R. Bras. Zootec., 55:e20250161, 2026
https://doi.org/10.37496/rbz5520250161

Forage crops
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Phytochemical potential of *Desmodium incanum* and *Paspalum notatum* in native subtropical grasslands: tannins and tocopherol content

Cesar Henrique Espírito Candal Poli[1]*, Jalise Fabíola Tontini[1], Aline Pericolo Sgiers[1], Melissa Oda-Souza[2], Renius de Oliveira Mello[3], Juliano Henriques da Motta[1], Filipe Martin de Campos[1], James Pierre Muir[4]

[1] Universidade Federal do Rio Grande do Sul, Departamento de Zootecnia, Porto Alegre, RS, Brasil.
[2] Universidade Estadual do Piauí, Centro de Ciências Agrárias, Teresina, PI, Brasil.
[3] Universidade Federal de Santa Maria, Departamento de Tecnologia e Ciência dos Alimentos, Santa Maria, RS, Brasil.
[4] Texas A&M AgriLife Research, Stephenville, TX, USA.

ABSTRACT - Condensed tannins and α-tocopherol are important antioxidants produced by forage plants, with potential benefits for grassland sustainability and ruminant productivity. The objective of this study was to quantify the concentrations of phenolic compounds in *Desmodium incanum* DC. and *Paspalum notatum* Flüggé in subtropical grasslands of the Brazilian Pampa and Atlantic Forest. Pasture samples were randomly collected from eight sites selected to represent different climatic conditions of the southernmost grasslands of Brazil. Six sites were located in the Pampa biome and two in the Atlantic Forest biome. The sampling protocol included collections during different periods of the year, representing the seasons as follows: summer (Jan-Feb), autumn (May-Jun), winter (Jul-Aug), spring (Sep-Oct), and spring-summer (Nov-Dec). *D. incanum* and *P. notatum* samples were collected and immediately stored in liquid nitrogen, then lyophilized, ground and the compounds were extracted for subsequent analysis. Condensed tannin concentration was estimated as bioreactive protein-precipitable phenolics (PPP). Different concentrations of total phenolics (TP), PPP and α-tocopherol were observed in *D. incanum* leaves across seasons. TP ranged from 63.91 to 242.71 g/kg dry matter (DM), PPP from 57.94 to 154.64 g/kg DM, and α-tocopherol from 435.06 to 905.12 mg/kg DM. In *P. notatum* leaves, TP ranged from 37.85 to 59.53 g/kg DM, while α-tocopherol varied from 10.89 to 95.56 mg/kg DM throughout the year. Air temperature, precipitation, relative humidity and solar radiation did not influence α-tocopherol levels (P>0.05). Air temperature and solar radiation were positively correlated with TP and PPP concentrations in *D. incanum* leaves. These findings indicate that *D. incanum* is a significant source of phenolic compounds and α-tocopherol in subtropical grasslands. Its antioxidant profile suggests a potential role in improving the quality of ruminant products, however seasonal and ontogenic variations must be considered when evaluating its nutritional value.

Keywords: forage bioactive compounds, grazing ruminant nutrition, growth performance, meat quality, plant antioxidant

***Corresponding author:**
cesar.poli@ufrgs.br

Received: August 18, 2025
Accepted: February 25, 2026

How to cite: Poli, C. H. E. C.; Tontini, J. F.; Sgiers, A. P.; Oda-Souza, M.; Mello, R. O.; Motta, J. H.; Campos, F. M. and Muir, J. P. 2026. Phytochemical potential of *Desmodium incanum* and *Paspalum notatum* in native subtropical grasslands: tannins and tocopherol content. Revista Brasileira de Zootecnia 55:e20250161. https://doi.org/10.37496/rbz5520250161

Editors:
Gustavo José Braga
André Fischer Sbrissia

1. Introduction

Native subtropical grasslands are among the largest and richest biodiverse areas of the world (Roesch et al., 2009). In South America, these grasslands are mainly found in Brazil, Argentina, Uruguay, and Paraguay, where they play a central role in livestock production systems. The Pampa and Atlantic Forest biomes comprise some of the main native subtropical grassland biomes. Unfortunately, these native grasslands are declining due to the adoption of short-term economic activities and incentives (Foucher et al., 2023). There are reports of a 25% reduction in the Pampa biome in the last 15 years (Mengue et al., 2020), with significant losses of plant and animal biodiversity. Despite these pressures, grazing with ruminants can be a sustainable alternative when appropriately managed (Nabinger et al., 2009). The Pampa biome and Atlantic Forest biome are characterized by natural pastures that support extensive livestock systems and provide ecosystem services essential for environmental sustainability (Nabinger et al., 2009). The diversity of plants in ruminant diets can result in meat with characteristic flavor, aroma, and fatty acid profile, which are conducive to improved human health and opportunities for value-added production (Devincenzi et al., 2012).

