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Sustainability of *Lolium multiflorum* L. 'Cajamarquino Ecotype', Associated with *Trifolium repens* L., at Three Cutting Frequencies in the Northern Highlands of Peru

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Abstract: Livestock farming feed in the northern highlands of Peru is based on the association of ryegrass (*Lolium multiflorum* L.) ecotype cajamarquino–white clover (*Trifolium repens* L.) Ladino variety, which constantly varies in its agronomic characteristics and nutritional value due to management considerations and its association with the soil and the animal. The objective of this study was to evaluate yield, plant height, growth rate, tillering, tiller number, spikelets, basal diameter in ryegrass, elongation rate, internode length and decline points in clover over one year. Nutritive value was represented by crude protein (CP), neutral detergent fibre (NDF), in vitro digestibility of dry matter (IVDDM) and metabolisable energy (ME) at three cutting frequencies (30, 45 and 60 days). Better yield (5588 kg DM ha) and plant height (47.1 cm) were shown by the 60-day cutting frequency; however, there was no difference ($p > 0.05$) between the three cutting frequencies in annual yield. There were no differences between the number of tillers and basal diameter. Clover height, elongation rate and internode length were higher at 60 days. The highest CP concentration and the lowest NDF value ($p < 0.05$) were achieved by clover at 30 and 45 days. Producers should consider the results when deciding when to use this association in dairy cattle feeding.

Keywords: cajamarquino ecotype; botanical composition; cutting frequencies; nutritional value; *Trifolium repens*

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1. Introduction

With the establishment of the 'Nestlé' milk collection company in the Cajamarca region in the mid-20th century [1], an important economic source was created for rural families in the northern highlands of Peru, whose extensive areas of *Lolium multiflorum* L. and *Trifolium repens* L. and proximity to the equator have provided favourable climatic conditions for the development of this dairy activity. Thus, there are 173,517 cows in production and 401,010 tonnes of milk per year, with an average yield of 6.3 kg per cow per day [2], which is low considering the good genetic potential of cattle in a grazing system. In this context, it has been observed for several decades that the consumption of this association by dairy cattle takes place at an advanced stage of maturity (50 to 90 days of regrowth), which affects not only the milk yield of the cows but also the productive behaviour, nutritional value and agronomic characteristics of these pastures, not to mention the contribution to the greenhouse effect through increased production of methane gas [3].

It is essential, therefore, to recognise that pastures serve to control soil carbon cycling [4] and nutrient recycling, mainly of nitrogen [5]—nutrients that remain accumulated in soils for up to decades [6], forming a soil–pasture interaction suitable for the grass–legume association environment [7].

This grass–legume association, characterised by complementing each other very well in terms of yield and nutritional value [8–10], has remained in most of the farms in this area for several decades, without being renewed, and is, therefore, considered a perennial life cycle grass and identified as *Lolium multiflorum* L. "cajamarquino ecotype". The adaptation and persistence of this grass in the northern highlands of Peru is due to the low frequency of grazing or cutting that is practised, which is done during the reproductive stage. In some of the high Andean plains of our region, it is grazed 90 days after regrowth [11].

The coexistence between grasses and legumes, in combination with the frequency of cutting, affects the morphological and productive behaviour of Italian ryegrass [12,13], as well as its botanical composition, particularly when cutting or harvesting is completed after 28 days; it is recommended that this frequency 42 days [14] when its yield is positively correlated with the mass of stolons. Long intervals between cuts allow for advanced pasture maturity, reflected in increased fibre [13] and higher aerial biomass yield. Still, this allows for low protein concentration, digestibility and energy [15–17], due to wall thickening and reduced cell content [18,19].

In addition to pasture management, the climatic characteristics of the time of year also affect the productive performance of the ryegrass–white clover association ([20–22], with ryegrass showing more limitations in its growth during drought events [23] compared to white clover, which, thanks to its variability, responds with visible phenological changes to new environmental conditions, competing successfully against other plants [24,25]. White clover is characterised by its essential contribution of protein in the diet of animals [18] and by its capacity to fix nitrogen and improve the productivity of this association [25]; however, it has been found that values lower than 20% of white clover in associated pastures seriously affect these indicators, in addition to restricting the total daily intake of nutrients. The presence of white clover in pastures should not be above 50% to meet the nutritional requirements of animals and the release of nitrogen to the environment [26,27].

For Tilus et al. (2022) [13], forage yield increases linearly as the time of cutting is prolonged. Thus, yields have been obtained in this association of 1470 kg DM ha^{-1} when this is conducted around 10 cm in height with a remnant greater than 4 cm [8], 2000 kg DM ha^{-1} if the height is 13 cm, and 2781 kg DM ha^{-1} if the ryegrass is 29.8 cm [28], and 3974 kg DM ha^{-1} when the height is 20 cm high and post-grazing remnant is 5 cm [29]. For white clover, heights of 22 cm have been found when cutting every 60 days [30]. Cumulative yields ranged from 13,039 to 27,290 kg DM ha^{-1} $year^{-1}$ [8,22,29,31].

