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Technical note

Botanical composition and nutritive value of the diet consumed by cattle in an area invaded by natal grass [*Melinis repens* (Willd.) Zizka]

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Abstract:

The objective was to evaluate the botanical composition and nutritional value in the diet of bovine cattle in areas invaded by natal grass [*Melinis repens* (Willd.) Zizka]. The research was conducted at the Salinas Ranch, in the municipality of Satevó, Chihuahua, in a brush grassland. Botanical composition of the area was determined by the line-point intercept method. Sampling was conducted from August 2013 to February 2014. The botanical composition of the diet (microhistological technique) and the nutritional value were determined using two esophageal-fistulated Hereford/Angus heifers (350 ± 5 kg). The data were subjected to a variance analysis, and the chemical composition of the diet was fitted using the PROC MIXED procedure of SAS to a mixed model. The average available forage during the four phenological stages was 1,279 kg DM ha^{-1}, with a presence of 87.5 % natal grass (1,119.13 kg DM ha^{-1}). The highest preference indexes were for *Aristida divaricata* (8.43) and *Croton pottsii* (12.95) during the growing stage; whereas the least preferred

species was *Melinis repens* (0.33 to 0.41). The highest crude protein content was observed during the growing (13.23 %) and flowering stages (10.71 %). During this study the best quality of the diet was determined during the growing and flowering stages and was mainly composed of *Melinis repens* during four stages.

Key words: Preference index, Voluntary intake, *Melinis repens*.

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Beef production in extensive systems depends mainly on the quantity and quality of available forage that are present in the pasturelands and are the cheapest food for the cattle. In northern Mexico, rainfalls usually occur from June to September (80 %), the period with the highest weight gains and the highest profitability[1]. One of the most important economic activities for livestock in the state of Chihuahua is the cow/calf system, which consists in the production of calves for export[2,3]. However, in recent years, the cattle industry has suffered losses due to droughts and to poor management of the grasslands. The result has been a loss in the output of the grassland and a reduction of the rangeland, due to forced sales and to the slaughter of the livestock under poor conditions[4,5]. Furthermore, large grassland areas have undergone changes in their structure due to the introduction of, and invasion by non-native species, which have an ecological impact through the loss of native forage species[6,7].

Natal grass (*Melinis repens*) is an opportunistic species that has spread in the last 30 yr across the Mexican territory[7], as it establishes quickly and displaces native species with an ecological, economic and livestock importance[8]. Its forage value ranges between fair and bad during the latency period, when it can attain raw protein (RP) values of 4 %[7]. However, the degree of preference by grazing animals is unknown. In Chihuahua, this species has moved from the center-south to the north of the state and is located mainly in areas that have been degraded due to overgrazing and in abandoned lands that were opened to agricultural use.

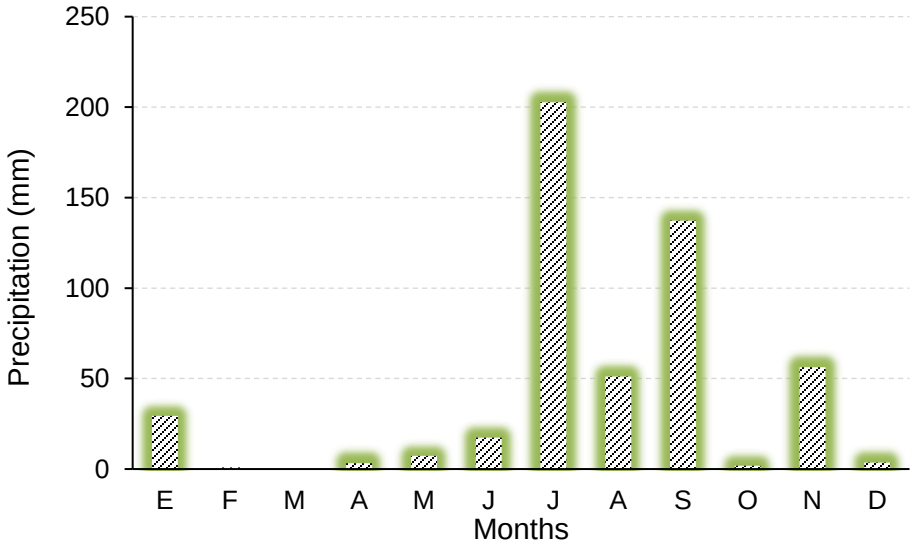
The studies on the diets of grazing bovine cattle in Chihuahua date back to the 1980s; these studies were performed in areas of the central-northern region and at a time when the invasion by *M. repens* was at its initial stage or when this species was not yet present[9,10,11]. Knowledge of the composition of the diet and of the nutritional contribution of forage plants in invaded areas may be helpful for designing and executing schemes of use and management of both the grasslands and the grazing cattle.