Beyond their structural and nutritional attributes, forage from subtropical grasslands has biochemical and physical properties that can impact animal product quality. Critical biochemical components of these forages (especially legumes) include condensed tannins (CT; Tedeschi et al., 2021a) and α-tocopherol (Tontini et al., 2019). However, information on the occurrence and variability of these compounds in native grasslands remains limited. These plant secondary compounds may vary throughout the year and across different environments (Gebrehiwot et al., 2002; Haring et al., 2007; Yang et al., 2018; Maxin et al., 2020).

These bioactive compounds primarily accumulate as adaptive responses to environmental and biotic stressors. Environmental stressors include factors such as temperature, humidity, and radiation (Schariatipour et al., 2022, 2023). These compounds are frequently polyphenolic in nature and may act as antioxidants by scavenging reactive oxygen species, as observed for tocopherols, or by forming complexes with proteins, as occurs with tannins (Tedeschi et al., 2021b). Although seasonal and environmental effects on secondary metabolites have been described in other ecosystems (Gebrehiwot et al., 2002; Haring et al., 2007; Yang et al., 2018; Maxin et al., 2020), such patterns have not been well characterized in subtropical native grasslands.

In ruminant production systems, these secondary compounds may exert beneficial effects. Condensed tannins can improve nitrogen utilization by reducing ruminal protein degradation, mitigate methane emissions, and contribute to the control of gastrointestinal parasites (Poli et al., 2025; Mueller-Harvey et al., 2019). Tocopherols play a key role as antioxidants in animal tissues, improving oxidative stability and shelf life of meat and milk products, while also contributing to animal health providing potential benefits (Riasat et al., 2018; Jacondino et al., 2024).

Among the dominant species of the lower stratum of native grasslands in the Pampa biome, *Desmodium incanum* DC. (Creeping beggarweed) and *Paspalum notatum* Flüggé (bahiagrass) stand out as representative legume and grass species, respectively (Boldrini, 2009; Caumo et al., 2021). *Desmodium incanum* is a perennial legume well adapted to continuously grazed systems under controlled stocking rates, partly due to the adhesive nature of its seeds, which favors persistence in pasture environments. Its forage availability is highest during spring, although dry matter accumulation extends into summer. Previous studies have identified *D. incanum* as a potential source of condensed tannins and α-tocopherol in Pampa grasslands (Tontini et al., 2019), highlighting its relevance for animal nutrition and meat quality (Descalzo and Sancho, 2008; Piluzza et al., 2014). *Paspalum notatum*, commonly known as bahiagrass, is a prostrate, dense perennial grass characterized by underground stems connected through rhizomes, propagating both by seeds and rhizomes, showing high adaptability to sandy and low-fertility soils, and presenting greater forage production during the warmer months of the year (Wallau et al., 2019).

We hypothesized that *D. incanum* will exhibit higher concentrations of phenolic compounds and α-tocopherol than *P. notatum*, and that these compounds will vary in response to seasonality and

environmental conditions, potentially influencing the nutritional quality of forage for ruminants. Therefore, the objective of this study was to determine the effects of season and regional characteristics on the concentrations of total phenolics, condensed tannins, and α-tocopherol in one of the main grass and legume species of the Pampa and Atlantic Forest biomes. This study provides essential insights into the biochemical composition of native forage species and their seasonal variations. The native grasslands harbor significant antioxidant compounds that remain largely unexplored and offer great potential for enhancing animal performance, improving the quality of animal products (meat and milk), and contributing to environmental sustainability. Additionally, we believe that this study provides valuable baseline data to support future research on native forage species and their role in grazing systems.

2. Material and methods

We analyzed biochemical characteristics of *D. incanum* and *P. notatum* leaves, collected in subtropical native Brazilian grasslands. Sample collections were carried out monthly from January 2019 to February 2020 in eight different areas, located in different physiographic regions of the southernmost state of Brazil, Rio Grande do Sul (RS). All the sites were areas that have been grazed by beef cattle.

