Article

Yield of white clover associated with ovillo grass at different grazing frequencies

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Abstract

The objective of the study was to evaluate the behavior of white clover associated with ovillo grass at four grazing frequencies. The treatments were fixed frequencies of 28 d in spring-summer and 35 d in autumn-winter and when the grassland intercepted 95 and 100% of intercepted radiation. Treatments were assigned to experimental units according to a randomized block design in divided plots with three replications. The variables evaluated were dry matter yield (DMY), botanical and morphological composition (BMC, %), growth rate (GR), intercepted radiation (IR, %) and height. The frequency of grazing during the winter modified the accumulation of dry matter (3885 kg DM ha^{-1}; $p< 0.05$). The GR was higher in the grazing frequency at 28 d in spring (70 kg DM ha^{-1} d^{-1}; $p< 0.05$). The highest height was reached at 28 d in the summer (26 cm; $p< 0.05$). Grazing frequency did not affect Grassland yield, except in winter when the best yield was obtained by grazing at 95% IR. The IR and the height of the plant are indicative of the dry matter yield and optimal harvest time.

Keywords: grass, grazing frequency, legume.

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Introduction

In the temperate zone of Mexico there are animal production systems that base their feeding on pure and associated grasslands (Rojas *et al*., 2016a). The most used grasses in this area are: ovillo grass or orchard (*Dactylis glomerata* L.), annual ryegrass or ballico (*Lolium multiflorum* L.), tall fescue (*Festuca arundinacea* Schreber), ballico or perennial ryegrass (*Lolium perenne* L.) which are found as monoculture or associated with legumes such as white clover (*Trifolium repens* L.), lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.)

These species are sown in approximately 171 520 hectares, which represents 13% of the total surface of this area (Amendola *et al*., 2005). Some studies conducted in Mexico and other latitudes in the temperate zone world (Black *et al*., 2009) have reported that grass and legume associations increase biomass yield (Sanderson *et al*., 2013; Rojas *et al*., 2016a), forage quality is improved (Lee, 2018) and soil fertility due to the properties and physiology of legumes to fix nitrogen (N) symbiotically (Randazzo *et al*., 2013; Rojas *et al*., 2017).

It is necessary to consider a proportion of no more than 40% of white clover when establishing the crop (Rojas *et al*., 2016b), since competition for water, light and other nutrients can affect the performance of grasses in the grassland. Studies to evaluate the defoliation and use of forages is a complex subject, since the adequate use of a pure or mixed plot.

It requires knowing the growth dynamics of the species present (Velasco *et al*., 2001), since seasonal and annual biomass production is highly variable due to morphological adaptations and physiological changes experienced by grasses and legumes at a given time (Durand *et al*., 1999), for example, long periods of drought, low or high light intensities.

Extreme temperatures and availability of nutrients, likewise, the ecosystems of the pastures are changing, since there are them, annual, biennial and perennial, another important characteristic in grasses, are the type of photosynthetic pathway used, since the area pastures cold are usually called C_3 and warm areas C_4; however, in the face of climate change, its behavior may be variable (Stromberg, 2011; Abhishek *et al*., 2018).

Several directed studies (Moreno *et al*., 2015; Rojas *et al*., 2016a; Maldonado *et al*., 2017) in the Mexican central highlands, when evaluating different grass and legume associations, have shown that the highest dry matter yields have been achieved in the spring-summer season, however, the results are not always consistent.

Since the seasonal and annual performance of forage plants is a function of climatic and soil conditions. The objective of the present study was to evaluate the effect of defoliation frequency on forage accumulation, yield components and botanical composition of white clover associated with ovillo grass.

Materials and methods

The study was conducted in a white clover grassland associated with ovillo grass from March 2012 to April 2013 in the experimental field of the College of Postgraduates, in Montecillo, Texcoco, State of Mexico, at 19º 29’ north latitude and 98º 53’ west longitude at a height of 2 240 masl. The climate of the place is temperate sub-humid, with an average annual precipitation of 636.5 mm, and a rainy season in summer (from June to October) and an average annual temperature of 15.2 °C (García, 2004). The soil was analyzed in the Vegetable Nutrition Laboratory, SC in 2011 and was identified as sandy loam soil, with pH 8.4 and 3.5% organic matter.

