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# Agronomic evaluation and techno-economic analysis of kikuyo grass biostimulation

## Evaluación agronómica y análisis técnico-económico de la bioestimulación del pasto kikuyo

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## ABSTRACT

Milk production in the Colombian high tropics depends on forage availability, particularly kikuyu grass. Its yield can be increased through the use of various bioproducts. To this end, five biostimulant treatments were evaluated in this study: an organic conditioner, a trihormonal mixture, *Bacillus subtilis*, an organic conditioner supplemented with boron and molybdenum (B–Mo), and a trihormonal mixture supplemented with B–Mo. A conventionally managed control treatment was included for comparison. The experiment was established on six 1.2-ha plots at Finca Tesorito, Manizales, Colombia, and grazing was managed under 37-day rotational cycles for 13 consecutive periods, totaling approximately 481 days. Biomass was measured using small 0.5 m × 0.5 m sampling frames at five points in each plot. An economic analysis was also conducted, examining costs, the benefit–cost ratio, and the unit gross margin. The trihormonal mixture, either alone or combined with B–Mo, showed the best performance. It yielded the highest forage yield (exceeding 3,700 kg of dry matter per hectare in the final rotation) and demonstrated the best economic performance, with a benefit–cost ratio of 1.23 at higher stocking rates. Overall, biostimulation—particularly with growth regulator-based treatments—shows strong potential as a practical, cost-effective, and sustainable strategy for enhancing dairy production in high-tropical production systems.

**Keywords:** *Bacillus subtilis*; benefit/cost; bioproducts; *Cenchrus clandestinus*; forage biomass; growth regulators; high tropical livestock farming

## RESUMEN

La producción de leche en el trópico alto colombiano depende de la cantidad de forrajes, especialmente del pasto kikuyu. Su rendimiento puede incrementarse mediante el uso de diferentes bioproductos. En este estudio se evaluaron cinco tratamientos de bioestimulación: un acondicionador orgánico, una mezcla trihormonal, *Bacillus subtilis*, un acondicionador orgánico suplementado con boro y molibdeno (B–Mo), y una mezcla trihormonal suplementada con B–Mo. Se incluyó un tratamiento control de manejo convencional para comparación. El experimento se estableció en seis parcelas de 1,2 ha en Finca Tesorito, Manizales, Colombia, y el pastoreo se manejó bajo ciclos rotacionales de 37 días durante 13 períodos consecutivos, totalizando aproximadamente 481 días. La biomasa se midió utilizando pequeños marcos de muestreo de 0,5 m × 0,5 m en cinco puntos de cada parcela. También se llevó a cabo un análisis económico, considerando los costos, la relación beneficio-costo y el margen bruto unitario. La mezcla trihormonal, ya sea sola o combinada con B-Mo, mostró el mejor desempeño. Produjo la mayor cantidad de forraje (más de 3700 kg de materia seca por hectárea en la última rotación) y obtuvo los mejores resultados económicos, con una relación beneficio-costo de 1,23 en un escenario con mayores cargas ganaderas. En general, la bioestimulación, en particular con tratamientos basados en reguladores del crecimiento, demuestra un gran potencial como estrategia práctica, rentable y sostenible para mejorar la producción lechera en sistemas de producción de zonas tropicales elevadas.

**Palabras clave:** *Bacillus subtilis*; beneficio/costo; biomasa forrajera; bioproductos; *Cenchrus clandestinus*; ganadería tropical intensiva; reguladores del crecimiento

## INTRODUCTION

Milk production is a strategic agricultural activity in Colombia because of its contributions to food security, rural economic development, and the livelihoods of thousands of farming families (ICA, 2025). In 2024, the country reported a cattle population of approximately 30.3 million animals across more than 629,000 farms, and raw milk production reached 7.712 billion liters, a 7.97% increase from the previous year (ICA, 2025; FEDEGAN, 2025). Despite this growth, the sector continues to face significant challenges, including rising production costs, limited adoption of technological innovations, and increasing climate variability, all of which affect farm productivity and competitiveness (Jafri *et al*., 2024).