As shown in Figure 1, of the eight sites, six are located in the RS Pampa biome: 1) coastal plain (municipality of Tavares: 31°23'38.0" S and 51°9'22.3" W, 3 m above sea level); 2) central valley (Eldorado do Sul: 30°06'09.4" S and 51°40'56.0" W, 46 m); 3) southeast hills (Encruzilhada do Sul:

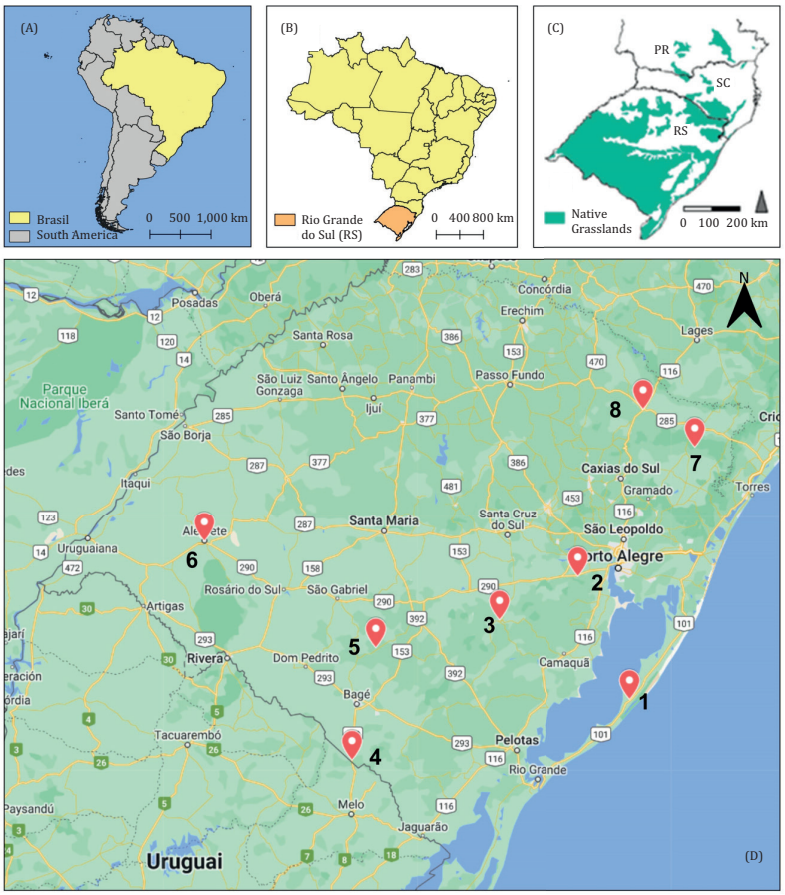


Figure 1 - (A) Brazil (yellow); (B) Rio Grande do Sul State (orange); (C) Pampa and Atlantic Forest biomes; (D) assessed areas of native grasslands in the Pampa Biome: (1) Tavares; (2) Eldorado do Sul; (3) Encruzilhada do Sul; (4) Aceguá; (5) Lavras do Sul; (6) Alegrete; and in the Atlantic Forest Biome: (7) Jaquirana; (8) Vacaria.

30°23'28.1" S and 52°28'02.3" W, 200 m); 4) south (Aceguá: 31°38'55.0" S and 54°09'26.0" W, 167 m); 5) southwest (Lavras do Sul: 30°41'55.0" S and 53°58'12.0" W, 330 m); and 6) west (Alegrete: 30°04'09.0" S and 55°59'28.0" W, 185 m). Two sites are located in the Atlantic Forest biome, a mountainous area in the north: 7) Jaquirana (29°05'43.0" S and 50°22'02.0" W, 815 m) and 8) Vacaria (28°11'08.19" S and 51°01'56.40" W, 970 m). There was no *D. incanum* in Aceguá and Vacaria, and no *P. notatum* in Tavares. The soil chemical analysis was carried out at a depth of 0 – 20 cm of all research sites in both biomes and the values are shown in Table 1.

The climate of the Pampa biome is classified as humid subtropical "Cfa", according to the Köppen (1948) classification system. In 2019, the annual accumulated precipitation averaged 1396 mm, and the annual temperature averaged 19.5 °C. In the mountain area (Atlantic Forest biome), the climate is classified as temperate humid "Cfb", according to the Köppen classification. In 2019, the average temperature was 15.1 °C, and the average accumulated precipitation was 1850 mm. Climatic data were summarized by season for each research site, including total precipitation, maximum, minimum, and mean air temperature, relative humidity, and solar radiation (Table 2). Seasonal averages and totals were used to characterize environmental conditions during the study period.

