Article

Productive behavior of perennial ryegrass alone and associated with ovillo grass and white clover

Sergio Iban Mendoza Pedroza[1]
Alfonso Hernández Garay[2]
Adelaido Rafael Rojas García[3§]
Humberto Vaquera Huerta[4]
Omar Ramírez Reynoso[5]
Rigoberto Castro Rivera[6]

[1]Department of Zootechnics-Autonomous University Chapingo. México-Texcoco Road km 38.5, Chapingo, Mexico State, México. (simpedroza@hotmail.com). [2]Genetic Resources and Productivity-Livestock-Postgraduate Collage. México-Texcoco Road km 35.5, Montecillo, Mexico State, México. (hernan@colpos.mx). [3]Faculty of Veterinary Medicine and Zootechnics Núm. 2-University Autnomus of Guerrero. Cuajinicuilapa, Guerrero, México. CP. 41940. [4] Statistics-Postgraduate Collage. México-Texcoco Road km 35.5, Montecillo, Mexico State, México. (hvaquera@colpos.mx). [5]Academic Unit of the Costa Chica Region-Autonomous University of Guerrero, Cruz grande, Florencio Villareal, Guerrero, México. (omarrr@hotmail.com). [6]Interdisciplinary Research Center for Regional Integral Development-National Polytechnic Institute-Oaxaca Unity. Hornos 1003, Sta. Cruz Xoxocotlán, Oaxaca, México. (rcastror@ipn.mx).

[§]Corresponding author: rogarcia_05@hotmail.com.

Abstract

The perennial ryegrass (*Lolium perenne* L.) was evaluated in monoculture and four associations with ovillo grass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.) in different proportions to maximize the yield of dry matter. The proportions in monoculture and the associations of perennial ryegrass, ovillo grass and white clover were: 100:00:00, 70:20:10, 50:00:50, 40:40:20 and 20:70:10, which distributed under a completely randomized block design with three repetitions. The yield of dry matter, botanical and morphological composition, leaf: stem and plant density ratio was evaluated. The association 50:00:50 exceeded 15% (3 671 kg MS ha^{-1}) to the association 70:20:10. The perennial ryegrass contributed more to dry matter yield in autumn and winter, while the ovillo grass and white clover in spring and summer. Regardless of the association, plant density was 29, 25, 20 and 11 m^{-2} plants for autumn, winter, spring and summer, respectively, with an average plant loss of 62%. The contribution of each species in the botanical composition of the associations was variable during the study period. Perennial ryegrass contributed 46, 40, 29 and 14% in autumn, winter, spring and summer, observing a decrease as the evaluation time elapsed ($p< 0.05$). The association that obtained the best characteristics of the yield was 50:00:50% of perennial ryegrass, ovillo grass and white clover.

Keywords: *Dactylis glomerata*, *Lolium perennial*, *Trifolium repens*, associated meadows, forage yield.

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Introduction

The main use provided to grasses has been as source of fodder for animal feed. The use of grasses through grazing with animals increases the productive efficiency of livestock farms, since they are the most economical source of nutrients compared to concentrates (Aguado *et al*., 2004). Therefore, availability must be guaranteed throughout the year (Arriaga *et al*., 1999), which can be achieved with good management of the prairie harvesting at short intervals and low intensities (Velasco *et al*., 2005; Mendoza *et al*., 2010). However, the production of forage in monoculture presents a marked seasonality (Rojas *et al*., 2016a; Maldonado *et al*., 2017).

It has been reported that the planting of grasslands associated with grasses and legumes are the best way to ensure a better distribution of forage production during the year, in addition to achieving a faster establishment and obtaining a better quality forage (Arriaga *et al*., 1999; Scheneiter *et al*., 2006). In addition, legumes fix atmospheric nitrogen, thereby reducing the use of nitrogenous chemical fertilizers (Gylfadottir *et al*., 2007; Pirhofer *et al*., 2012). However, for a good gramineae-legume association, it is necessary to consider the compatibility between species, as well as their adaptation to the climatic and edaphic conditions of the region (Arriaga *et al*., 1999).

