Nutritional characterization of *Larrea divaricata* Cav during winter and its potential as cattle and goats feed

Caracterización nutricional de *Larrea divaricata* Cav durante la temporada invernal y su potencial como alimento para bovinos y caprinos

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Abstract

The ever-increasing global demand for agricultural commodities and progressive climate change factors are displacing extensive beef cattle and goat ranching from temperate humid regions to peripheral regions with semi-arid characteristics. Extensive investigation is required on native desert plants to be safely incorporated into feed programs and to maintain the biodiversity and sustainability of these fragile ecosystems. *Larrea divaricata* is a native plant adapted to arid and semi-arid biomes of South and Western-South America. This research evaluates the nutritional composition of the browsing available canopy parts of *Larrea divaricata* during the winter season in a semi-arid region of Argentina. Its crude protein content resulted in 11.20% of dried matter and its soluble protein content resulted in nearly 80% of the crude protein. Acid detergent fiber fraction, ash-corrected neutral detergent fiber fraction, lignin, ash content, fat-like compounds, and non-fibrous carbohydrates resulted in 17.42, 35.51, 12.09, 9.96, 5.92 and 3.82% of dried matter, respectively. Essential bioelements Ca, Mg and K resulted within standard forage requirements. Total polyphenols and flavonoids resulted in 430 mg/g and 140 mg/g, respectively. These results demonstrate that *Larrea divaricata* can be an effective complement for winter-feeding beef cattle and goats in arid and semi-arid regions.

Keywords
native plant • shrub • jarilla • ruminant • desert • forage

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Resumen

La creciente demanda de productos agrícolas y el cambio climático, están desplazando la ganadería extensiva desde regiones templadas húmedas, hacia regiones periféricas semiáridas. Ello requiere de investigaciones exhaustivas respecto de plantas nativas, para incorporarlas eficientemente a los programas de alimentación y mantener la biodiversidad y la sostenibilidad de estos frágiles ecosistemas. La *Larrea divaricata*, es una planta nativa de las regiones áridas y semiáridas de Sudamérica. Este trabajo, evalúa la composición nutricional de las partes aéreas disponibles para el ramoneo de *Larrea divaricata* durante la temporada invernal. Su contenido de proteína cruda resultó 11,20% de la materia seca. La proteína soluble resultó cerca del 80% de la proteína cruda. La fracción de fibra detergente ácida, la fracción de fibra detergente neutra, la lignina, el contenido de cenizas, los compuestos grasos y los carbohidratos no fibrosos resultaron 17,42; 35,51; 12,09; 9,96, 5,92 y 3,82% de la materia seca, respectivamente. Bioelementos esenciales como Ca, Mg y K resultaron en niveles relevantes. Los polifenoles y flavonoides totales representaron 430 mg/g y 140 mg/g, respectivamente. Estos resultados, demuestran que *Larrea divaricata* puede ser un complemento eficaz para la alimentación invernal de ganado vacuno y caprino en regiones áridas y semiáridas.

Palabras claves

planta nativa • arbusto • jarilla • rumiante • desierto • forraje

Introduction

Larrea divaricata is a plant endemic to the southern and western territories of South America, including vast arid and semi-arid regions of Argentina, Bolivia, Chile and Peru (20, 30). *L. divaricata* is a perennial shrub, 0.9 to 1.8 meters tall, with resinous leaves that grow at the tips of the branches (7) and exhibit antifungal properties (4). This plant is closely related to *Larrea tridentata* (Sessé & Moc. ex DC.) Coville, which is endemic to western North America, including Mexico and the USA (32). Both species belong to the Zygophyllaceae family (32) and thrive only in their natural habitats (14, 27). Interestingly, countries where *L. divaricata* and *L. tridentata* are endemic account for 18% of world livestock inventory (31).