In pasture-based dairy systems, forage availability and quality are major determinants of animal performance and milk production. Kikuyu grass (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone) is the dominant forage species in medium- and high-altitude dairy regions of the Colombian Andes because of its high palatability, vigorous regrowth, and adaptability to acidic, humid soils (Vargas *et al*., 2018). However, its productivity and nutritional performance are strongly influenced by fertilization practices, environmental conditions, and overall pasture management (Xixi *et al*., 2019). Therefore, developing strategies that enhance forage productivity while maintaining environmental and economic sustainability is a priority for dairy production systems in the high tropics.

Biostimulants and beneficial microorganisms have emerged as promising tools for improving crop performance by stimulating key physiological processes. These products can enhance nutrient uptake, promote plant growth, increase tolerance to abiotic stress, and improve resource-use efficiency (Stella *et al*., 2025). For example, trihormonal formulations containing auxins, gibberellins, and cytokinins stimulate cell division, elongation, and root development (Alcantara *et al*., 2019), whereas *Bacillus subtilis* has been linked to plant growth promotion and biological control (González-León *et al*., 2022). Furthermore, integrating biostimulants with mineral nutrition strategies may yield synergistic effects that increase forage biomass production and nutrient-use efficiency while reducing reliance on conventional fertilizers (Rouphael & Colla, 2020).

Despite the growing body of evidence supporting the use of biostimulants in crops, information on their productive and economic effects in kikuyu-based dairy systems in tropical highland environments remains limited. Biostimulant applications were hypothesized to enhance kikuyu regrowth, biomass accumulation, and nutrient-use efficiency relative to conventional management, resulting in increased forage availability and improved economic returns. In particular, treatments combining growth-promoting compounds with boron and molybdenum were expected to yield greater agronomic and economic responses due to potential synergistic effects on plant metabolism and nutrient utilization. Therefore, this study aimed to evaluate the agronomic and techno-economic performance of different biostimulant strategies applied to kikuyu grass under tropical highland dairy production conditions.

## MATERIALS AND METHODS

### *Location and Plant Material*

The research was conducted at Tesorito Farm, owned by the University of Caldas, in the village of Maltería, municipality of Manizales, department of Caldas, Colombia. The site is located at an elevation of 2,280 m, with a mean annual temperature of 17 °C, relative humidity of 78%, and average precipitation of 1,800 mm (Granja Tesorito, 2025). The soils are classified as Andisols derived from volcanic ash, exhibiting a sandy loam texture and high organic matter content that enhance moisture retention and natural fertility (Granja Tesorito, 2025).

***Crop Management and Experimental Design***

The study was conducted as a production-scale field evaluation under commercial grazing conditions. A total of 7.2 ha of established kikuyu pasture was divided into six 1.2-ha paddocks, each assigned to one of five biostimulant treatments or a conventionally managed control. The paddocks were grazed by 40 dairy cows in 13 consecutive rotations (481 days), following 37-day grazing cycles comprising approximately 30 days of regrowth and 7 days of occupation. Bioproduct applications were carried out five days after the cattle left each plot and aligned with the production system's fertilization schedule, with re-entry occurring when the forage reached 30 cm in height (Martins *et al*., 2021). The application volume was 200 L ha$^{-1}$, delivered using a Royal Condor manual backpack sprayer.

Because each treatment was applied to a single paddock, the study lacked independent, replicated experimental units. Therefore, paddocks served as management units rather than replicated experimental plots. Within each paddock, five quadrat samples were collected during each rotation to characterize forage production; these measurements were treated as subsamples rather than independent treatment replicates. Consequently, the results should be interpreted as responses under the farm's specific production conditions rather than as definitive estimates of treatment effects applicable to all production systems.

***Treatments***

All treatments received a baseline application of urea fertilizer at 100 kg ha$^{-1}$, and the bioproducts were applied as foliar treatment according to the specifications in Table 1.