Taking into account that the chemical composition presents a high variability due to the time of harvest or maturity stage [18], when evaluating ryegrass independently, values of 10.01 to 14.67% CP; 37.64 to 47.52% NDF, 67.65 to 71.04% IVDDM, and 2.36 to 2.49 Mcal kg^{-1} DM of ME have been obtained [32,33]. In white clover, concentrations of 24.8% CP and 40.9% NDF were found for five cutting frequencies and three cuts of 20.6% CP and 42.9% NDF were obtained [16]. When Ladino white clover was cut between 16 to 18 cm in height, Vallejos et al. (2021) [30] found 23.06% CP, 11.6% ash, 25.8% NDF, and 75.04% IVDDM. In this same species, Fonseca-López et al. (2020) [34] found 10.9% ash.

As part of the agronomic characteristics of this association, Ganderats and Hepp (2003) [35] obtained 3368 tillers m^2 and Balocchi et al. (2013) [36] found 5800 to 7000 tillers m^2; this quantity probably influences the greater basal diameter. In this regard, Han et al. (2022) [37] add that to increase the number of tillers and basal diameter of ryegrass, the soil must be well managed so that the microbial population develops well. In white clover, Dous-soulin et al. (2018) [38] found no difference in stolon elongation rate (0.3–0.5 cm day^{-1}), and Lluga-Rizani et al. (2021) [24] obtained values from 9 to 60 flower heads. When the mowing frequency is between 56 and 84 days, a marked reduction in white clover is observed in the floristic composition [14], probably due to the shading generated by the ryegrass [8,10]. Conversely, high mowing frequencies favour ryegrass and make white clover more competitive [39]. Although Vallejos (2009) [40] finds no difference between mowing frequencies about weeds, these can vary between 17.6 and 36.3% [41]. The objective of this work was to determine the sustainability for production yield, forage biomass, plant height, floristic composition (basically ryegrass, clover and weeds), morphology of ryegrass and white clover in association and nutritive value, at different phenological stages of associated ryegrass-clover pastures in the northern highlands of Peru.

2. Materials and Methods

2.1. Location

The research was carried out at the Centro de Investigación y Promoción Pecuaria "Huayrapongo" of the Universidad Nacional de Cajamarca, Peru (Latitude 07º09′49″ S, Longitude 78º30′00″ W) located at 2718 masl; it covered the period from May 2018 to April 2019, in an area of 10 hectares (Figure 1). The pastures were installed approximately 40 years ago, and reseeding is done every five years for ryegrass and clover. A randomized complete block experiment was carried out, where three ages of poly pasture cut (30, 45 and 60 days old); for this, 12 plots of 30 m^2 each were selected for each treatment and evaluated for a whole year in the two agro-meteorological stations present in the study area.

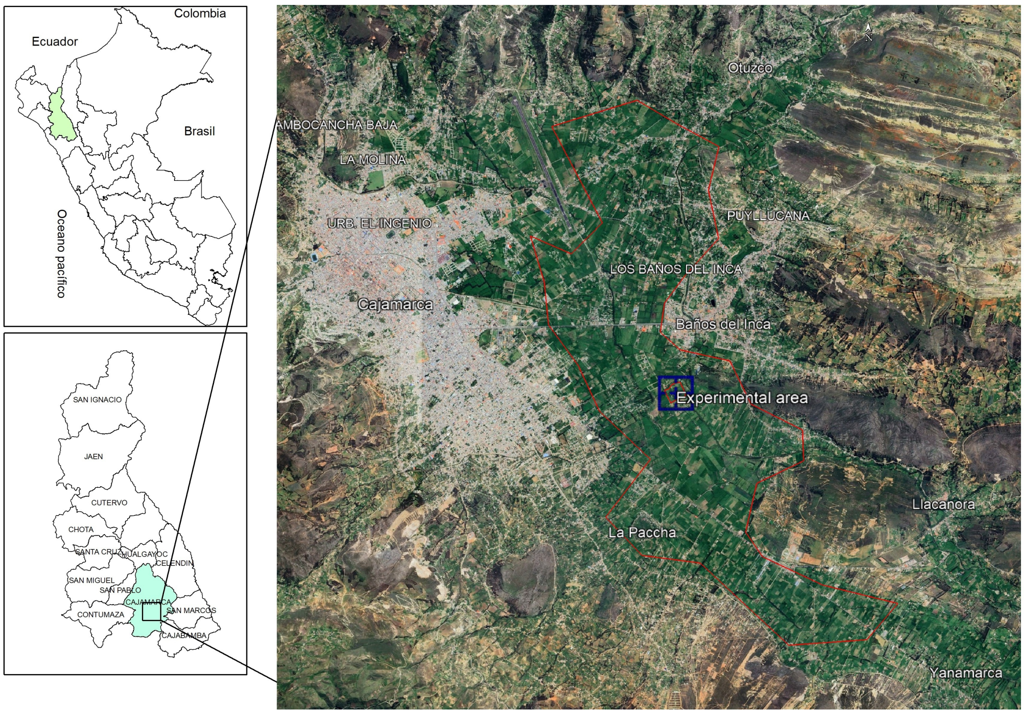

Figure 1. Ubication and direct area of influence of the experiment.