Grassland management and treatment

To know the effect of the grazing frequency on the accumulation of seasonal and annual dry matter, botanical and morphological composition, growth rate of the crop (GR), intercepted radiation (IR) and height of the grassland, three treatments were proposed that consisted grazing every 28 days, 95 and 100% intercepted radiation (IR) during spring and summer, and every 35 days, 95 and 100% intercepted radiation during autumn and winter.

The sowing was carried out broadcasting in February 2009 with a density of pure and viable seed at a density of 6 kg ha^{-1} of white clover, while for ovillo grass it was 20 kg ha^{-1}, with this proportion a mixture of 40-60 white clover and ovillo grass, respectively. Nine 9 x 7 m plots were used, where the treatments were distributed under a randomized complete block design with three replications.

Before starting the investigation and in the middle of each season in 2012, uniform grazing was carried out, as defoliators, sheep of the Suffolk x Dorset breed were used, until leaving a remaining leaf area of 5 cm above ground level and for a better management was established electric fence in the experimental plots. During the dry season, the grasslands were gravity watered at field capacity every two weeks and were not fertilized.

Climate data

The monthly averages for outdoor temperature (maximum and minimum) and monthly precipitation during the study period were obtained from the Agrometeorological Station of the Postgraduate College, located 100 m from the experimental site (Figure 1). The monthly maximum temperature ranged from 22.1 to 30.2 °C, meanwhile, the minimum temperature was -2.6 to 11 °C.

The highest temperature occurred in spring, with the highest recorded in April being 30.2 °C, the lowest temperature was recorded in winter with -2.6 in December. Accumulated precipitation from March 2012 to April 2013 was 613 mm, of which 75.8% occurred in the months of June, July, August, September and October 2012, accumulating precipitation of 465 mm.

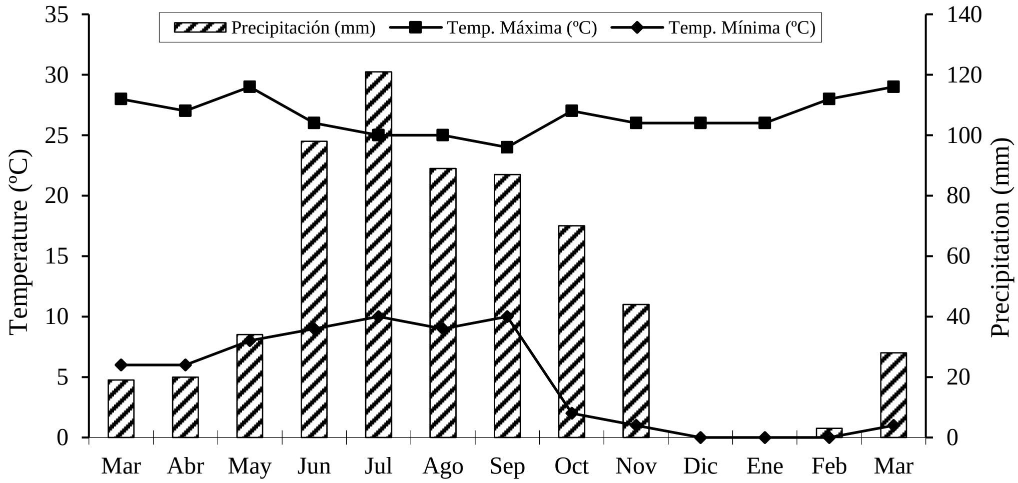

Figure 1. Maximum and minimum monthly average temperature and accumulated precipitation during the study period.

Dry matter yield

After the uniform grazing, three 0.25 m^2 squares were cut at a height of 5 cm starting from the soil in each plot and repetition depending on the grazing frequency. The forage harvested in each quadrant was washed and dried in labeled paper bags in a forced air stove at 55 °C for 72 h to estimate the amount of dry matter per hectare.

Botanical and morphological composition

To know the botanical composition of the forage, it was taken from the sample for dry matter yield and separated into white clover and ovillo grass. The morphological components of white clover (leaf, petiole, stolon and flower) and ovillo grass (leaf, stem and spike) were separated. Each component was dried in a labeled paper bag and remained in a forced air oven at 55 °C for 72 h to determine its dry weight.

Growth rate

The growth rate was calculated with the performance data obtained in each cut, in each of the repetitions, with the following formula: GR = R/T.