**Table 1.** *Specifications and characteristics of the treatments evaluated*

| Treatment | Bioproduct | Composition | Dose |
|---|---|---|---|
| 1 | Organic conditioner | Water-soluble potassium ($K_2O$): 51.70 g L$^{-1}$<br>Water-soluble iron (Fe): 5.60 g L$^{-1}$<br>Water-soluble manganese (Mn): 1.37 g L$^{-1}$<br>Water-soluble zinc (Zn): 4.20 g L$^{-1}$<br>Total oxidizable organic carbon (TOOC): 50.0 g L$^{-1}$<br>Free amino acids: 53.0 g L$^{-1}$<br>*Contains the following amino acids: aspartate, glutamate, serine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine, lysine, hydroxyproline, and proline. | 2 mL L$^{-1}$ |
| 2 | trihormonal | Cytokinin (Kinetin) 0.091 g L$^{-1}$<br>Gibberellic acid ($GA_3$) 0.051 g L$^{-1}$<br>Indole-3-butyric acid (IBA) 0.051 g L$^{-1}$ | 0.5 mL L$^{-1}$ |
| 3 | *Bacillus subtilis* | 1 x $10^{12}$ CFU kg$^{-1}$ | 5 mL L$^{-1}$ |
| 4 | T1 + B-Mo | T1 + Boron (B) 126 g L$^{-1}$ - Molybdenum (Mo) 0.1 g L$^{-1}$ | 4 mL L$^{-1}$ |
| 5 | T2 + B-Mo | T2 + Boron (B) 126 g L$^{-1}$ - Molybdenum (Mo) 0.1 g L$^{-1}$ | 4 mL L$^{-1}$ |
| 6 | Control | Conventional management – Urea (46-0-0) | 100 kg ha$^{-1}$ |

### *Evaluated Variables*

The variables evaluated in this study included post-grazing recovery and aboveground forage biomass production. Aboveground biomass was quantified using a destructive sampling method. In each experimental plot, a 0.5 m x 0.50 m (0.25 $m^2$) PVC quadrat was randomly placed at five sampling points (*n*= 5). Herbage within each quadrat was harvested at the residual height corresponding to actual cattle grazing level. All collected samples were weighed immediately in the field using a calibrated digital scale.

To account for the quadrat surface area, the mean fresh biomass per square meter ($B_m$, kg $m^{-2}$) was calculated as (equation 1):

$$B_m = \frac{\sum_{i=1}^{n} Wi}{n \text{ x } A} \quad \text{e1}$$

Where $W_i$ is the fresh weight of individual subsample *i* (kg), *n* is the total number of sampling points per plot (*n* = 5), and *A* is the quadrat area (0.25 $m^2$). Total green forage yield ($Y_b$, kg $ha^{-1}$) was extrapolated to the hectare scale as follows (equation 2):

$$Y_b = Bm\ x\ 10{,}000 \quad \text{e2}$$

Where 10,000 represents the conversion factor from square meters to hectares ($m^2$ $ha^{-1}$). All biomass harvests were performed after midday to minimize confounding effects from residual morning dew, ensuring reliable fresh-weight determinations.

For the economic evaluation, monthly direct production costs were recorded per treatment, including inputs, labor, machinery, and external services (Durán & Zolano, 2019). The unit gross margin (UGM, USD $L^{-1}$) and the benefit–cost ratio (B/C) were computed as (equation 3):

$$UGM = \frac{I_L - C_v}{v_m}$$

$$B/C = \frac{N_b}{C_t} \quad \text{e3}$$

Where $I_L$ is the gross income from milk sales, $C_v$ is the total variable cost, $V_m$ is the total volume of milk produced (L), $N_b$ is the net economic benefit, and $C_t$ is the total investment cost. All monetary indicators were reported in standardized currency units.