2.2. Soil Characteristics and Weather Conditions

The pasture corresponded to an association of Italian ryegrass (*Lolium multiflorum* L.) "cajamarquino ecotype"–white clover (*Trifolium repens* L.) var. Ladino, which has a marked presence of Kikuyu (*Pennisetum clandestinum*), chicory, and wild plantain. Soil analysis indicated clay texture, neutral pH 6.8, organic matter 8.04%, phosphorus 2.5 ppm, and 320 ppm potassium. The ambient temperature and rainfall conditions during the months of evaluation are detailed in Figure 2.

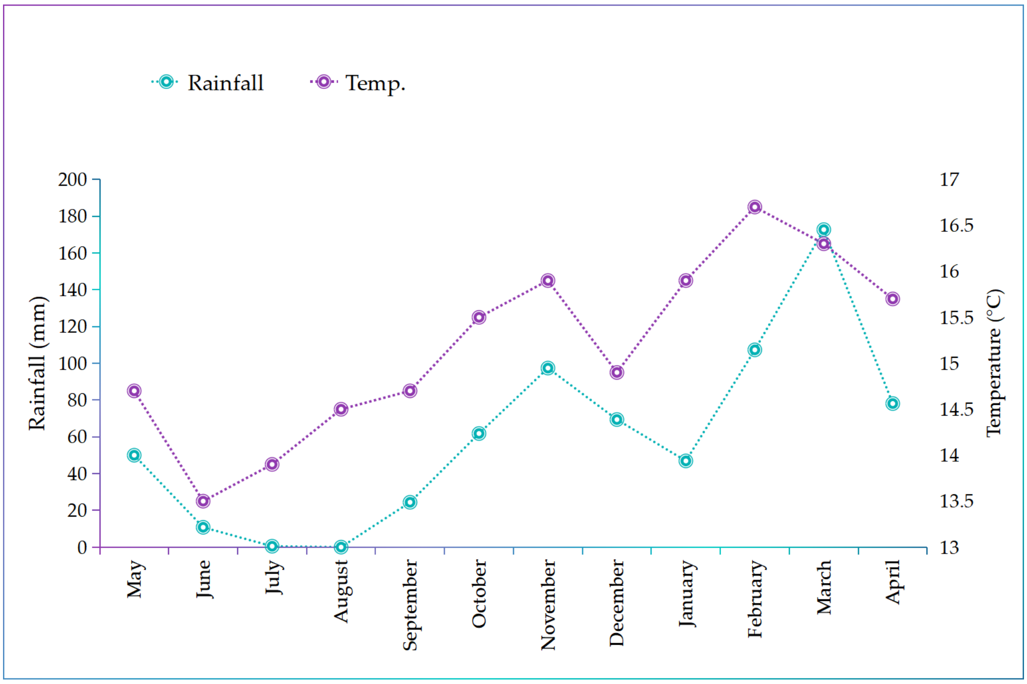

Figure 2. Average ambient temperature and rainfall during the assessment. Source: Senamhi (2024) [42].

As shown in Figure 2, the dry season from May to October and the rainy season from November to April are considered. With this, the two climatic seasons have been differentiated and are associated with fodder availability for livestock.

2.3. Sample Collection

One square metre of quadrats was placed inside each plot, obtaining three representative samples through the cut, 5 cm above the ground. The samples were placed in plastic bags, identified and weighed on an OHAUS electronic balance (±0.5 g), then transported to the Soil, Water, Fertilizer and Pasture Service Laboratory of the National Institute for Agrarian Innovation (INIA). Then, 100 g of each sample was used to determine the percentage of dry matter, in a forced air cooker (cooker equipment, MRC) at 105 °C for 24 h and constant weight, and 300 g of the samples of each treatment were separated for the analysis of the nutritional value.

2.4. Parameters Assessed

From the weights obtained from the green forage, the yield in kg of dry matter (DM) per cut and per year in one hectare was determined according to the cutting frequency. Twelve cuts were made during the year for 30-day frequencies, eight for 45-day frequencies and six for 60-day frequencies. From these values and the time elapsed in each cutting, the growth rate was expressed in kg DM ha^{-1} day^{-1}. Plant height was also measured for ryegrass and white clover. The height at the time of cutting or harvesting was measured within each quadrant, taking as a reference the ground level up to the maximum height at which the most significant number of leaves was concentrated (more than 70%). A 70 cm metal ruler was used.