Extensive husbandry methods for beef cattle and goats are undergoing accelerated changes. The ever-increasing global demand for agricultural commodities, coupled with progressive climate change factors, is displacing extensive livestock ranching from temperate humid regions to peripheral areas with semi-arid characteristics (28). The situation in South America is no exception. Record prices of soybean, corn, wheat and sunflower, among other agricultural commodities (8), have driven extraordinary profitability of the cultivated land in the humid Pampa region of Argentina (3). As a result, extensive beef cattle and goat activities are being displaced from the humid Pampa region to the semi-arid Pampa region (34). The semi-arid Pampa region is located in central-south Argentina, between the humid Pampa region to the north and the Patagonia region to the south. The climate of the semi-arid Pampa region is dry with temperate summers and cold, harsh winters (43). Here, beef cattle and goat ranching are adapted to silvopastoral systems and incorporate native forests and wild pastures into the diet (6, 43). Figure 1 (page 165), shows the distribution of *L. divaricata* in Argentina, the direction of agricultural expansion and extensive livestock displacement toward semi-arid zones. This situation presents an opportunity for exploiting native vegetation as forage, without using agrochemicals, soil tillage, or artificial irrigation and with the advantageous approval of Carbon Neutral Meat Certifications.

Native forage species should be palatable, have adequate nitrogen content and exhibit high digestibility (15, 33, 39). However, extensive research is needed to safely incorporate native plants into livestock feeding programs, while maintaining the biodiversity and sustainability of the land (14, 15, 38, 40).

Figure 1. Distribution of *L. divaricata* in Argentine territory and displacement of extensive livestock as a consequence of agriculture.

Figura 1. Distribución de la *L. divaricata* en el territorio argentino y desplazamiento de la ganadería extensiva como consecuencia de la presión agrícola.

In the semi-arid Pampa region, beef cattle and goats browsing on *L. divaricata* are considered essential for nutrition during winter seasons. Here, pastures are dry, nutrient-poor, and scarce, either due to overgrazing, fires, or snow cover (13). However, there is little scientific information on the nutritional value of *L. divaricata* during the winter season (41).

This article studies the nutritional value of L. divaricata canopy leaves collected from specimens in the semi-arid Pampa region, during winter. It focuses on key nutritional parameters, including nutritional content, energy value, mineral composition, and digestibility. The analysis followed standard procedures recommended for assessing the nutritional requirements of beef cattle and goats. The discussion highlights the potential of *L. divaricata* as winter forage for beef cattle and goats.

Materials and Methods

Plant material

Samples of *L. divaricata* were obtained from two fields in the Province of La Pampa, Argentina. One field is located in the Department of Loventué, at an elevation of 308 meters above sea level, with coordinates 36°11’00” S latitude and 65°18’00” W longitude, while the other is in the Department of Chalileo, at 306 meters above sea level, with coordinates 36°13’32” S latitude and 66°56’25” W longitude. The samples were obtained from 30 randomly selected specimens located within a radius of 200 meters from the above-mentioned geographical references (15 specimens from Loventué and 15 specimens from Chalileo). All samples were taken during the winter season (late August). Only plant organs compatible with browsing habits of cattle and goats were collected. All specimens appeared healthy and homogeneous. Leaf samples were taken without considering the size or age of the plant.

Sample conditioning

Samples of 100 g of leaves were collected from 30 randomly selected *L. divaricata* specimens until a total weight of 3 kg was reached. The obtained material was immediately placed in a dark dry place. The samples were mixed and dried under ambient conditions for 7 days until constant weight. Natural drying was preferred due to the presence of resin on the surface of the leaves. The dry matter (DM) and moisture percentages were determined gravimetrically. The dried material was ground in a blade mill, passed through a 2-mm mesh sieve, homogenized, and subdivided into several fractions for various types of analysis.

Other materials

A 95% ethanol aqueous solution (Purocol, Almirante Brown, Argentina) was used as solvent for polyphenol and flavonoid extraction. Distilled water was used as medium for extract resuspension. Folin-Ciocalteu reagent (Sigma Chemical Co.) and sodium carbonate (Alcalis de la Patagonia, San Antonio Oeste, Argentina) were used for polyphenols characterization. Reagent-grade sulfuric acid (Biopack, Buenos Aires, Argentina) was used for lignin determination. Analytical grade dichloromethane (CH_2Cl_2), methanol (MeOH) and N-hexane (Cicarrelli, San Lorenzo, Argentina) were used as extraction solvents for isolating *L. divaricata* resin.

