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Romy Vallejos-Cacho , Luis Asunción Vallejos-Fernandez , [Wuesley Yusmein Alvarez-Garcia](#) <sup>\*</sup> ,  
Eduardo Alberto Tapia-Acosta , Sylvia Saldanha-Odriozola , [Carlos Enrique Quilcate-Pairazaman](#)

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## Article

# Sustainability of *Lolium multiflorum* L. "Ecotype Cajamarquino", Associated with *Trifolium repens* L., at Three Harvest Times in the Northern Highlands of Peru

Romy Vallejos-Cacho <sup>1,†</sup> , Luis A. Vallejos-Fernández <sup>1,†</sup> , Wuesley Y. Alvarez-García <sup>2,†,\*</sup> , Eduardo Alberto Tapia-Acosta <sup>1</sup> , Sylvia Saldanha-Odrizola <sup>3</sup> and Carlos Quilcate-Pairazaman <sup>2</sup> 

<sup>1</sup> Unidad de Posgrado de Ingeniería en Ciencias Pecuarias, Universidad Nacional de Cajamarca, Av. Atahualpa 1050, Cajamarca 06003. Peru; lvallejos@unc.edu.pe; etapia@unc.edu.pe

<sup>2</sup> Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria (INIA), Jr. Wiracocha s/n, Baños del Inca, Cajamarca 06004. Perú; walvarez@inia.gob.pe, walvarezg@cip.org.pe

<sup>3</sup> Facultad de Agronomía, Universidad de la Republica de Uruguay, Garzón 780, 12900 Montevideo, Uruguay

\* Correspondence: walvarez@inia.gob.pe, +51 933 636 673 (W.Y.A-G); lvallejos@unc.edu.pe; Tel.: +51 969 697 019 (L.V-F.);

† These authors contributed equally to this work.

**Abstract:** Livestock in the northern highlands of Peru is fed on the association of ryegrass (*Lolium multiflorum* L.) ecotype cajamarquino - white clover (*Trifolium repens* L.) Ladino variety constantly varies in its agronomic characteristics and nutritional value due to management considerations and its association with the soil and the animal. The objective was to evaluate yield, plant height, growth rate, number of tillers, spikelets, basal diameter in ryegrass, elongation rate, internode length, and decrease points in clover over one year. Nutritional 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. Better yield (5588 kg DM ha) and plant height (47.1 cm) showed the 60-day cutting frequency; however, there was no difference ( $p>0.05$ ) among the three cutting frequencies in annual yield. There was no difference between number of tillers and basal diameter. Clover height, elongation rate and internode length were higher at 60 days. The highest CP concentration and lowest NDF value ( $p<0.05$ ) were achieved by clover at 30 and 45 days. The producers should consider the findings obtained to decide the time of use of this association in dairy cattle feeding.

**Keywords:** Ecotype cajamarquino; Botanical composition; Cutting frequencies; Nutritional value; *Trifolium repens*.

## 1. Introduction

Pastures are used worldwide to feed livestock; however, the anatomy and digestive physiology of these animals means that methane is generated as an end product of this digestive process, the greenhouse effect of which is more harmful than other gases, and the state of maturity of the pasture influences methane production [1]. It is essential, therefore, to recognise that pastures serve to control soil carbon cycling [2] and nutrient recycling, mainly of nitrogen [3]; nutrients that remain accumulated in soils for up to decades [4], forming a soil-pasture interaction suitable for the grass-legume association environment [5]. Italian ryegrass-white clover pastures, introduced in the northern highlands of Peru from 1950 onwards, still constitute the basis of the diet of dairy cattle, the main economic activity of the rural population in this area. This grass-legume association, characterised by complementing each other very well in terms of yield and nutritional value [6–8], 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 [9].

The coexistence between grasses and legumes, in combination with the frequency of cutting, affects the morphological and productive behaviour of Italian ryegrass [10,11], as well as its botanical composition, particularly when cutting or harvesting is done after 28 days; it is recommended that this frequency be done at 42 days [12] when its yield is positively correlated with the mass of stolons. Long intervals between cuts allow for advanced pasture maturity, reflected in increased fibre [11] higher aerial biomass yield. Still, low protein concentration, digestibility and energy [13–15], due to wall thickening and reduced cell content [16,17].