The floristic composition (Figure 3) was quantified using the same samples obtained for yield, separating and classifying them according to the species present (ryegrass, clover and weeds). We considered the green forage weights of each of the species considered. For ryegrass morphology, the number of tillers and ears of ryegrass per square metre was considered, and these values were obtained by counting each one of them for each ryegrass plant contained in the 1 square metre quadrant in three representative places within the subplot. To determine the basal diameter, a tape measure (1.5 m) was used and placed around the base of the ryegrass plant. Depending on the treatments, stolon length and internode length were measured using a ruler for white clover morphology. The growing points per square metre were counted from where the leaves emerged along the stolons, and the number of flowers (flower heads) contained in the quadrants was counted.

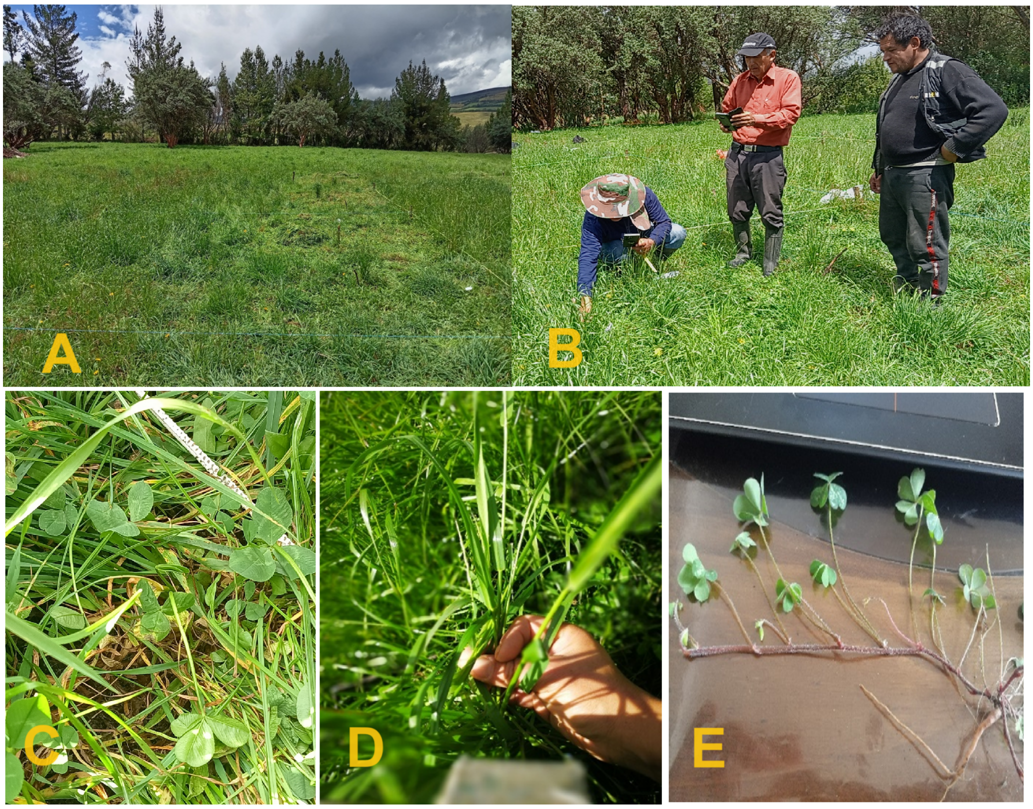

Figure 3. Photographs of the evaluated pastures. (**A**) Experiment location and plot delimitation. (**B**) Evaluation of the floristic composition and agronomic indicators of the ryegrass–white clover association. (**C**) Floristic composition of the ryegrass–clover association. (**D**) Evaluation and counting of ryegrass tillers. (**E**) Evaluation of buds and stolons of white clover.

2.5. Nutritional Value

The determination of crude protein was carried out at the Soil, Water, Fertilizer and Pasture Service Laboratory of INIA, Cajamarca, according to AOAC 928.08 [43]; NDF according to AOAC 962.09 [44] and ash according to AOAC 942.05 [45]. The determination of neutral detergent fibre [46] was carried out with a fibre analyzer kit, FIWE, VELP and the in vitro digestibility of dry matter–IVDDM with the digester kit (ANKOM, Macedon, NY, USA) [47]. These analyses were conducted at the Laboratory of Animal Nutrition and Food Bromatology of the National University Torîbio Rodriguez de Mendoza of Amazonas.

2.6. Statistical Analysis

An exploratory analysis of the data was carried out to determine normality and homogeneity of variances, using the Shapiro–Wilks ($p < 0.05$) and Levene ($p < 0.05$) tests, respectively. The Analysis of Variance (ANOVA) was carried out to determine the differences in dry matter yield, plant height and other morphogenetic characteristics corresponding to Italian ryegrass ecotype cajamarquino and white clover variety Ladino, as well as their nutritional value, using the GNU of the RStudio platform (v. 2024.04.2 Build 764) of the R Project [48]. For the comparison of means, Tukey's multiple range test was used ($p < 0.05$).