Phytochemical determinations

Qualitative determinations of phytocompounds, including polyphenols and flavonoids, were conducted according to conventional spectrophotometry techniques (2, 5).

The total polyphenolic content was determined using the Folin-Ciocalteu method (37). Briefly, a sample of 1 g of dried material was extracted with 20 mL of 95% ethanol solution at room temperature for 24 h. Subsequently, the obtained extract was filtered and concentrated under vacuum to obtain 500 mg of dry extract. The dry extract was resuspended in 150 mL of distilled water and incubated in a water bath for 30 min at 90°C. A 2 mL aliquot of this resuspended extract was withdrawn and mixed with 1 mL of Folin-Ciocalteu reagent and 10 mL of distilled water. After vortexing, 12 mL of a sodium carbonate aqueous solution (290 g/L) were added to the mixture and left to react for 30 min in the dark. The absorbance at 760 nm of the blue-colored solution was recorded. Results were converted to total polyphenol content expressed in mg of gallic acid equivalents per 100 g of dry sample (mg GAE/100 g). Four random samples were taken from the dry and homogenized material for each polyphenol analysis. Then, three aliquots were taken from the stock solution obtained from each of these four samples. Finally, the absorbance readings of the aliquots were carried out twice.

The total flavonoid content is expressed as milligram quercetin equivalent per gram of extract (mg QE/g) and determined according to the Quercetin standard curve method (36). The aluminum chloride colorimetric method determined flavonoid content in *L. divaricata* extracts. Absorption readings at 425 nm were taken after 30 minutes of incubation in the dark. All analyses were performed in triplicate, and the results were averaged.

Evaluation of nutritional assets of *L. divaricata*

Core nutrients, minerals, and digestibility of *L. divaricata* were measured according to standard recommended techniques (46).

In cattle, forage crude protein (CP) indicates potential growth and productivity enhancement. CP is expressed as a percentage of DM, and determined by measuring nitrogen (N) content, using GAFTA and ISO methods (10, 21). It is important to note that the CP fraction includes non-protein nitrogenous substances such as amines, amides, urea, nitrates, peptides and isolated amino acids. These compounds are soluble, highly degradable and have less nutritional value than true proteins. A high level of CP does not always indicate a good protein level.

Soluble protein (SP) is reported as a percentage of the CP and indicates how much of the total CP is available for digestion. The acid detergent insoluble CP (ADICP) measures the tightly bound protein not available for digestion. ADICP is a fraction of the acid detergent fiber fraction (ADF) bounded to the protein content. ADF measures the content of cellulose, lignin, Maillard compounds, silica and cutin in the DM. ADF is an indirect indicator of the degree of digestibility of the forage. The higher the ADF, the less digestible is the forage. It is expressed as a percentage of the DM, and determined according to ISO 13906 (25). Neutral detergent insoluble CP (NDICP) measures the amount of protein that is bound to the neutral detergent fiber fraction (NDF). It is expressed as a percentage of the DM, and determined according to ISO 16472 (26). NDF is a measure of the cell wall content in the DM and includes the content of hemicelluloses, cellulose and lignin. ADF is part of NDF. Both ADF and NDF vary with plant phenotype, age and season. The NDF corrected for ash content (aNDF) is expressed as a percentage of DM. High aNDF value generally occurs in plant species with low CP values (45).

Lignin is a polyphenol produced by the plant during its maturation. Lignin is responsible for plant rigidity and support. It is not digestible by ruminants and acts as a barrier to the ruminal microbial digestion of cellulose and hemicelluloses. The lignin content is expressed as a percentage of DM, and measured according to an adapted method based on ISO 13906 (25).

Non-fibrous carbohydrates (NFC) are sugar-related carbohydrates and starches. NFC are nearly 100% digestible. Starch is the most important fraction of the NFC. NFC are necessary for the growth of intestinal bacteria and the production of high-quality bacterial proteins (19). NFC is expressed as a percentage of DM. NFC is determined by measuring the total glucose content, after cleaving sugar-related carbohydrate and starch molecules into individual glucose molecules.

The above-mentioned parameters were determined using a Fibertec™ automated system (Gerber Instruments, Effretikon, Switzerland). The Fibertec™ automated system used programmed standard reference methods.

