

Article

Agronomic and Nutritional Potential of Ryegrass (*Lolium multiflorum* Lam.) Accessions as Raw Material for Silage in the Tropical Andes of Peru

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Abstract

In the tropical Andes, rangeland degradation has become one of the main threats to the sustainability of livestock production in the face of climate change. In this context, optimizing the yield and nutritional quality of raw material for silage is essential to sustain livestock productivity. The aim of this study was to identify local accessions (LM) of *Lolium multiflorum* Lam. with greater forage potential through evaluations in consecutive cuts made at the anthesis phenological stage, using a randomized complete block design with four replicates and ten local accessions (LM1, LM2, LM3, LM4, LM6, LM7, LM8, LM11, LM12 and LM13). The statistical analysis, based on linear mixed models, showed that cuts at anthesis had a significant effect among accessions, revealing high variability in agronomic and nutritional performance across cuts. In LM4, plant height at the fourth cut was 2.48-fold higher than at the first cut. Likewise, LM4 and LM13 were identified as the latest accessions to reach anthesis in the first cut, with a decreasing trend across cuts and stabilization from the third cut onward. These accessions also showed the greatest basal coverage area, increasing 9.94- and 8.18-fold in the fourth cut relative to the first. Fresh forage yields in LM4 and LM13 increased 13.2- and 10.1-fold, and dry matter yields 13.98- and 9.86-fold, compared with the first cut. They also exhibited the highest average daily dry matter accumulation rate. By contrast, the fresh forage and dry matter yields of the remaining accessions were significantly lower than those of LM4 and LM13. The main difference between these two accessions was observed in dry matter percentage, with higher values and a stable trend in LM4 across all cuts. In terms of nutritional quality, LM4 presented crude protein of 24.2% in the second cut and 24.0% in the fourth cut, while digestibility was 86.2% in the second cut and 85.0% in the fourth cut. In conclusion, although the ensiling process was not evaluated in this study, LM4 showed the most stable

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and outstanding values in both agronomic and nutritional performance, thus emerging as a promising accession for selection and use as raw material for silage production in the tropical Andes.

Keywords: *Lolium multiflorum* Lam.; forage grasses; phenological phases; yield and nutritional composition

1. Introduction

Cattle farming plays a fundamental role in food security, providing proteins and micronutrients that are essential in the human diet [1,2]. In Peru, there are more than 881,000 cattle producers, over 40% of whom live in poverty and operate farms smaller than 5 ha [3,4]. In the Amazonas region, around 16% of the land area has been affected by anthropogenic activities, and more than 70% of this area is degraded, with low-quality native grasslands, deteriorated improved pastures, and reduced availability of fallow land due to the conversion of grasslands and scrublands into cropland [5–7].

Currently, climate change is one of the main challenges for cattle production, as it reduces food supply through prolonged droughts, strong seasonality, temperature fluctuations, and an increased presence of pests and diseases, thereby increasing the vulnerability of small-scale cattle producers [8,9]. In addition, extensive pastoralism practices, which require high labor demand and large areas of pasture, have driven the transition to stall systems [10,11]. As a result, livestock farmers have become increasingly dependent on imported feed supplements, which has raised production costs and undermined their financial sustainability [12,13].

Faced with this problem, many producers resort to using *Pennisetum clandestinum*, a species considered invasive and used mainly as a secondary forage resource [14]. This situation implies the need for forages with greater agronomic and nutritional potential, in order to help reduce risk factors in production. However, the indiscriminate introduction of exotic or highly improved fodder genotypes can reduce local biodiversity and accelerate the degradation of ecosystems [15]. The genus *Lolium*, although not native to the Tropical Andes of Peru, stands out for its resistance and tolerance to different altitudinal zones; its acclimatization to cold reveals a valuable genetic reservoir against frost and drought [16], demonstrating an adaptive capacity between 1800 and 3600 m above sea level, with low levels of damage and incidence of pests and diseases [17].

It should be noted that yields and nutritional composition vary among *Lolium* species [18]. *Lolium multiflorum* Lam. stands out for its usefulness in the phytoremediation of contaminated soils, accelerating the degradation of polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene, and reducing their half-life [19]. It also increases nitrogen (N) adsorption in the soil and significantly reduces nitrate leaching compared to *Lolium perenne* [20], and has superior ensilability, with greater stability and quality than other species of the genus [21]. However, the growth cycle of ryegrass depends on both the genotype and the environment in which it develops, revealing differences between varieties in days to flowering, days to anthesis, persistence, longevity, yield, and nutritional composition [22–25]. These differences may be associated with responses to photoperiod and vernalization requirements [26,27], as well as functional traits such as root architecture and regrowth capacity, which determine tolerance to water and heat stress and production stability between harvests [28–30].

In this context, one alternative is to evaluate locally sourced ryegrass (*Lolium multiflorum* Lam.) varieties from other regions of the country, selected for their origin in environments with altitudinal zones and agroecological conditions similar to those of the study

area. In the Peruvian Tropical Andes, there is still limited scientific evidence on the agronomic and nutritional performance of local ryegrass accessions, justifying the need to establish a scientific basis that can guide the future sustainability of livestock systems [31,32].

The lack of appropriate technologies for forage conservation during critical periods of scarcity restricts producers' options for dealing with climate variability. In response, producers often increase animal stocking rates to compensate for yield losses, which reduces the forage available per head and makes system performance more dependent on herd size than on individual performance, thus reducing meat and milk yields [33,34]. In addition, as forage matures, its digestibility decreases and it becomes less viable for consumption as fresh forage, being better utilized in silage [35]. Therefore, the initial evaluation of the harvested material is key to determining its suitability during the silage process and future losses of dry matter and nutritional value at each stage, allowing for improved planning to cope with critical periods and reduce forage losses, thus contributing to the stability of the system [36,37].

There is wide range of unexplored genetic diversity regarding how its agronomic and nutritional characteristics vary when used as raw material for silage under the conditions of the tropical Andes of Peru. In this context, the objective of this study was to identify local accessions of *Lolium multiflorum* Lam. with greater forage potential through evaluations in consecutive cuts made at the anthesis phenological stage. The hypothesis was that, even when evaluated at anthesis and under similar agroecological conditions, the accessions differ consistently in yield and nutritional composition, and that the changes resulting from consecutive cuts do not occur to the same extent in all of them. In particular, it is expected that at least one accession will combine higher yield and nutritional value with greater stability between cuts, expressed as less variation of these attributes throughout the cuts, positioning itself as a promising candidate as raw material for forage conservation strategies. This study generated an experimental database describing the behavior of little-explored local accessions. It is important to note that this study only evaluated the harvested forage as silage raw material (agronomic and nutritional characteristics) and did not assess the ensiling process or fermentation quality. Therefore, our study was limited to the silage potential inferred from the raw material attributes and highlights the need for future research on silage quality and the management of these accessions under different conditions in the Peruvian tropical Andes. Our results can guide producers' decision-making to reduce the impact of climate change without compromising ecosystems in the Tropical Andes, thus contributing to the sustainability of livestock systems.

2. Materials and Methods

2.1. Description of the Research Area

The study was conducted in the province of Chachapoyas, Amazonas region, Peru, at an altitude of 2446 m above sea level, with coordinates 77°51'43.82" W longitude and 6°12'27.35" S latitude. During the experimental period, from July 2024 to June 2025, meteorological data were collected using a Vantage Pro2-Davis station (Davis Instruments Corp., Hayward, CA, USA). As shown in Figure 1, data were recorded for the following parameters: temperature (maximum: 21.16 ± 1.94 °C; minimum: 10.37 ± 1.76 °C) (Figure 1b,c), relative humidity ($78.15 \pm 8.21\%$), and precipitation (2.26 ± 5.12 mm day⁻¹) (Figure 1a,d). Values are reported as mean \pm standard deviation (SD).