Various techniques have been utilized to gain knowledge of the botanical composition of the diet of grazing cattle. One of these is the microhistology, which allows identifying and

quantifying the botanical composition of the diet consumed[11,12]. Furthermore, it is important to know the nutritional value of the diet of the grazing cattle in order to establish the necessary supplementation during the critical time, when the requirements are not met by the grazing animals. For these reasons, and because there is no information on grasslands invaded by *M. repens*[13], this study evaluated the botanical composition and the nutritional value of the diet of the grazing cattle in an area invaded by *M. repens*.

This study was conducted at the "Salinas" Ranch, located in the municipality of Satevó, Chihuahua, located at 27° 57' 00" N and 106° 07' 00" W, and at an altitude of 1,540 m asl. Average annual temperature is 18.1 °C, and its historical mean annual precipitation, 464 mm[14]. However, in the year 2013, according to CONAGUA[15], there was a mean precipitation of 550 mm; the rainfalls during the study year were mainly distributed from July to September and November (Figure 1). Climate of the area is classified, according to Köppen, as BWh[16]. The soils are shallow (0 to 25 cm), regular internal drainage, and a pH of 5.3 to 6.6[16].

Figure 1: Mean precipitation (mm) in the year 2013 during the study period[14]



This study was conducted in an area of 200 ha and has a rangeland coefficient of 4 ha UA^{-1}, which was determined based on the amount of usable forage (1,470 kg ha^{-1}) and the requirements of the type of animal[10,17]. This area was selected because it is representative of the grasslands invaded by *M. repens*. The vegetation type is short-midgrass grassland invaded by *M. repens*, and with the presence of shrub species like *Mimosa biuncifera* and *Prosopis glandulosa*, and grasses like *Bouteloua gracilis*, *Bouteloua curtipendula*, among others (Table 1)[16,18].

Table 1: Botanical composition of the area of study during the vegetative stage

Grasses	Coverage (%)[1]	Shrubs	Coverage (%)[1]	Herbaceous species	Coverage (%)[1]
Bouteluoa curtipendula	0.41	*Aloysia wrightii*	1.46	*Bulbostilis juncoides*	0.63
Bouteluoa gracilis	2.50	*Calliandra eriophylla*	9.60	*Dichondria argentea*	0.41
Melinis repens	63.72	*Condalia* sp.	0.21	*Euphorbia* sp.	0.21
		Juniperus monosperma	1.25	*Evolvolus alsynoides*	9.60
		Mimosa biuncifera	1.67	*Haploppapues gracilis*	0.21
		Prosponis glandulosa	2.50	*Macrosiphonia hypoleuca*	0.21
		Tecoma stands	0.21	*Millia biflora*	0.62
				Sida procumbens	3.76
				Croton pottsii	1.00

[1] Determined with the point intercept method[18].

The botanical composition and the quality of the diet were conducted during four sampling stages (August, 2013, to February, 2014), corresponding to the phenological stages of the grasses (vegetative, reproductive, post-reproduction and dormant season) present in the area (Table 2). The first sampling was carried out during the vegetative stage (August 3 to 7, 2013); the second, during the reproductive stage (October 1 to 5, 2013); the sampling for the post-reproduction stage was carried out on December 3 to 7, 2013, and the dormant season was conducted during February 1 to 5, 2014. Two Hereford/Angus heifers with an average weight of 350 ± 5 kg, fistulated (with a Bar Diamond^{TM} esophageal fistula)[19,20,21]. Holechek[12] mentions that the botanical composition in grasslands with a very homogeneous vegetation can be determined using two animals during two to three days; for this reason, two fistulated animals were utilized during a 5-d grazing period.