In addition to pasture management, the climatic characteristics of the time of year also affect the productive performance of the ryegrass-white clover association ([18–20], with ryegrass showing more limitations in its growth during drought events [21] compared to white clover, which thanks to its variability responds with visible phenological changes to new environmental conditions, competing successfully against other plants [22,23]. White clover is characterised by its essential contribution of protein in the diet of animals [16] and by its capacity to fix nitrogen and improve the productivity of this association [23]; 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 [24,25]

For Tilus et al. (2022) [11], forage yield increases linearly as the time of cutting is prolonged thus yields have been obtained in this association of 1470 kg DM ha<sup>-1</sup> when this is done around 10 cm in height with a remnant greater than 4 cm [6]; 2000 kg DM ha<sup>-1</sup> if the height is 13 cm and 2781 kg DM ha<sup>-1</sup> if the ryegrass is 29.8 cm [26]; 3974 kg DM ha<sup>-1</sup> when the height is 20 cm high and post-grazing remnant of 5 cm [27]. For white clover, heights of 22 cm have been found when cutting every 60 days [28]. Cumulative yields ranged from 13039 to 27290 kg DM ha<sup>-1</sup> year<sup>-1</sup> [6,20,27,29].

Taking into account that the chemical composition presents a high variability due to the time of harvest or maturity stage [16], when evaluating ryegrass independently, values of 10.01 to 14.67 % CP; 37.64 to 47.52 % NDF; 67.65 to 71.04 % DIVMS and 2.36 to 2.49 Mcal kg<sup>-1</sup> DM of ME have been obtained [30,31]. In white clover, concentrations of 24.8 % CP and 40.9 % NDF were found for five cutting frequencies and three cuts 20.6 % CP and 42.9 % NDF were obtained [14]; when Ladino white clover was cut between 16 to 18 cm in height, Vallejos et al. (2021) [28] found 23.06 % CP, 11.6 % ash; 25.8 % NDF and 75.04 % DIVMS; in this same species Fonseca-López et al., (2020) [32], determined 10.9 % ash.

As part of the agronomic characteristics of this association, Ganderats and Hepp (2003) [33] obtained 3,368 tillers m<sup>2</sup> and Balocchi et al. (2003) [34], 5,800 to 7,000 tillers m<sup>2</sup>; this quantity probably influences the greater basal diameter. In this regard Han et al. (2022) [35] 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, Doussoulín et al. (2018) [36] found no difference in stolon elongation rate (0.3 - 0.5 cm day<sup>-1</sup>); Lluga-Rizani et al. (2021) [22] obtained values from 9 to 60 flower heads. When the mowing frequency is between 56 and 84 days, a marked reduction of white clover is observed in the floristic composition [12], probably due to the shading generated by the ryegrass [6,8]. Conversely, high mowing frequencies favour ryegrass and make white clover more competitive [37]. Although Vallejos (2009) [38] finds no difference between mowing frequencies about weeds, these can vary between 17.6 and 36.3 % [39]. 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.

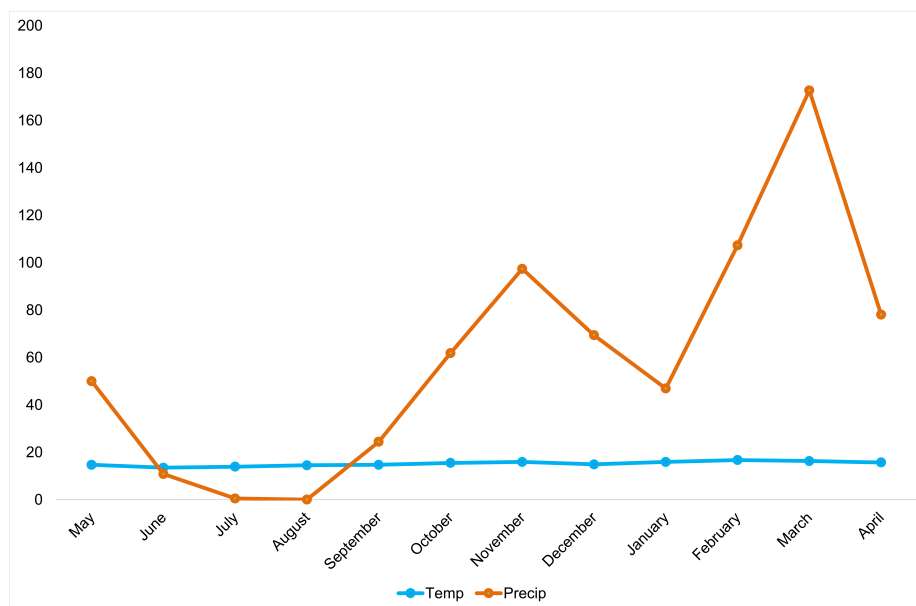
## 2. Materials and Methods

### 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 07°09'49" "S", Longitude 78°30'00" "W") located at 2,718 masl; it covered the period from May 2018 to April 2019, in an area of 10 hectares. The pastures were installed approximately 40 years ago, and reseeded 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<sup>2</sup> each were selected for each treatment, which were evaluated for a whole year in the two agro-meteorological stations present in the study area.