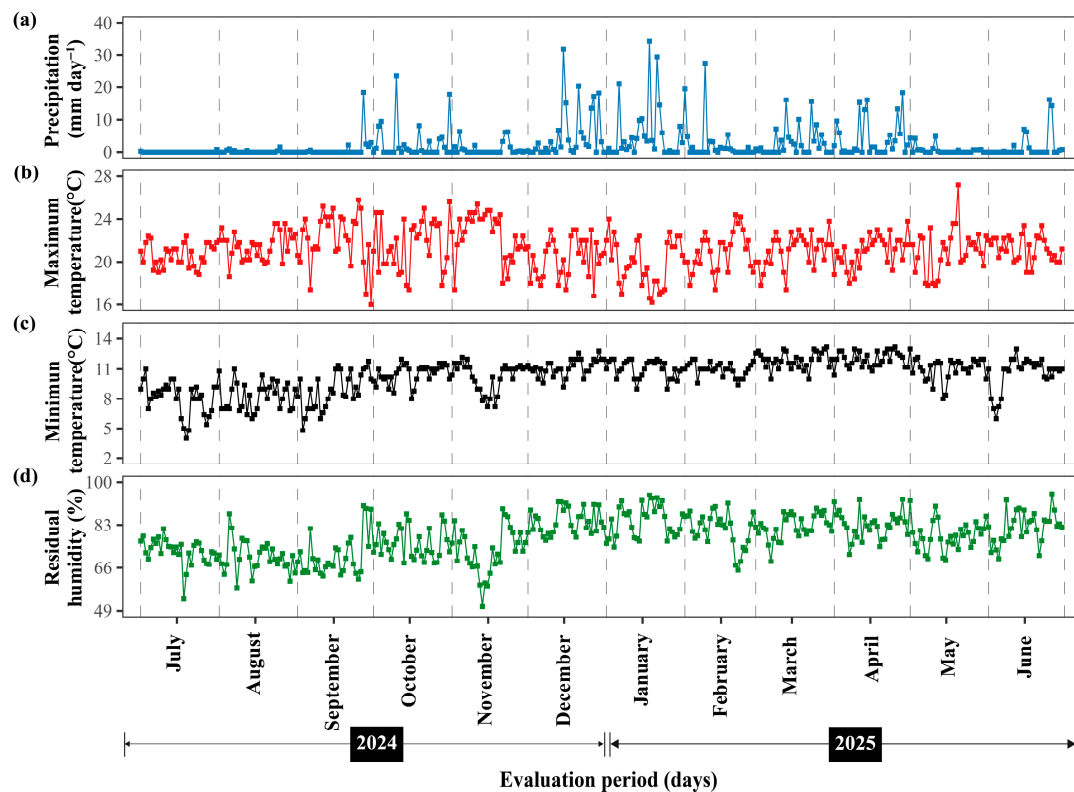


Figure 1. Meteorological conditions during the experimental period. (a) precipitation; (b) maximum temperature; (c) minimum temperature; and (d) relative humidity.

Likewise, soil sampling was carried out based on the Peruvian state's soil study regulations DS No. 013-2010-AG [38]. In the total area of the investigation (525 m²), a zigzag route was carried out marking 10 sampling points. The surface of each point was cleaned and a shovel was inserted to a depth of 32 cm. The extracted subsamples were mixed and homogenized and, applying the quartering method, a composite sample of 1 kg was formed [39]. Next, the samples were then coded and transferred to the Laboratorio de Investigación de Suelos y Aguas (LABISAG) at the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, accredited by the Instituto Nacional de Calidad, Ministerio de Producción, in accordance with standard NTP-ISO/IEC 17025:2017 [40]. The physicochemical parameters were analyzed according to the Bazán methodology [41], indicating a clayey-sandy texture (sand 48%, silt 10% and clay 42%). In addition, the pH (5.5), electrical conductivity (0.07 dS m⁻¹), organic matter content (4.47%), carbon (2.59%), nitrogen (0.22%), phosphorus (10.19 ppm) and potassium (175.21 ppm) were determined.

2.2. Obtaining Plant Material

The ten ryegrass (*Lolium multiflorum* Lam.) accessions were acquired from an altitude of 2667 m above sea level, with geographic coordinates 78°27'07" W longitude and 07°09'56" S latitude. The field evaluation, however, was conducted at 2446 m above sea level; this altitudinal difference was considered moderate and not expected to markedly affect plant establishment under the study conditions. These accessions were under ex situ conservation in the Programa Nacional de Investigación en Pastos y Forrajes del Instituto Nacional de Innovación Agraria (INIA), located at the Estación Experimental Agraria Baños del Inca in the department of Cajamarca. All accessions were collected in the department of Cajamarca for conservation in the germplasm bank; the origin characteristics of each accession are described in Table 1. Among the characteristics of the conservation area was a relatively stable day length throughout the year, with a maximum photoperiod of

approximately 12.1 h. The propagation method in our study was vegetative, using clumps (planting points) of eight 12 cm-long tillers taken from vigorous mother plants in optimal phytosanitary conditions to obtain identical plants. The accessions were selected for their fresh forage yields exceeding 800 kg ha⁻¹ and for being considered local accessions. For transport, the tillers were removed with the corresponding portion of soil adhering to the roots and placed in Kraft paper bags containing moistened paper towels to prevent dehydration of the plant material during transport prior to planting.

Table 1. Origin of accessions within the germplasm bank.

Accessions	Code	Collection Site	Province	EAST Coordinate	NORTH Coordinate
<i>Lolium multiflorum</i> Paccha—LM1	LM1	Paccha	Chota	78°48'40.85" W	6°19'43.16" S
<i>Lolium multiflorum</i> Cutervo—LM2	LM2	Cutervo	Cutervo	78°49'15.76" W	6°22'34.56" S
<i>Lolium multiflorum</i> Tacabamba—LM3	LM3	Tacabamba	Chota	78°36'36.29" W	6°23'37.05" S
<i>Lolium multiflorum</i> Tacabamba—LM4	LM4	Tacabamba	Chota	78°36'36.29" W	6°23'37.05" S
<i>Lolium multiflorum</i> Calquis—LM6	LM6	Calquis	San Miguel	78°58'26.35" W	6°55'15.54" S
<i>Lolium multiflorum</i> El Agrario—LM7	LM7	El Agrario	San Miguel	78°50'55.37" W	7°0'2.52" S
<i>Lolium multiflorum</i> Bambamarca—LM8	LM8	Bambamarca	Hualgayoc	78°29'52.3" W	6°40'42.1" S
<i>Lolium multiflorum</i> Sendamal—LM11	LM11	Sendamal	Celendín	78°10'52.49" W	6°57'54.96" S
<i>Lolium multiflorum</i> Sendamal—LM12	LM12	Sendamal	Celendín	78°10'52.49" W	6°57'54.96" S
<i>Lolium multiflorum</i> Micuypampa—LM13	LM13	Celendín	Cajamarca	78°12'45.07" W	7°1'32.91" S

2.3. Experimental Design

The study was conducted using a randomized complete block design (RCBD) with four replicates (blocks). The local accessions factor included 10 levels: LM1, LM2, LM3, LM4, LM6, LM7, LM8, LM11, LM12, and LM13. In each block, one plot was established per accession (10 plots per block), for a total of 40 experimental units (Figure 2). Four successive cuts were made on each experimental unit, considering four levels: first, second, third, and fourth cuts; each cut was made when each accession reached anthesis. Agronomic parameters were evaluated in the four cuts, while nutritional parameters were evaluated only in the second and fourth cuts.