Table 2: Botanical composition (%) of the grasses in the study area

Species	Sampling stages			
	Vegetative	Reproductive	Post-reprod.	Dormancy
Aristida divaricata	0.57[1]	--[2]	--[2]	0.61[1]
Bothriochloa barbinodis	--	4.12	1.15	0.81
Bouteloua chondrosioides	--	4.67	0.37	0.99
Bouteloua curtipendula	9.58	7.61	3.26	3.60
Bouteloua gracilis	10.15	7.35	4.89	3.49
Bouteloua hirsuta	--	2.44	1.75	1.32
Heteropogon contortus	--	--	1.01	1.48
Leptochloa dubia	--	1.11	--	0.90
Muhlenbergia phleoides	--	1.01	0.61	0.86
Melinis repens	79.68	71.68	86.96	85.94

[1] Values calculated based on the biomass production.

[2] Not observed in the reading.

Heifers were given a 3-d adaptation period in the study area before each sampling. Heifers were put in a corral during the night before sampling, fasting from water and food to avoid sampling contamination. In the morning, they were placed in the study area during 45 to 60 min, after which the bags were withdrawn with the collected sample. The samples were taken to the animal nutrition laboratory of the Faculty of Zootechnics and Ecology, to be dried in a forced air stove at 60 °C during 72 h, and subsequently ground using a Wiley™ mill with a 1 mm mesh (Arthur H. Tomas, Philadelphia, PA, USA)[22,23].

Dry matter yield. In order to estimate the dry matter yield (kg MS ha^{-1}), 25 points were selected at random, and a 0.25 m^2 quadrant was utilized to cut the forage. The grasses (separated by species) in each quadrant were cut at ground level. The forage samples were dried in a stove at 75 °C during 72 h and weighed at the Laboratory of Animal nutrition of the Faculty of Zootechnics and Ecology of the Universidad Autonoma de Chihuahua (FZyE-UACH). The percentage of each species was calculated based on the weights obtained during the four phenological stages, using a Tor Rey™ L-EQ scale, and the dry weight was extrapolated to the production of DM per hectare, according to the following formula:

$$\text{DM ha}^{-1} = \frac{weight\ of\ the\ dry\ sample\ \ (g) * 4}{1000\ g} * 10{,}000$$

Botanical composition of the area**.** The botanical composition was measured in two different ways: A general study of the present vegetation in July was described using the line-point intercept method[18], in order to have a reference of the species present in the study area. The measurement was taken only in July because the composition of the shrubs does not change with the various stages[17]. The second vegetation measurement was done counting the presence or absence of grasses during each phenological stage using a 0.25 m^2 quadrant.

Botanical composition of the diet. The vegetal tissues of the species present in the diet of the cattle were identified using the microhistological technique modified by Peña and Habib[11], which reads five tissue slides, and 20 fields in each of these. The work was carried out in the Water-Soil Plant Laboratory of the Colegio de Posgraduados, COLPOS), Campus SLP.

Preference index. The index of preference by the species was estimated based on the botanical composition of the diet and of the area, using the formula proposed by Van Dyne and Heady[24].

$$\text{PI} = \frac{\text{Botanical composition of the diet}}{\text{Botanical composition of the area}}$$

Higher values represent a greater preference by the cattle, and smaller values, a lower level of preference. After the samples were prepared (30 for each sampling period) were prepared, they were subjected to chemical analyses in order to determine their raw protein content and *in vitro* digestibility. These were conducted at the laboratory of animal nutrition of FZyE-UACH. The crude protein content (CP) was determined using the Kjeldahl method: the amount of nitrogen (N) in the sample was multiplied by 6.25[22]. Furthermore, the *in vitro* digestible organic matter was determined in a Daisy II incubator, following the methodology proposed by Ankom™ (Ankom Technology, Fairport, NY, USA)[23]. The neutral (NDF) and acid detergent fiber (ADF) were determined using the methodology proposed by Van Soest *et al*[25].

In order to carry out the statistical analysis of the botanical composition of the diet, only the category of grasses, herbs and shrubs was included. The data were subjected to a variance analysis in a completely randomized design (α= 0.05), using the MIXED procedures of the SAS software[26]. The equation of the statistic model utilized was:

$$y_{ij} = \mu + P_i + e_{ij}$$

Where:

$\boldsymbol{y_{ij}}$= value of the response variable (for each species and for the groups) observed at the i^{th} phenological stage;

μ= effect of the overall mean;

$\boldsymbol{P_i}$= fixed effect of the i^{th} phenological stage;

$\boldsymbol{e_{ij}}$= term of the random error associated with the observations, where $e_{ij} \sim NIID(0, \sigma^2)$.