Sanderson *et al*. (2005) reported that forage yield is higher in meadows associated with more than three species compared to two species (7 600 vs 4 800 kg MS ha^{-1}). In this regard, Camacho and García (2003) found that the association alfalfa, white clover, perennial rye, tall fescue and ovillo grass had an annual yield of 23 454 kg MS ha^{-1}, with a seasonal distribution of 23, 22.6, 25.4 and 29% for winter, autumn, summer and spring, respectively. Also, Castro *et al*. (2012) when evaluating different associations found that the highest yield of forage (17 275 kg MS ha^{-1}) occurred with the proportion 40, 20 and 40% of white clover, ovillo grass and perennial ryegrass, respectively.

In Mexico, the grass-legume association on forage production is not well studied. Therefore, the objective of this study was to evaluate the forage yield of perennial ryegrass alone and associated with ovillo grass and white clover.

Materials and methods

The study was conducted from September 2010 to September 2011, in the Experimental Field of the Postgraduate Collage, Montecillo, Texcoco, State of Mexico, located at 19º 29' north latitude and 98º 53' west longitude, at a height of 2 240 meters above sea level The climate of the place is temperate subhumid, the driest of the subhumid, with rain in summer and an average annual precipitation of 636.5 mm and rain regime in summer, (June to October) and average annual temperature of 15.2 ºC (García, 2004). The soil of the area is sandy loam and slightly alkaline with pH 7-8 (Ortiz, 1997).

The grasslands were established in February 2010. The sowing of the grasses was carried out in rows separated by 30 cm, while the legume was sown perpendicular to a distance between rows of 30 cm. The sowing density was 30, 20 and 5 kg ha^{-1} for perennial ryegrass, ovillo grass and white clover, respectively. The grasslands were not fertilized. During the dry season, irrigations were carried out at field capacity every two weeks.

Before starting the study, a uniformity cut was made by grazing sheep at an approximate height of 5 cm. During the evaluation period, grazing was carried out every four weeks in spring-summer and every 5 and 6 weeks during autumn and winter, respectively. The sheep were only used as defoliators, which were used in the experimental plots through the use of electric fences.

The association of grasses and legumes was carried out using the Minitab package (2006), with a design of vertices with three components of the mixture, with restriction to the legume in 10 and 50% minimum and maximum, respectively; and having a 100% treatment for ryegrass. The following associations were evaluated: 100:00:00, 70:20:10; 50:00:50; 40:40:20; y 20:70:10% of perennial ryegrass, ovillo grass and white clover, respectively. The treatments were randomly randomized to a completely randomized block design, with three repetitions per treatment. The size of the experimental plot was 8 × 9 m. The evaluated variables were: forage yield, botanical and morphological composition, leaf: stem ratio and density of plants by surface.

To estimate the forage yield, at the start of the experiment in each plot, two fixed squares of 0.25 m^2 were randomly placed, where the forage was harvested before grazing. The harvested forage was deposited in paper bags. Subsequently, the forage was washed and dried in a forced air oven, at a temperature of 55 °C for 72 h. After harvesting the forage, the meadows were grazed by sheep at a height of 5 cm. After grazing, in each of the plots, a square of 0.25 m^2 was randomly harvested to estimate the amount of residual forage. To determine the total forage yield per cut and per unit area (kg MS ha^{-1}), the forage was added to the forage present before grazing. The accumulated yield per station and annual was obtained by adding the yield per cut.

For the botanical and morphological composition, half of each season, of the harvested forage a sub sample of approximately 20% of the total forage was taken. Said sub-sample was separated into the different species of plants: perennial ryegrass, ovillo grass, white clover and weeds. For the morphological composition, each of the desirable species was separated into its morphological components: leaf, stem, flower and senescence. Subsequently, each component was dried in a forced air oven, at a temperature of 55 °C, for 72 h.