Where: GR= seasonal average growth rate (kg DM ha^{-1} d^{-1}); R= seasonal forage yield (kg DM ha^{-1}); T= days elapsed in each cut.

Intercepted radiation

The intercepted radiation was carried out before each grazing using the wooden ruler method described by Rojas *et al*. (2016b). To do this, five readings were taken in each experimental unit at random, consisting of sliding a wooden ruler 1 m long under the canopy with a south-north orientation. Subsequently, the shaded centimeters were counted, which represented the percentage of radiation intercepted by the plant canopy.

Grassland height

The height of the plant was measured before grazing and 20 samples were taken at random throughout the experimental unit with a graduated ruler 50 cm long and 1 mm precision above the canopy and slid until it touched the first morphological component of the association and the data was recorded (Adams *et al*., 1977).

Statistical analysis

Treatments were assigned to experimental units according to a randomized block design in divided plots with three replications, where the large plot was the association and the treatments the grazing frequencies. The data obtained by cutoff was organized by station and analyzed with PROC GLM from SAS (SAS Institute, 2009); the averages were compared with the Tukey test (α= 0.05).

Results and discussion

Dry matter yield

Table 1 shows the yield data for white clover associated with ovillo grass. In general, it is observed that the accumulation of dry matter was not modified (p> 0.05) in spring, summer and autumn at different grazing frequencies, however, in winter the highest accumulation of dry matter (p> 0.05), was presented at 95% IR (3 885 kg DM ha^{-1}), which exceeded 1 925 kg DM ha^{-1} (equivalent to 98%) at the grazing frequency of 100% IR (1 960 kg DM ha^{-1}) and 1 635 kg DM ha^{-1} (equivalent to 73%) at the frequency of 35 days (2 250 kg DM ha^{-1}).

Table 1. Yield (kg DM ha^{-1}) of white clover (*Trifolium repens* L.) associated with ovillo grass (*Dactylis glomerata* L.) at three grazing frequencies.

Season	Grazing frequency					
	28-35 (d)	IR (%)	95% of IR	Interval (d)	100% of IR	Interval (d)
Spring	5 740 a	92	4 385 a	27	5 667 a	30
Summer	5 797 a	93	4 658 a	26	5 341 a	30
Autumn	2 674 b	92	2 915 b	32	3 297 b	39
Winter	2 250 b	84	3 885 a	38	1 960 c	56
Anual average	4 115		3 960		4 066	
SEM	326		198		242	
Significance	**		**		**	
Annual yield	16 461		15 843		16 265	

Lowercase letter averages between columns represent significant difference (Tukey, 0.05). *= p< 0.05; **= p< 0.01; d= days.

On the other hand, the annual yield was not modified in the different grazing frequencies ($p > 0.05$). Other researchers (Castro *et al*., 2012) obtained a yield of 17 275 kg DM ha^{-1} in mixed grasslands with white clover, ovillo grass and perennial ryegrass, which are similar to what was found in the present investigation, which were 17 296 and 17 100 kg DM ha^{-1} at the frequency of 28-35 d and 100% IR, respectively. On the other hand, Rojas *et al*. (2016b) reported annual productions of 17 589 when evaluating different associations of clew, ryegrass and white clover grass, where the highest seasonal values were found in spring-summer, which were 7 292 and 5 072 kg DM ha^{-1}, respectively.

In the present experiment, in a very similar way, the highest seasonal yield averages were presented in spring-summer, which were statistically very similar ($p > 0.05$) but different from autumn-winter ($p < 0.05$).

The contribution of white clover and ovillo grass to dry matter yield in kg DM ha^{-1} per year and seasonally is found in Table 2. In general, white clover contributed approximately 65% of total annual dry matter, although there was no difference significant statistic ($p > 0.05$) per treatment.

Table 2. Annual and seasonal yield (kg DM ha^{-1}) by desirable species of white clover (*Trifolium repens* L.) associated with ovillo grass (*Dactylis glomerata* L.) at three grazing frequencies.