The chemical composition data were subjected to a variance analysis, adjusting a mixed model with the PROC MIXED procedure of the SAS software[26], with the following equation:

$$y_{ijk} = \mu + P_i + V_j + \theta_{ij} + e_{ijk}$$

Where:

$\boldsymbol{y_{ijk}}$= value of the response variable (OM, CP, *iv*DOM, NDF, ADF) observed at the i^{th} phenological stage in the j^{th} fistulated animal;

μ= effect of the overall mean;

$\boldsymbol{P_i}$= fixed effect of the i^{th} phenological stage;

$\boldsymbol{V_j}$= random effect of the j^{th} fistulated animal;

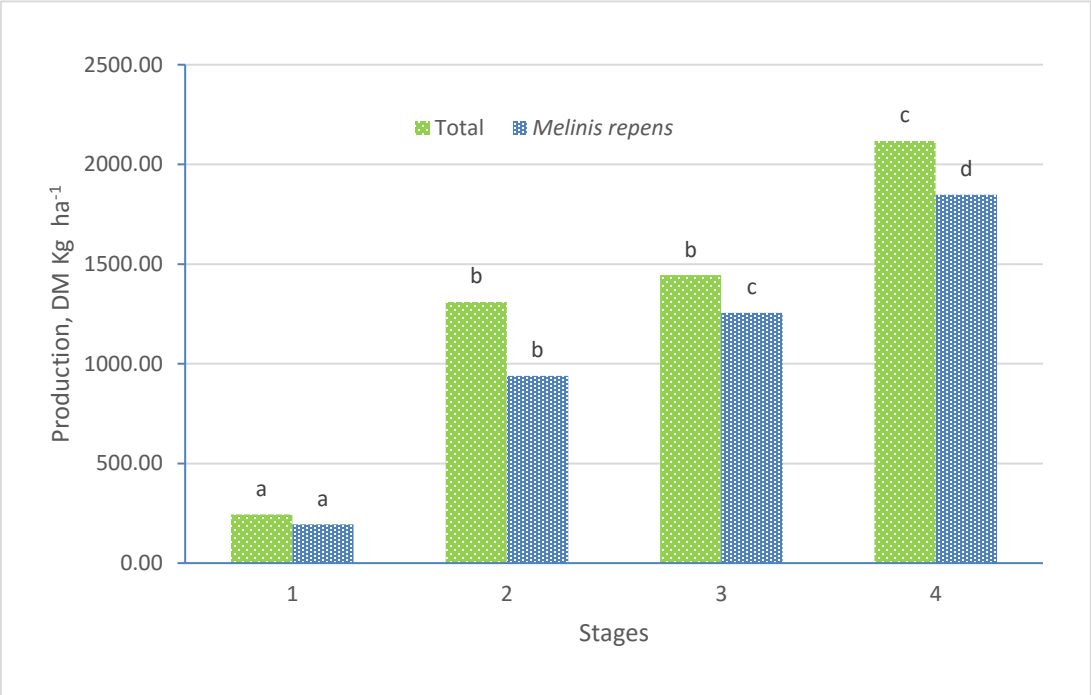
$\boldsymbol{\theta_{ij}}$= random effect of the interaction between the i^{th} phenological stage and the j^{th} fistulated animal;

$\boldsymbol{e_{ijk}}$= term of the random error associated with the observations, where $e_{ijk} \sim NIID(0, \sigma^2)$.

Where a significant effect ($P<0.05$) of the phenological stage was observed, the least significant difference (LSD) test was utilized for the mean comparison ($\alpha = 0.05$).

The dry matter yields were different ($P<0.05$) between stages (Figure 2). The greatest output (2,119 kg MS ha^{-1}) occurred at the latency stage, due perhaps to the residual humidity of the November rainfalls. Furthermore, Figure 3 shows the forage yield of *M. repens*, which represents 72.38 to 88.55 % of the botanical composition of the area. However, another study[27] reports a dry matter production of 2,913 kg ha^{-1} in grasslands invaded by *M. repens* during a rainy year in Aguascalientes, Mexico. However, the production in dry years was 1,488 kg ha^{-1}. This shows that the amount of precipitation and humidity in the soil are important factors to consider. The mean precipitation in the study area during the study year was 510 mm (considered atypical), while the historical annual mean for these areas is 462.4 mm[14].

Figure 2: Least square means of the total yield and of the yield of *Melinis repens* during the phenological stages



[abcd] Different letters between columns indicate a difference ($P<0.05$).