The leaf:stem ratio was estimated from the morphological components, using the following formula: leaf:stem= H/T; where H= leaf dry weight (kg MS ha^{-1}) and T= stem dry weight (kg MS ha^{-1}).

The density of plants m^{-2} was quantified in a fixed square of 1 m^2 placed, at the beginning of the experiment, randomly in each of the experimental plots. The counting of plants present by species was carried out the day before each grazing.

An analysis of variance was performed with the Mixed Models procedure (SAS, 2009) for all variables to test differences between treatments, based on the statistical design of randomized complete blocks. The mean comparison of the treatments was carried out using the adjusted Tukey test (α= 0.05).

Results and discussion

Forage yield

The annual and seasonal forage yield of perennial ryegrass and its association with ovillo grass and white clover are shown in Table 1. Differences between treatments were found ($p< 0.05$), where forage yields were 23 837, 22 133, 21 971, 21 840 and 20 165 kg MS ha^{-1} for the associations 50:00:50, 40:40:20, 100:00:00, 70:20:10 and 20:70:10, respectively. It was observed that the maximum performance obtained with the 50:00:50 association was 15.4% higher than that obtained with the 20:70:10 association ($p< 0.05$). These results confirm the point made by Camacho and García (2003), who reported for alfalfa associated with white clover, perennial ryegrass, tall fescue and ovillo grass an annual yield of 23 454 kg MS ha^{-1}. While Castro *et al*. (2012) and Rojas *et al*. (2016a) for the same species evaluated in the present study, average yields of 17 t MS ha^{-1} were obtained.

Table 1. Annual and seasonal forage yield (kg MS ha^{-1}) of perennial ryegrass (*Lolium perenne* L.) alone and associated with ovillo grass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.).

Association (Perennial ryegrass-ovillo grass-white clover)	Year 2010	Year 2011			EEM	Sig.	Annual
	Autumn	Winter	Spring	Summer			
100:00:00	8742 Aa	3793 c	5271 b	4165 Cc	501	***	21971 AB
70:20:10	8310 Aa	2740 c	5629 b	5161 Ab	595	***	21840 AB
50:00:50	8337 Aa	4938 bc	5950 b	4612 Bc	452	***	23837 A
40:40:20	6880 Ab	2642 c	7569 a	5042 Bb	581	***	22133 AB
20:70:10	6088 B	3744	5666	4667 B	369	ns	20165 B
Average	7670 a	3571 c	6016 b	4729 c		***	
EEM	285	300	231	101			
Sig.	***	ns	ns	***			

abcd= means with the same lowercase literal in the same row, they are not different ($p> 0.05$); ABCD= means with the same capital letter in the same column, not different ($p> 0.05$); EEM= standard error of the mean; Sig.= Significance; ***= $p< 0.05$; ns= not significant.

The proportion of the seasonal distribution of forage yield of the best association 50:00:50 was 35, 25, 19 and 21% for the seasons of autumn, spring, summer and winter, respectively. While the association that showed the lowest yield of dry matter did not present significant differences between seasons. It was observed that independently of the association, the proportion of the dry matter yield distribution was 35, 16, 27 and 22% for the autumn, winter, spring and summer seasons, respectively ($p< 0.05$). The highest forage yield recorded during the fall was attributed to the rapid growth of perennial ryegrass.

While in summer, the decrease in perennial ryegrass plants caused a lower yield of dry matter. The lower yield during the winter was attributed to the low temperatures (Figure 1), since according to Hernández *et al*. (1997) said temperatures cause a reduction in growth and, consequently, the rate of forage accumulation is reduced, due to the low rate of appearance

and leaf expansion (Horrocks and Vallentine, 1999). It has been indicated that the optimum temperature for the growth of perennial ryegrass and ovillo gras is 18 to 21 °C; while for white clover 24 °C (Brock and Tilbrook, 2000).