Season	Grazing frequency			SEM	Sig.	Average
	28-35 days	95% of IR	100% of IR			
	White clover (kg DM ha^{-1})					
Spring	3 034 a	2 905 a	3 816 a	324	ns	3 252 a
Summer	2 175 b	1 745 b	1 925 b	180	ns	1 948 b
Autumn	1 266 c	1 550 b	1 527 b	196	ns	1 086 c
Winter	1 504 bc	1 464 b	1 184 b	198	ns	1 384 c
Anual average	1 994	1 916	2 113	162	ns	
SEM	170	100	176			106
Significance	**	**	**			**
Annual yield	7 979	7 665	8 452	805	ns	
	Ovillo grass (kg DM ha^{-1})					
Spring	1 603 a	757 b	1 157 b	218	ns	1 172 b
Summer	1 770 a	1 695 a	2 288 a	291	ns	1 918 a
Autumn	644 b	939 ab	1 032 bc	118	ns	654 c
Winter	287 b	426 b	401 c	57	ns	371 d
Anual average	1 076	954	1 219	117	ns	
SEM	102	162	132			54
Significance	**	**	**			**
Annual yield	4 304	3 816	4 879	586	ns	

Means with different capital letters between rows represent significant difference and averages with lower case letters between columns represent significant difference (Tukey, 0.05). Sig.= significance; *= $p < 0.05$; **= $p < 0.01$. Int= Interval between grazing, in days. ns= not significant.

During spring the production of white clover was higher (3 252 kg DM ha^{-1}; p<0.05), which exceeded in 199, 135 and 67% the forage produced in autumn (1 086 kg DM ha^{-1}) winter (1 384 kg DM ha^{-1}) and summer (1 984 kg DM ha^{-1}), respectively. On the other hand, ovillo grass produced more forage in the summer (1 918 kg DM ha^{-1}; p< 0.05), which exceeded in 417, 193 and 64% the forage produced in winter (371 kg DM ha^{-1}), autumn (654 kg DM ha^{-1}) and spring (1 172 kg DM ha^{-1}), respectively.

During the spring-summer the climatic conditions were more favorable for the good performance of the grasslands (Figure 1), in addition the elevated temperatures favored a greater light interception, allowing the canopy to reach a higher leaf area index faster than in autumn-winter (Rojas *et al*., 2016a).

On the other hand, the horizontal arrangement of white clover leaflets helps to reestablish its leaf area more quickly than the ovillo grass (Maldonado *et al*., 2017), consequently, the little contribution of the ovillo grass to the annual yield could be due to the fact that when this species is subjected to a severe grazing intensity and is left with a minimum of remaining leaves, and carbohydrate reserves at the root, the regrowth is slow, therefore, it is recommended to leave three leaves per stem, to reactivate the photosynthesis process and ensure a greater regrowth in the shortest possible time (Turner *et al*., 2006).

Botanical and morphological composition

Figure 2 shows seasonal changes in the botanical and morphological composition of grasslands under three grazing frequencies. Regardless of the grazing frequency, white clover contributed more than 50% during spring, 60% during autumn-winter and only 40% in summer. During this time, ovillo grass provided the highest percentage (40%) during the year (p< 0.01).

But also, the highest percentage of other grasses and weeds was presented, a component that had the highest contribution in spring, followed by summer, winter and the lowest, in autumn (p< 0.05). Similar results were found in a study, where white clover was the main component (26 to 66%) in the associations evaluated as a consequence of its greater ability to compete with erectly growing species and tolerate defoliation (Flores *et al*., 2015).

On the other hand, Castro *et al*. (2012) in five associations of white clover, ovillo grass and perennial ballico reported white clover with the highest contribution of the total yield of 50%, perennial ballico with 28% and ovillo grass with 12%; the remaining 10% was made up of dead material, other grasses and weeds.

White clover tends to dominate in the associated grasslands over time due to its stoloniferous growth habit, obtaining greater advantage compared to the ovillo grass and ballico grasses that are tufted and erect (Rojas *et al*., 2017). As can be seen in this investigation, it is not the exception, since the initial mixture of the grassland was 40-60 of white clover and ovillo, three years after planting.