3. Results and Discussion

3.1. Productive Performance

The productive yield characteristics, considering the biomass and plant height of both desirable species, are shown in Table 1, where it is highlighted that the biomass shows differences in each cutting but not for the growth rate or biomass per day, as well as for the annual yield. Yield is not affected by cutting frequency but by the time of year. Plant height varies with mowing age, considering that the phenology of the plant is different for both ryegrass and clover.

Table 1. Productive performance of the ryegrass–white clover association, according to treatment and time of year.

Factors	Biomass (Kg × ha^{-1})			Plant Height (cm)	
	Day	**Cut**	**Year**	**Ryegrass**	**Clover**
Grazing frequency (days)					
30	84.37	2531.20 ^{c}	30,796.7	20.35 ^{c}	10.23 ^{c}
45	88.69	3991.00 ^{b}	32,371.7	32.54 ^{b}	14.54 ^{b}
60	91.85	5511.26 ^{a}	33,527	47.12 ^{a}	18.25 ^{a}
SE	3.83	138.91	1398.76	3.13	0.91
p value	*0.4562*	*0.0003*	*0.4565*	*0.0002*	*0.0001*
Time of year					
Rainy	96.11 ^{a}	4360.33	35,140.27 ^{a}	32.49	14.96
Dry	79.89 ^{b}	3648.22	29,592.55 ^{b}	34.18	13.7
SE	3.71	496.77	1036.54	4.8	1.41
p value	*0.008*	*0.328*	*0.0002*	*0.8071*	*0.542*

SE: standard error. Different letters in the column for each factor mean significant differences (HSD Tukey, $p < 0.05$).

Table 1 shows the highest biomass yield per cut at 60 days (5511.26 kg DM ha^{-1}), followed by grazing frequencies at 45 days and 30 days with 3991 kg DM ha^{-1} and 2531.20 kg ha^{-1}, respectively, thus confirming the excellent production generated by the association of these pastures coupled with the frequency of grazing [8–10,12]. The results obtained exceed the amounts found by Egan et al. (2018) [8], Rojas et al. (2016) [29] and López et al. (2021) [28], probably due to the different times at which the cuts or grazing were carried out, as well as the physicochemical and biological characteristics of the soil [13,37]. Meanwhile, the annual biomass (kg DM ha^{-1} yr^{-1}) obtained in our study shows that there is no significant difference ($p > 0.05$) among the three grazing frequencies, an essential result for producers in the northern highlands of the country since the values found (kg DM ha^{-1}) at 60 days of cutting was double those of 30 days, the annual production is the same. However, the quality of the pastures is better when they are less mature [18,19]. Gierus et al. (2012) [16] point out that the cutting frequency influences the yield of the ryegrass–white clover association; thus, the more significant number of cuts per year favours the productive behaviour of these associated pastures. This statement is confirmed by the study conducted, in which the yield per cut (kg DM ha^{-1}) obtained at 60 days is higher than at 30 and 45 days of cutting ($p < 0.05$); however, the accumulated yield (kg DM ha^{-1} yr^{-1}) is similar ($p > 0.05$), without considering the more significant contribution of kg of CP and ME concentration (kcal/kg DM) obtained with cutting frequencies of 30 and 45 days.

Our values exceed the annual yields of Mendoza et al. (2018) [22], Rojas et al. (2017) [29], Egan et al. (2018) [8] and Annicchiarico and Tomasoni (2010) [31], probably related to age due to the establishment of these pastures for several decades. The climatic conditions [20,21] that occur in the inter-Andean valley of the northern highlands of Peru are characterised by two well-defined climatic seasons during the year, rainfall and low water or drought, conditions that influence the productive behaviour of this forage association. From another perspective, when considering the time of year and the rainy season, the evaluated forage floor has a higher biomass accumulated per day, but there is no difference per cut. It should be considered that in rainy conditions, both the yield in kg DM ha^{-1} cut-1 and kg DM ha^{-1} year-1 was higher than the results referred by Claffey et al. (2019) [20], Tozer et al. (2014) [21] and Mendoza et al. (2018) [22]. So in the rainy season, the biomass productivity was higher than in the dry season by 18.74%, a period that lasts about six months, adding that the areas under study during the dry season were irrigated fortnightly by flooding, but rainfall water affects productivity.

The best height size (cm) for '*Lolium multiflorum* L.' was obtained ($p < 0.05$) at 60 days (47.12 cm), followed by 45 days with 32.54 cm and 30 days with 20.35 cm. Although the height increases as the plants mature, this level is not maintained, it decreases gradually. Thus, from 30 to 45 days of cutting, a 39.5% increase was obtained, and from 45 to 60 days, it was 28%. Considering that these results were higher than the values found by Tilus et al. (2022) [13], Egan et al. (2018) [8], Rojas et al. (2017) [29] and López et al. (2021) [28], probably due to the grazing age, season and soil management, as the evaluated pastures were established more than 30 years ago, as detailed in the methodology; this shows the sustainability of the pasture for dairy productivity. We must also give importance to the organic matter content of the soil [6], which is installed in the pasture.