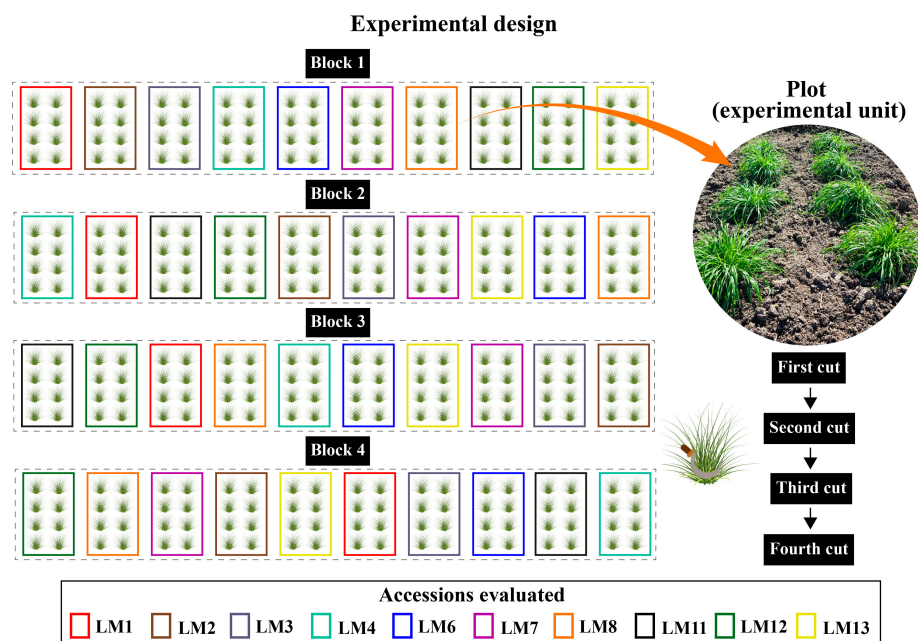


Figure 2. Experimental design and field layout. The diagram illustrates the Randomized Complete Block Design (RCBD) with four blocks. The bottom legend (Accessions evaluated) identifies the ten

accessions corresponding to the color-coded plots. The circular insert details the experimental unit (plot), and the flow chart on the right depicts the repeated measures sampling strategy (four sequential cuts) performed on the same plots.

2.4. Establishment of the Experimental Area

Land preparation began with primary tillage using a disc plow at a depth of 32 cm to loosen the soil. Secondary tillage was carried out with two cross-passes of an offset disc harrow to break up soil clods, followed by a final pass with a leveling harrow to even out the surface and prepare the land for planting. The layout of the experimental units and the opening of furrows were performed manually using stakes, string, and hand tools. Each experimental unit consisted of a 2 m × 3 m (6 m²) plot, with 1.5 m spacing between plots and between blocks. Planting was carried out manually on 1 July 2024, using groups of eight tillers for each ryegrass (*Lolium multiflorum* Lam.) accession. Planting was uniform, with 0.75 m between clumps (planting points) and 0.75 m between rows. Each experimental unit contained eight clumps, and each clump was established with eight tillers. The plant material was provided by the Germplasm Bank of the Programa Nacional de Investigación en Pastos y Forrajes del Instituto Nacional de Innovación Agraria (INIA). Fertilization consisted of applying chicken manure at a rate of 300 g m⁻², containing 2.67% nitrogen, 3.74% phosphorus, and 2.19% potassium. Irrigation was applied using a sprinkler system with a flow rate of 25 L s⁻¹. During the first two weeks after sowing, irrigation was applied twice per week, with each irrigation event lasting 20 min. This resulted in an irrigation depth of approximately 46 mm per event, equivalent to a total weekly irrigation of 92 mm (equivalent to 922 m³ ha⁻¹). Subsequently, irrigation frequency was reduced and adjusted according to rainfall conditions. Weed control was carried out manually every 10 days based on prior monitoring. For pest and disease management, carbendazim (200 mL ha⁻¹) was applied to control *Puccinia graminis*, and cypermethrin (300 mL ha⁻¹) to control *Dalbulus maidis*.

2.5. Evaluation Parameters

2.5.1. Qualitative Morphological Characterization

The morphological characterization of the ten accessions was performed to identify possible variations associated with the study conditions and their agronomic usefulness. Growth habit was evaluated to describe plant architecture; leaf color was evaluated as a visual indicator of chlorophyll status and vigor; and traits such as basal node and texture were evaluated to differentiate between accessions and possible implications for palatability. The evaluation was performed following the criteria of Maity et al. [42], as shown in Table 2.

Table 2. Evaluation criteria for qualitative characteristics.

Attributes	Classification
Growth habit	A scale of 1 to 5 was established to rate the angle of the stem or main tillers, taking the horizontal axis as a reference, where erect (greater than 60°) (1), semi-erect (between 30° and 60°) (3), and prostrate (less than 30°) (5)
Leaf color	Leaf color was rated on a scale of 1 to 5 as light green (1), green (3), and dark green (5)
Basal node color	The color of the basal node was rated on a scale of 1 to 5 as light green (1), green (3), and reddish (5)
Texture of leaf	The texture of leaf was evaluated on a scale of 1 to 5: very smooth (1), smooth (3), and rough (5)

2.5.2. Plant Height

Plant height was recorded for each accession when it reached anthesis. Measurements were taken using a Bahco flexometer (SNA Europe SAS, Éragny-sur-Oise, France), selecting six groups at random and measuring from the base of the stem (ground level) to the tip of the tallest leaf [43].

2.5.3. Days to Anthesis

Days to anthesis were defined as when 70% of the clumps in each experimental unit had flowers with fully emerged anthers and stigmas [44].

2.5.4. Basal Coverage Area

Basal coverage area was assessed by selecting six groups per experimental unit. In each group, the major and minor diameters were measured at ground level, and the cover area was calculated using the formula for the area of an ellipse [45]. The formula Equation (1) is expressed as:

$$\text{Basal coverage area (cm}^2\text{)} = \pi \left(\frac{\text{LD}}{2}\right) \left(\frac{\text{MD}}{2}\right) \quad (1)$$

where LD: largest diameter (cm) and MD: minor diameter (cm).

2.5.5. Yield Parameters

Fresh forage yield was determined by harvesting a 1 m² quadrat located in the central area of each 2 × 3 m plot, avoiding plot borders to minimize edge effects. The harvest was carried out at a height of 5 cm from ground level and the forage obtained was weighed in situ using an electronic scale (precision ± 5 g). The data were recorded in a field book in units of kg m⁻². In addition, 1000 g of fresh forage was extracted from each sample and placed in an oven for 72 h at a temperature of 60 °C until a constant weight was reached. The dry matter (%) was calculated by dividing the dry weight by the fresh weight and multiplying the result by 100. To calculate the dry forage yield (t ha⁻¹), the fresh forage yield was first extrapolated to t ha⁻¹ and multiplied by the dry matter percentage (%).

2.5.6. Dry Matter Accumulation Rate

The average dry matter accumulation rate was calculated by dividing the dry matter yield by the number of days elapsed until anthesis for each accession in each cut. The results were expressed in kg ha⁻¹ day⁻¹ [46].

2.5.7. Nutritional Composition

Nutritional analysis was performed at the Laboratorio de Nutrición Animal y Bromatología (LABNUT) de la Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, where neutral detergent fiber (NDF), acid detergent fiber (ADF), protein, and digestibility were evaluated. Samples were transported from the field to the laboratory in labeled Kraft paper bags. The samples were then divided into 5- to 10 cm segments, and 250 to 500 g of chopped sample were collected for each experimental unit. The resulting material was placed in new, pre-weighed Kraft paper bags. The initial fresh weight was recorded on a gram scale, and the sample was subsequently dried at 60 °C for 48 h in a forced-air oven until a constant weight was reached. Once the dried sample was obtained, its dry weight was recorded, and it was then ground in a hammer mill with a 2 mm screen for subsequent analysis.