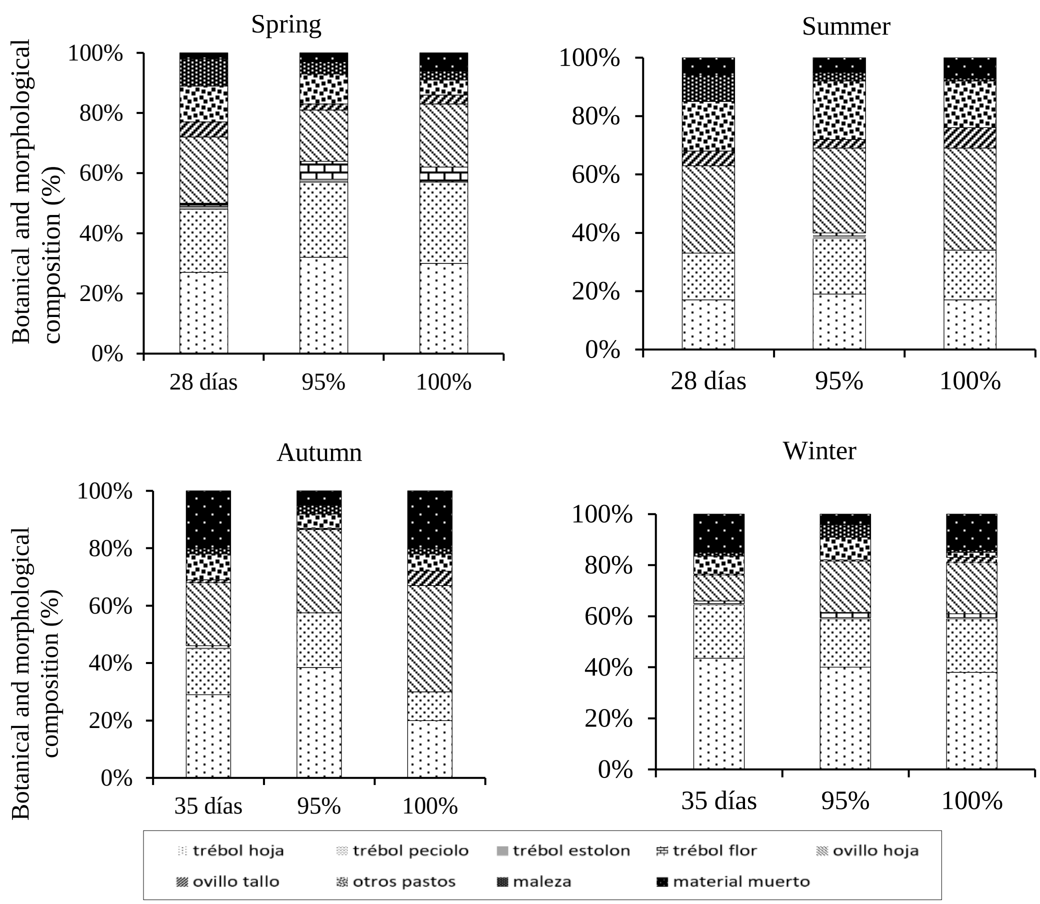

Figure 2. Seasonal changes in the botanical and morphological composition (%) of white clover (*Trifolium repens* L.) associated with ovillo grass (*Dactylis glomerata* L.) at three grazing frequencies.

Crop growth rate

The growth rate of white clover associated with ovillo grass when varying the grazing frequency is observed in Table 3. The highest seasonal growth rate occurred in the frequency of 28 days during spring (70 kg DM ha^{-1} d^{-1}; $p< 0.05$), which exceeded the frequency of 95% IR (52 kg DM ha^{-1} d^{-1}) in 18 kg DM ha^{-1} d^{-1} (equivalent to 35%).

Table 3. Seasonal changes in the growth rate (kg DM ha^{-1} day^{-1}) of white clover (*Trifolium repens* L.) associated with ovillo grass (*Dactylis glomerata* L.) at three grazing frequencies.

Season	Grazing frequency			SEM	Significance	Average
	28-35 days	95% of IR	100% of IR			
Spring	70 aA	52 aB	66 aAB	5	*	63 a
Summer	67 a	56 a	56 ab	4	ns	60 a
Autumn	31 b	31 b	40 bc	5	ns	34 b
Winter	27 b	41 ab	25 c	4	ns	31 b

Season	Grazing frequency			SEM	Significance	Average
	28-35 days	95% of IR	100% of IR			
Annual average	49	44	46	2	ns	
SEM	6	4	4			3
Significance	**	*	**			**

Means with different capital letters between rows represent significant difference and averages with lower case letters between columns represent significant difference (Tukey, 0.05). Sig.= significance; *= $p< 0.05$; **= $p< 0.01$. Int= interval between grazing, in days. ns= not significant.