At each research site, three paddocks measuring approximately 3 to 10 hectares were selected for the monthly collections of *D. incanum* and *P. notatum*. Sampling was consistently conducted in the same paddocks throughout the experimental period in order to reduce spatial variability. Leaf sampling of both species was performed randomly within each paddock, with sampling points distributed across the entire paddock area to adequately represent spatial heterogeneity. Plants were sampled at variable distances from each other, avoiding collections concentrated in a single location. For each identified plant, between 10 and 20 fully expanded leaves were collected from multiple individuals distributed throughout the paddock. Correct taxonomic identification of the species was carried out in the field using a botanical identification guide (Nabinger and Dall'Agnol, 2019), based on diagnostic morphological traits. Both species are perennial and reproduce predominantly through vegetative propagation under natural conditions, which may result in relatively homogeneous populations within paddocks. Nevertheless, sampling prioritized the collection of leaves from numerous individual plants to minimize potential intraspecific variability. For each species, a single composite sample per site and per month was formed, consisting of approximately 200 g of fresh material, corresponding to at least 50 g of dry matter (DM). This amount was obtained by collecting leaves from dozens of individual plants, as only leaves were harvested, and entire plants were not removed. This sampling strategy was considered sufficient to represent the plant population within each paddock while minimizing disturbance to the vegetation.

Table 1 - Soil chemical attributes of the research sites located in the Pampa biome and Atlantic Forest biome from samples collected at a depth of 0–20 cm

Site	Soil pH	P	K	Al^{3+}	Ca^{2+}	Mg^{2+}	Base*	Al**
	in water	(mg kg^{-1})		(cmolc kg^{-1})			saturation (%)	
Pampa biome								
Aceguá	5.6	2.2	119.3	1.1	6.0	2.7	58.4	11.3
Alegrete	5.4	2.4	142.3	0.3	11.1	4.4	68.0	2.1
Eldorado do Sul	4.5	2.3	53.3	1.9	1.2	1.7	32.3	38.9
Encruzilhada do Sul	4.8	1.9	33.0	1.2	0.9	0.2	25.7	49.3
Lavras do Sul	4.9	5.3	134.7	0.8	4.2	1.7	52.5	10.8
Tavares	4.8	3.9	28.7	0.8	0.2	1.3	31.7	33.3
Atlantic Forest biome								
Jaquirana	4.8	3.6	106.7	5.9	1.2	0.1	12.6	78.8
Vacaria	5.0	2.6	70.3	3.7	1.3	0.6	23.5	62.0

* Base saturation = (Ca + Mg + K)/(Al + Ca + Mg + K) × 100.
** Al saturation = Al/(Al + Ca + Mg + K) × 100.

Table 2 - Seasonal climate data of accumulated precipitation, air temperature (minimum, maximum, and mean), humidity and radiation for each research site in 2019, obtained from the weather stations of the Brazilian National Institute of Meteorology (INMET) closest to each research site

Site	Season	Precipitation (mm)	Max. Temp. (°C)	Min. Temp. (°C)	Mean Temp. (°C)	Humidity (%)	Radiation (kJ/m^2)
Aceguá	Summer	553.2	27.30	3.90	14.40	79.10	1330.85
	Autumn	233.9	32.00	10.80	4.80	66.06	2217.50
	Winter	388.2	25.20	2.50	14.40	80.56	806.85
	Spring	538	27.20	6.40	15.70	84.47	1048.01
Alegrete	Summer	729.6	29.30	4.50	16.10	77.36	544.33
	Autumn	261	31.30	4.10	18.30	83.41	1321.67
	Winter	270	24.50	1.20	13.40	82.03	880.51
	Spring	426.2	26.40	3.30	15.30	83.58	1113.47
Eldorado do Sul	Summer	294.2	28.20	4.60	16.40	85.38	1409.01
	Autumn	505.4	30.30	5.10	17.90	88.90	1368.19
	Winter	358.8	24.60	1.80	13.80	79.05	990.97
	Spring	387.4	25.40	5.30	14.90	88.95	1188.36
Encruzilhada do Sul	Summer	33.3	31.60	8.40	18.60	73.39	1661.25
	Autumn	274.6	34.80	6.90	20.40	76.79	1391.26
	Winter	534.2	26.00	3.20	12.60	89.87	741.33
	Spring	517	30.40	7.30	17.40	77.91	1031.38
Jaquirana	Summer	462.4	33.8	11.7	20.9	70.85	1628.4
	Autumn	497	36.5	10.7	23.1	75.47	1311.9
	Winter	309.4	30	5.7	16	83.71	789.34
	Spring	654.8	32.8	10.5	20.6	74.59	1021.24
Lavras do Sul	Summer	524.4	33.40	7.60	18.50	72.15	1064.9
	Autumn	322.6	33.20	8.00	22.20	71.68	1135.48
	Winter	535	26.00	3.20	12.60	89.87	741.33
	Spring	573.8	28.60	9.10	19.20	75.27	1034.10
Tavares	Summer	136	32.80	6.50	19.00	70.69	1167.2
	Autumn	361	36.00	3.90	20.80	73.42	1492.22
	Winter	406.6	22.40	6.00	11.60	82.27	591.34
	Spring	316.2	30.50	7.30	17.60	77.47	1067.57
Vacaria	Summer	456	32.30	8.70	18.70	70.37	1655.96
	Autumn	435.6	34.40	6.70	20.70	75.64	1389.5
	Winter	287.6	27.70	4.30	13.80	86.86	776.3
	Spring	546.6	30.50	7.10	17.80	78.52	1030.8

