

Effect of sugarcane (*Saccharum officinarum*) silage and rumen activator supplement on milk yield and quality in high Andean tropical dairy systems of Colombia

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Abstract

The limited availability and quality of forages in the Colombian high Andean tropics generate a chronic energy deficit in cattle diets, forcing small-scale producers to rely on commercial concentrates. This study evaluated the effect of supplementation with whole sugarcane silage and a rumen activator supplement (RAS) made from sugarcane by-products on milk yield and composition in Holstein crossbred cows. The study was conducted in two localities of Nariño, Colombia: El Encano, Pasto (L1, 2,830 m a.s.l.) and Sandoná (L2, 2,400 m a.s.l.). Six cows per locality were assigned to a replicated 3×3 Latin square design with three 15-day periods: T1 = grazing + mineralized salt; T2 = grazing + 12 kg cow⁻¹ day⁻¹ whole sugarcane silage; T3 = grazing + silage + 0.5 kg cow⁻¹ day⁻¹ RAS. In L1, T2 produced the highest milk yield (9.15 L cow⁻¹ day⁻¹, $p < 0.001$), and T2 and T3 exceeded the control in protein and total solids yield ($p < 0.001$); milk urea nitrogen was higher in T2 and T3 ($p = 0.014$). In L2, T3 produced the highest milk yield (11.81 L cow⁻¹ day⁻¹), protein yield (328.9 g cow⁻¹ day⁻¹) and total solids yield (1,382.5 g cow⁻¹ day⁻¹) ($p < 0.001$). Milk composition (g/100 ml) was similar among treatments in both localities ($p > 0.05$). The RAS response occurred only in L2, showing that its effect depends on the animals' productive potential and the system's energy deficit. Sugarcane silage supplementation is a viable, economical, and sustainable alternative for these small-scale producers.

Introduction

In Colombia, cattle farming accounts for about 78% of agricultural land use and is a pillar of the rural economy for food security, employment, and income (Osorio-Giraldo et al. 2024). The national herd comprises beef (43%), specialized dairy (12%) and dual-purpose (45%) systems; farms with better management produce 45–50% more milk with a lower carbon footprint (González-Quintero et al. 2022), revealing a productivity gap that feeding strategies can close.

Specialized dairy systems are concentrated in the high tropics—Cundinamarca, Boyacá, Antioquia, Nariño, and the Coffee Region—where altitude and climate favor forage production but also impose chronic energy constraints on cattle diets (Durana et al. 2023).

In Nariño, 18% of the territory lies in the cold thermal zone with a minifundium-based structure and rugged topography, where specialized dairy farming is the main rural activity (Molano-Bernal et al. 2021; Durana et al. 2023; Núñez Pérez 2021). The departmental dairy chain provides ~ 27% of the regional agricultural GDP and involves ~ 40,000 producers—80% peasant or indigenous—producing 1,300,000 L of milk per day in 2025 (Patiño-Burbano et al. 2023; SAGAN 2025; Holmann et al. 2004).

Pastures are based on kikuyu grass (*Cenchrus clandestinus*), cocksfoot (*Dactylis glomerata*), Yorkshire fog (*Holcus lanatus*) and ryegrass (*Lolium* spp.), with a predominance of the Holstein breed; 50% of the herds have fewer than 15 cows in milk (Cardona-Iglesias et al. 2022; Nieto-Sierra et al. 2020b).

The introduction of forage species with high protein and low fiber has generated an energy deficit and dietary imbalance (Zambrano Burbano 2019), worsened by climate variability, soil degradation, and

inadequate management, which forces producers to use costly commercial concentrates (Cardona-Iglesias et al. 2022; Castro-Rincón et al. 2020). Strategic supplementation is therefore key to closing the energy gap during critical periods (Avellaneda-Avellaneda et al. 2014), and silages made from non-conventional local resources reduce concentrate dependence while improving profitability and sustainability (Cardona-Iglesias et al. 2024; Castro-Rincón et al. 2020; Halmemies-Beauchet-Filleau et al. 2018).

Panela sugarcane is Colombia's second most important crop after coffee, involving ~ 350,000 families and ranking the country as the world's second-largest producer of panela after India (Jiménez-Carvajal et al. 2024; Gutiérrez-Mosquera et al. 2018). The trapiche (sugar mill) generates large volumes of bagasse, filter cake (cachaza) and melote with limited use, offering an opportunity for valorization in animal feeding (Gutiérrez-Mosquera et al. 2018; Ordóñez-Díaz and Rueda-Quiñónez 2017).

The main by-products of *Saccharum officinarum* used in cattle are tops, bagasse, molasses, filter cake, and melote; of the whole plant, only 24% is used to produce panela (Cardona-Iglesias et al. 2024; Díaz-Montilla et al. 2022; Lagos-Burbano et al. 2022; Lagos-Burbano and Castro-Rincón 2019). Whole sugarcane used as forage can reach in vitro dry matter digestibility (IVDMD) of 65% or more (Salazar-Ortiz et al. 2018); however, its low protein content and rapid alcoholic fermentation make it necessary to add urea and mineralized salt to improve its conservation and nutritional value (Sousa-Alves et al. 2020).

In the high tropics of Nariño, supplementation with sugar-mill by-products has produced increases of 12.5–15% in milk production (Lagos-Burbano et al. 2021; Kawashima et al. 2002; Martín 2005). Pachón et al. (2005) documented increases of 0.848 vs. 0.769 kg/day in steers supplemented with multnutritional blocks of panela sugarcane, and Morales Lara and Herrera Maradiaga (2011) reported + 1.43 kg milk cow⁻¹ day⁻¹ in tropical grazing with returns above 350%, confirming the economic potential of these by-products for small-scale producers. Cardona-Iglesias et al. (2024) found that sugarcane-top silage in dual-purpose cows has an NDF of 65% and an IVDMD of 48%, making it necessary to add additives to improve the digestibility of the fibrous fraction and enhance the productive response.

The incorporation of rumen-activating additives—rapidly fermentable carbohydrates, NPN, and elemental sulfur—optimizes the energy:protein synchrony, maximizes microbial protein synthesis and improves conversion efficiency in grazing systems (Serbest 2025; Lopes et al. 2024). The melote and bagasse of *Saccharum officinarum* are suitable sources of fermentable carbohydrates because of their rapid degradation profile and wide local availability (Palani et al. 2025; Edirisinghe et al. 2024; Ojeda et al. 1997). Recent studies additionally show that sugarcane polyphenols and flavonoids exert immunomodulatory effects on the ruminal microbiome and on dairy product quality (Edirisinghe et al. 2024; Marino et al. 2025).