### 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 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 1.



**Figure 1.** During the assessment, average ambient temperature and rainfall Source: Senamhi (2024) [40].

As shown in Figure 1, 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 ( $\pm 0.5$  g), then transported to the Soil, Water, Fertilizer and Pasture Service Laboratory of the National Institute for Agrarian Innovation (INIA); 100 g of each

sample were 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, 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<sup>-1</sup> day<sup>-1</sup>. 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 were concentrated (more than 70 %). A 70 cm metal ruler was used.

The floristic composition was quantified using the same samples obtained for yield, separating and classifying them according to the species present (ryegrass, clover and weeds). Considering 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.

#### 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 [41]; NDF according to AOAC 962.09 [42] and ash according to AOAC 942.05 [43]. The determination of neutral detergent fibre [44] was carried out with a fibre analyzer kit, FIWE, VELP and the *in vitro* digestibility of dry matter - DIVMS with the digester kit (ANKOM, USA) [45]. These analyses were conducted at the Laboratory of Animal Nutrition and Food Bromatology of the National University Toribio Rodríguez 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 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, out using the GNU of the RStudio platform of R Project [46]. For the comparison of means, Tukey's multiple range test was used ( $p < 0.05$ ).

### 3. Results

#### 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 association ryegrass - white clover, according to treatment and time of year.

Factors	Biomass (Kg x ha <sup>-1</sup> )			Plant height (cm)	
	Day	Cut	Year	Ryegrass	Clover
Grazing frequency (days)					
30	84.37	2531.20 <sup>c</sup>	30796.7	20.35 <sup>c</sup>	10.23 <sup>c</sup>
45	88.69	3991.00 <sup>b</sup>	32371.7	32.54 <sup>b</sup>	14.54 <sup>b</sup>
60	91.85	5511.26 <sup>a</sup>	33527	47.12 <sup>a</sup>	18.25 <sup>a</sup>
SE	3.83	138.91	1398.76	3.13	0.91
<i>p value</i>	0.4562	0.0003	0.4565	0.0002	0.0001
Time of year					
Rainy	96.11 <sup>a</sup>	4360.33	35140.27 <sup>a</sup>	32.49	14.96
Dry	79.89 <sup>b</sup>	3648.22	29592.55 <sup>b</sup>	34.18	13.7
SE	3.71	496.77	1036.54	4.8	1.41
<i>p value</i>	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 2.** Stolon elongation rate, growing points, internode length and number of flowers of white clover, according to cutting frequency.

Factors	<i>Lolium multiflorum</i> L.			<i>Trifolium repens</i>			
	Number of tillers	Number of ears	Basal diameter	Elongation rate (cm x day <sup>-1</sup> )	Growing points (m <sup>2</sup> )	Internode length (cm)	Number of flower heads (m <sup>2</sup> )
Grazing frequency (days)							
30	101	3.54 <sup>b</sup>	34.00 <sup>a</sup>	0.44 <sup>c</sup>	15.33	0.91 <sup>b</sup>	22.02
45	174.89	11.30 <sup>b</sup>	44.11 <sup>b</sup>	0.59 <sup>b</sup>	20.0	1.32 <sup>b</sup>	42.11
60	128.44	30.61 <sup>a</sup>	43.44 <sup>b</sup>	0.89 <sup>a</sup>	24.0	2.28 <sup>a</sup>	44.44
SE	24.48	2.51	1.41	0.01	1.99	0.136	8.38
<i>p value</i>	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).

3.2. Plant Morphology

Table 2 shows the values for the number of tillers and ears, the basal diameter for ryegrass, 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.

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, estimated from each species' proportions.

3.4. Nutritional Composition

Table 4 shows the composition of crude protein (CP), ash, neutral detergent fibre (NDF), *in vitro* digestibility, 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.

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

Factors	Ryegrass	Clover	Malezas	Rate R:C
Grazing frequency (days)				
30	62.93 <sup>ab</sup>	20.21	16.81 <sup>a</sup>	3.34
45	60.49 <sup>a</sup>	21.66	17.61 <sup>a</sup>	3
60	74.94 <sup>b</sup>	16.67	8.61 <sup>b</sup>	5.47
SE	3.26	2.52	1.84	0.77
<i>p value</i>	0.0174	0.3822	0.0074	0.0816
Time of year				
Rainy	69.46	15.20 <sup>a</sup>	15.31	5.13 <sup>b</sup>
Dry	62.78	23.82 <sup>b</sup>	13.37	2.73 <sup>a</sup>
SE	3.27	1.38	2.08	0.57
<i>p value</i>	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 4.** Floristic composition (%) of the ryegrass - white clover association according to grazing frequency and time of year.