Crude protein content was processed using AOAC Method No. 928.08 [47]. This procedure involved digesting the sample with sulfuric acid and converting the nitrogen to ammonia, followed by quantification by titration with hydrochloric acid. The nitrogen

obtained was then multiplied by 6.25 to estimate the crude protein concentration. NDF and ADF were determined using the ANKOM A200 procedure [48]. NDF was quantified using a neutral detergent solution in the presence of alpha-amylase and sodium sulfite, followed by rinsing with acetone and oven drying. ADF was measured using an acidic detergent solution, followed by filtration and oven drying. In vitro digestibility was determined using the ANKOM DAISY II Incubator system, following the standardized protocol of ANKOM Technology for forage and fibrous materials. The dry, ground samples were weighed at 0.50 g in ANKOM F57 filter bags that had been pre-washed with acetone for 5 min and completely dried at room temperature to remove surfactants that could inhibit microbial activity. The bags were heat-sealed and placed in the digestion jars in the incubator, including one blank filter bag per jar for subsequent correction. Two buffer solutions were used: buffer solution A, composed of per KH_2PO_4 (10 g L^{-1}), $\text{MgSO}_4 \cdot 7 \text{ H}_2\text{O}$ (0.5 g L^{-1}), NaCl (0.5 g L^{-1}), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 g L^{-1}), and reagent-grade urea (0.5 g L^{-1}), and buffer solution B, composed of per Na_2CO_3 (15 g L^{-1}) y $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (1 g L^{-1}). Both solutions were preheated to $39 \text{ }^\circ\text{C}$ and mixed in a 1:5 ratio (B: A) until a final pH of 6.8 was reached. For each digestion beaker, 1600 mL of the buffer mixture was added, and the beaker were placed in the incubator, allowing thermal stabilization for 30 min before the addition of the ruminal inoculum.

The ruminal fluid was obtained from a single bovine. Collection was performed using thermos preheated with water at $39 \text{ }^\circ\text{C}$ to preserve microbial activity. At the time of collection, the hot water was removed to introduce the ruminal fluid. It was transported to the laboratory within 30 min of collection, where the ruminal fluid was extracted from the thermos and homogenized in a blender (previously heated and purged with CO_2) for 30 s. This procedure allowed the microorganisms adhering to the fibrous fraction to be detached. The material was then filtered through four layers of gauze to obtain the clarified ruminal fluid in a previously heated container, purged with CO_2 during the transfer of the inoculum.

Once the digestion beaker had reached thermal stability, they were individually removed from the incubator, and 400 mL of rumen fluid was added to each beaker. Subsequently, each beaker was purged with CO_2 for approximately 30 s, creating a gaseous atmosphere over the contents without allowing direct bubbling in the liquid medium, and then hermetically sealed. Incubation was carried out for 48 h in an ANKOM DAISY II incubator, which provides continuous stirring and automatic temperature control, maintaining a temperature of $39.5 \pm 0.5 \text{ }^\circ\text{C}$. At the end of the incubation period, the beaker were drained, and the filter bags were gently rinsed twice with cold distilled water to remove excess digestion solution and soluble compounds. Subsequently, the bags were treated with a neutral detergent solution in an ANKOM fiber analyzer to remove microbial residues and remaining soluble fractions. In vitro digestibility was calculated from the final NDF residue, applying the correction for white bags, according to the standard equations of the DAISY II method [49].

2.6. Data Analysis

Data analysis was performed using Rstudio software version 4.5.0 for Windows. Statistical analysis was based on a linear mixed-effects model using the lmer function of the lme4 package [50]. The model included accessions and cuts as fixed effects, as well as their bidirectional interaction (accessions \times cut). Likewise, the block and the plot nested within the block were included as random terms in order to capture possible variability between experimental units. The assumptions of normality and homogeneity of variances of the model were validated by visual inspection of diagnostic graphs. To determine statistical differences in fixed effects, a type III analysis of variance (ANOVA) was used with the Kenward-Roger method using the lmerTest package [51]. When significance was found,

simple effects were analyzed by comparing estimated marginal means with the emmeans package [52] applying Sidak's adjustment. Data not meeting model assumptions were natural log-transformed prior to analysis. For biological interpretation, table values are reported as back-transformed estimated marginal means on the original scale using the exponential function.

3. Results

3.1. Parameters' Morphology

3.1.1. Qualitative Morphological Evaluation

The ten accessions presented different qualitative morphological characteristics, indicating diversity in physical attributes between accessions over the course of their development, as shown in Table 3.

Table 3. Qualitative morphological characteristics in ten ryegrass accessions.

Accession	Growth Habit	Leaf Color	Basal Node Color	Texture of Leaf
LM1	5	1	5	3
LM2	3	1	5	3
LM3	1	1	5	3
LM4	3	5	5	5
LM6	1	3	5	5
LM7	3	1	1	3
LM8	1	1	3	3
LM11	3	3	1	3
LM12	3	1	1	3
LM13	3	5	1	5

Morphological classification: growth habit: erect (1), semi-erect (3), prostrate (5); leaf color: light green (1), green (3), dark green (5); basal node color: light green (1), green (3), reddish (5); and leaf texture: very smooth (1), smooth (3), rough (5).

Figure 3 shows the differences in the coloration of the basal node of three local accessions, which reveals marked variation between them.

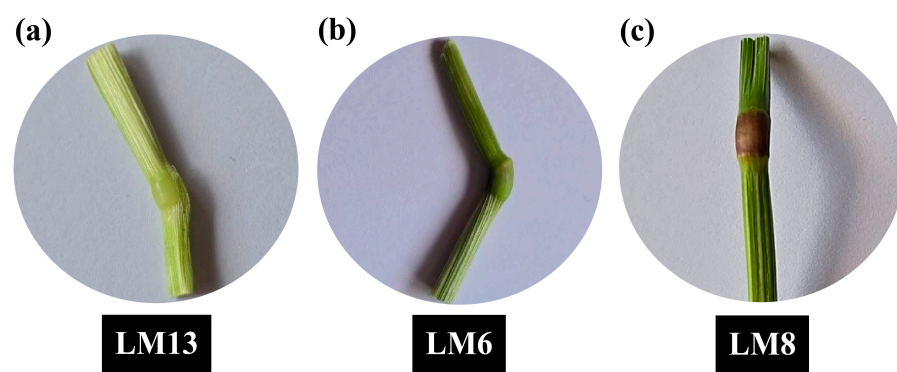


Figure 3. Differences in the coloration of the basal node of each accession. (a) Accession LM13 with a light green basal node, (b) accession LM6 with a green basal node, and (c) accession LM8 with a reddish basal node.

3.1.2. Plant Height

Plant height revealed significant effects of the accessions ($p < 0.001$) and cuts ($p < 0.001$), as well as their interaction ($p < 0.001$). During the first cut, LM12 had the greatest regrowth height (31.5 ± 0.5 cm); however, regrowth height decreased to 20.4 ± 0.3 cm by

the fourth cut. Accession LM13 had the greatest plant height in the second cut (33.7 ± 0.3 cm) and in the third cut (60.1 ± 0.7 cm), but this upward trend was not maintained in the fourth cut, where regrowth height decreased to 53.8 ± 0.4 cm. In contrast, LM4 showed a sustained increase in plant height across cuts, reaching the greatest value in the fourth cut (57.7 ± 0.6 cm), which was 2.48 times greater than in the first cut (Table 4).

Table 4. Evaluation of plant height.