### *Statistical Analysis*

Data collected across the 13 grazing rotations were analyzed using a repeated-measures approach to describe temporal patterns in forage production and economic performance among treatments. Mean values and measures of variability were calculated for each treatment throughout the evaluation period. Because each treatment was applied to a single paddock, independent experimental replication was not available. Therefore, the statistical analysis was intended to characterize treatment responses over time rather than to provide formal inferential comparisons among treatments. Results should be interpreted as exploratory evidence obtained under commercial production conditions. All analyses were conducted using SigmaPlot (Systat Software Inc.).

## RESULTS

### *Forage Assessments*

The trihormonal treatment (T2) and T2 + B–Mo (T5) consistently produced the highest forage biomass throughout the evaluation period. Both treatments increased progressively from rotation 4 onward and exceeded 3,700 kg ha$^{-1}$ in the final rotation (Figure 1). In contrast, the organic conditioner (T1), *Bacillus subtilis* (T3), and the organic conditioner + B–Mo (T4) showed intermediate responses, while the conventionally managed control maintained relatively stable biomass between 2,400 and 2,500 kg ha$^{-1}$ across rotations. These observations suggest a positive association between growth regulator-based biostimulants and forage accumulation under the evaluated production conditions.

Beginning in approximately the fifth rotation, biomass production under the *Bacillus subtilis* treatment showed a gradual increase, suggesting a delayed response compared with the hormone-based treatments.

The control (T6), managed with conventional fertilization, maintained a constant value between 2,400 and 2,500 kg ha$^{-1}$, indicating that fertilization alone did not produce progressive increases in biomass under the conditions of this study, even though the application of chemical fertilizers has been a long-standing practice.

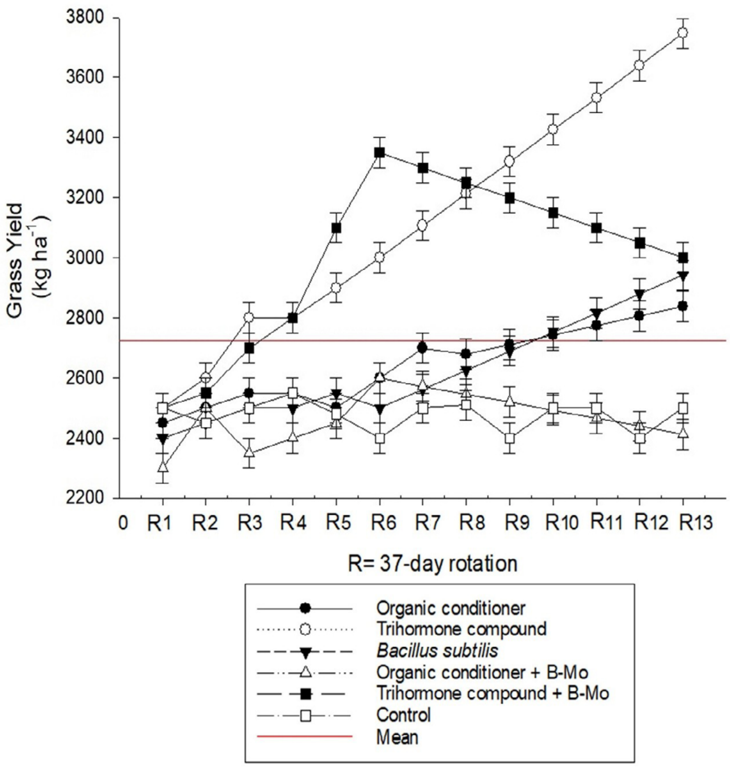


**Figure 1.** *Forage production behavior as a function of treatment, with rotations every 37 days for a total of 13 rotations.*

***Cost Structure in a Dairy System Using Kikuyu Grass***

The cost structure showed that direct costs accounted for the largest share of expenses, at 84.14% of total monthly outflows (Table 2). Among the direct costs, cattle feeding (41.47%) and labor (5.44%) were the most significant. Indirect costs, represented by public services (water and electricity), made up the remaining 11.7%.