Ethereal extract (EE) is the lipid fraction of the DM. It is mainly composed of oils, fats and other high-energy nutrients. EE values greater than 14% can be toxic to rumen bacteria. Additionally, EE compounds are prone to becoming rancid during storage. It is expressed as a percentage of DM and determined according to an ether extraction method (9, 23).

The mineral profile in forage is an important factor for extensive livestock production. The ash fraction accounts for the macro and micro inorganic elements of the plant and those acquired from the environment. The ash fraction of common forages is usually less than 10% of DM. Ash contents higher than 10% are considered likely contaminated with soil materials. The ash content is expressed as a percentage of DM and measured gravimetrically, by comparing the weight before and after burning the sample (11, 22). Total mineral in ash was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (NexION 2000 ICP Mass Spectrometer, Perking Elmer, Boston, USA). The elemental analysis was performed using an Atomic Absorption Spectrometer (AAS) (AANALIST 200, Perkin Elmer, USA), according to the standard methods (1, 12, 24). The following elements were determined: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorous (P), manganese (Mn), sulfur (S), zinc (Zn), copper (Cu), and iron (Fe).

Evaluation of nutritional assets of *L. divaricata* resin

Subsamples of *L. divaricata* were withdrawn from the original dried sample and used to extract the resin. Fifty g of dried samples were immersed in 200 ml of CH_2Cl_2/MeOH (3:1) at 25°C for 24 h. The supernatant was recovered, filtered and the solvent was evaporated. Subsequently, the dried extract was immersed in N-hexane. The resin was dissolved in the upper N-hexane phase. The N-hexane phase was evaporated to isolate the resin material. The evaluation of the nutritional assets of the *L. divaricata* resin was carried out using the same procedures and methods described in the previous section.

Feedstuff *in vitro* rumen NDF digestion procedure and energy calculations

The total digestible nutrient content (TDN) is calculated as a fraction of the NDF content. Fiber digestibility (NDFD) is important to estimate how much fiber can be digested. NDFD is expressed as a percentage of NDF. The NDFD value is determined using the "traditional" Goering and Van Soest method (tNDFD) (1970). 30 h of digestion time was selected to define the extent of fiber digestion. The indigestible fiber content (uNDF) is expressed as a percentage of DM and defined as the part of the fiber that could not been digested after

30 h of digestion time. Fiber digestibility parameters were determined using a Fibertec™ automated system (Gerber Instruments, Effretikon, Switzerland).

The net energies for lactation (NEL), maintenance (NEM), and growth (NEG) account for the energies needed for milk production, physical activities such as breathing and walking, and muscle and bone formation, respectively. They are expressed in Mcal/kg and calculated according to standard procedures (35, 47).

The metabolizable energy (ME) is accurately determined through in vitro assays, following the traditional Goering and Van Soest method (1970) with the modifications proposed by Goeser (17, 18). This technique determines the feedstuff fiber digestibility in animal nutrition models. Feed samples were digested for 30 h using the standard procedure. The fiber digestion fraction was determined by the difference between intact and digested samples, according to Equations (1) and (2):

$$NDF = 100\frac{(bag + res) - (bag \cdot cf)}{(bag + S) - bag} \tag{1}$$

$$NDFD = 100\frac{1 - NDF_{res}}{NDF_0} \tag{2}$$

were:
S, *bag*, and *res* = the weight of the sample
bag = containing the sample and its residue, respectively
NDF_{res} = the NDF residue after digestion
NDF_0 = the initial NDF (*i.e.*, digestion time = 0 h).

In this test, each sample was analyzed in duplicate.

Statistical analysis

The total polyphenol content was calculated as the mean ± SD. Final mean concentration of polyphenols was calculated using a gallic acid standard curve. Comparison of mean values of measured parameters was performed by a one-way ANOVA (Infostat Software, version 2020) using Duncan's multiple range test, with a level of significance $p < 0.05$.

Results and Discussion

Phytocompounds in *L. divaricata* during winter

The total content of polyphenols and flavonoids in *L. divaricata* extracts resulted in 430.9 ± 2.27 mg GAE/g dry extract and 140.4 ± 2.81 mg GAE/g dry extract, respectively. Phytocompounds have beneficial effects on ruminants. They form tannin-protein complexes that protect proteins from microbial activity in the rumen. As a result, the proteins can reach the intestine and dissociate at the appropriate pH (36, 44).