Accessions	Plant Height (cm ²)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	25.2 ± 0.1 cB	20.3 ± 0.3 eC	26.7 ± 0.2 cdeA	26.4 ± 0.3 dAB
LM2	23.1 ± 0.1 dA	19.9 ± 0.5 eB	18.3 ± 0.3 fC	23.4 ± 0.2 eA
LM3	28.1 ± 0.1 bA	28.0 ± 0.5 bA	25.4 ± 0.2 eB	25.8 ± 0.3 dB
LM4	23.3 ± 0.5 dD	29.5 ± 0.2 bC	54.0 ± 0.5 bB	57.7 ± 0.6 aA
LM6	29.3 ± 0.2 bA	25.5 ± 0.2 cC	27.9 ± 0.3 cB	23.4 ± 0.4 eD
LM7	20.8 ± 0.5 eD	24.6 ± 0.3 cdC	27.1 ± 0.3 cdB	28.9 ± 0.4 cA
LM8	28.6 ± 0.3 bA	28.6 ± 0.3 bA	19.0 ± 0.2 fB	18.6 ± 0.2 gB
LM11	26.1 ± 0.4 cB	25.5 ± 0.3 cB	27.8 ± 0.4 cA	23.1 ± 0.2 eC
LM12	31.5 ± 0.5 aA	23.6 ± 0.2 dC	25.7 ± 0.4 deB	20.4 ± 0.3 fD
LM13	28.1 ± 0.4 bD	33.7 ± 0.3 aC	60.1 ± 0.7 aA	53.8 ± 0.4 bB

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

3.1.3. Basal Coverage Area and Days to Anthesis

Basal coverage area and days to anthesis showed significant effects of accessions ($p < 0.001$) and cuts ($p < 0.001$), as well as their interaction ($p < 0.001$). Both variables showed different patterns among accessions in the different cuts (Table 5). Basal coverage area increased significantly in LM4 and LM13, which showed the largest and most consistent increases from the first to the fourth cut (LM4: 33.8 ± 0.9 to 336.0 ± 5.8 cm²; LM13: 47.2 ± 3.3 to 386.2 ± 5.9 cm²), while the lowest coverage in the fourth cut was recorded for LM1 (17.5 ± 0.9 cm²) and LM3 (17.3 ± 1.2 cm²). In terms of days to anthesis, most accessions remained below 50 days in all cuts, while LM4 and LM13 were markedly later in the first cut (120.8 ± 2.4 and 132.2 ± 2.6 days, respectively) and then showed pronounced reductions in subsequent cuts, with smaller changes between the third and fourth cuts (LM4: 65.5 ± 1.8 vs. 70.0 ± 0.0 ; LM13: 57.8 ± 1.7 vs. 57.2 ± 1.4 days). When summarizing the pattern as the average of the second to fourth cuts in relation to the first cut, LM4 decreased from 120.8 to 74.5 days on average, and LM13 from 132.2 to 63.3 days, while the previous accessions, such as LM1, LM3, and LM6, showed comparatively smaller differences between the first cut and the average of the second to fourth cuts. Overall, LM4 and LM13 combined the greatest basal expansion with a marked reduction in time to anthesis after the first cut, while the remaining accessions generally maintained earlier flowering and lower Basal coverage area.

Table 5. Morphological evaluation of basal coverage area and days to anthesis.

Accessions	Basal Coverage Area (cm ²)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	14.7 ± 0.8 eC	14.2 ± 0.7 gC	22.1 ± 0.8 dA	17.5 ± 0.9 fB
LM2	23.3 ± 1.0 cdB	24.1 ± 1.6 deB	40.7 ± 1.5 bA	42.8 ± 0.5 cA
LM3	6.8 ± 0.3 gC	7.6 ± 0.4 hC	14.0 ± 0.9 eB	17.3 ± 1.2 fA
LM4	33.8 ± 0.9 bD	151.9 ± 4.0 aC	268.9 ± 10 aB	336.0 ± 5.8 aA
LM6	28.0 ± 1.2 bcC	65.2 ± 2.1 bA	38.3 ± 1.4 bB	69.8 ± 2.5 bA
LM7	10.8 ± 0.3 fD	15.2 ± 0.8 fgC	22.6 ± 1.3 dB	40.7 ± 2.0 cdA

LM8	28.8 ± 1.0 bcAB	31.7 ± 1.2 cA	26.4 ± 0.6 cdB	32.2 ± 0.4 deA
LM11	15.6 ± 0.6 eC	19.1 ± 0.9 efB	28.6 ± 1.4 cA	29.1 ± 1.1 eA
LM12	22.0 ± 0.9 dC	26.4 ± 1.1 cdAB	24.2 ± 4.1 cdBC	30.1 ± 1.1 eA
LM13	47.2 ± 3.3 aD	168.8 ± 6.4 aC	286.6 ± 6.5 aB	386.2 ± 5.9 aA
	Days to Anthesis			
Accessions	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	40.8 ± 1.5 cdA	35.0 ± 0.8 defgA	39.8 ± 1.7 bA	40.2 ± 1.0 cdA
LM2	46.2 ± 1.1 cA	42.2 ± 2.7 cdAB	34.5 ± 1.0 bcC	37.0 ± 1.0 cdeBC
LM3	43.5 ± 1.5 cdA	40.2 ± 1.3 cdefA	37.0 ± 1.8 bcA	41.5 ± 1.2 cA
LM4	120.8 ± 2.4 bA	88.0 ± 2.3 aB	65.5 ± 1.8 aC	70.0 ± 0.0 aC
LM6	36.0 ± 0.6 dA	32.8 ± 1.7 efgA	39.8 ± 7.9 bA	34.5 ± 1.6 cdeA
LM7	49.0 ± 1.2 cA	41.2 ± 1.1 cdeB	38.5 ± 1.8 bB	36.8 ± 1.2 cdeB
LM8	35.0 ± 0.0 dA	28.0 ± 0.0 gA	28.5 ± 0.5 cA	29.2 ± 0.8 eA
LM11	45.5 ± 1.4 cAB	49.0 ± 1.2 cA	38.5 ± 1.4 bBC	31.5 ± 0.6 deC
LM12	40.8 ± 3.1 cdA	31.5 ± 1.3 fgB	31.0 ± 1.6 bcB	31.5 ± 0.6 deB
LM13	132.2 ± 2.6 aA	74.8 ± 2.5 bB	57.8 ± 1.7 aC	57.2 ± 1.4 bC

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

3.2. Parameters' Yield

3.2.1. Fresh Forage and Dry Matter Yields

Fresh forage and dry matter yields (Table 6) showed significant effects of accessions ($p < 0.001$) and cuts ($p < 0.001$), as well as their interaction ($p < 0.001$). Fresh forage yield ($t\ ha^{-1}$) in all accessions did not exceed the threshold of $0.80\ t\ ha^{-1}$ in any cut, with the exception of LM13 and LM4, which easily exceeded the threshold across all cuts. During the first cut, LM13 ($1.75 \pm 0.04\ t\ ha^{-1}$) showed the highest average, being statistically superior to LM4 ($1.01 \pm 0.05\ t\ ha^{-1}$) by 73.27% in fresh forage. However, after the second cut onwards, LM4 and LM13 consistently showed the highest averages without statistical difference and with a sustained upward trend between cuts, where LM4 and LM13 increased by 13.2 and 10.1 times more than in the first cut. For dry matter yield ($t\ ha^{-1}$), a similar pattern was observed, with the same LM4 and LM13 accessions standing out with values exceeding $0.200\ t\ ha^{-1}$ and a tendency to increase between cuts, showing values of 3.751 ± 0.219 and $2.888 \pm 0.288\ t\ ha^{-1}$ in the fourth cut, with an increase of 13.98 and 9.86 times compared to their initial value in the first cut. Furthermore, there was no statistically significant difference between the two accessions in the third and fourth cuttings. Overall, LM4 and LM13 are among the alternatives with the highest biomass for silage production, showing an upward trend with each cut.

Table 6. Evaluation of fresh forage ($t\ ha^{-1}$) and dry matter yield ($t\ ha^{-1}$).

