.05$). For initial soil characterization, the confidence interval of the means ($\alpha=0.05$) was used as a dispersion statistic. All data were processed using SPSS statistical software, version 25 (15).

RESULTS AND DISCUSSION

Table 2 presents the influence of treatments on selected chemical properties of the soil, 90 days after the application of lime and phosphate limestone. A significant effect was observed from lime and phosphorus fertilizer on the levels of exchangeable Ca and available phosphorus, respectively. In the case of lime, increases in pH and reductions in exchangeable acidity were also recorded.

The effect of lime on reducing acidity can be attributed to the contribution of Ca from the liming material and, consequently, to the displacement of H^+ ions from cation exchange sites into the soil solution. It is known that the reaction mechanisms of liming amendments neutralize H^+ ions in the soil solution through OH^- ions generated when lime comes into contact with water, resulting in a reduction in acidity (12, 16).

The effect of phosphorus fertilizer on increasing available P content is attributed to the initially low levels of this element in the soil and to the applied dose through fertilization.

Table 2. Effect of lime, phosphorus fertilization, and phosphate limestone on soil chemical properties 90 days after treatment application

Treatments	OM (g kg^{-1})	P (mg kg^{-1})	pH H_2O	H + Al	Ca	Mg	K
				(cmol$_c$ kg^{-1})			
Control	25.8	5.1 b	4.7 d	3.57 a	3.52 b	1.15	0.21
Lime	26.1	5.3 b	5.8 b	2.17 b	4.79 a	1.17	0.23
50 kg ha^{-1} P_2O_5	25.6	6.9 a	4.9 d	3.53 a	3.55 b	1.16	0.21
Lime + 50 kg ha^{-1} P_2O_5	26.0	6.8 a	6.0 a	2.18 b	4.81 a	1.19	0.23
Phosphate limestone	25.9	7.2 a	5.9 a	2.15 b	4.88 a	1.17	0.22
SE ±	1.5	0.1	0.1	0.20	0.32	0.12	0.04
P Value	0.36	0.00	0.00	0.00	0.00	0.25	0.32

Means with different letters in the same column differ significantly according to Tukey's test ($P<0.05$)

This phenomenon has also been demonstrated under acidic soil conditions, where the application of P quantities exceeding the soil's phosphate fixation capacity leads to increased availability (17).

Phosphate limestone produced effects similar to those observed with lime application, both in increasing pH and exchangeable Ca content, and in reducing exchangeable acidity. This is likewise explained by its Ca contribution and its role in lowering soil acidity. Additionally, it led to increases in available phosphorus content comparable to those obtained with phosphorus fertilization, consistent with its P contribution.

Phosphate limestone proved to be just as effective as lime the most commonly used liming material in Cuba, for reducing soil acidity. The increase in available phosphorus content in the soil observed in the treatment where phosphate limestone was applied is attributed to the nutrient content of the liming material, as previously mentioned, and to the soil's own acidity. The phosphorus contained in phosphate limestone is in a form that is not readily available to plants; however, the presence of H^+ ions in the soil solution may have contributed to the solubilization of phosphorus, making it available to plants (18).

In this regard, some authors (19), when using phosphate limestone as a phosphorus source for coffee cultivation in soils with varying degrees of acidity, observed that the increase in available phosphorus content was greater in soils with pH values below 6.

The treatments did not affect the soil's organic matter, exchangeable potassium (K), or magnesium (Mg) content. In the first case, this was because no organic sources were applied in any of the treatments. In the second case, although data on the content of both nutrients in the liming materials is not available, it is presumed that, if present, they were not in sufficient quantities to cause an increase in the soil, which aligns with results obtained by other authors using mineral amendments with low levels of both elements (20).

The results in Table 3 indicate the effect of the treatments on nutrient concentrations in the aerial biomass. In the first cut, the application of phosphate fertilizer either alone or combined with lime produced a significant increase in nitrogen (N) and phosphorus (P) concentrations. However, lime alone and phosphate limestone had no effect on these indicators. The treatments did not influence calcium (Ca) concentrations.