The aim of this study was to evaluate the effect of supplementation with whole panela sugarcane silage and a RAS made from agroindustrial by-products on the yield and compositional quality of milk in Holstein crossbred dairy cows in two localities of the department of Nariño, Colombia.

Materials and Methods

Study sites and agroclimatic conditions

The study was carried out in two localities of the department of Nariño, Colombia (Table 1). L1: Santa Clara village, El Encano district, Pasto (1°09'04.845" N / 77°07'41.365" W), 2,830 m a.s.l., lower montane very humid forest (Holdridge 2000). L2: Alto Jiménez village, Sandoná (1°20'55" N / 77°30'47" W), 2,400 m a.s.l., lower montane humid forest. Climate data: IDEAM (2025).

Table 1
Agroclimatic characteristics of the experimental localities.

Locality	Municipality	Village	Altitude (m a.s.l.)	Precip. (mm year ⁻¹)	Mean T (°C)	RH (%)
L1	Pasto — El Encano	Santa Clara	2,830	2,000	11	86.8
L2	Sandoná	Alto Jiménez	2,400	1,385	14	80.0

Experimental animals

The characteristics of the experimental animals are presented by locality (Table 2). In L1 (El Encano), six Holstein cows were used (live weight 550 ± 35 kg; initial body condition 3.5–4.0 on a 1–5 scale); in L2 (Sandoná), six crossbred Holstein cows were used (live weight 520 ± 40 kg; initial body condition 3.8–4.0). In both localities, the cows were in their 3rd to 5th calving and at 200 ± 15 days in milk (DIM) at the start. Baseline milk yield was 8.7 ± 1.7 L cow⁻¹ day⁻¹ in L1 (Bucheli farm records, April 2018, n = 26) and 9.2 ± 1.2 L cow⁻¹ day⁻¹ in L2. Water was provided ad libitum.

Table 2
Description of the experimental animals by locality.

Parameter	L1 — El Encano (2,830 m a.s.l.)	L2 — Sandoná (2,400 m a.s.l.)
No. of cows	6	6
Breed	Holstein	Holstein and crosses
Calvings (range)	3rd–5th	3rd–5th
Mean live weight (kg)	550 ± 35	520 ± 40
Days in milk at start (DIM)	200 ± 15	200 ± 15
Baseline yield (L cow ⁻¹ day ⁻¹)	8.7 ± 1.7 ¹	9.2 ± 1.2 ²
Initial body condition (1–5)	3.5–4.0	3.8–4.0

¹ Bucheli farm records, El Encano, April 2018 ($n = 26$ cows; mean = 8.7 ± 1.7 L day⁻¹). ² Manuel Tobar farm records, Alto Jiménez, Sandoná, 2018 (mean = 9.2 ± 1.2 L day⁻¹).

Cows in L1 grazed pastures dominated by kikuyu grass (*Cenchrus clandestinus*) and white clover (*Trifolium repens*) under rotational grazing with electric fencing, two daily moves (06:00 and 16:00 h) and a 50-day rest period. Cows in L2 grazed Setaria grass (*Setaria* sp.) with a 40-day rest period.

Experimental design and treatments

A replicated 3×3 Latin square design was used in each locality, with six cows (two squares of three), three 15-day periods (7 days of adaptation + 8 days of measurement) and random assignment of treatment sequences:

- T1 (control): grazing + mineralized salt.
- T2: grazing + 12 kg cow⁻¹ day⁻¹ of whole sugarcane silage (fresh basis).
- T3: grazing + 12 kg cow⁻¹ day⁻¹ of whole sugarcane silage + 0.5 kg cow⁻¹ day⁻¹ of RAS.

Supplement formulation and management

Before the experiment, a nutritional balance based on the NRC (2001) was performed to quantify the energy deficit of the grazing diets. The supplement formulation is detailed below (Table 3). Supplements were offered in individual troughs during milking (06:00 and 16:00 h). The silage was made from 13-month-regrowth whole sugarcane with the addition of 3% urea and 3.5% mineralized salt on a dry-matter basis (Sánchez Matta 2005).

Table 3
Formulation of supplements made from *Saccharum officinarum* by-products. Values as % of dry matter.

Ingredient	RAS (% DM)	Silage (% DM)
Sugarcane melote	45.0	—
Sugarcane bagasse	8.0	—
Agricultural lime	15.0	—
Barley bran	10.0	—
Urea / ammonium sulfate (9:1)	8.0	—
Mineralized salt (10%)	7.0	3.5
Flowers of sulfur	1.0	—
Nacadero (<i>Trichanthera gigantea</i>)	5.0	—
Yeast	1.0	—
Whole sugarcane (13-month regrowth)	—	93.5
Urea	—	3.0
Total	100.0	100.0

Measured variables and feed analysis

During the measurement periods, grass samples were taken in the paddock by manual cutting (Bonnet et al. 2011). Samples were dried at 65°C for 72 h, ground through a 1.0 mm screen and analyzed by NIRS DS 2500 (FOSS Analytical, Denmark) (Ariza-Nieto et al. 2018). Raw materials and the RAS were analyzed using AOAC (2005) techniques: crude protein by Kjeldahl (NTC 4657); NDF according to Van Soest (AOAC 973.18); ADF (AOAC 962.09); ether extract (AOAC 973.18); ash (AOAC 942.05); calcium by EDTA complexometry; phosphorus by UV-VIS spectrometry (NTC 498). Milk yield was recorded daily by weighing (Tru-Test Milk Meters, New Zealand). Milk samples for composition (fat, protein, total solids, and MUN) were analyzed by infrared spectroscopy (AOAC 972.16) with a FOSS MilkoScan 7RM (FOSS Analytical A/S, Denmark) at the milk laboratory of the AGROSAVIA Obonuco Research Center (Lagos-Burbano et al. 2021).

Statistical analysis

Data were analyzed with a linear mixed model (LMM): $Y_{ijkl} = \mu + T_i + P_j + V_k(SI) + \epsilon_{ijkl}$, where T_i = treatment (fixed effect), P_j = period (fixed effect), $V_k(SI)$ = cow nested within square (random effect) and ϵ_{ijkl} = residual error. Least-squares means (LSM) were compared with Tukey's test ($\alpha = 0.05$). Analyses were performed with the lme4 v. 1.1–35, lmerTest, and emmeans packages in R v. 4.3.2 (R Core Team

2023). To quantify the magnitude of the treatment effect on each response variable, Cohen's d effect size was calculated as the difference between the treatment LSM and the control LSM divided by the residual standard deviation of the LMM; values of $|d| \geq 0.2$, ≥ 0.5 and ≥ 0.8 were interpreted as small, medium, and large effects, respectively (Cohen 1988). To explore the multivariate structure of the response, a principal component analysis (PCA) was performed on the four independent variables (milk yield, %fat, %protein, and MUN) for each locality, after standardization (mean = 0, SD = 1).