Immediately after collection, samples were placed in plastic bags and stored in a liquid N to avoid tissue oxidation and degradation. These samples were then freeze-dried and kept under refrigeration in the dark to determine total phenolic compounds (TP), protein-precipitable phenolics (PPP), and α-tocopherol. The freeze-dried samples were ground in coffee grinders and stored in 15-g plastic bottles with seal-type lid, wrapped in aluminum foil, and refrigerated.

To represent each season, samples of each species were pooled as follows: samples collected in January and February were combined to represent summer; May and June represented autumn; July and August represented winter; September and October represented spring; and November and December represented the spring–summer period. After laboratory processing, the two monthly samples corresponding to each season were pooled to form a single composite sample. The legume *D. incanum* was in the reproductive phase (flowering + fruiting) from October to May, while it remained in the vegetative phase from June to September. This species is capable of both self-fertilization (autogamy) and cross-fertilization (allogamy) under natural conditions. The grass *P. notatum* exhibited

a reproductive period (flowering) from November to April and a vegetative period from May to October, and most naturally occurring biotypes of this species reproduce predominantly by apomixis, although sexual reproduction via cross-fertilization may also occur.

2.1. Total phenolic compounds and condensed tannin analyses

Following Naumann et al. (2013), species-specific standards were created for each plant species using CT extracts purified on Sephadex LH-20 (GE Healthcare Bio-Sciences Corp, Piscataway, NJ, USA) and lyophilized to recover purified CT. For CT extraction, 20 g of plant tissue was processed using 250 mL of an acetone-water mixture (7:3, v/v). The aqueous phase, which retained the CTs, was separated, and the remaining acetone was eliminated under reduced pressure. The extract was then combined with Sephadex LH-20 in a slurry prepared with an equal mixture of methanol and water (1:1, v/v). To remove unwanted compounds, multiple washes were performed with the same methanol-water solution until the absorbance at 280 nm dropped to 0.10 or lower. The CT bound to Sephadex were subsequently eluted using acetone-water (7:3, v/v), and any remaining acetone was evaporated with an air stream or vacuum. Finally, the aqueous fraction containing the CT was frozen at −80 °C and lyophilized for further analysis.

Condensed tannin biological activity was estimated through the analysis of PPP. As described by Cooper et al. (2014) and Naumann et al. (2014), the PPP concentrations of warm-season legumes are highly correlated (R^2 = 0.81) to the percentage of total CT. Therefore, in this study, PPP was used as an estimate of the concentration of bioreactive CT. The PPP was analyzed by the modified method of Hagerman and Butler (1978), and a more reduced-scale methodology was used, as described by Cooper et al. (2014). To prepare the extracts, 50 mg of plant tissue was mixed with 1 mL of a 1:1 (v/v) methanol-water solution and shaken for 30 minutes on a G10 Gyrotory® shaker (New Brunswick Scientific Co., Inc., Edison, NJ, USA). The mixture was then centrifuged at 16,070× *g* for 5 minutes. For PPP analysis, 50 µL of the supernatant was combined with 250 µL of buffer A (0.20 M acetic acid, 0.17 M sodium chloride, pH 4.9), 50 µL of bovine serum albumin, and 50 µL of methanol-water (1:1, v/v). The solution was incubated at room temperature for 30 minutes and then centrifuged at 16,070× *g* for 5 minutes. The supernatant was removed by vacuum aspiration, and the protein-phenolic pellet was washed with 250 µL of buffer A before undergoing another centrifugation and aspiration step. The final protein-phenolic pellet was dissolved in 800 µL of an SDS/TEA solution (1% w/v sodium dodecyl sulfate and 5% v/v triethanolamine), followed by the addition of 200 µL of $FeCl_3$ (0.01 M in 0.01 M HCl). After 30 minutes, absorbance was measured at 510 nm, and results were quantified using species-specific standards.