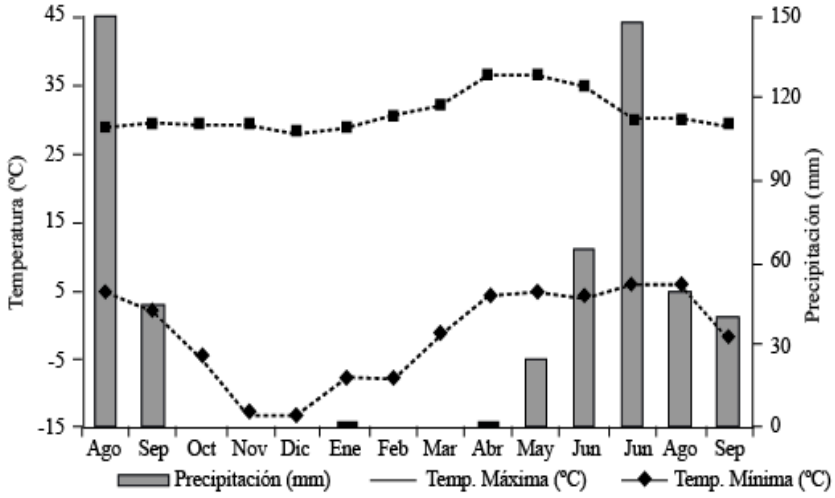

Figure 1. Precipitation and maximum and minimum monthly temperature recorded during the study period.

Botanical and morphological composition

The botanical and morphological composition of perennial rye grass alone and associated with grass and white clover is shown in Figure 2. There was a difference between treatments in the seasons ($p< 0.05$) for the botanical composition, where perennial ryegrass presented values of 46, 40, 29 and 14% for autumn, winter, spring and summer, respectively ($p< 0.05$).

The perennial rye grass in pure cultivated, during the autumn, contributed in the yield of forage with 98% (8 170 kg MS ha^{-1}), whereas in the summer it contributed 41% (1 891 kg MS ha^{-1}), the rest corresponded to weeds and dead material. The association 70:20:10, during the summer, presented a proportion of 26% of weeds and a decrease of perennial ryegrass of 80%. A behavior similar to the previous one was observed for the 50:00:50 association, where in the summer 25.5% of weeds and a 60% decrease of perennial ryegrass were found. The decrease in the proportion of perennial ryegrass, as the season elapsed, could be due to the harvest intensity, since being a species of erect growth was more susceptible to the loss of growth meristems, compared to the ovillo grass and white clover (Hodgson, 1990).

The grass for all associations contributed to forage yield with 4, 10, 18 and 23% for autumn, winter, spring and summer, respectively. The association 20:70:10 was the one that contributed the most with grass, with values of 11, 36, 47 and 61% for autumn, winter, spring and summer, respectively. While in the association 40:40:20 the contribution of the ovillo grass to the forage yield was 10, 14, 30 and 50% for autumn, winter, spring and summer, respectively. A behavior similar to the previous one was observed with the association 70:20:10, where the contribution of the ovillo grass to the yield was 2, 11, 30 and 31% for the seasons of autumn, winter, spring and summer, respectively.