Results

Chemical composition of the feeds

The chemical composition of the feeds is summarized below (Table 4). Whole sugarcane silage with 3% urea had a DM of 28.7%, CP of 15.0%, NDF of 51.4% and ADF of 18.93% DM. The RAS had 48.4% DM, 28.8% CP and 40.8% NDF. Kikuyu grass (L1) and Setaria grass (L2) had an NDF of 61.3 and 60.4% DM and NEL of 1.28 and 1.13 Mcal kg⁻¹ DM, respectively, determined by NIRS (Lagos-Burbano et al. 2021).

Table 4
Chemical composition of the feed resources used in the study.

Parameter	Unit	Kikuyu ¹ (L1)	Setaria ² (L2)	Sugarcane silage ³	RAS ⁴	Method
Dry matter (DM)	%	21.13	19.13	28.74	48.36	Thermograv.
Crude protein (CP)	% DM	10.32	9.37	14.99	28.81	Kjeldahl
Ether extract (EE)	% DM	—	2.41	0.85	13.74	AOAC 973.18
NDF	% DM	61.28	60.43	51.42	40.80	Van Soest
ADF	% DM	35.62	34.96	18.93	20.60	AOAC 962.09
Hemicellulose	% DM	24.92	25.47	32.49	20.20	Calculated
Lignin	% DM	8.12	8.40	4.65	1.93	AOAC
Ash	% DM	11.53	—	6.76	8.24	AOAC 942.05
Calcium (Ca)	% DM	0.29	0.21	0.62	0.28	Complexom.
Phosphorus (P)	% DM	0.25	0.22	0.29	0.20	UV-VIS
NEL*	Mcal kg ⁻¹ DM	1.28	1.13	—	—	NIRS/NRC 2001

¹ Kikuyu (*C. clandestinus*): mean of NIRS samples, AGROSAVIA Nariño. ² Setaria (*Setaria* sp.): mean for the Nariño zone, determined by NIRS. ³ Whole sugarcane silage: mean of 4 analyses, AGROSAVIA 2018. ⁴ RAS:

mean of 2 formulations, AGROSAVIA 2018. * NEL estimated with NRC (2001) equations. — = data not available.

Milk yield and quality — Locality 1 (El Encano, 2,830 m a.s.l.)

In L1, T2 produced the highest milk yield (9.15 L cow⁻¹ day⁻¹, +19.0% over the control; $p < 0.001$), followed by T3 (8.22 L cow⁻¹ day⁻¹, +6.9%) and T1 (7.69 L cow⁻¹ day⁻¹); T3 did not differ from either T2 or T1 (Tukey, $p > 0.05$) (Table 5; Fig. 1). Supplementation (T2 and T3) increased protein and total solids yield relative to the control ($p < 0.001$), with no change in fat yield ($p = 0.112$) (Fig. 2). Milk percentage composition was similar among all treatments ($p > 0.05$). MUN was higher in T2 (9.14 mg/100 ml) and T3 (8.98 mg/100 ml) than in T1 (7.31 mg/100 ml) ($p = 0.014$; Fig. 3).

Table 5

Milk yield and composition in L1 — El Encano (2,830 m a.s.l.). Least-squares means (LSM) $\pm$ SEM.

Variable	T1 Control	T2 Silage	T3 Silage + RAS	SEM	p-value
Milk yield (L cow ⁻¹ day ⁻¹)	7.69 b	9.15 a	8.22 ab	0.685	< 0.001***
FY — Fat (g cow ⁻¹ day ⁻¹)	269.2	328.7	317.2	35.22	0.112 ns
PY — Protein (g cow ⁻¹ day ⁻¹)	237.8 b	287.9 a	271.2 ab	16.77	< 0.001***
TSY — Total solids (g cow ⁻¹ day ⁻¹)	813.9 b	1104.4 a	1039.4 a	83.12	< 0.001***
— Milk composition —					
Fat (g/100 ml)	3.68	3.69	3.88	0.284	0.619 ns
Protein (g/100 ml)	3.41	3.19	3.31	0.173	0.107 ns
Total solids (g/100 ml)	11.37	12.22	12.58	0.503	0.083 ns
MUN (mg/100 ml)	7.31 b	9.14 a	8.98 a	1.237	0.014 *

a, b: means with different letters in the same row differ by Tukey's test ($p \leq 0.05$). T1 = grazing + mineralized salt; T2 = grazing + sugarcane silage (12 kg fresh basis cow⁻¹ day⁻¹); T3 = grazing + silage + RAS (0.5 kg cow⁻¹ day⁻¹). SEM = standard error of the mean. ns = not significant. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. MUN = milk urea nitrogen.

Milk yield and quality — Locality 2 (Sandóná, 2,400 m a.s.l.)

In L2, T3 produced the highest milk yield (11.81 L cow⁻¹ day⁻¹; +17.5% over the control; $p < 0.001$), above T2 (10.77) and T1 (10.05 L cow⁻¹ day⁻¹), which did not differ from each other (Tukey, $p > 0.05$) (Table 6; Fig. 1). Fat yield was higher in T2 and T3 than in the control (357.7 and 397.2 vs. 312.4 g cow⁻¹ day⁻¹; $p =$

0.004), and for protein and total solids yield T3 reached the highest values (+ 22% and + 26% over the control; $p < 0.001$) (Fig. 2). Milk percentage composition and MUN were similar among treatments ($p > 0.05$; Fig. 3). Figures 2 and 3 illustrate the response patterns by locality.

Table 6

Milk yield and composition in L2 – Sandoná (2,400 m a.s.l.). Least-squares means (LSM) $\pm$ SEM.