To quantify total phenols (TP), 50 µL of the supernatant from the crude plant extract was mixed with 850 µL of an SDS/TEA solution. Then, 200 µL of $FeCl_3$ was added. After 30 minutes, absorbance was measured at 510 nm, and TP concentration was determined using external standards, following the same procedure as the PPP assay.

2.2. α-tocopherol analysis

The α-tocopherol content was determined according to the methodology adapted from Prates et al. (2006), using 0.2 g of plant tissue, and it was expressed as mg/kg of sample DM weight. Briefly, the ground samples were saponified with potassium hydroxide, ethyl alcohol, and water. The solution was vortexed for 2 min and heated to 80 °C in a water bath, stirred for 15 min, and then cooled in ice water. Three mL of distilled water were added and the mixture was again vortexed for 2 min. Then, the solution was centrifuged at 2500 rpm for 5 min to separate it into phases. The supernatant was collected and added to another 15 mL Falcon tube containing anhydrous sodium sulfate. Subsequently, this supernatant solution was also collected and added to a microcentrifuge tube, dried under nitrogen at low temperature and then diluted in an acetonitrile, methanol, and MTBE solution, and then the dilution was injected into the HPLC.

The determination was performed using high-performance liquid chromatography (HPLC; SHIMADZU), with MeOH:H_2O (93:7, v/v) as the mobile phase and a 30-cm NovaPak 4-µm column (Górnas et al., 2014). The analyses were performed using fluorescence detection (excitation at 295 nm and emission at 325 nm). The limit of detection (LoD) and the limit of quantification (LoQ) were 0.052 and 0.159 ppm, respectively.

2.3. Forage chemical composition

Carbon (C) and crude protein (CP) contents were analyzed according to the AOAC methods (AOAC, 1995; method numbers: 972.43-1975 and 990.03, respectively). Neutral detergent fiber (NDF) concentration was determined according to Van Soest et al. (1991), and acid detergent fiber (ADF) was quantified according to Goering and Van Soest (1970).

2.4. Statistical analyses

Multivariable analysis including principal component analysis (PCA) (Jolliffe, 1986) and clustering, using Euclidean distance and the unweighted pair-group average method, were applied to *D. incanum* and *P. notatum*. The two multivariable analyses were carried out for each plant species. Pearson's correlations were performed among plant chemical and environmental characteristics.

Principal component analysis (PCA) was used to explore the interrelationships among plant biochemical traits and environmental variables, allowing the identification of major gradients of variation in phytochemical composition. Hierarchical cluster analysis was applied to group sampling sites and seasonal observations according to their overall biochemical and environmental profiles. These multivariate approaches were employed to identify patterns relevant to forage quality and antioxidant availability in native grasslands.

The multivariable analysis included plant biochemical characteristics and seasonal climatic variables: TP (g/kg plant), PPP (only for *D. incanum*), CP, NDF, ADF, α-tocopherol (mg/kg DM), mean temperature (Tmean) (°C), minimum temperature (Tmin) (°C), maximum temperature (Tmax) (°C), precipitation (mm), humidity (%) and radiation (kJ/m^2). Multivariable analyses were performed with the statistical computing software R (R Core Team, 2020), using the "FactoMineR" (Lê et al., 2008) and "factoextra" (Kassambara and Mundt, 2020) packages.

3. Results

Only the *D. incanum* legume contained PPP (Tables 3 and 4). The minimum and maximum TP values found in *D. incanum* were 126.24 g/kg DM (Lavras do Sul) and 283.09 g/kg DM (Tavares), respectively. PPP values ranged from 96.55 g/kg DM (Eldorado do Sul) to 193.30 g/kg DM (Tavares). The α-tocopherol concentration varied from a minimum of 308.6 mg/kg DM (Jaquirana) to a maximum of 1275.52 mg/kg DM (Tavares). In *P. notatum*, TP and α-tocopherol concentrations were numerically lower than in the legume but still showed variation. The minimum TP and α-tocopherol values were 42.23 g/kg DM (Alegrete) and 4.53 mg/kg DM (Aceguá), respectively. The maximum values were 71.58 g/kg DM (Eldorado do Sul) for TP and 76.68 mg/kg DM (Lavras do Sul) for α-tocopherol.