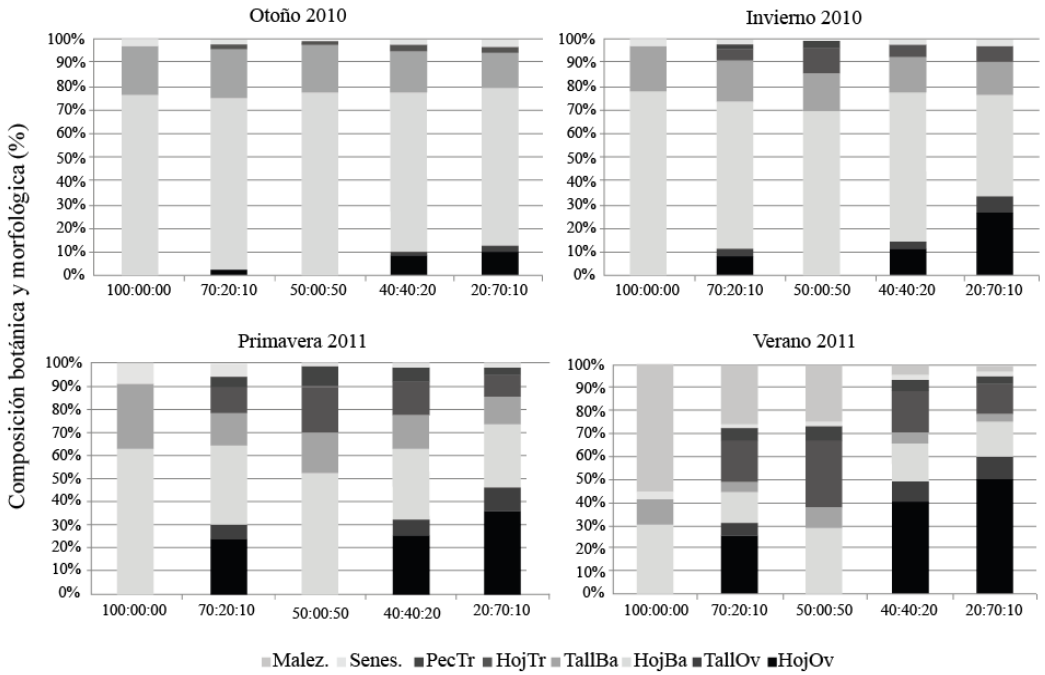

Figure 2. Botanical and morphological composition of perennial ryegrass alone and associated with grass and white clover. Malez= weed; Senes= senescence; PecTB= white clover petiole; HojTr= clover leaf; TallBa= rye stem; HojBa= rye leaf; TallOv= ovillo grass stem; HojOv= leaf ovillo grass.

The white clover increased as the seasons passed, where its contribution to forage yield for all associations was 1, 4, 10 and 12% for autumn, winter, spring and summer, respectively. The association with the highest contribution of white clover to forage yield was that of 50:00:50, with values of 1, 14, 28 and 35% in autumn, winter, spring and summer, respectively.

The increase of the ovillo grass and white clover, as the seasons passed, was attributed to the slow growth of these species, as well as to the habit of semierect growth of the ovillo grass and stoloniferous grass of the white clover, which favored a lesser removal of the meristems of growth, during grazing. In addition, with the decrease in perennial ryegrass there was less competition between species for light and nutrients, which caused a greater growth of the ovillo grass and clover grass (Durand *et al*., 1999; Rojas *et al*., 2016a; Maldonado *et al*., 2017).

The dead material morphological component showed significant differences ($p< 0.05$) for the associations in the different seasons of the year, where the highest values were 4 and 2% in the spring and summer seasons, respectively. It was found that perennial ryegrass in pure culture presented the highest amount of dead material with values of 8 and 3% ($p< 0.05$), during the spring and summer seasons, respectively.

The proportion of weeds was only significant ($p< 0.05$) during the summer season, with values of 55.8, 26, 25.5, 4.1 and 3% for the associations 100:00:00, 70:20:100, 50:00:50, 40:40:20 and 20:70:10, respectively. Pérez *et al*. (2002); Garduño *et al*. (2009) reported that severe perennial ryegrass crops reduced their persistence as a result of eliminating a larger quantity of growth meristems.

Stalk leaf ratio

The leaf:stem ratio of perennial ryegrass and its association with ovillo grass and white clover are shown in Table 2. No significant differences were found in the leaf: stem ratio for the associations evaluated (p>0.05). The effect was significant between the seasons (p>0.05), where the leaf: stem ratio was 5.9, 4.6, 3.9 and 3.6 for autumn, summer, winter and spring, respectively. In this regard, Zaragoza *et al*. (2009) pointed out that the leaf: stem relationship of the ovillo grass varies with the season of the year and the age of regrowth with average values in summer of 2.5 and 2.8 at the first and eighth week after regrowth, respectively.

Table 2. Relationship leaf: stem of perennial ryegrass (*Lolium perenne* L.) alone and associated with ovillo grass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.).