Regarding white clover height (Table 1), values increase with maturity stage [18]; thus, at cutting frequency ($p < 0.05$) at 60 days, it reaches 18.25 cm, at 45 days it reaches 14.54 cm and at 30 days it is 10.23 cm. Expressing the superiority of height in percentage, the cutting frequency of 45 days over 30 days is 31%, and the cutting frequency of 60 days versus 45 days is 17%, concluding that as the maturity stage of the plant advances, the increase in height continues but with a gradual decrease, probably due to the genetic characteristics of the species and the phenology and reproductive cycle of the plant. Comparing the values of the study with those found by Vallejos et al. (2021) [30] of 21.95 cm, their results are lower, which may be due to soil and environmental conditions, among other factors, such as soil quality and management. During the two seasons of the year, no difference was found ($p > 0.05$); this considers that the plant can grow, and there is no difference considering the effect of the season, taking the average of the three grazing moments.

3.2. Plant Morphology

Table 2 shows the values for the number of tillers and ears, the basal diameter for ryegrass and the elongation rate, growing points, internode length and flower heads for white clover, considering mowing frequency as an evaluation factor. It is essential to consider aspects of morphology because they are related to the productivity and nutritional characteristics of the plant.

Table 2. Stolon elongation rate, growing points, internode length and number of flowers of white clover, according to cutting frequency.

Factors	***Lolium multiflorum* L.**			***Trifolium repens***			
	Number of Tillers	**Number of Ears**	**Basal Diameter**	**Elongation Rate (cm × day^{-1})**	**Growing Points (m^2)**	**Internode Length (cm)**	**Number of Flower Heads (m^2)**
Grazing frequency (days)							
30	101	3.54 ^{b}	34.00 ^{a}	0.44 ^{c}	15.33	0.91 ^{b}	22.02
45	174.89	11.30 ^{b}	44.11 ^{b}	0.59 ^{b}	20.0	1.32 ^{b}	42.11
60	128.44	30.61 ^{a}	43.44 ^{b}	0.89 ^{a}	24.0	2.28 ^{a}	44.44
SE	24.48	2.51	1.41	0.01	1.99	0.136	8.38
p value	*0.2136*	*0.0037*	*0.0122*	*0.0000*	*0.0871*	*0.0048*	*0.2310*

SE: standard error. Different letters in the column for each factor mean significant differences (HSD Tukey, $p < 0.05$).

In Table 2, it is observed that there is no significant difference ($p > 0.05$) in the number of tillers for ryegrass according to cutting frequency. Likewise, these values are lower than those found by Ganderats and Hepp (2003) [35] and Balocchi et al. (2013) [36], probably because it is not a continuous practice of producers to perform good soil management, which favours the pasture and the microbial population of the soil [37]. The number of ears was higher at 60 days ($p < 0.05$) because the pasture was approaching its maximum reproductive stage.

Our study on white clover growth points to the dynamic nature of plant responses. We observed that morphological changes continue until 60 days, with the highest values obtained at this frequency ($p < 0.05$), surpassing those at 45 and 30 days. This finding contrasts with the results of Doussoulin et al. (2018) [38], who obtained relatively lower levels, possibly due to the different cutting times [14]. The number of flower heads in our work falls within the range found by Lluga-Rizani et al. (2021) [24], suggesting that the morphological variability [25] of this species in different environmental conditions of the highlands could be a contributing factor. It is important to note that the age of the plant influences the length and growing points, but the aspects related to the elongation rate are primarily dictated by the environmental conditions.

3.3. Floristic Composition

The floristic composition shows the proportions of desirable species present, mainly composed of ryegrass, clover, and weeds, Kikuyu. As shown in Table 3, estimating the ratio between ryegrass and clover is essential, as estimated from each species' proportions.

Table 3. Floristic composition (%) of the ryegrass–white clover association according to grazing frequency and time of year.

Factors	Ryegrass	Clover	Weeds	Rate R:C
Grazing frequency (days)				
30	62.93 ^{ab}	20.21	16.81 ^{a}	3.34
45	60.49 ^{a}	21.66	17.61 ^{a}	3
60	74.94 ^{b}	16.67	8.61 ^{b}	5.47
SE	3.26	2.52	1.84	0.77
p value	*0.0174*	*0.3822*	*0.0074*	*0.0816*
Time of year				
Rainy	69.46	15.20 ^{a}	15.31	5.13 ^{b}
Dry	62.78	23.82 ^{b}	13.37	2.73 ^{a}
SE	3.27	1.38	2.08	0.57
p value	*0.1702*	*0.0006*	*0.5187*	*0.0102*

SE: standard error. Different letters in the column for each factor mean significant differences (HSD Tukey, $p < 0.05$).