The category "Inputs for pasture management" accounted for 29.83% of total costs, with foliar and compound fertilizers being the most significant. The use of biostimulants—such as the organic conditioner, trihormonal compound, B-Mo, and Bacillus subtilis—represented 2.22% of expenses, with the trihormonal compound accounting for the largest individual share. These results highlight biostimulants as low-cost investments with high potential for productive returns.

**Table 2.** *Cost structure of production in USD ha$^{-1}$ per month in the dairy system*

| | Monthly | | | |
|---|---|---|---|---|
| **Direct Costs** | **Quantity** | **Unit** | **Total value (USD)** | **Participation (%)** |
| **Crop labor** | | | | |
| Fertilization | 3 | Workday | | |
| Spraying | 1 | Workday | | |
| Cattle transport | 2 | Workday | | |
| Cleaning | 2 | Workday | | |
| Milking | 2 | Workday | $ 252 | 5.44% |
| Application of vaccines/vitamins/antibiotics | 2 | Workday | | |
| Dewormer application | 2 | Workday | | |
| Fence repair | 1 | Workday | | |
| Paddock clearing | 1 | Workday | | |
| **Total** | | | **$ 252** | **5.44%** |
| **Pasture management inputs** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |
| Foliar fertilizer | 100 | Kg | $ 66.30 | 1.43% |
| Fertilizer | 25 | Bag (50 kg) | $ 721.03 | 15.58% |
| Insecticide | 1 | 4 L | $ 180.54 | 3.90% |
| Fungicide | 1 | 4 L | $ 34.25 | 0.74% |
| Herbicide | 2 | 4 L | $ 61.20 | 1.32% |
| Silicone adhesive | 1 | 4 L | $ 215 | 4.64% |
| Organic conditioner | 1 | L | $ 10.20 | 0.22% |
| Trihormonal compound | 1 | L | $ 61.20 | 1.32% |
| *Bacillus subtilis* | 1 | L | $ 16.83 | 0.36% |
| B-Mo | 1 | L | $ 14.75 | 0.32% |
| **Total** | | | **$ 1,381.30** | **29.83%** |
| **Cattle feeding** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |

| Monthly | | | | |
|---|---|---|---|---|
| **Pasture management inputs** | **Quantity** | **Unit** | **Total value (USD)** | **Participation (%)** |
| Concentrate | 90 | Bag (50 kg) | $ 1,760.26 | 38.07% |
| Mineralized salt | 7 | Bag (50 kg) | $ 157.50 | 3.40% |
| **Total** | | | **$ 1,917.76** | **41.47%** |
| **Medications and medical supplies** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |
| Vaccines | 80 | mL (2-3 cm) | $ 63.75 | 1.38% |
| Dewormers | 2 | L | $ 19.38 | 0.42% |
| Antibiotics | 2 | Bottles 250 mL | $ 21.93 | 0.47% |
| Vitamin B12 | 2 | Bottles 250 mL | $ 43.86 | 0.95% |
| Reagents for analysis | 1 | Gallon (4 L) | $ 10 | 0.22% |
| California reagent | 1 | 1.5 L | $ 20 | 0.43% |
| Gloves | 4 | Box | $ 22.44 | 0.48% |
| Syringes | 1 | Box | $ 3.57 | 0.08% |
| Sleeves | 1 | Box | $ 7.65 | 0.16% |
| **Total** | | | **$ 212.58** | **4.60%** |
| **Cattle management supplies** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |
| Acid detergent | 1 | 20 L | $ 23.97 | 0.52% |
| Alkaline detergent | 1 | 20 L | $ 23.97 | 0.52% |
| Neutral detergent | 1 | 20 L | $ 18.36 | 0.40% |
| Chlorinated sanitizer | 1 | 20 L | $ 15.30 | 0.33% |
| Ixer sealant | 1 | 20 L | $ 26.01 | 0.56% |
| Disinfectant | 1 | 20 L | $ 21.16 | 0.46% |
| **Total** | | | **$ 128.77** | **2.80%** |
| **Indirect costs** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |
| **Other costs** | | | | |
| Water service | 1 | Bill | $ 71.40 | 1.54% |
| Electricity service | 1 | Bill | $ 586.50 | 12.67% |
| **Total** | | | **$ 657.90** | **14.21%** |
| **Expenses** | **Quantity** | **Unit** | **Total value (USD)** | **Part (%)** |
| Gasoline | 20 | 4 L | $ 77.52 | 1.67% |
| **Total** | | | **$ 77.52** | **1.67%** |
| **Income** | **Unit** | | **Total value (USD)** | |
| Milk production | L | | $ 9,550.30 | |
| Sale of cows/bulls/calves | Kg | | $ 200 | |
| **Total** | | | **$ 9,750.30** | |