Variable	T1 Control	T2 Silage	T3 Silage + RAS	SEM	p-value
Milk yield (L cow ⁻¹ day ⁻¹)	10.05 b	10.77 b	11.81 a	0.860	< 0.001***
FY – Fat (g cow ⁻¹ day ⁻¹)	312.4 b	357.7 a	397.2 a	35.71	0.004 **
PY – Protein (g cow ⁻¹ day ⁻¹)	269.6 b	290.2 b	328.9 a	13.82	< 0.001***
TSY – Total solids (g cow ⁻¹ day ⁻¹)	1097.7 b	1201.3 b	1382.5 a	92.33	< 0.001***
– Milk composition –					
Fat (g/100 ml)	3.28	3.45	3.51	0.208	0.329 ns
Protein (g/100 ml)	2.85	2.85	2.82	0.121	0.731 ns
Total solids (g/100 ml)	11.50	11.71	11.75	0.211	0.183 ns
MUN (mg/100 ml)	10.94	11.20	10.63	0.675	0.590 ns

*a, b: means with different letters in the same row differ by Tukey's test ($p \leq 0.05$). T1 = grazing + mineralized salt; T2 = grazing + sugarcane silage (12 kg fresh basis cow⁻¹ day⁻¹); T3 = grazing + silage + RAS (0.5 kg cow⁻¹ day⁻¹). SEM = standard error of the mean. ns = not significant. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. MUN = milk urea nitrogen.*

Effect size and multivariate analysis

The effect sizes (Cohen's d) confirm and quantify the LMM results (Fig. 4). In L1, T2 (silage) produced the largest-magnitude effects on TSY ($d = + 1.00$; large effect), PY ($d = + 0.91$; large) and milk yield ($d = + 0.82$; large); T3 (silage + RAS) produced medium effects on TSY ($d = + 0.76$), PY ($d = + 0.61$) and FY ($d = + 0.43$), but a small effect on milk yield ($d = + 0.33$). In L2 the pattern is reversed: T3 produced the largest effects on PY ($d = + 1.08$; large), TSY ($d = + 1.04$; large) and FY ($d = + 0.91$; large), all greater than those of T2 on the same variables ($d = + 0.38$ – 0.42). In both localities and for both comparisons, milk fat and protein percentages showed small to negligible effects ($|d| < 0.40$), confirming that supplementation acts mainly on production volume rather than on the concentration of milk components.

The PCA of the four independent variables (milk yield, %fat, %protein, and MUN) captured 74.1% and 68.3% of the total variation in L1 and L2, respectively (PC1: ~41%; PC2: ~30%) (Fig. 5). In both localities, PC1 is strongly associated with milk yield (negative loading) and milk protein percentage (positive

loading), reflecting the inverse relationship between production volume and protein concentration that is characteristic of the dilution effect. PC2 mainly captures the variation in %fat and MUN. Treatment centroids separate along the PC1 axis: in L1 the control occupies the position of highest %protein and lowest milk yield, with T2 displaced toward higher production; in L2, T3 occupies the position of maximum milk yield with lower %protein, confirming the same gradient but through the most intensive treatment.

The magnitude of the response to the RAS differed between localities (Fig. 6): in L1, milk yield followed an arch-shaped pattern with a peak at T2, whereas in L2 it increased progressively through T3; protein and total solids yield showed the same locality-dependent trend.

Discussion

Whole sugarcane silage with 3% urea had a DM of 28.7%, CP of 15.0% and NDF of 51.4% DM, within the range reported by Sousa-Alves et al. (2020) for urea-treated sugarcane silages (CP: 11–18%; NDF: 45–58% DM). The addition of urea improved the nitrogen supply relative to untreated sugarcane (CP: ~4% DM), favoring the energy:protein synchrony in the rumen (Sousa-Alves et al. 2020; Arredondo-Pérez 2011). Mendonça et al. (2004) and Cardona-Iglesias et al. (2024) noted that untreated sugarcane has a high lignin content that limits ruminal digestibility, which non-protein nitrogen corrects while improving the use of fermentable carbohydrates. Jesus (2011) confirmed that sugarcane diets with urea as the nitrogen source raise milk fat content (4.06% vs. 3.5% with soybean meal). The RAS had a high CP (28.8% DM) and an estimated NEL of 2.1 Mcal kg⁻¹ DM, well above that of kikuyu grass (1.3 Mcal kg⁻¹ DM), increasing the energy density of the total diet (Lagos-Burbano et al. 2021).

In L1, supplementation increased milk yield ($p < 0.001$), but only T2 exceeded the control (+ 19.0%; Tukey, $p < 0.001$); T3 and T1 performed similarly, indicating no additional benefit from the RAS in this locality (Fig. 1).

For protein and total solids yield, T2 and T3 exceeded the control ($p < 0.001$) with similar yields to each other (Fig. 2). Milk percentage composition remained similar among treatments ($p > 0.05$), consistent with Lagos-Burbano et al. (2021), Molavian et al. (2020) and Neves Neto (2009), who attribute the higher total component output to greater milk volume rather than to changes in concentration. Andrade et al. (2016) and Magalhães et al. (2004) corroborated that additive-treated sugarcane silage can match maize silage (~ 22 kg cow⁻¹ day⁻¹) without affecting milk composition.

MUN was higher in T2 and T3 than in T1 ($p = 0.014$; Fig. 3). The values for T2 and T3 lie within the optimal range of 8–12 mg/100 ml for grazing cows on balanced diets (Pardo et al. 2009), whereas the control was below it, evidencing a ruminal nitrogen deficit in the absence of supplementation. The increase in MUN reflects the greater availability of ruminal N derived from the silage urea (3% DM), which stimulates microbial protein synthesis (Sousa-Alves et al. 2020). Nieto-Sierra et al. (2020a) documented similar results in the high Andean tropics of Nariño when supplementing with faba bean silage, which reinforces the consistency of these findings.

In L2, T3 exceeded T1 and T2 in milk yield (+ 17.5%; $p < 0.001$), in contrast to L1, where the RAS produced no additional response. Notably, the control in L2 ($10.05 \text{ L cow}^{-1} \text{ day}^{-1}$) already exceeded the maximum production of L1 ($T2 = 9.15 \text{ L cow}^{-1} \text{ day}^{-1}$), reflecting substantial differences in baseline productive potential between systems (2,400 vs. 2,830 m a.s.l.). Morales Lara and Herrera Maradiaga (2011) reported increases of $1.43 \text{ kg cow}^{-1} \text{ day}^{-1}$ when supplementing with sugarcane in tropical grazing systems, reaffirming the potential of *Saccharum officinarum* by-products when base forages are energy-limiting (Bargo et al. 2003; Andrade et al. 2016).

For fat yield, T2 and T3 exceeded the control ($p = 0.004$), and for protein and total solids T3 was superior to T1 and T2 ($p < 0.001$) (Fig. 2). Milk percentage composition and MUN remained similar among treatments ($p > 0.05$), consistent with Lagos-Burbano et al. (2021), Molavian et al. (2020) and Moreira et al. (2014). Ahmed et al. (2023) showed under commercial conditions that *Saccharum officinarum* extract increased milk yield by 8% and reduced somatic cell count by 47%, evidencing that the effects of sugarcane derivatives extend to udder health and product quality.