Table 3 shows the proportion of the main species evaluated—ryegrass 'Ecotype cajamarquino', white clover 'Ladino variety' and weeds. Two cut-off lines have been drawn; the lower one indicates the appropriate level of 30% [27], and the upper one is 70%, both represent the average value in which the percentage of clover and ryegrass should be. However, it can be seen that with cutting frequencies of 30 and 45 days, the ryegrass does not reach 70% and at 60 days ($p < 0.05$) it exceeds this limit. This coincides with Tillus et al. (2022) [13] and Vallejos (2020) [32] in that the lower the frequency of grazing or cutting, the higher the percentage of ryegrass. Regarding white clover, the three mowing frequencies ($p > 0.05$) occupy approximately 2/3 of the value indicated by the marked line (30%) for white clover, probably due to the effect of the time of year and fertilization. Similarly, it has been observed ($p < 0.05$) that weeds tend to decrease as plants mature [13], reaching the lowest level in our study at 60 days, a lower value than that found by Vallejos (2009) [40] and Vallejos (2019) [41], probably due to the effect of shade and competition generated by the height of the plant.

Table 3 provides a comprehensive comparison of the two seasons of the year and their impact on the average of the three cutting frequencies. It reveals that during the rainy season, the yield of the Cajamarca ecotype ryegrass is significantly higher ($p < 0.05$) than during the dry season [23]. Conversely, the yield of white clover is higher ($p < 0.05$) in the dry season, demonstrating its adaptability to adverse environmental conditions [24,25], in this case to fortnightly irrigation. The presence of weeds ($p > 0.05$), mainly represented by *Pennisetum clandestinum*, *Taraxacum officinale* and plants of the genus Plantago, remained constant throughout the year, highlighting their resilience to seasonal variations.

The ratio of ryegrass/clover expresses values that should be taken into account as an indicator of pasture management in a dairy farm; as part of good management, we had a floristic composition constituted by 55% of ryegrass, 40% white clover. Moreover, for 5% weeds, we obtain a ratio of 1.4; in the case of 60% ryegrass, 30% white clover [26] and 10% weeds, the ryegrass/white clover ratio would be 2.0. In our study, the values found are higher than 3; under these conditions, the intake of the animals would probably be affected and, as a consequence, there would be a low yield, as can be observed in our environment [27]. Compared to the rainy season, the best ratio value is observed in the dry season. To improve the floristic composition, the reseeding of white clover could be evaluated during the last third of the rainy season. An interaction between the time of year and mowing frequency on the percentage of weeds was observed, reflecting that both factors influence the productive response of the weeds.

3.4. Nutritional Composition

Table 4 shows the composition of crude protein (CP), ash, neutral detergent fibre (NDF), in vitro digestibility of dry matter (IVDDM), metabolisable energy (ME) and estimated protein production per year for ryegrass species, clover, weeds (mainly Kikuyo) and the overall association of the estimated total forage floor intake.

In Table 4, it can be seen that the highest contribution in kg ha^{-1} $year^{-1}$ of CP ($p < 0.05$) in ryegrass corresponds to the cutting frequency of 60 days; however, this same frequency presents the lowest value in clover and weeds. It can be expressed that as an association, the best time of use is between 30 and 45 days of regrowth ($p < 0.05$), and it could be improved even if the percentage of white clover is increased (30 to 40%) in these associated pastures. These results should be complemented with an evaluation of the chemical composition of the soil, both in nitrogen (N) and sulphur (S), considering that the latter nutrient, in addition to being part of the protein molecule, improves the homeostasis of plant tissue through physiological tolerance mechanisms [49] in acid soils with the presence of Al^{3+}, characteristic of the soils of the northern highlands of Peru. Regarding the nutritive value of *Lolium multiflorum*, *Trifolium repens* and weeds (*Pennisetum clandestinum*, *Taraxacum officinale* and plants of the genus Plantago), according to the frequency of cutting, it is observed that when this is increased in the three groups of species evaluated, the concentration of nutrients varies [18]. Thus, CP and IVDDM ($p < 0.05$) decrease [15,16] and NDF ($p < 0.05$) increases [13], a consequence of a thickening of the cell wall that is compensated by a decrease in cell content [18,19]. The highest CP value and lowest NDF were achieved by 30- and 45-day white clover; the best IVDDM level corresponded to 30- and 45-day cutting frequencies for ryegrass, white clover and weeds.

The nutrient values found in this study for *Lolium multiflorum* are consistent with those found by Vallejos et al. (2020) [41] and Oliva et al. (2018) [33], providing a solid foundation for practical application. The average of the values found in white clover Ladino variety for CP, according to the average of the three cutting frequencies (24.9%), doubles that of ryegrass (12.5%) and significantly exceeds weeds (15.4%), highlighting the potential of this species (*Pennisetum clandestinum*, 30%) to enhance the quality of the diet [18] and the productivity of this association [25]. The CP of white clover at 30 and 45 days of cutting is higher than those found by Gierus et al. (2012) [16] and Vallejos et al. (2021) [30] but lower in NDF, suggesting the importance of timing in cutting. Likewise, ash concentration and IVDDM are in line with those obtained by Fonseca-López et al. (2020) [34] and Vallejos et al. (2021) [30], further reinforcing the practical implications of these findings.