The TP, PPP, and α-tocopherol concentrations in plants varied among different seasons (Table 4). In winter the minimum concentrations of TP and PPP were observed for both plant species. The *D. incanum* plants had the maximum levels of TP in spring-summer (242.71 g/kg DM) and *P. notatum* in autumn (59.5 g/kg DM). The minimum and maximum α-tocopherol concentration of *D. incanum* varied from 435.06 mg/kg DM (spring) to 905.12 mg/kg DM (autumn). The values found for α-tocopherol were numerically lower than those of the legume, with the maximum value being 95.56 mg/kg DM (summer) and the minimum value being 10.89 mg/kg DM (spring).

Table 3 - Average concentration of secondary compounds in leaves of *Desmodium incanum* and Bahiagrass (*Paspalum notatum*) at different sites of native grasslands of southern Brazil

Sites[1]	*Desmodium incanum*			*Paspalum notatum*[2]	
	TP	PPP	Tocopherol	TP	Tocopherol
Aceguá	NP	NP	NP	53.89 (46.2 – 62)	4.53 (0.16 – 19.2)
Alegrete	166.84 (42.8 – 235.3)	106.30 (24 – 146.1)	799.04 (128.1 – 2052.1)	42.23 (27 – 57.2)	15.52 (0.7 – 39.4)
Eldorado do Sul	147.16 (68.6 – 214.3)	96.55 (40.9 – 135.5)	360.67 (87.2 – 625.6)	71.58 (49.8 – 98.9)	10.12 (0 – 30.8)
Encruzilhada do Sul	160.37 (49.6 – 225.4)	109.49 (33.3 – 155.2)	554.41 (143.8 – 1295.5)	44.61 (21.8 – 74.5)	15.08 (0– 66.12)
Jaquirana	240.18 (187.2 – 313.8)	146.62 (106.9 – 172.1)	308.66 (124.2 – 648.5)	47.2 (39.3 – 59.3)	35.5 (0 – 154.83)
Lavras do Sul	126.24 (3.6 – 229.6)	105.20 (62.2 – 146.5)	440.69 (10.2 – 1595.9)	46.53 (20.7 – 57.9)	76.68 (0 – 322.4)
Tavares	283.09 (155 – 362.1)	193.30 (133.6 – 236)	1275.52 (275.9 – 2066.1)	NP	NP
Vacaria	NP	NP	NP	44.9 (28.9 – 59.8)	52.9 (0 – 140.9)
SEM	16.615	7.897	261.760	5.832	25.157