The differential response to the RAS between localities is the most relevant finding of this study. In L1, the milk-yield curve shows an arch-shaped pattern: $T1 (7.69) \rightarrow T2 (9.15) \rightarrow T3 (8.22 \text{ L cow}^{-1} \text{ day}^{-1})$, where T2 was the only treatment that exceeded the control ($p < 0.001$), while T3 and T1 performed similarly (Tukey, $p > 0.05$). In L2, by contrast, the curve is strictly increasing— $T1 (10.05) \rightarrow T2 (10.77) \rightarrow T3 (11.81 \text{ L cow}^{-1} \text{ day}^{-1})$ —and T3 exceeded T2 and T1 ($p < 0.001$). The same pattern is visible for protein and total solids (Fig. 6). The magnitude of the increase of T3 over the control was + 6.9% (ns) in L1 versus + 17.5% ($p < 0.001$) in L2, a difference that quantifies the modulating effect of each system's conditions on the response to the rumen activator.

To interpret this result, the mechanism of action of the RAS must be considered. Its effectiveness rests on the synchrony of three ruminal factors acting in a coordinated way: (1) rapidly fermentable carbohydrates (melote, 45% DM) that provide the energy substrate for microbial proliferation; (2) non-protein nitrogen at an N:S ratio of 9:1 (urea/ammonium sulfate), which simultaneously supplies N and S for the synthesis of sulfur amino acids by rumen microorganisms; and (3) sources of medium-degradability protein (nacedero, barley bran) that extend N availability beyond the carbohydrate fermentation peak (Serbest 2025; Lopes et al. 2024).

Recent evidence supports these mechanisms at the microbiome and fermentation level. Marino et al. (2025) showed that supplementation with sugarcane derivatives in Holstein cows increases the proportion of propionogenic bacteria (*Prevotella spp.*) and the ruminal propionate concentration, which directly improves the efficiency of metabolizable energy use. Along the same lines, Palani et al. (2025) confirmed that molasses—a component analogous to the melote of the RAS—optimizes N:S synchrony and supplement palatability when combined with urea, maximizing the productive response in grazing ruminants.

The absence of a response to the RAS in L1 is explained because silage alone ($12 \text{ kg fresh basis cow}^{-1} \text{ day}^{-1}$) already supplied enough fermentable energy and urea N (3% DM) to reach the ruminal N:S

synchrony required by the cows in that locality. In other words, in L1 the RAS was redundant: the deficit of the base forage had been covered by the silage before the activator could exert any additional effect.

In L2, by contrast, the greater productive potential of the animals (Holstein crosses, 520 kg live weight, baseline yield of 9.2 ± 1.2 L day⁻¹) generated a metabolic demand that silage alone did not fully meet. The RAS compensated for that deficit by enhancing ruminal fermentation and microbial protein synthesis, which translated into a significant response in milk yield as well as in protein (+ 22%) and total solids (+ 26%) yield. These results confirm that the animals' genetic level, the quality of the base forage, the altitude of the system, and the level of supplement intake act as key modulators of the response to rumen activators, and that their effect is expressed only when there is an energy–protein deficit that silage alone cannot correct (Bargo et al. 2003; Cardona-Iglesias et al. 2022; Cardona-Iglesias et al. 2021).

Supplementation with whole panela sugarcane silage treated with urea proved to be an effective strategy to improve milk yield and total milk components in grazing systems of the high Andean tropics, without compromising the compositional quality of the milk. The RAS provided an additional productive benefit only in the system with the greater genetic potential and higher metabolic demand (L2), showing that its effectiveness is conditioned by the level of energy deficit that silage alone cannot cover.

MUN was sensitive to supplementation in L1–T2 and T3 reached the optimal range (8.98–9.14 mg/100 ml) while T1 (control) showed a ruminal N deficit (7.31 mg/100 ml; $p = 0.014$)—confirming that urea-treated silage corrects the energy:protein imbalance typical of kikuyu-based systems in the high Andean tropics and can be used as an indicator for managing supplementation.

The by-products of the panela agro-industry—sugarcane silage and a RAS made from melote, bagasse, and sugar-mill by-products—constitute a viable, low external-cost, and sustainable supplementation strategy for the dairy systems of the high Andean tropics of Nariño, with the potential to reduce dependence on commercial concentrates and add value to local residues.

Statements and Declarations

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Competing interests. The authors declare that they have no competing interests.

Author contributions. Conceptualization and experimental design were performed by Edwin Castro-Rincón. Data collection was performed by Edwin Castro-Rincón, Elizabeth Lagos-Burbano, Juan Leonardo Cardona-Iglesias, and Yeferson Andrés Bran-Miranda. Data and information analysis was performed by Edwin Castro-Rincón, Elizabeth Lagos-Burbano, Juan Leonardo Cardona-Iglesias, Diego Hernán Meneses-Buitrago, and Yeferson Andrés Bran-Miranda. The manuscript was written by Edwin Castro-Rincón, Elizabeth Lagos-Burbano, Diego Hernán Meneses-Buitrago, and Yeferson Andrés Bran-Miranda. All authors read and approved the final manuscript.

Data availability. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval. This study did not involve experimental procedures requiring approval by an animal ethics committee. The animals were kept under normal commercial management on their farms of origin, and only non-invasive, routine measurements (milk yield recording and milk sampling, and body condition scoring) were performed with the informed consent of their owners; no painful or invasive procedures were applied. All animal handling followed standard husbandry and welfare practices. This study did not involve human participants.