### *Economic Evaluation of the Production System*

The cost structure of the evaluated production system showed a monthly operating cost of USD 4,627.84, while monthly revenues reached USD 9,750. This yielded a net cash flow of USD 5,122.16 for the overall analysis. Table 3 presents cost variations per treatment and includes a hypothetical economic scenario associated with increased forage production. An exploratory economic scenario was developed to illustrate the potential economic implications of the additional forage produced under the trihormonal treatment. This exercise assumed that the observed increase in forage production could translate into higher milk output under appropriate grazing and nutritional management. However, actual increases in stocking rate were not directly evaluated and would depend on factors including dry matter content, forage utilization efficiency, grazing losses, animal nutrient requirements, and seasonal forage availability (Table 4). Under this scenario, revenues increased to USD 10,234, with a net cash flow of USD 5,648 and a benefit–cost ratio of 1.2, compared with 1.1 for the other treatments.

**Table 3.** *Profitability analysis of pastures enhanced by biostimulants*

| Treatment | Income (USD) | Expenses (USD) | Net flow (USD) | B/C | Unit price (USD) | UGM | CUP |
|---|---|---|---|---|---|---|---|
| | | | Monthly (USD) | | | | |
| Organic conditioner | $ 9,750 | $ 4,535 | $ 5,215 | 1.15 | $ 0.55 | $ 0.30 | $ 0.25 |
| Trihormonal compound | $ 9,750 | $ 4,586 | $ 5,164 | 1.13 | $ 0.55 | $ 0.29 | $ 0.26 |
| *Bacillus subtilis* | $ 9,750 | $ 4,540 | $ 5,210 | 1.15 | $ 0.55 | $ 0.30 | $ 0.25 |
| Organic conditioner + B-Mo | $ 9,750 | $ 4,552 | $ 5,198 | 1.14 | $ 0.55 | $ 0.29 | $ 0.26 |
| Trihormonal compound + B-Mo | $ 9,750 | $ 4,603 | $ 5,147 | 1.12 | $ 0.55 | $ 0.29 | $ 0.26 |
| Control | $ 9,750 | $ 4,525 | $ 5,225 | 1.15 | $ 0.55 | $ 0.30 | $ 0.25 |
| Hypothetical economic scenario associated with increased forage production (table 4) | $10,234 | $4,586 | $5,648 | 1.23 | $ 0.55 | $ 0.29 | $ 0.26 |

Furthermore, based on the experimental data, an exercise was conducted to estimate the potential increase in stocking rate per paddock. Assuming a producing cow contributes 30 liters of milk per day of USD 0.53 per liter, and considering that 3 liters of the trihormonal compound were applied across the six plots (½ liter per plot), with a unit cost of USD 61.2 per liter, the difference between production value and input cost was calculated for the general balance (Table 4).

**Table 4.** *Analysis of the increase in stocking rate (1) for the trihormonal compound treatment (T2) in contrast with the increase in dry matter.*

| Stocking rate | | | |
|---|---|---|---|
| **Production** | | **Input** | |
| Animal in production | 30 L/ Milk | Quantity | 3 L |
| Time | 30 days | Quantity | 6 plots (½ L/plot) |
| Milk price | USD 0.537 | Price | USD 61.12 |
| Total | USD 483.75 | Total | USD 183.6 |
| USD 483.75 - USD 183.6 = USD 300.15 | | | |

## DISCUSSION

Trihormonal compounds (auxins, gibberellins, and cytokinins) exert positive effects from the establishment stage of forage, promoting high levels of dry-matter accumulation (Rocha *et al*., 2020). These results are consistent with the greater biomass accumulation observed in the trihormonal treatments under the evaluated conditions. These findings suggest that hormone-based biostimulants may increase forage productivity in high-tropical dairy systems.