Association (Perennial ryegrass-ovillo grass-white clover)	Year 2010		Year 2011		Average	EEM	Sig.
	Autumn	Winter	Spring	Summer			
Ovillo grass (H/T)							
100:00:00	-	-	-	-	-	-	-
70:20:10	5.6 a	3 b	3.7 ab	4.4 ab	4.2	0.3	***
50:00:50	-	-	-	-	-	-	-
40:40:20	5.8 a	4.1ab	3.5 b	4.7 ab	4.5	0.4	***
20:70:10	6.3 a	4.5 ab	3.5 b	4.7 ab	4.7	0.4	***
Average	5.9 a	3.9 b	3.6 b	4.6 ab			***
EEM	0.7	0.5	0.5	0.6			
Sig.	ns	ns	ns	ns	ns		
Perennial ryegrass (H/T)							
100:00:00	3.6 ab	4.2 a	2.1 b	2.8 ab	3.2	0.2	***
70:20:10	3.4	3.7	2.5	2.9	3.1	0.3	ns
50:00:50	3.9	4.4	2.9	3	3.6	0.3	ns
40:40:20	3.8 ab	4.2 a	2.1 b	3.1 ab	3.3	0.2	***
20:70:10	4.3 a	3.2 ab	2.1 b	4.1 ab	3.4	0.3	***
Average	3.8 a	3.9 a	2.3 b	3.2 ab			***
EEM	0.2	0.2	0.3	0.2			
Sig.	ns	ns	ns	ns			
White clover (H/T)							
100:00:00	-	-	-	-	-	-	-
70:20:10	3.2	2.8	2.7	3.1	2.9	0.3	ns
50:00:50	2.5 b	3 b	2.6 b	4.6 a	3.1	0.3	***
40:40:20	2.8	3.1	2.5	3.2	2.9	0.2	ns
20:70:10	3.5 ab	3.5 ab	2.4 b	4.5 a	3.4	0.3	***
Average	3	3.1	2.6	3.8			ns
EEM	0.2	0.2	0.3	0.2			
Sig.	ns	ns	ns	ns	ns		

abcd= means with the same lowercase literal in the same row, they are not different (p> 0.05); ABCD= means with the same capital letter in the same column, they are not different (p> 0.05); EEM= standard error of the mean; Sig.= significance; ***= p< 0.05; ns= not significant.

No significant differences were found in the leaf: stem ratio of perennial ryegrass between associations ($p> 0.05$). However, the effect was significant ($p> 0.05$) between seasons, where the minor leaf: stem relationships were registered during the spring in the 100:00:00, 40:40:20 and 20:70:10 associations, with 2.1. These results can be attributed to the fact that in spring the temperature was optimal, which favored a greater growth and the entry of the reproductive phase of ryegrass. In this regard, Velasco *et al*. (2005) pointed out that the cutting frequency and the season of the year directly affect the amount of leaf and stem of this species, registering the highest stem values in spring and summer. Pérez *et al*. (2002) reported for perennial ryegrass, at five months of age, values of the leaf:stem ratio of 1.4 and 1.1 harvesting at a height of 3 and 15 cm, respectively.

In white clover the leaf: stem relationship had no significant effect ($p> 0.05$) for all associations. However, the effect was significant between the seasons of the year ($p> 0.05$) for the 50:00:50 association, where values of 4.6, 3, 2.6 and 2.5 were obtained during Summer, Winter, Spring and Autumn, respectively. A behavior similar to the previous one was obtained with the association 20:70:10, where the leaf: stem ratio values were 4.5, 3.5, 3.5 and 2.4 for the Summer, Autumn, Winter, and Spring seasons, respectively.