Nutritional and mineral assets of *L. divaricata* during winter

Table 1 (page 169) summarizes the nutritional composition and mineral content of *L. divaricata*. It also compares *L. divaricata* values with typical nutritional and mineral assets of common cattle feeds (35). The water content resulted in 6.10 wt%. This value is nearly half of the humidity of Alfalfa-R, Corn-G, Sorghum-G, and Barley-G. This result suggests the potential for long-term silage of *L. divaricata* leaves. Low moisture content reduces the risk of fungal proliferation and helps preserve nutritional value of plant material for extended periods.

Table 1. Nutritional profile of browsing available canopy leaves of *L. divaricata* and other forages during winter.

Tabla 1. Activos nutricionales de las hojas de la copa disponibles para el ramoneo de *L. divaricata* durante el invierno y otros forrajes.

	L. divaricata	**Alfalfa-P**	**Alfalfa-R**	**Corn-G**	**Sorghum-G**	**Barley-G**
Humidity	**%WT**					
DM	93.9	21.0	88.9	88.0	86.1	88.0
H_2O	6.10	79.0	11.1	12.0	13.9	12.0
Assets	**%DM**					
CP	11.20	22.8	16.4	9.8	8.3	13.5
ADF	17.42	36.7	31.0	27.3	36.0	23.3
aNDF	35.51	40.0	43.0	12.0	54.9	21.0
EE	5.92	1.4	1.3	3.8	2.2	2.1
Ashes	9.96	11.6	10.0	1.3	1.7	2.3
Lignin	12.09					
NFC	3.82	18.4	22.0	65.0	34.5	62.7
Losses	4.08					
Protein	**%CP**					
SP	79.3-64.7	50	60	60	40	70
ADICP	20.71					
NDICP	35.27					
Minerals	% DM	% NRC	Mineral Content in Common Feed Supplements			
Ca	1.44	0.43-0.66	RGT, corn, sunflower, flax: 0.06–0.38			
K	0.78	0.90-1.00	barley, sunflower, sorghum, wheat: 0.76–1.08			
S	0.37	~0.20	RGT: 0.15, corn in plant: 0.20			
P	0.09	0.28-0.41	RGT: 0.26, corn: 0.22			
Mg	0.09	0.20-0.25	RGT: 0.16, corn in plant: 0.14			
Na	0.0447	~0.18	Corn: 0.06			
Fe	0.0229	0.005	Corn: 0.0048			
Mn	0.00563	0.004	Corn: 0.0010			
Zn	0.00312	0.004	Corn: 0.0046			
Cu	0.00232	0.001	Corn: 0.0013			

Alfalfa-P: alfalfa-based pastures in winter, Alfalfa-R: alfalfa rolls, Corn-G: corn in grains, Sorghum-G: sorghum in grains, Barley-G: barley in grains. NRC recommendations, RGT: RYE GRASS TAMA.

Alfalfa-P: pasturas de base alfalfa en invierno, Alfalfa-R: rollos de alfalfa, Maíz-G: maíz en grano, Sorghum-G: sorgo en grano, Barley-G: heno en granos. Recomendaciones de la NRC, RGT: RYE GRASS TAMA.

The composition of *L. divaricata* in terms of nutritional parameters resulted in 11.20% of CP, 17.42% of ADF, 35.51% of aNDF, 5.92% of EE, 9.96% of Ashes, 12.09% of lignin, and 3.82% of NFC. 4.08% of the material was lost during the analysis.

The CP of *L. divaricata* resulted higher than that of Corn-G and Sorghum-G, and lower than that of Alfalfa-R and Barley-G. In addition, the SP of *L. divaricata* resulted higher than that of Alfalfa-R, Corn-G and Sorghum-G, and lower than that of Barley-G. Interestingly, the SP ranged from 64.73% to 79.29% of the CP, confirming the potential of *L. divaricata* as protein source and nitrogen contributor to bacterial growth and microbial protein synthesis in the rumen (42). Insoluble fractions of CP accounted for 35.27% and 20.71% of CP for NDICP and ADICP, respectively. Thus, *L. divaricata* can be included in cattle diets without significant variations in digestibility and forage intake. *L. divaricata* meets the protein requirements for cattle growth and productivity. This is the first time these values have been reported in scientific literature.