In the Caribbean, Bonilla *et al.* (2021) observed low dry matter values during initial cycles, suggesting that longer application periods are required to unlock biological responses. This parallels the findings of the present study, in which the effects of *Bacillus subtilis* became more notable beginning in the fifth rotation. Choudhary *et al*. (2016) provide mechanistic insight, noting that these bacteria synthesize phytohormones, improve soil structure through exopolysaccharides, and reduce ethylene-induced stress via ACC deaminase activity. Additionally, *Bacillus subtilis* induces systemic resistance, enhancing long-term plant resilience. These features help explain the delayed but positive effects observed here and reinforce the conclusion that *Bacillus subtilis* is a promising medium-term alternative for livestock production systems.

Although mineral fertilizers can enhance pasture quality—as shown by Ortiz *et al*. (2021) using P and N in combination with mycorrhizae—biological inputs also deliver substantial benefits. Vargas *et al*. (2018) demonstrated that microbial biofertilizers improve kikuyu persistence and quality, while Domingues *et al*. (2020) found that plant growth–promoting bacteria increase dry-matter production in tropical grasses. The present study adds further evidence that biostimulants not only sustain biomass production but do so at a minimal cost increase (1.32%), thereby enhancing the dairy system's benefit–cost ratio. This economic efficiency is central to the study's conclusions.

The findings also align with Villalobos *et al*. (2013), who reported that agricultural inputs accounted for the largest share of pasture system costs (85%), comparable to the cost structure observed here. Their reported dry matter yields of 3,517 kg ha$^{-1}$ are similar to the 3,800 kg ha$^{-1}$ achieved with the trihormonal treatment, strengthening the conclusion that this compound was associated with biomass levels comparable to those reported in other high-altitude forage systems. Additionally, Villalobos *et al*. (2013) found that farms investing more in forage management achieved greater milk yields per hectare. This mirrors the current study, where modest investments in biostimulants were associated with higher productivity and improved economic indicators.

Similar trends have been observed internationally. In Bulgaria, moderate applications of organic fertilizers such as Lumbrex® and Lumbrical® increased biomass and profitability, with strong correlations among yield, gross income, and costs (Churkova & Churkova, 2025). This aligns with global efforts to reduce reliance on synthetic fertilizers. Rojo *et al*. (2023) emphasize that biological alternatives reduce production costs and environmental impacts, enhancing competitiveness under climate change—echoing the sustainability component of this study's conclusions. Beer *et al*. (2024) similarly highlight that biostimulants improve nutrient-use efficiency, while Quintero *et al*. (2022) show that integrating bio-input strategies lowers operational costs and environmental burdens. Collectively, these studies reinforce the conclusion that biostimulants are both productive and sustainable options for high-tropical dairy systems.

Mechanistically, the trihormonal compound and its combination with B-Mo supported sustained biomass production through growth regulators that promote cell division and elongation, consistent with Rouphael and Colla (2020). The synergistic effects of B-Mo may stem from boron and molybdenum's roles in photoassimilate transport and enzymatic functioning (Win *et al*., 2025). Although *Bacillus subtilis* is recognized for its biostimulatory properties (Hashem *et al*., 2019), the biomass production under this treatment did not surpass that of the hormonal treatments — likely due to edaphoclimatic constraints. However, its positive medium-term response is consistent with the conclusion that this bacterium represents a promising longer-term strategy. Other bacterial genera such as *Azotobacter* sp. and *Azospirillum* sp. have also been shown to improve kikuyu vigor (Vivianco-Galván *et al*., 2021), further supporting the viability of biological alternatives in forage systems.