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Figures

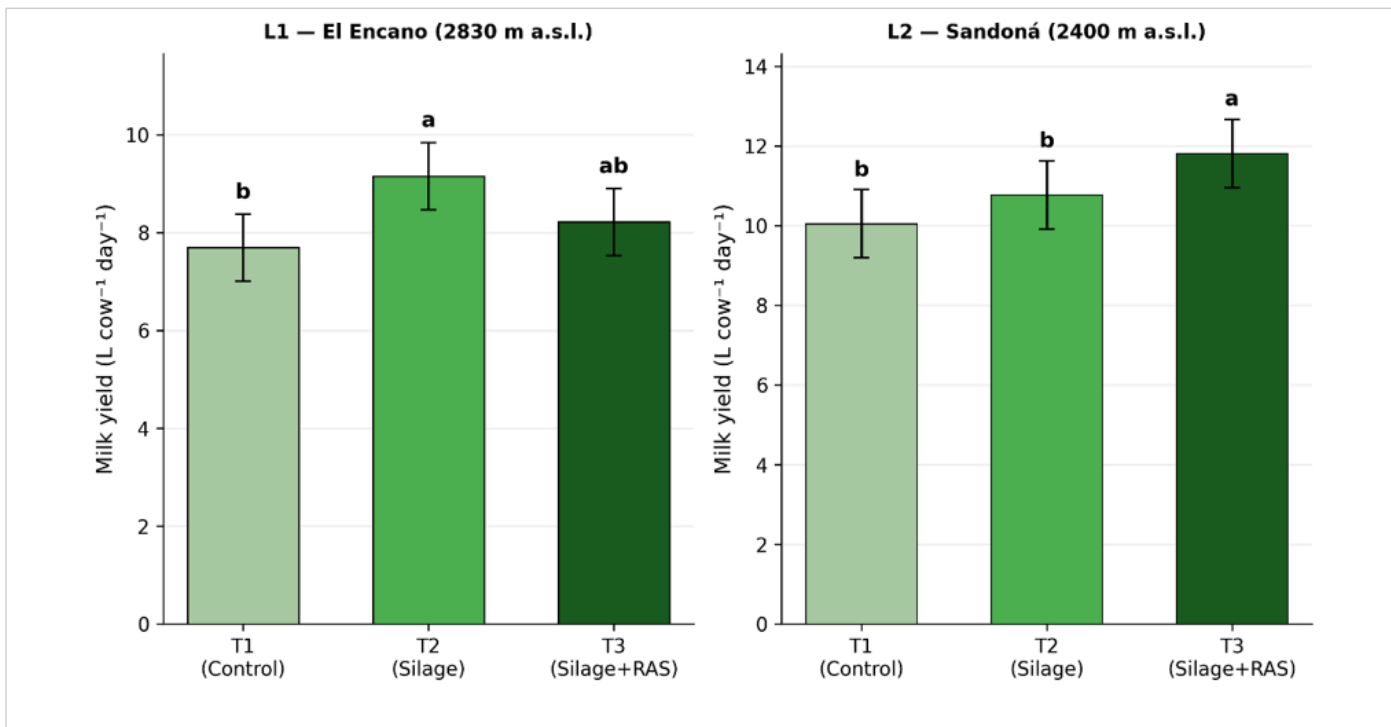


Figure 1

Milk yield (L cow⁻¹ day⁻¹) by treatment and locality. Bars = LSM means $\pm$ SEM. Different letters above the bars indicate significant differences among treatments (Tukey, $p \leq 0.05$). T1 = control; T2 = sugarcane silage; T3 = silage + RAS.

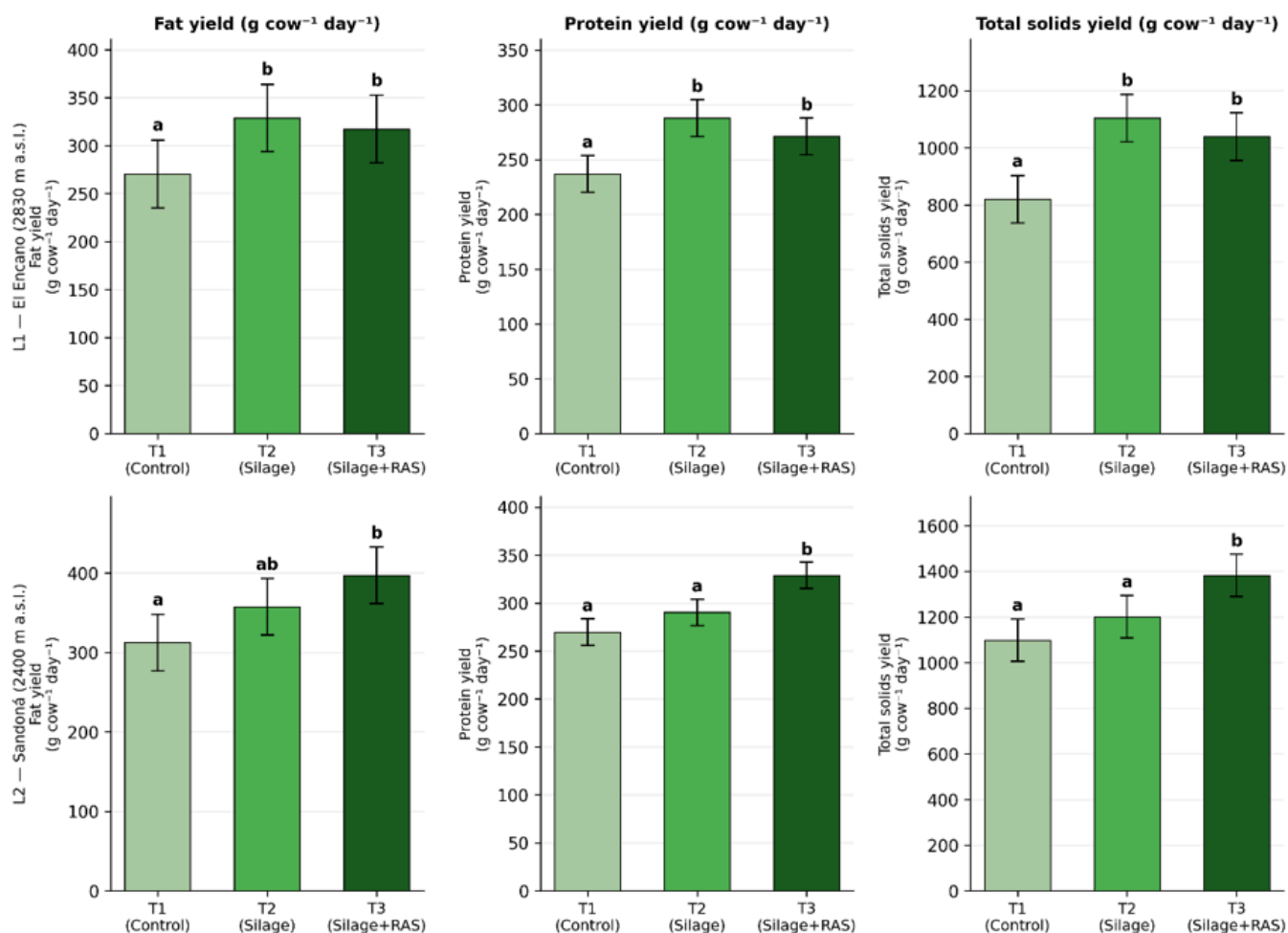


Figure 2

Milk component yield (g cow⁻¹ day⁻¹) by treatment and locality. FY = fat; PY = protein; TSY = total solids. Different letters = significant differences (Tukey, $p \leq 0.05$). Panels without letters = not significant.