During the sampling period, no statistical difference ($p> 0.05$) was observed in the other seasons of the year in the different grazing frequencies and annual average; however, although the trend was similar, on average there was a higher growth rate in spring (63 kg DM ha^{-1} d^{-1}; $p< 0.05$), which was 103% higher in the winter (31 kg DM ha^{-1} d^{-1}), 85% in the fall (34 kg DM ha^{-1} d^{-1}) and only exceed 5% in the summer (60 kg DM ha^{-1} d^{-1}).

These results coincide with those observed by Velasco *et al*. (2001) who in pure grasslands of ovillo recorded the highest GR in the third week of spring, which was 78 kg DM ha^{-1} d^{-1}, while for summer it was in the fourth week with 50 kg DM ha^{-1} d^{-1}. Similarly, the results obtained coincide with other work led by Velasco *et al*. (2002) when evaluating the growth curve of the perennial ryegrass, they reported the highest growth rates in spring, followed by summer with values of 98 and 53 kg DM ha^{-1} d^{-1}, respectively.

Seasonal changes in growth rate (kg DM ha^{-1} d^{-1}) is closely linked to the amount of radiation intercepted, largely determined by the leaf area index (IAF), photosynthetically active intercepted radiation (PAR; 400-700 nm) and due to the interaction with numerous environmental factors (Ewert, 2004; Abhishek *et al*., 2018) and physiological characteristics (Durand, 1999).

As observed in Figure 1, the environmental conditions were not favorable for the autumn-winter seasons, since it was in those sampling times that the lowest temperatures occurred and there was a higher incidence of frost, affecting the performance of the grassland (Rojas *et al*., 2016a).

Grassland height

Grassland height is an indirect method that provides estimates of the forage mass present in the grassland and is a useful tool that helps to make management decisions of pasture and legume associations efficiently and allows optimizing the costs of production (Adams *et al*., 1977).

The height in white clover grasslands associated with ovillo grass when varying the grazing frequencies is observed in Table 4. The highest height of the grassland was presented in the grazing frequency of 28 days in summer with 26 cm, exceeding 5 cm at the grazing frequency of 95% IR with 21 cm, in the same way, winter showed higher height in the frequency of 100% IR with 17 cm and lower grazing frequency of 35 days with 11 cm ($p= 0.05$).

Table 4. Seasonal changes in height (cm) of white clover (*Trifolium repens* L.) associated with ovillo grass (*Dactylis glomerata* L.) at three grazing frequencies.

Season	Grazing frequency			SEM	Significance	Average
	28-35 d	95% of IR	100% of IR			
Spring	18 ab	17 a	19 b	1.3	ns	18 b
Summer	26 aA	21 aB	25 aA	0.7	*	24 a
Autumn	14 b	13 b	16 b	1	ns	14 c
Winter	11 bB	12 bB	17 bA	0.5	**	13 c
Anual average	17 B	15 C	19 A	0.5	**	
SEM	1.8	0.8	0.8			1
Significance	*	**	**			**

Means with different capital letters between rows represent significant difference and averages with lower case letters between columns represent significant difference (Tukey, 0.05). *= $p< 0.05$; **= $p< 0.01$. Int= Interval between grazing, in days. ns= not significant.

The results obtained coincide with Flores *et al*. (2015), who reported the highest average heights in summer (38 cm) followed by spring (18 cm), autumn (14 cm) and winter (13 cm), respectively. Grassland height is positively related to dry matter yield (Castro *et al*., 2012) and intercepted radiation.

As observed in the present experiment, where the highest height found in the summer and spring coincided with the highest seasonal dry matter yield, as has been demonstrated in other works directed by Flores *et al*. (2015) and Rojas *et al*. (2016b) in associations of white clover, ovillo grass and perennial ryegrass.

Conclusions

The annual dry matter yield, growth rate, height of white clover forage associated with ovillo grass was higher in spring-summer and grazing yield set at 28 days after grazing. Regardless of the grazing frequencies, the contribution of white clover was higher compared to ovillo grass in autumn and winter. It is recommended to graze the association of white clover and ovillo grass when the grassland reached 95% of intercepted radiation, regardless of the season of the year.

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