Accessions	Fresh Forage Yield ($t\ ha^{-1}$)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	0.30 ± 0.02 dB	0.12 ± 0.01 eC	0.61 ± 0.01 bA	0.72 ± 0.05 cA
LM2	0.15 ± 0.01 eC	0.13 ± 0.01 eC	0.30 ± 0.02 cB	0.43 ± 0.03 deA
LM3	0.06 ± 0.00 fC	0.16 ± 0.01 deB	0.29 ± 0.03 cA	0.36 ± 0.02 eA
LM4	1.01 ± 0.05 bC	4.26 ± 0.26 aB	15.20 ± 0.78 aA	13.41 ± 0.69 aA
LM6	0.38 ± 0.02 cdC	0.49 ± 0.02 bB	0.60 ± 0.03 bB	1.00 ± 0.03 bA
LM7	0.15 ± 0.00 eC	0.24 ± 0.02 cB	0.54 ± 0.11 bA	0.51 ± 0.01 dA
LM8	0.42 ± 0.03 cA	0.23 ± 0.02 cC	0.33 ± 0.01 cAB	0.32 ± 0.02 eB
LM11	0.17 ± 0.00 eC	0.19 ± 0.01 cdC	0.25 ± 0.02 cB	0.32 ± 0.02 eA
LM12	0.34 ± 0.02 cdB	0.48 ± 0.02 bA	0.28 ± 0.05 cB	0.31 ± 0.01 eB

LM13	1.75 ± 0.04 aC	5.46 ± 0.11 aB	19.98 ± 0.43 aA	17.68 ± 0.79 aA
Accessions	Dry Matter Yield (t ha ⁻¹)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	0.058 ± 0.004 cdB	0.023 ± 0.002 gC	0.142 ± 0.01 bA	0.182 ± 0.016 bA
LM2	0.047 ± 0.004 deC	0.036 ± 0.001 fC	0.072 ± 0.003 dB	0.100 ± 0.008 cdA
LM3	0.011 ± 0.001 gC	0.043 ± 0.001 efB	0.073 ± 0.004 dA	0.077 ± 0.005 deA
LM4	0.268 ± 0.015 aC	1.218 ± 0.107 aB	4.327 ± 0.212 aA	3.751 ± 0.219 aA
LM6	0.087 ± 0.008 bB	0.092 ± 0.006 bcB	0.165 ± 0.014 bA	0.215 ± 0.016 bA
LM7	0.031 ± 0.002 fC	0.06 ± 0.002 deB	0.120 ± 0.026 bcA	0.112 ± 0.003 cA
LM8	0.098 ± 0.010 bA	0.079 ± 0.007 cdA	0.080 ± 0.004 cdA	0.082 ± 0.003 cdeA
LM11	0.032 ± 0.002 efC	0.044 ± 0.004 efB	0.059 ± 0.004 dA	0.069 ± 0.008 eA
LM12	0.082 ± 0.003 bcB	0.122 ± 0.014 bA	0.072 ± 0.01 dB	0.074 ± 0.004 deB
LM13	0.292 ± 0.010 aC	0.870 ± 0.045 aB	3.107 ± 0.158 aA	2.888 ± 0.288 aA

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

3.2.2. Daily Accumulation Rate and Dry Matter Percentage

The average daily accumulation rate of dry matter and dry matter percentage showed significant effects of accessions ($p < 0.001$) and cuts ($p < 0.001$), as well as their interaction ($p < 0.001$), as shown in Table 7. The highest dry matter percentage values were recorded in LM4 in all cut, being statistically higher than LM13. However, it did not differ from LM2 in the first cutting, LM3 and LM8 in the second cut, and in the third and fourth cut only showed differences with LM13. Based on the average daily accumulation rate LM4, LM6, LM8, and LM13 showed the highest accumulations, but did not differ from LM12 during the first cut. Likewise, LM4 and LM13 showed the highest values from the first to the fourth cut in a stable manner between cuts with statistically higher values compared to the other accessions from the second cut onwards.

Table 7. Evaluation of daily accumulation rate and percentage of dry matter.

Accessions	Dry Matter (%)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	19.6 ± 0.91 cdB	19.0 ± 2.09 cdeB	23.3 ± 1.44 aAB	25.6 ± 1.94 aA
LM2	31.8 ± 1.49 aA	27.3 ± 2.38 bAB	24.0 ± 1.90 aB	23.4 ± 1.93 aB
LM3	19.5 ± 0.40 cdC	28.1 ± 1.94 abA	25.7 ± 1.95 aAB	21.7 ± 0.37 abBC
LM4	26.7 ± 1.01 abA	28.5 ± 0.79 abA	28.5 ± 0.62 aA	28.0 ± 0.55 aA
LM6	22.5 ± 1.07 bcdAB	18.8 ± 1.75 deB	27.2 ± 1.44 aA	21.5 ± 1.14 abB
LM7	21.1 ± 0.90 bcdA	25.1 ± 1.97 bcdA	22.2 ± 0.48 abA	22.1 ± 0.38 abA
LM8	23.5 ± 1.84 bcdB	34.7 ± 1.38 aA	24.2 ± 1.32 aB	26.1 ± 1.21 aB
LM11	18.8 ± 0.66 cdA	23.2 ± 1.42 bcdA	23.9 ± 1.18 aA	21.5 ± 1.16 abA
LM12	24.4 ± 0.97 bcA	25.6 ± 2.37 bcA	26.6 ± 3.00 aA	24.2 ± 1.58 aA
LM13	16.8 ± 0.53 dA	15.9 ± 0.60 eA	15.5 ± 0.48 bA	16.2 ± 0.94 bA
Accessions	Dry Matter Accumulation Rate (kg ha ⁻¹ day ⁻¹)			
	First Cut	Second Cut	Third Cut	Fourth Cut
LM1	1.4 ± 0.1 bcB	0.7 ± 0.1 eC	3.6 ± 0.4 bcA	4.5 ± 0.5 bcA
LM2	1.0 ± 0.1 cdB	0.9 ± 0.0 deB	2.1 ± 0.1 deA	2.7 ± 0.2 deA
LM3	0.3 ± 0.0 fC	1.1 ± 0.1 cdB	2.0 ± 0.1 deA	1.9 ± 0.2 eA
LM4	2.2 ± 0.2 aC	13.9 ± 1.3 aB	66.1 ± 2.9 aA	53.6 ± 3.1 aA
LM6	2.4 ± 0.3 aC	2.8 ± 0.3 bcC	4.5 ± 0.7 bB	6.2 ± 0.4 bA
LM7	0.6 ± 0.1 eC	1.5 ± 0.1 cB	3.1 ± 0.6 bcdA	3.1 ± 0.1 cdA
LM8	2.8 ± 0.3 aA	2.8 ± 0.3 bA	2.8 ± 0.2 cdA	2.8 ± 0.1 deA

LM11	0.7 ± 0.0 deC	0.9 ± 0.1 deC	1.5 ± 0.2 eB	2.2 ± 0.2 deA
LM12	2.0 ± 0.2 abB	3.9 ± 0.5 bA	2.4 ± 0.4 deB	2.4 ± 0.1 deB
LM13	2.2 ± 0.0 aC	11.7 ± 0.8 aB	53.8 ± 2.3 aA	50.7 ± 5.7 aA

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

3.3. Nutritional Composition

3.3.1. Acid Detergent Fiber and Neutral Detergent Fiber

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) showed significant effects of accessions ($p < 0.001$) and cuts ($p < 0.001$), as well as their interaction ($p < 0.05$) (Table 8). In general, LM8 (together with LM12) consistently presented the lowest ADF values, suggesting a less fibrous profile, while LM7 recorded the highest ADF values and showed no differences between cuts; in the fourth cut, LM3 did not differ from LM7. Regarding NDF, the highest values were observed in LM3 and LM13, indicating generally more fibrous accessions, with increases from the second to the fourth cut. Taken together, these patterns differentiate accessions with lower fiber (LM8 and LM12) from those with higher fiber (LM7 and LM3 for FDA; LM3 and LM13 for NDF) throughout the harvests.

Table 8. Evaluation of neutral detergent fiber and acid detergent fiber.