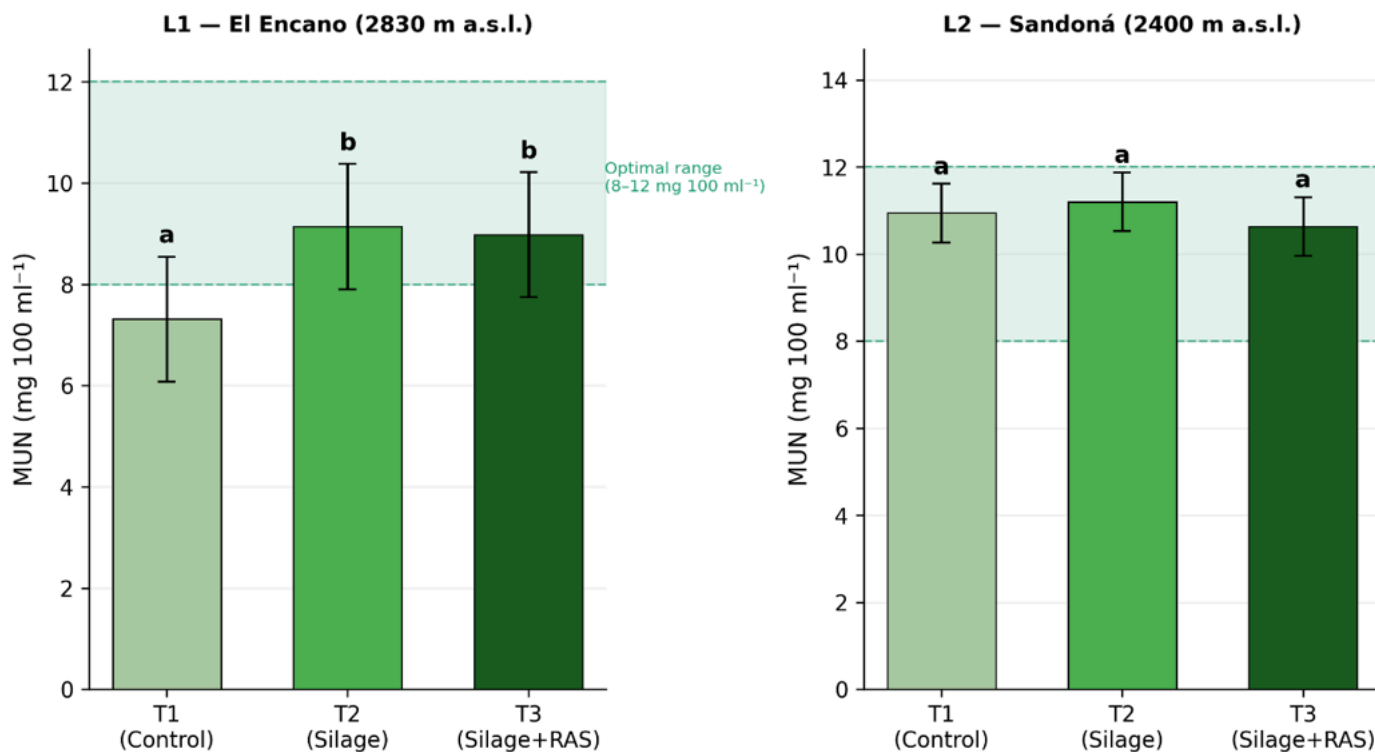


Figure 3

Milk urea nitrogen (MUN, mg/100 ml) by treatment and locality. Green band = optimal range for grazing cows (8–12 mg/100 ml). Different letters = significant differences in L1 (Tukey, $p = 0.014$). L2: $p = 0.590$ ns.

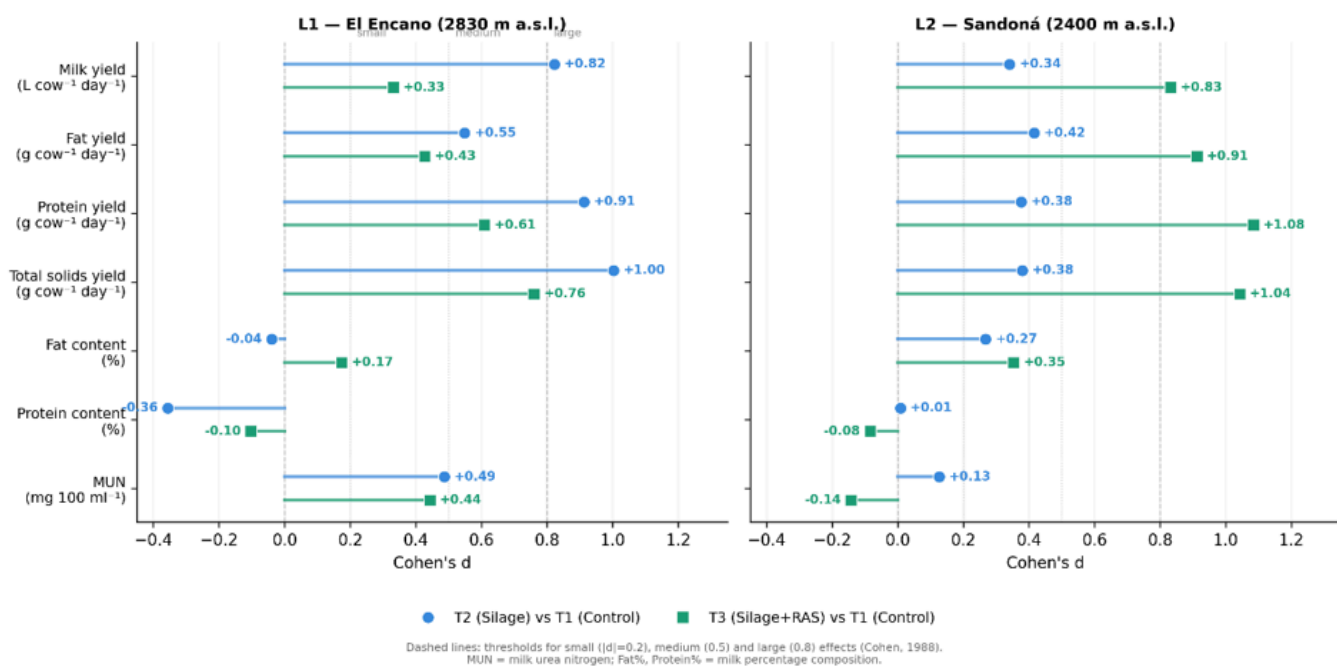


Figure 4

Treatment effect sizes (Cohen's d) on each response variable, by locality. Circles = T2 (Silage) vs. T1 (Control); squares = T3 (Silage+RAS) vs. T1. Dashed lines indicate the small ($|d| = 0.2$), medium (0.5) and large (0.8) thresholds according to Cohen (1988). Milk yield; FY, PY, TSY = fat, protein, and total solids yield (g cow⁻¹ day⁻¹); Fat%, Protein% = percentage concentration; MUN = milk urea nitrogen.