In the second harvest, the treatments exhibited different behavior. Unlike the first harvest, the application of phosphate limestone resulted in increased concentrations of N and P, similar to those observed in the treatment where lime was applied in combination with phosphorus fertilizer. Lime and phosphorus fertilizer applied independently also increased N concentrations in the biomass, although their effects were less pronounced than in treatments where both were applied together. Phosphate limestone led to increases in Ca concentrations comparable to those achieved with lime applications, likely due to its contribution of this nutrient to the soil, as shown in Table 2.

Several aspects deserve attention when evaluating the effects of treatments on nutrient concentrations in biomass, based on the results of both samplings. Unlike phosphorus fertilization, whose effects were evident from the first harvest due to the nutrient being applied in a plant-available form, the influence of lime and phosphate limestone on increasing N, P, and Ca concentrations in biomass was not observed until the second harvest.

This indicates that both mineral amendments, which supply nutrients in forms not readily available to plants and whose solubilization occurs gradually (20), required time to interact with the soil in order to improve the nutritional status of the plants. It should be noted that although the liming materials were mixed with the soil fifteen days prior to Siratro sowing and the pots were kept moist to ensure their reaction with the soil, the first harvest was conducted 40 days after sowing. Apparently, this period was insufficient for either amendment to produce an increase in nutrient concentrations in the aerial biomass of the plants.

However, the second harvest was conducted 35 days after the first, meaning that 90 days had passed since the application of the amendments. This reaction time, under the conditions of this study, ensured greater effectiveness in improving the nutritional status of the plants, as reflected in the soil effects shown in Table 2.

Some authors (21) recommend applying lime well in advance of crop sowing, especially for short-cycle crops, to ensure effective interaction with the soil and greater agronomic efficiency.

Table 3. Effects of lime, phosphorus fertilization, and phosphate limestone on nutrient concentrations in the aerial biomass of Siratro

Treatments	Nutrient concentrations in biomass (g kg^{-1})					
	First harvest			Second harvest		
	N	P	Ca	N	P	Ca
Control	30.9 b	1.7 b	15.9	30.1 c	1.7 b	15.9 b
Lime	31.1 b	1.9 b	16.1	32.9 b	1.9 b	20.5 a
50 kg ha^{-1} P_2O_5	33.8 a	2.4 a	15.7	32.7 b	2.3 a	15.7 b
m+ 50 kg ha^{-1} P_2O_5	33.5 a	2.5 a	16.3	36.1 a	2.4 a	20.8 a
Phosphate limestone	31.3 b	1.8 b	15.8	35.5 a	2.3 a	21.1 a
SE	0.4	0.1	0.4	0.4	0.1	0.4
P Value	0.00	0.00	0.41	0.00	0.00	0.00

Means followed by different letters in the same column differ significantly according to Tukey's test (P<0.05)

The effect of phosphorus fertilization on increasing N concentrations in biomass may be attributed to the role of P in transporting photosynthetic products from leaves to roots, where biological N fixation occurs, and in the movement of N-containing compounds from nodules to plant growth sites for the formation of new tissues (22).

Similarly, the influence of lime on increasing N content in biomass may be related to improved biological N fixation, as the rhizobium-legume symbiosis is known to be restricted in highly acidic soils. Liming allows plants to fix greater amounts of atmospheric N due to the proper development of the bacteria involved in this process (23).

The fact that the highest N concentrations in biomass were achieved in treatments where lime and P were applied together or phosphate limestone was used indicates the need for liming to be accompanied by adequate P supply to enhance nitrogen nutrition in Siratro. Furthermore, the effect of phosphate limestone on increasing Ca and P concentrations in biomass observed in the second harvest confirms that this amendment can serve as a source of both nutrients for plants. This finding aligns with results from other studies (24) and corresponds with its effects on increasing Ca and P contents in the soil.