Accessions	ADF (%)	
	Second Cut	Fourth Cut
LM1	28.4 ± 0.30 cdB	30.3 ± 0.23 bA
LM2	29.3 ± 0.54 bcA	30.0 ± 0.36 bcA
LM3	30.9 ± 0.53 bB	33.4 ± 0.75 aA
LM4	27.0 ± 0.51 dB	28.2 ± 0.64 cdA
LM6	29.6 ± 0.58 bcA	30.5 ± 0.49 bA
LM7	33.3 ± 0.13 aA	34.4 ± 0.33 aA
LM8	24.5 ± 0.23 eA	24.7 ± 0.22 eA
LM11	28.0 ± 0.06 cdB	31.1 ± 0.64 bA
LM12	26.6 ± 0.32 deA	26.2 ± 0.27 deA
LM13	28.3 ± 0.45 cdA	29.2 ± 0.64 bcA
Accessions	NDF (%)	
	Second Cut	Fourth Cut
LM1	53.9 ± 0.19 bcdA	55.1 ± 0.36 bcdA
LM2	52.5 ± 0.71 cdA	53.1 ± 0.21 dA
LM3	57.1 ± 0.14 aB	58.6 ± 0.44 aA
LM4	52.9 ± 0.35 bcdA	54.1 ± 0.75 cdA
LM6	54.2 ± 0.52 bcB	56.4 ± 0.64 bcA
LM7	54.0 ± 0.58 bcdB	55.4 ± 0.19 bcA
LM8	50.1 ± 0.11 eA	50.7 ± 0.47 eA
LM11	53.7 ± 0.35 bcdA	54.3 ± 0.30 cdA
LM12	51.8 ± 0.32 deA	50.5 ± 1.09 eB
LM13	54.8 ± 0.32 abB	57.0 ± 0.51 abA

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

3.3.2. Protein and In Vitro Digestibility

Protein content and in vitro digestibility showed significant effects of accession ($p < 0.001$) and cut ($p < 0.001$), as well as their interaction ($p < 0.05$), as shown in Table 9. Overall,

LM4 presented the most favorable profile, registering the highest crude protein values and high in vitro digestibility in both cuts. Crude protein remained unchanged from the second to the fourth cut in LM4, LM8, LM12, and LM13, while it decreased in the remaining accessions, with LM3 showing the lowest protein content in both cuts. Regarding digestibility, most accessions showed no change between cuts; however, it decreased from the second to the fourth cut in LM2, LM7, and LM12. Although LM8 showed the highest digestibility, not differing from LM4 in both cuts, LM7 registered the lowest in both cuts.

Table 9. Protein and digestibility assessment.

Accessions	Protein (%)	
	Second Cut	Fourth Cut
LM1	17.4 ± 0.05 dA	15.0 ± 0.25 deB
LM2	14.0 ± 0.47 fgA	12.5 ± 0.32 fB
LM3	12.6 ± 0.09 gA	10.5 ± 0.30 gB
LM4	24.2 ± 0.62 aA	24.0 ± 0.38 aA
LM6	17.0 ± 0.48 deA	15.9 ± 0.42 dB
LM7	13.7 ± 0.35 fgA	11.4 ± 0.34 fgB
LM8	20.2 ± 0.51 bcA	19.5 ± 0.50 bcA
LM11	15.3 ± 0.48 efA	13.2 ± 0.29 efB
LM12	18.4 ± 0.50 cdA	17.9 ± 0.59 cA
LM13	21.1 ± 0.65 bA	20.4 ± 0.49 bA
Accessions	In vitro digestibility (%)	
	Second cut	Fourth cut
LM1	78.7 ± 0.37 cdA	79.3 ± 0.47 cA
LM2	81.0 ± 0.36 cA	79.2 ± 0.33 cB
LM3	78.0 ± 0.15 dA	77.7 ± 0.56 cdA
LM4	86.2 ± 0.20 abA	85.0 ± 0.94 abA
LM6	80.0 ± 0.56 cdA	79.6 ± 0.46 cA
LM7	78.2 ± 0.40 dA	76.8 ± 0.75 dB
LM8	88.0 ± 0.36 aA	87.2 ± 0.51 aA
LM11	78.5 ± 0.20 dA	78.6 ± 0.35 cdA
LM12	85.9 ± 0.38 abA	83.8 ± 0.32 bB
LM13	85.1 ± 0.54 bA	84.0 ± 0.52 bA

Note. Means ± standard error of the sample mean is presented. Different lowercase letters in the same column indicate significant differences between accessions within each cut, while different uppercase letters in the same row indicate significant differences between cuts for the same accession.

4. Discussion

Our study revealed high variability in basal coverage area among accessions within each cut [53]. This could explain why accessions LM4 and LM13 exhibited the highest performance at the end of the fourth cut, exceeding the basal coverage area recorded in the first cut by more than eight times. This increase may also be linked to a high adaptive capacity to the thermal regime [24]. Among the advantages that accessions LM4 and LM13 could offer by presenting greater basal coverage area is weed suppression, helping to mitigate competition for resources [54,55]. They could also contribute to reducing soil erosion [56,57].

With regard to days to anthesis, accessions LM4 and LM13 exceeded 120 days in the first cut, exhibiting a decreasing trend until stabilizing in the third cut. In contrast, accessions LM1, LM3, LM6, and LM8 showed phenological stability throughout the cuts with shorter days. The record of longer and shorter days until anthesis in our study can be attributed to genetic variation in the reproductive phenology of each accession, influenced by sensitivity to photoperiod [58]. This variation in days to anthesis is relevant because it

allows early and late accessions to be differentiated, which is useful for selection and improvement aimed at synchronizing flowering with cutting windows and improving production stability between harvests under tropical Andean conditions [59–61].

In turn, the origin of the accessions conditions the type of inductive requirement [62]. For example, Cooper [63] revealed that 50% of the population of *Lolium multiflorum* Lam. requires induction by cold and short days, while the remaining 50% shows a quantitative response with no induction. In this context, the behavior recorded in LM4 and LM13 could be associated with obligate or partial vernalization requirements. This could be supported by the initial delay and subsequent stabilization, indicating that the requirements were progressively met after cumulative exposure to winter conditions during successive cuts, allowing for a faster reproductive transition in subsequent cycles. This could be corroborated by the study by Adhikari et al. [25] with the selection of plants and the crossing of early and late groups in *Lolium* species, where they observed variations that differed by up to 28 days between populations. Our results allowed us to identify the phenological cycle of each accession under the conditions of the Tropical Andes, facilitating the planning of cuts and conservation. In this regard, LM4 and LM13 represent a late group with a subsequent sharp reduction in time to anthesis, while LM1, LM3, LM6, and LM8 maintain early and consistent behavior, information that can be used in selection schemes and management decisions.

Regarding fresh forage yields, our study showed that, in accessions that were later to anthesis, yields were significantly higher, with a fourth cut yield 13.2 and 10.1 times higher than the value recorded in the first cut in accessions LM4 and LM13. These results are consistent with the study by Choi [64], which indicates that the longer the days until anthesis, the longer the active growth period, leading to greater light interception and biomass accumulation. He also points out that the most optimal cuts for greater productivity of late-cycle *Lolium multiflorum* Lam. are during the heading and flowering stage, with no significant differences, at which point there is a balance between quantity and quality. Previous research has also shown that greater plant height is associated with higher fresh forage yields [65,66]. This is consistent with our results, where the greatest plant height recorded between cuts was observed in LM4 and LM13. In addition, LM4 and LM13 obtained the highest daily dry matter accumulation rates in a stable manner between cuts. This is supported by the study by Gaytán Valencia et al. [46], who obtained higher fresh forage and dry matter yields in *Medicago sativa* L. when cutting at four weeks, showing a higher daily accumulation of dry matter compared to cutting at three weeks. On the other hand, yield according to the planting method may vary [67]. Based on our study, it has the limitation that only the vegetative propagation method was used, which implies future studies in the same local accessions using different propagation methods. Among the qualitative traits shared by LM4 and LM13 was the dark green color of their leaves. This characteristic could be associated with genetic or environmental factors, which play an important role in leaf senescence by interfering with yellowing through alteration of the chlorophyll decomposition pathway, allowing stay-green genotypes to remain green longer and part of the photosynthetic apparatus (thylakoid membrane proteins) to remain intact for longer [68].