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

CP: Crude Protein; NDF: Neutral detergent fibre; DIVMS: ; ME: Metabolisable energy;SE: Standard error. Different letters in the column for each factor mean significant differences (HSD Tukey, *p*<0.05).

4. Discussion

4.1. Productive Performance

Table 1 shows the highest biomass yield per cut at 60 days (5511.26 kg DM ha<sup>-1</sup>), followed by grazing frequencies at 45 days and 30 days with 3991 kg DM ha<sup>-1</sup> and 2531.20 kg ha<sup>-1</sup> respectively,

thus confirming the excellent production generated by the association of these pastures coupled with the frequency of grazing [6–8,10]. The results obtained exceed the amounts found by Egan et al. (2018) [6], Rojas et al. (2016) [27] and López et al. (2021) [26], 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 [11,35]. Meanwhile, the annual biomass ( $\text{kg DM ha}^{-1} \text{ yr}^{-1}$ ) obtained in our study shows that there is no significant difference ( $p>0.05$ ) between the three grazing frequencies, an essential result for producers in the northern highlands of the country since the values found ( $\text{kg DM ha}^{-1}$ ) at 60 days of cutting double those of 30 days, the annual production is the same; with the addition that the quality of the pastures (Table 4) is better when they are less mature [16,17]. These results reaffirm Gierus et al. (2012) [14] that 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; in our study, we found that in annual yield ( $\text{kg DM ha}^{-1} \text{ year}^{-1}$ ) with cutting frequency from 30 to 45 days, there is an increase of 6 %. From 45 to 60 days, it is reduced to 2 %, probably indicating that with frequencies of 60 days, the ryegrass would be close to the ceiling of its yield.

Our values exceed the annual yields of Mendoza et al. (2018) [20], Rojas et al. (2017) [27], Egan et al. (2018) [6] and Annicchiarico and Tomasoni (2010) [29], probably due to the establishment of these pastures associated for several decades - related to age, climatic conditions [18,19] that occur in the inter-Andean valley of the northern highlands of Peru, which is 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  $\text{kg DM ha}^{-1} \text{ cut}^{-1}$  and  $\text{kg DM ha}^{-1} \text{ year}^{-1}$  was higher than the results referred by Claffey et al. (2019) [18]; Tozer et al. (2014) [19] and Mendoza et al. (2018) [20], 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.

#### 4.2. Plant Height

The most excellent 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, 28 %. Considering that these results were higher than the values found by Tilus et al. (2022) [11], Egan et al. (2018) [6], Rojas et al. (2017) [27] and López et al. (2021) [26], probably due to the grazing age, season and soil management, considering that 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 [4], which is installed in the pasture.

Regarding white clover height (Table 1), values increase with maturity stage [16]; thus, at cutting frequency ( $p<0.05$ ) at 60 days, it reaches 18.25 cm, at 45 days, 14.54 cm and at 30 days 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) [28] of 21.95 cm, their results are lower, which may be due to soil and environmental conditions, among other factors, 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.

### 4.3. Crop Morphology

In Table 2, it is observed that there is no significant difference ( $p > 0.05$ ) in number of tillers for ryegrass according to cutting frequency; likewise, these values are lower than those found by Ganderats and Hepp (2003) [33] and Balocchi et al. (2013) [34], probably because it is not a continuous practice of producers to perform a good soil management, which favours the pasture and the microbial population of the soil [35] that favours tillering. The number of ears was higher at 60 days ( $p < 0.05$ ) because the pasture is reaching the maximum reproductive stage at this phenological 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) [36], who obtained relatively lower levels, possibly due to the different cutting time [12]. The number of flower heads in our work falls within the range found by Lluga-Rizani et al., (2021) [22], suggesting that the morphological variability [23] of this species in different environmental conditions of the highlands could be a contributing factor. It's 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.