When analyzing Siratro nodulation, it was observed that both the number of nodules and nodulation effectiveness were higher in treatments where lime and phosphorus fertilizer were applied together, and in those with phosphate limestone (Table 4). When lime or phosphorus fertilizer were applied individually, increases in both indicators were also observed, but their effects were significantly lower than in the aforementioned treatments.

These results demonstrate that although Siratro was inoculated with an efficient rhizobium strain at sowing, it was necessary to correct soil acidity and ensure phosphorus supply either through synthetic fertilizer or phosphate limestone to improve nodulation effectiveness. This improvement was also reflected in the N concentrations of the aerial biomass of the plants.

Both liming and phosphorus fertilization promote the nodulation process in forage legumes (25), as confirmed by the treatments that benefited from these amendments. Correcting soil acidity leads to increased microbial activity, which, in the case of rhizobia, translates into a higher number and effectiveness of nodules and, consequently, greater efficiency in atmospheric nitrogen fixation (26). The positive effects of phosphorus fertilization on Siratro nodulation have also been demonstrated (3).

The effect of treatments on Siratro biomass yield is presented in Table 5. The aerial biomass at the time of the first harvest showed a pattern similar to that observed in nutrient concentrations in the biomass at that stage; that is, significant increases were found only with the application of phosphorus fertilizer. This indicated that Siratro grown in soil with very low P content may require a supply of this nutrient from the early growth stages.

In the second harvest, where the positive influence of liming materials on soil and plant nutritional status was already evident, according to the results presented in Tables 2 and 3. It was observed that although lime and phosphorus fertilization applied independently increased biomass production, the greatest effects were obtained when lime was combined with phosphorus fertilization and with phosphate limestone. No significant differences were found between these two treatments, clearly indicating that the use of phosphate limestone as a source of Ca and P can contribute to improving Siratro productivity.

Table 4. Effects of lime, phosphorus fertilization, and phosphate limestone on nodulation performance in Siratro

Treatments	Nodules per pot	Nodulation effectiveness (%)
Control	18.72 d	63 d
Lime	23.51 c	74 c
50 kg ha^{-1} P_2O_5	32.83 b	85 b
Lime + 50 kg ha^{-1} P_2O_5	45.45 a	96 a
Phosphate limestone	44.31 a	97 a
ES ±	1.4	2.9
P Value	0.00	0.00

Means followed by different letters in the same column differ significantly according to Tukey's test ($p < 0.05$)

Table 5. Effects of lime, phosphorus fertilization, and phosphate limestone on biomass yield (g pot^{-1}) of Siratro: aerial (ABY), root (RBY), and total (TBY)

Treatments	First harvest	Second harvest		
	ABY	ABY	RBY	TBY
Control	3.31 b	4.12 d	4.07 d	8.20 d
Lime	3.55 b	5.59 c	5.19 c	10.77 c
50 kg ha^{-1} P_2O_5	5.62 a	6.73 b	6.41 b	13.14 b
Lime + 50 kg ha^{-1} P_2O_5	5.59 a	7.91 a	7.69 a	15.60 a
Phosphate limestone	3.47 b	7.93 a	7.72 a	15.65 a
SE ±	0.20	0.25	0.23	0.31
P Value	0.00	0.00	0.00	0.00

TBY (ABY + RBY of the second harvest). Means with different letters in the same column differ significantly according to Tukey's test ($p < 0.05$).

CONCLUSIONS

The use of phosphate limestone represents an agronomically effective alternative for correcting soil acidity and increasing the levels of plant-available calcium and phosphorus. Its application improves the nutritional status and biomass yield of Siratro, producing effects similar to those obtained with liming using calcium carbonate and synthetic phosphorus fertilizer. It is recommended that the results obtained in this experiment be validated under field conditions.

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