The relatively low ADF and aNDF values further reinforce that *L. divaricata* is a good candidate for inclusion in cattle diets. Similar results have been reported for other forage trees, including *Larrea cuneifolia*, (aNDF = 18.30%) and *Prosopis torquata* (aNDF = 33%) (41). Moreover, the highly energetic EE and NFC fractions did not show excessive values that could disturb the bacterial balance in the rumen. However, the NFC content of *L. divaricata* resulted an order of magnitude lower than the reference forages, suggesting the need for starch-based feed complements.

The most abundant mineral elements in *L. divaricata* were Ca, K, S, P and Mg. In addition, it contained several essential and valuable microminerals, including Fe, Mn, Zn, and Cu. Concerning mineral content, *L. divaricata* meets the forage requirement for Ca, K, Fe, Mn, Zn, and Cu. But, it is deficient in P, Mg and Na.

Nutritional and mineral assets of *L. divaricata* resin during winter

The content of resinous secretions of *L. divaricata* ranged from 10% to 25% of the DM. The high resin content limits the use of *L. divaricata* as a large-scale forage plant (29). Table 2 presents the nutritional composition and mineral content of *L. divaricata* resin. The average values of ash and total EE resulted in 1.05% and 0.70%, respectively. The content of K, Na, Fe, Mg, Ca, Zn, Cu and Mn resulted in 65.00, 31.40, 23.30, 12.70, 5.75, 0.82, 0.67 and 0.14 mg% of resin, respectively. Interestingly, the Fe content in the resin and the leaves were almost identical. S and P were not detected. These results suggest that *L. divaricata* can be a good source of minerals, especially during winter.

Table 2. Chemical profile of *L. divaricata* resin during winter.

Tabla 2. Perfil químico de la resina de *L. divaricata* durante el invierno.

Resin Content	**10 - 25 %**
Components	% of resin
EE	0.70
Ash	1.05
Minerals	**mg% of resin**
K	65.00
Na	31.40
Fe	23.30
Mg	12.70
Ca	5.75
Zn	0.82
Cu	0.67
Mn	0.14
P	ND
S	ND

Note. N/D: Not Detected.

Nota. N/D: No Detectado.

Feedstuff *in vitro* rumen NDF digestion and energy values of *L. divaricata* during winter

The NFC and TDN content resulted in 41.37% and 60.2%, respectively. The NEL, ENG and ENM energy parameters are presented in table 3 (page 171). Concerning forage quality, *L. divaricata* shows values lower than the recommended standards. However, these findings are significant because *L. divaricata* provides a low-cost source of nutrients. This is particularly valuable for animal husbandry practiced in the semi-arid Pampa region during winter. Results are consistent with previously reported literature (41).

Table 3. Digestibility and energy calculations of *L. divaricata* during winter.
Tabla 3. Digestibilidad y cálculos de energía de *L. divaricata* durante el invierno.

DIGEST*	Values
NDFD 30, %FDN	12.8
uNDF 30	30.9
uNDF30om	27.4
NFC	41.3
NRC 2001 Energy calculations Dairy	
TDN 1X	60.2
ENL 3X Mcal/kg	1.35
ENG Mcal/kg	0.78
ENM Mcal/kg	1.36
EM 3X NRC2001 (Mcal/kg)	2.18
EM 1X NRC2001 (Mcal/kg)	2.23

Conclusions

The nutritional value of *L. divaricata* during winter was studied and characterized. The results of this study highlight the significant nutritional potential of *L. divaricata* as a supplementary feed for beef cattle and goats in the semi-arid Pampa region during winter. *L. divaricata* offers a balanced protein content, adequate EM, digestible NDF, and appropriate levels of EE, NFC, and minerals. This makes *L. divaricata* a suitable feed for beef cattle and goats during challenging periods and geographies where pastures are dry, poor in nutrients and scarce.

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