### 4.4. Floristic Composition

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 % [25], and the upper one 70 %, both represent the average value in which the percentage of clover and ryegrass should be, respectively; 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, coinciding with Tillus et al, (2022) [11] and Vallejos (2019) [30], 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 [11], reaching the lowest level in our study at 60 days, a lower value than that found by Vallejos (2009) [38] and Vallejos (2019) [39], 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 [21]. Conversely, the yield of white clover is higher ( $p < 0.05$ ) in the dry season, demonstrating its adaptability to adverse environmental conditions [22,23], 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 Ryegrass: Clover express values that should be taken into account as an indicator of pasture management in a dairy farm; if, as part of good management, we had a floristic composition (constituted by 55 % of ryegrass, 40 % white clover). Moreover, for 5% of weeds, we will obtain a ratio of 1.4; in the case of 60 % ryegrass, 30 % white clover [24] 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, a low yield, as can be observed in our environment[25]. 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. 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.

#### 4.5. Nutritional Value

In Table 4, it can be seen that the highest contribution in  $\text{kg ha}^{-1} \text{ year}^{-1}$  of CP ( $p < 0.05$ ) in rye grass 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 [47] in acid soils with the presence of  $\text{Al}^{3+}$ , 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 [16]; thus, CP and DIVMS ( $p < 0.05$ ) decrease [13,14] and NDF ( $p < 0.05$ ) increases [11], a consequence of a thickening of the cell wall that is compensated by a decrease in cell content [16,17]. The highest CP value and lowest NDF were achieved by 30- and 45-day white clover; the best DIVMS 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) [39] and Oliva et al. (2018) [31], 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*) at 30 %, to enhance the quality of the diet [16] and the productivity of this association [23]. The CP of white clover at 30 and 45 days of cutting is higher than those found by Gierus et al. (2012) [14] and Vallejos et al. (2021) [28], but lower in NDF, suggesting the importance of timing in cutting. Likewise, ash concentration and DIVMS are in line with those obtained by Fonseca-López et al. (2020) [32] and Vallejos et al., (2021) [28], further reinforcing the practical implications of these findings.

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 findings demonstrate that the yield and annual protein production can be significantly improved when the pastures are used between 30 and 45 days of cutting frequency. In fact, including the three associated species can lead to a production of more than 5219 kg of protein per hectare per year. This underscores the need for further study on the use and cycling of nutrients in the soil under this production system, offering a hopeful path towards enhanced productivity and sustainability.

#### 5. Conclusions

The highest yield per cut ( $p < 0.05$ ) of the ryegrass ecotype cajamarquino-white clover var. Ladino ( $\text{kg DM ha}^{-1} \text{ cut}$ ) corresponded to the cutting frequency of 60 days; however, in annual accumulated yield ( $\text{kg DM ha}^{-1} \text{ yr}^{-1}$ ), there was no difference ( $p > 0.05$ ) between 30, 45 and 60 days of cutting. During the rainy season, the yield of this association is 16.7 % higher than during the dry season under fortnightly irrigation. The morphological characteristics of ryegrass and white clover are affected ( $p < 0.05$ ) by cutting frequencies, highlighting that the older the plant, the more significant the morphological difference. The percentage of white clover and weeds in the floristic composition tends to decrease, and the Cajamarca ryegrass ecotype increases at mowing frequencies of 60 days, which is nutritionally expressed in the lower CP concentration in ryegrass. The CP concentration of Ladino white clover was higher ( $p < 0.05$ ) than that of ryegrass ecotype Cajamarca at 30, 45 and 60 days

of cutting; the lowest level of NDF was found in clover at 30 and 45 days, and the DIVMS was better at 30 and 45 days. The annual protein accumulated in the forage association is higher ( $p < 0.05$ ) for the cutting frequency of 30 and 45 days, with 5309.29 and 5219.19 kg, respectively. With these results, it is considered that the sustainability of the association of ryegrass and clover is viable due to the production and nutritive value they present under highland conditions.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, L.V.F. and R.F.C.; methodology, R.F.C., L.V.F. and W.Y.A.G.; software, W.Y.A.G., E.A.T.A. and S.S.O.; validation, C.E.Q.P., R.V.C. and L.V.F.; formal analysis, W.Y.A.G.; C.E.Q.P.; E.A.T.A. and L.A.V.F.; investigation, R.V.C., L.A.V.F., S.S.O. and E.A.T.A.; resources, C.E.Q.P., L.A.V.F.; data curation, R.V.F., W.Y.A.G. and E.A.T.A.; writing—original draft preparation, R.V.C., L.A.V.F. and W.Y.A.G.; writing—review and editing, L.A.V.F., W.Y.A.G. and R.V.F.; visualization, W.Y.A.G.; supervision, L.A.V.F. and C.E.Q.P.; project administration, R.V.C., L.A.V.F. and C.E.Q.P.; funding acquisition, L.A.V.F. and C.E.Q.P. All authors have read and agreed to the published version of the manuscript".

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