Table 3 shows the species consumed by the grazing cattle in different sampling stages; during the dormant stage, *M. repens* was the species with the highest content in the diet (35.5 %), compared with the vegetative, reproductive and post-reproductive stages (27, 29 and 29 %, respectively). The content of this invasive grass in the diet exhibited a difference ($P<0.05$); this may be due to the occurrence of a regrowth, during the dormant season, that was consumed by the cattle. Furthermore, other studies report that the abundance of a species in a particular area may influence the diet of grazing animals[28,29]. The diet consumed by the bovine cattle grazing in areas invaded by *M. repens* consisted primarily of grasses (74.05 ± 1.66 %) during all four phenological stages. These findings agree with those reported by other authors[30], who pointed out that grasses are the main food consumed by grazing cattle.

Table 3: Botanical composition (%) of the diet of bovine cattle in an area invaded by *Melinis repens*

Species	Sampling stages			
	Vegetative	**Reproductive**	**Post-reprod.**	**Dormancy**
Grasses				
Aristida divaricata	4.81[a]	5.01[a]	4.63[a]	3.17[a]
Bothriochloa barbinodis	0.74[a]	3.29[b]	3.39[b]	3.63[b]
Bouteloua chondrosioides	4.06[a]	4.96[a]	6.02[a]	4.97[a]
Bouteloua curtipendula	8.84[a]	10.14[a]	11.19[a]	11.12[a]
Bouteloua gracilis	9.23[a]	13.59[b]	12.07[ab]	10.87[ab]
Bouteloua hirsuta	2.38[a]	4.24[b]	3.85[b]	4.08[b]
Heteropogon contortus	0.36[a]	2.86[b]	3.01[b]	2.37[b]
Leptochloa dubia	0.36[a]	3.72[b]	2.58[b]	2.71[b]
Muhlenbergia phleoides	0.71[a]	2.87[a]	1.77[a]	2.29[a]
Melinis repens	26.67[a]	29.27[a]	28.86[a]	35.53[b]
Total	58.16[a]	79.95[b]	77.37[b]	80.74[b]
Herbaceous species				
Croton pottsii	12.95[a]	5.54[b]	4.72[b]	4.19[b]
Total	12.95	5.54	4.72	4.19
Shrubs				
Calliandra eriophylla	15.85[a]	8.58[b]	11.08[ab]	8.95[b]
Prosopis glandulosa	13.03[a]	5.93[b]	6.83[b]	6.13[b]
Total	28.88[a]	14.51[b]	17.91[b]	15.08[b]

[ab] Means with different letters in the same row indicate a statistical difference ($P<0.05$).

Melinis repens was the species with the lowest preference index (ranging between 0.33 and 0.41) during the four stages, despite its high density in the study area. However, *Bouteloua chondrosioides* presented a high rate (16.26) at maturity, while *Aristida divaricata* presented an index of 8.43 during the stage of growth (Table 4). Despite the low density of these species in the study area, they presented the highest preference rates. This performance is supported by previous studies[28,31], where it was observed that species that showed greater preference index, were less present in the study area.

Table 4: Preference index in an area invaded *Melinis repens*

Species	Sampling stages			
	Vegetative	**Reprodcutive**	**Post-reprod.**	**Dormancy**
Grasses				
Aristida divaricata	8.43[1a]	--[2]	--	5.19[a]
Bothriochloa barbinodis	--	0.80[a]	2.95[ab]	4.49[b]
Bouteloua chondrosioides	--	1.06[a]	16.26[b]	5.02[a]
Bouteloua curtipendula	0.92[a]	1.33[a]	3.43[b]	3.09[b]
Bouteloua gracilis	0.91[a]	1.85[b]	2.47[bc]	3.11[c]
Bouteloua hirsuta	--	1.74[a]	2.20[ab]	3.09[b]
Heteropogon contortus	--	--	2.98[a]	1.60[a]
Leptochloa dubia	--	3.36[a]	--	3.01[a]
Muhlenbergia phleoides	--	2.84[a]	2.91[a]	2.66[a]
Melinis repens	0.34[a]	0.41[a]	0.33[a]	0.41[a]
Herbaceous species				
Croton pottsii	12.95[a]	5.54[b]	4.72[b]	4.19[b]
Shrubs				
Calliandra eriophylla	1.65[a]	0.89[b]	1.15[ab]	0.93[ab]
Prosopis glandulosa	5.21[a]	2.37[b]	2.73[b]	2.45[b]

[1] High values represent a high preference by the cattle.