Density of plants

Statistical differences were found ($p< 0.05$) between treatments for the plant density variable. For perennial ryegrass, the association 100:00:00 recorded the highest density of plants, with a value of 28 plants m^{-2}, while the lowest density was obtained in the association 40:40:20 with 14 plants m^{-2}. A decrease in the density of plants m^{-2} was observed as the seasons passed, where the values were 29, 25, 20 and 11 plants m^{-2} for Autumn, Winter, Spring and Summer, respectively (Table 3). Rojas *et al*. (2016b) reported a three-year-old perennial ryegrass prairie with a lower density of plants, as the age of the pasture decreases its persistence. In ovillo grass, the association 20:70:10 was the one that registered the highest number of plants, with an average of 33 plants m^{-2}, followed by the association 40:40:20 with 21 plants m^{-2} and 70:20:10 with 10 plants (Table 3).

For white clover the highest density was found in the association 50:00:50 with an average of 10 plants m^{-2}, followed by the association 40:40:20 with 9 plants m^{-2} and 20:70:10 with five plants (Table 3). In a similar study, Rojas *et al*. (2016b) for these same associated species reported densities of ovillo grass-bearing grass of 24 and 23 plants m^{-2} in the first and second year of evaluation, respectively; while the density in white clover was 41 and 38 m^{-2} plants, for the first and second year, respectively. Chapman and Lemaire (1993) noted that grasslands respond differently to management, where harvest intensity or cutting interval affect their forage persistence and yield. In the present study, the perennial ryegrass was affected to a greater extent and the ovillo grass and white clover benefited.

Table 3. Density of plants (plants m^{-2}) of perennial ryegrass (*Lolium perenne* L.) alone and associated with ovillo grass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.).

Association (Perennial ryegrass- ovillo grass-white clover)	Year 2010		Year 2011		Average	EEM	Sig.
	Autumn	Winter	Spring	Summer			
			Ovillo grass (plants m^{-2})				
100:00:00	-	-	-	-	-	-	-
70:20:10	12 C	10 C	10 C	9 C	10 C	0.5	ns
50:00:50	-	-	-	-	-	-	-
40:40:20	22 B	22 B	21 B	21 B	21 B	0.6	ns
20:70:10	35 A	34 A	31 A	31 A	33 A	1	ns
Average	23	22	21	20			ns
EEM	3.8	3.7	3.6	3.6			
Sig.	***	***	***	***	***		
			Perennial ryegrass (plants m^{-2})				
100:00:00	40 Aa	35 Aab	26 Ab	12 c	28 A	3.3	***
70:20:10	31 Ba	27 ABa	21 ABb	9 c	22 AB	2.6	***
50:00:50	25 Bba	21 Ba	19 ABa	10 b	19 B	1.8	***
40:40:20	21 Ba	20 Ba	18 Ba	9 b	14 B	1.8	***
20:70:10	25 Ba	24 Bab	17 Bbc	13 c	19 B	1.7	***
Average	29 a	25 ab	20 b	11 c			***
EEM	1.8	1.8	1.6	0.8			
Sig.	***	***	***	ns	***		
			White clover (plants m^{-2})				
100:00:00	-	-	-	-	-	-	-
70:20:10	4 B	4 B	4 B	4 B	4 B	0.2	ns
50:00:50	11 A	10 A	10 A	10 A	10 A	0.3	ns
40:40:20	9 A	9 A	9 A	8 AB	9 A	0.3	ns
20:70:10	5 B	5 B	5 B	5 B	5 B	0.2	ns
Average	7	7	7	7			ns
EEM	1	0.9	0.9	0.9			
Sig.	***	***	***	***	***		

abcd= means with the same lowercase literal in the same row, they are not different ($p> 0.05$); ABCD= means with the same capital letter in the same column, they are not different ($p> 0.05$); EEM= standard error of the mean; Sig.= significance; ***= $p< 0.05$; ns= not significant.

Conclusions

The association perennial rye, ovillo grass and white clover in the proportion 50:00:50% had the highest yield of dry matter. In the autumn season the maximum production of forage was obtained and the lowest in winter. The perennial ryegrass was the species that contributed most to the yield in the fall, winter and spring seasons; while the ovillo grass and white clover did it in summer. According to the management of the prairies in the study, perennial ryegrass was the species with the least persistence.

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