The economic analysis confirmed a favorable margin of approximately 62% per cow, comparable to findings in Bolivia where nutritional balance assessments linked pasture supply to herd productivity (Cao *et al*., 2019). The economic analysis indicated favorable profitability indicators for the trihormonal treatment. Although increased forage production may create opportunities to improve stocking capacity, this possibility was not directly quantified in the present study. Future evaluations should incorporate measurements of dry matter intake, forage utilization efficiency, grazing losses, and carrying capacity to determine whether biomass gains translate into sustainable increases in livestock numbers.

The findings of this study reaffirm that biostimulants—particularly trihormonal formulations and growth-promoting bacteria such as *Bacillus subtilis*—represent a promising strategy for increasing the productivity, economic efficiency, and environmental sustainability of high-tropical dairy systems. Their ability to enhance biomass accumulation, improve nutrient-use efficiency, and support plant tolerance to stress reinforces their value as complementary tools within modern pasture management. However, despite their evident potential, the use of biostimulants is not exempt from limitations that must be acknowledged to ensure their appropriate and effective implementation.

A primary challenge is the variability in field responses. Biological products are highly sensitive to edaphoclimatic conditions, soil microbiome composition, and pasture management practices. As observed in this study, *Bacillus subtilis* had effects only in the medium term, suggesting that microbial establishment may require time and adequate ecological conditions to produce measurable responses. This temporal variability may create uncertainty for producers expecting short-term returns. Additionally, hormone-based products, while consistently effective at stimulating growth, may show diminishing responses under nutrient deficiencies or adverse climatic stress if not supported by balanced fertilization and appropriate agronomic practices.

An important limitation of this study is the absence of independent plot replication. Since each treatment was assigned to a single paddock, treatment effects cannot be fully separated from paddock-specific characteristics. Consequently, the observed differences among treatments should be interpreted as indicative responses under the environmental and management conditions evaluated rather than definitive evidence of treatment superiority. Future research should include replicated experimental units across multiple sites and seasons to validate the agronomic and economic responses observed in this field evaluation.

## CONCLUSIONS

Under the environmental and management conditions evaluated, the trihormonal treatment and the trihormonal treatment supplemented with B–Mo produced the highest forage biomass, exceeding 3,700 kg ha$^{-1}$ during the final grazing rotation. Incorporating biostimulants represented a relatively small share of production costs and was associated with favorable economic indicators relative to the dairy system's overall operating budget. The greater forage availability observed in the trihormonal treatments suggests opportunities to improve stocking capacity. However, this hypothesis was not directly evaluated and requires validation through studies that measure forage utilization, dry matter intake, and carrying capacity. *Bacillus subtilis* showed a delayed but positive response during the latter grazing rotations, indicating potential value as a biological alternative for pasture management in high-tropical dairy systems. Because each treatment was evaluated in a single paddock, the results should be interpreted as indicative responses under commercial production conditions rather than definitive evidence of treatment superiority. Future studies with replicated experimental units are necessary to confirm the agronomic and economic responses observed in this field evaluation.

## AUTHOR CONTRIBUTIONS

Conceptualization, H-S.A.; B.J. and C-A.N.; Methodology, H-S.A. and B.J.; Software, C-A.N.; L.K.; and N.J.; Validation, H-S.A. and C-A.N.; Formal Analysis, H-S.A.; K.L.; N.J.; B.J.; and C-A.N.; Investigation, H-S.A.; N.J. and L.K.; Resources C-A.N.; Data Curation, L.K.; and N.J.; Writing—Original Draft Preparation, H-S.A.; L.K. and N.J.; Writing—Review & Editing, H-S.A.; C-A-N. and B.J.; Visualization, H-S.A.; L.K. and N.J.; Supervision, H-S.A.; C-A.N. and B.J.; Project Administration, H-S.A.; C-A.N. and B.J.; Funding Acquisition, H-S.A.; C-A.N. and B.J.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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