In general terms, dry matter yield (t ha^{-1}) was less than 0.200 t ha^{-1} for most accessions and cuts, with the exception of the results presented in LM4 and LM13. According to studies in local accessions of *Lolium multiflorum* Lam. under chemical fertilization ($160\text{-}130\text{-}66 \text{ kg of N-P}_2\text{O}_5\text{-K}_2\text{O ha}^{-1} \text{ year}^{-1}$) dry matter yields were obtained that ranged between 3.64 and 4.49 t ha^{-1} with cuts made every 60 days [31]. Based on our results, yields of 3.7 and 2.8 t ha^{-1} were obtained at 57.2 and 70 days to anthesis in the fourth cutting, competitive ranges close to those obtained with synthetic fertilization, with stability from the third cutting onward under organic fertilization. These findings are promising, considering that

chemical fertilization with nitrogen (N) could double biomass yields, as reported by Vásquez et al. [43] in the INIA 910—Kumymarca variety at a N dose of 180 kg ha⁻¹. Consequently, LM4 and LM13 are emerging as the accessions with the greatest biomass yield potential in the Tropical Andes. Our results suggest future research evaluating the response of LM4 and LM13 to different nitrogen gradients, in order to enhance key physiological components for performance such as tiller density and leaf area index [69].

The percentage of dry matter varied between accessions and cuts. The LM4 accession presented the most stable values between cuts, with values ranging from 26.7 to 28.5%, higher than LM13. In terms of ensilability, dry matter contents below 25% (equivalent to 75% moisture) are associated with a higher risk of poor fermentation, high pH, and effluent losses during ensiling; while values between 25% and 30% dry matter ($\geq 70\%$ moisture) still reflect a wet raw material and correlate with greater damage due to bacterial proliferation and deterioration of nutritional quality under certain conditions [70,71]. In our study, most accessions harvested at anthesis showed dry matter values below the recommended levels, except for LM2 in the first cut and LM8 in the second, confirming a high moisture content in the raw material. Therefore, strategies such as pre-wilting and the use of inoculants (e.g., *Lactobacillus plantarum*) may be necessary to increase dry matter and improve silage quality, as reported by Lio et al. [72]. From a practical standpoint, accession LM4, which has higher dry matter content and lower moisture content than the other accessions, may require a shorter pre-wilting period to achieve suitable silage conditions, potentially shorter than for more moist accessions. This management can be complemented with inoculants to promote more stable fermentation and obtain better quality silage.

Nutritional analysis revealed the highest acid detergent fiber (ADF) contents in LM7 ($33.3 \pm 0.13\%$ and $34.4 \pm 0.33\%$). These results were similar to those reported by Yavuz et al. [73] in the evaluation of ryegrass lines, where they obtained values ranging from 31.41 to 34.75% ADF using the half-sib family selection breeding method. Likewise, among accessions, it was observed that certain accessions increased the percentage of ADF in the fourth cut, while others remained stable. This could be related to the genetic expression of each accession in response to seasonal changes or environmental variability [74]. It should be noted that our study was limited to nutritional composition evaluations during anthesis only. The literature mentions that during this stage, plant senescence is greater, evidencing greater lignification, which could have an impact on the increase in neutral detergent fiber (NDF) [75]. Based on our findings, NDF values exceeded 50% in all our accessions. In contrast, Alende et al. [76] reported values below 46.05% in intermediate tetraploids and short-cycle diploids of *Lolium multiflorum* Lam. On the other hand, there are studies that indicate that rumination time is quadratically related to the NDF concentration in the diet of cattle and to digestibility, indicating that NDF regulation affects ruminal function [77]. The results obtained in our study serve as a starting point for future evaluations, with the aim of reducing the NDF content above 50% in all accessions, to fill the knowledge gap on the performance of these accessions in evaluations with earlier cuts before anthesis.

Based on protein content, accession LM4 presented the highest stable values among cuts, with figures ranging between 24.2 and 24% protein. However, these values may vary depending on topography [78] and under different silvopastoral systems in interaction with the season [79]. In addition, it has been reported that some lines of *Lolium multiflorum* Lam. may show different responses (high or low) to nitrogen uptake, although these differences are not always consistent and may be reversed in subsequent cycles [80,81]. This behavior could have contributed to the variability observed in our experiment, as nitrogen is directly related to increased protein content in *Lolium multiflorum* Lam. Plants [82]. In this sense, N use efficiency can translate into differential responses in yield and nutritional quality depending on the availability of nitrogen in the soil, and would be conditioned by traits such as plant architecture and root biomass development [83,84].

The results shown in our study on LM4 are more remarkable than studies with phosphate fertilization applications, which showed protein values of 17.87% in *Panicum maximum* [85]. Similarly, it was higher than the results presented in the evaluation of six forage grasses, where the highest value reached 14.23% [86]. However, the high protein content observed in LM4 could make it susceptible to greater protein degradation during the silage process, but the higher percentage of dry matter (%) compared to the other accessions could contribute to moderating proteolysis and, consequently, reducing the formation of soluble nitrogen during silage [87]. Studies indicate that intrinsic plant proteases can initiate the early stages of forage proteolysis after cutting, even in the absence of ruminal microorganisms, contributing to the initial formation of peptides and soluble nitrogen [88]. This suggests that the outstanding protein and dry matter values in LM4 need to be further studied in evaluations at each stage of silage, and how this affects its performance in terms of its contribution to quality.

On the other hand, digestibility in LM4 and LM8 remained stable in the second and fourth cut, with statistically high values. These results are promising, suggesting a possible trend toward increased meat and milk production if these values remain stable during ensiling [89]. It should be noted that a high lignin content can affect digestibility, acting as a physical barrier to microbial degradation [90]. Furthermore, the results obtained in our study were superior to those of other local grasses [78]. This reveals the broad potential of local *Lolium multiflorum* Lam. accessions in the Tropical Andes, based on LM4.

One of the main limitations of this study is that it was conducted during a single annual cycle (July 2024 to June 2025) and at a single location, so the consistency of the trends observed could vary in years with different precipitation patterns or at sites with different Andean elevations. In addition, the evaluation was restricted to four cuts, so longer trials are required to corroborate the stability of each accession over time. Furthermore, the forage was evaluated only as raw material, without considering its behavior during the silage process, which highlights the need for additional studies that include the preparation and evaluation of silage to more accurately quantify its contribution to quality. Taken together, these results constitute a preliminary reference for the performance of local accessions under the agroecological conditions evaluated in the Tropical Andes and lay the foundation for future research.

5. Conclusions

Agronomic performance was significantly higher in LM4 and LM13. Accession LM4 had the highest plant height at the fourth cut (57.7 ± 0.6 cm), which was 2.48 times higher than at the first cut. In terms of days to anthesis, LM4 and LM13 were the latest accessions, with values decreasing at the third cut and remaining stable in subsequent cuts, along with greater basal coverage area than the other accessions between cuts. Compared to the first cut, fresh forage yield in LM4 and LM13 increased 13.2 and 10.1 times during the fourth cut; likewise, dry matter yield increased 13.98 and 9.86 times, with a tendency to increase between cuts. This was associated with a higher average daily accumulation of dry matter. However, LM13 had a low percentage of dry matter compared to LM4, being lower in all cuts. In addition, LM4 showed a stable trend between cuts, with no variability in the percentage of dry matter. In terms of nutritional content, LM4 showed superiority in protein content between the second and fourth cuts, with stability between the two. It also revealed the greatest stability and highest digestibility values between cuts. Overall, LM4 was identified as a promising candidate for future evaluations as a silage feedstock under field conditions, including assessments throughout the process; in addition, it requires evaluations in multiple environments and over several years to confirm the extrapolation of our data. Given the relatively high moisture content of the harvested biomass in some accessions, wilting prior to ensiling is recommended to achieve an optimal dry

matter range and reduce the risk of effluent losses and suboptimal fermentation. LM4 appears to be the option closest to the optimum moisture point, thus reducing wilting time.

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