[2]Not consumed by the cattle or not observed in the reading.

[abc] Means with different letters in the same row indicate a difference ($P<0.05$).

The content of crude protein differed ($P<0.05$) between phenological stages. The highest values were found during the vegetative and reproductive stages (13 and 11 %, respectively). In contrast, the lowest values occurred during the dormancy stage (6.5 %; Table 5). However, another study reports CP values of 10.49 % during the summer, and 5.49 % during the spring; this may account for the fact that the animals are unable to meet their nutritional requirements[32]. Besides, other authors report that, during the rainy season, the bovine cattle can satisfy its minimum maintenance requirements thanks to the phenology of the grasses[33]; however, the nutrients diminish in winter, and therefore these requirements are unmet during this season[34]. The CP values registered in the present research during the vegetative and reproductive stages were probably due to the consumption of species with high CP values, such as *Bouteloua gracilis* and *Bouteloua curtipendula*, shrubs like *Prosopis glandulosa* and *Calliandra eriophylla*, and the herbaceous species *Croton pottsii*[35,36,37]. Moreover, the season of the year is another factor to be considered in this type of studies[38]. Thus, it may

be assumed that the reported CP values were not a result of the consumption of *Melinis repens*, as the CP values of this species range are 4 to 6 %[7].

Table 5: Means (±SE) of the chemical composition of the diet consumed by grazing bovine cattle in an area invaded by *Melinis repens*, during the phenological stages

Variable (%)	Phenological stage			
	Vegetative	**Reproductive**	**Post-reprod.**	**Dormancy**
CP	13.76±0.92^{a}	10.72±0.92^{b}	8.61±0.92^{bc}	6.559±0.92^{c}
OM	85.86±0.607^{a}	85.95±0.607^{a}	81.39±0.607^{b}	81.10±0.607^{b}
*iv*DOM	41.36±1.86^{a}	38.53±1.86^{a}	43.08±1.86^{a}	36.56±1.86^{a}
NDF	70.71±1.54^{a}	71.19±1.54^{a}	71.02±1.54^{a}	72.64±1.54^{a}
ADF	42.80±1.38^{a}	42.46±1.38^{a}	47.74±1.38^{a}	42.70±1.38^{a}

CP= crude protein; OM= organic matter; *iv*DOM= *in vitro* digestibility of the organic matter.

NDF: ADF= neutral and acid detergent fiber.

^{abc} Means with different letters in the rows indicate difference (P<0.05).

The *iv*DOM ranged between 36.56 and 43.08 % (P>0.05; Table 5). This may be due to the structural change of the grasses, herbs, and shrubs during the development of the plants, which affects the amount of digestible tissue present in them[39]. Other authors have reported values of 52.3 to 54.9 % in areas invaded by *Melinis repens* during the dormancy stage[13]. This result agrees with those reported in another study, in which the *iv*DOM values were 67.34 and 58.23 % in the summer and the spring, respectively[32].

Table 5 shows the data obtained for the neutral detergent fiber (NDF), for which no differences were found (P>0.05); however, these results differed from those of another study[32], which reported NDF values of 64 to 74 % in a grassland where *Melinis repens* was present. Other researchers[40,41] reported values of 69.2 to 70.1 %, which were similar to those estimated in the present study. Furthermore, Murillo[42] reported similar NDF values (70.4%) to ours during the dormancy stage. Likewise, the ADF was estimated, and no significant differences were found (P>0.05). However, other authors report values above those obtained in this study[32]. The content of lignified polysaccharides increases with maturity, and therefore the ADF value also increases[43]. Probably for this reason, the ADF values in the diet found in the present research ranged between 42.7 and 47.74 %. A recent study reports similar ADF values (42.3 %) to those obtained in this study.

The botanical composition of the diet was constituted mainly by *Melinis repens* during the four phenological stages, as this was the most abundant grass in the study area. The grasses were preferred by the animals during the four sampling periods, and there was only one

herbaceous species (*Croton pottsii*) during the sampling. The highest preference indices for *M. repens* were obtained during the dormancy and reproductive stages. The nutritional value of the diet of grazing bovine cattle in areas invaded by *Melinis repens* does not meet the requirements for the production and maintenance during the post-reproductive and dormancy stages. It is advisable to carry out studies with a focus on the planning and design of utilization schemes in grasslands invaded by exotic species.

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