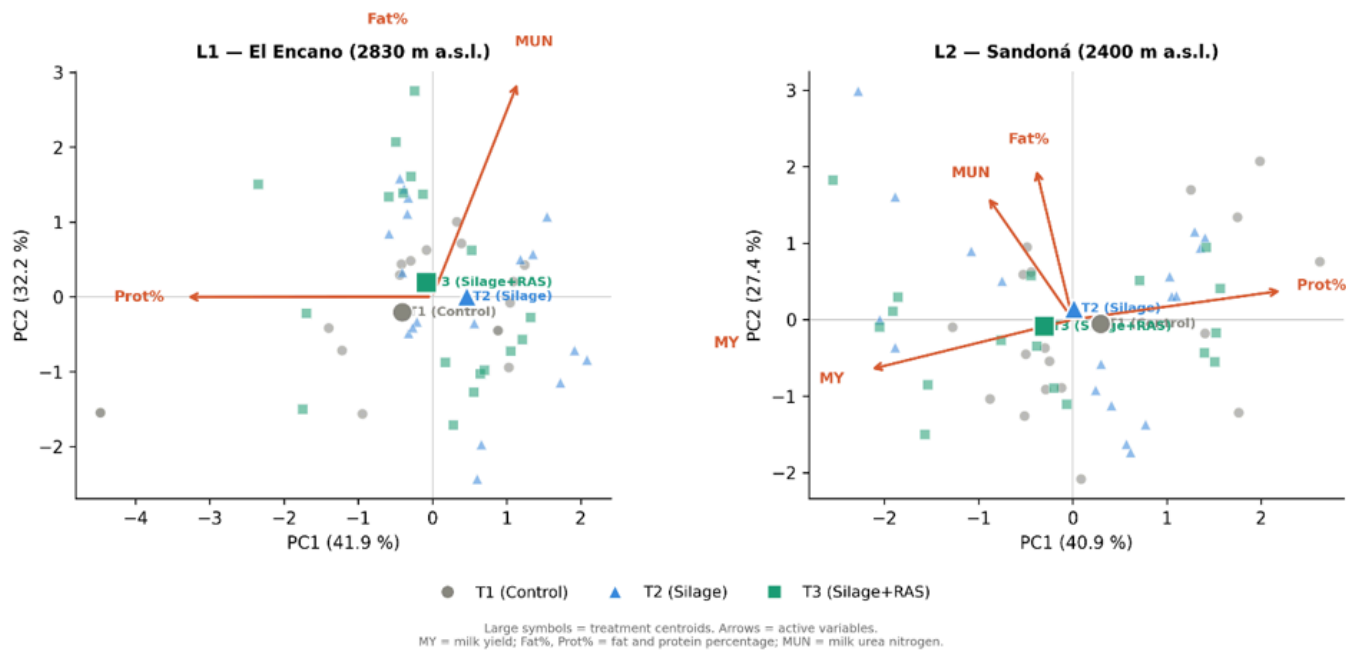


Figure 5

Principal component analysis (PCA) biplots by locality on four standardized independent variables. Points = individual observations; large symbols = treatment centroids; red arrows = active variables. Milk yield; %Fat, %Protein = milk concentration; MUN = milk urea nitrogen. L1: 74.1% of variance explained (PC1 + PC2). L2: 68.3%.

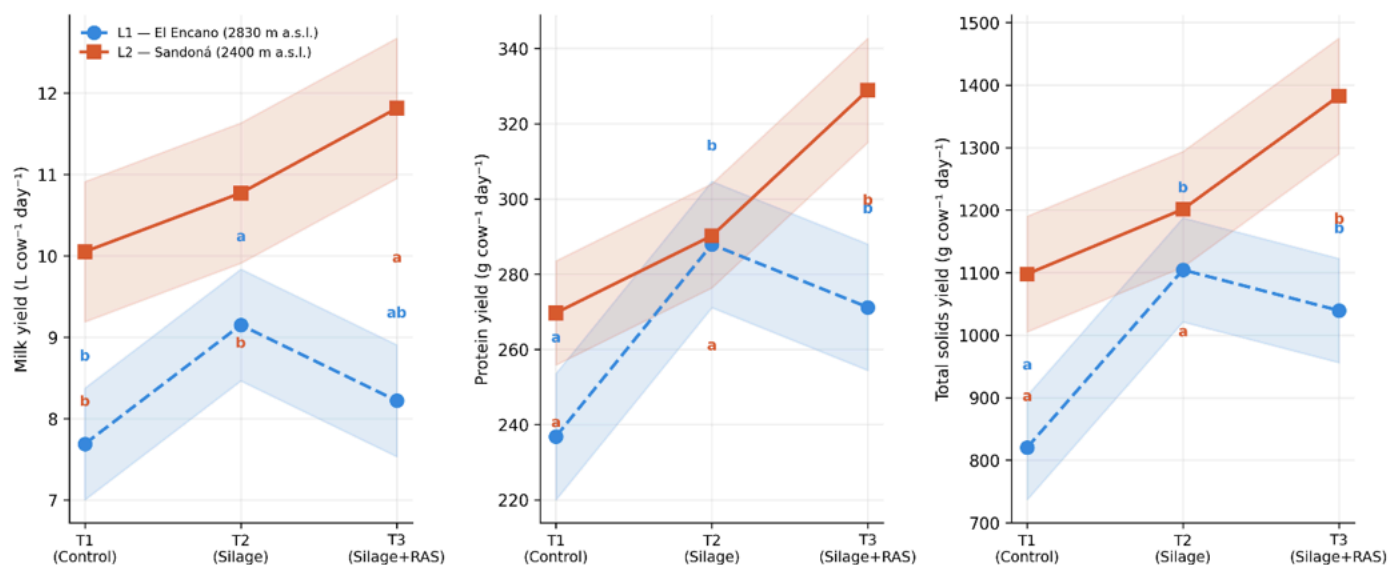


Figure 6

Differential response to the RAS between localities. Milk, protein, and total solids yield (LSM $\pm$ SEM) by treatment. Blue = L1 El Encano (2,830 m a.s.l.); orange = L2 Sandóná (2,400 m a.s.l.). The differential pattern illustrates the modulating effect of productive level and altitude on the response to the rumen activator.