Table 4. Floristic composition (%) of the ryegrass–white clover association according to grazing frequency and time of year.

Species/Grazing Frequency (Days)	CP (%)	Ash (%)	NDF (%)	IVDDM (%)	ME (Mcal/kg MS)	Kg CP × ha × Year
Ryegrass						
30	13.99 [a]	12.42 [a]	36.90 [a]	72.74 [a]	2.75 [a]	2710.57 [ab]
45	12.34 [ab]	9.79 [b]	40.84 [a]	69.92 [a]	2.61 [a]	2416.27 [a]
60	11.36 [b]	8.60 [b]	45.87 [b]	61.91 [b]	2.26 [b]	2855.20 [b]
SE	0.38	0.52	1.05	0.86	0.04	83.75
p value	*0.008*	*0.0052*	*0.0028*	*0.0003*	*0.0002*	*0.026*
Clover						
30	27.32 [a]	11.03	22.34 [a]	75.96 [a]	2.91 [a]	1700.57 [a]
45	26.43 [a]	12.58	28.33 [b]	72.71 [b]	2.77 [b]	1853.40 [a]
60	21.43 [b]	11.41	35.34 [c]	69.59 [c]	2.61 [c]	1199.57 [b]
SE	0.88	0.71	1.07	0.66	0.03	51.97
p value	*0.0066*	*0.343*	*0.0004*	*0.0015*	*0.001*	*0.0003*
Weeds						
30	17.35 [a]	10.94	38.02 [a]	69.80 [a]	2.65 [a]	898.17 [a]
45	16.65 [ab]	13.58	43.16 [b]	68.35 [ab]	2.56 [ab]	949.53 [a]
60	12.64 [b]	12.08	54.08 [c]	64.08 [b]	2.37 [b]	365.07 [b]
SE	0.93	0.65	0.91	1.07	0.05	45.66
p value	*0.0237*	*0.062*	*0.0000*	*0.0221*	*0.0167*	*0.0002*
Association						
30	17.24 [a]	11.89 [a]	34.13 [a]	72.86 [a]	2.77 [a]	5309.29 [a]
45	16.12 [a]	11.04 [a]	38.44 [b]	70.08 [a]	2.63 [a]	5219.19 [a]
60	13.18 [b]	9.47 [b]	44.93 [c]	63.52 [b]	2.34 [b]	4419.87 [b]
SE	0.45	0.31	0.8	0.77	0.04	145.18
p value	*0.0017*	*0.0044*	*0.0002*	*0.0004*	*0.0004*	*0.0091*

CP: crude protein; NDF: neutral detergent fibre; IVDDM: in vitro digestibility of dry matter; ME: metabolisable energy; SE: standard error. Different letters in the column for each factor mean significant differences (HSD Tukey, $p < 0.05$).

These results offer a promising outlook for the stability of the production systems in the northern highlands of Peru. The nutritional contribution of the forage association, including the contribution of weeds, which is mainly composed of *Pennisetum clandestinum*, is a key factor in this stability. The sustainability of these pastures, which date back to an age of more than 40 years, is a testament to the potential for improvement in the system of using these pastures to enhance the profitability of livestock productivity. The results show that the biomass yield per year is similar for the three cutting frequencies. However, the annual protein production is significantly higher when cattle consume the grasses in the 30–45 day cutting frequency interval and the metabolisable energy intake. The three associated species contribute more than 5219 kg of CP per hectare per year. These findings highlight the need for further study of soil nutrient use and cycling under this grassland management system, offering a hopeful path towards greater productivity and sustainability.

4. Conclusions

The accumulated yield (kg DM ha^{-1} yr^{-1}) of the association of ryegrass ecotype cajamarquino and white clover variety Ladino was similar for the three cutting frequencies. The best nutritional quality corresponded to the 30 and 45-day cutting frequency pastures, as they contained higher crude protein (kg CP ha^{-1} yr^{-1}) and metabolisable energy concentration (Mcal per kg DM), compared to the 60-day cutting frequency. The floristic composition at 60 days showed an increase in ryegrass and a decrease in white clover, with a consequent lower nutritional intake. The sustainability of the ryegrass 'cajamarquino ecotype' and white clover Ladino variety association is viable due to its good productive performance and nutritional value in high altitude conditions at cutting frequencies of

30 and 45 days in the highlands of northern Peru. The results are precious for livestock farmers who have the ryegrass–clover association as a source, as it will allow them to obtain a more significant amount of biomass and protein at the optimum grazing time (30 to 45-day intervals). The findings of this study open the window for evaluating the benefits or limitations of mowing frequency and animal response on the behaviour of nutrients in highlighting their relationship with productivity yields and the accumulation of nutrients in the soil due to the already-known advantages of clover as a legume. This research will provide a better understanding of the closed system of pastures that have been in use for more than half a century in the northern highlands of Peru and its efficiency on the circular economy of livestock producers.

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