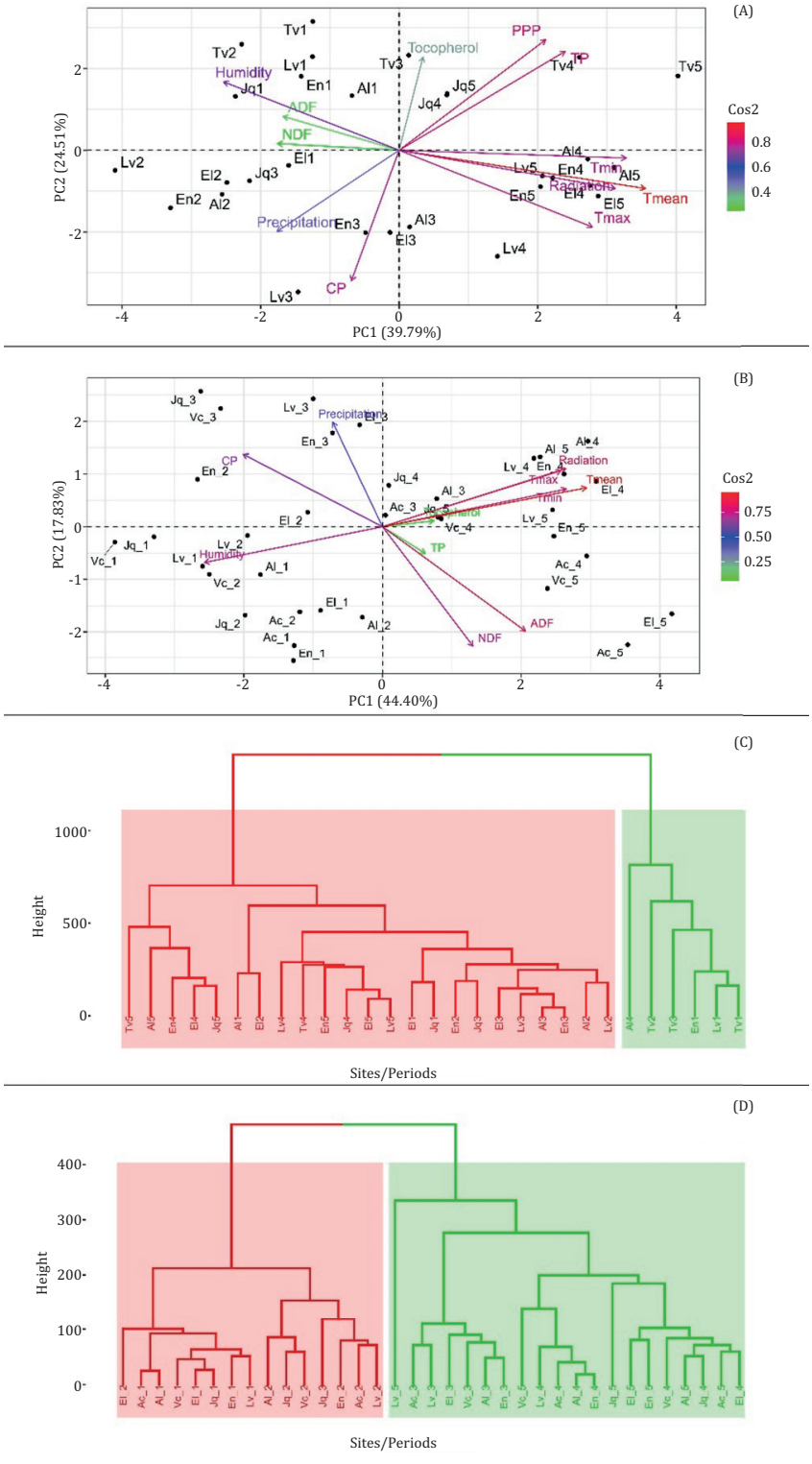
TP - total phenols (g/kg DM); PPP - protein-precipitable phenolics (g/kg DM); α-tocopherol (mg/kg DM); NP - species not present; SEM - standard error of the mean.
[1] The sites are located in Rio Grande do Sul State, southern Brazil, in the Pampa biome: Aceguá (southwest, 31°38'55.0" S and 54°09'26.0" W, 167 m above de sea level); Alegrete (west, 30°04'09.0" S and 55°59'28.0" W, 185 m); Eldorado do Sul (central valley, 30°06'09.4" S and 51°40'56.0" W, 46 m); Encruzilhada do Sul (southeast hills, 30°23'28.1" S and 52°28'02.3" W, 200 m); Lavras do Sul (west, 30°41'55.0" S and 53°58'12.0" W, 330 m) and Tavares (coastal plain, 31°23'38.0" S and 51°9'22.3" W, 3 m); in the Atlantic Forest biome: Jaquirana (mountain areas in the north, 29°05'43.0" S and 50°22'02.0" W, 815 m) and Vacaria (mountain areas in the north, 28°11'08.19" S and 51°01'56.40" W, 970 m).
[2] Bahiagrass did not present protein-precipitable phenolics.

Table 4 - Average concentration of secondary compounds in leaves of *Desmodium incanum* and Bahiagrass (*Paspalum notatum*) in different periods of 2019 at different sites of native grasslands of southern Brazil

Year periods	*Desmodium incanum*			*Paspalum notatum*[1]	
	TP	PPP	Tocopherol	TP	Tocopherol
Jan/Feb (summer)	229.24 (168.8 – 347.9)	154.64 (124.7 – 224.8)	534.28 (87.2 – 979.6)	46.95 (21.8 – 77.8)	95.56 (0.3 – 322.4)
May/Jun (autumn)	202.23 (124.9 – 248.2)	138.37 (90.9 – 196.9)	905.12 (124.2 – 1595.9)	59.53 (45.3 – 74.5)	11.52 (0.01 – 39.4)
Jul/Aug (winter)	63.91 (3.6 – 155)	57.94 (24 – 133.6)	621.60 (10.2 – 2066.1)	37.85 (20.7 – 62.8)	12.63 (0 – 66.1)
Sep/Oct (spring)	169.10 (81.3 – 302.3)	104.90 (62.2 – 175.2)	435.06 (125.4 – 1626.8)	50.26 (32.3 – 63.1)	10.89 (0 – 56.1)
Nov/Dec (spring-summer)	242.71 (132.3 – 362.1)	152.70 (82.5 – 236)	671.93 (131.1 – 2052.1)	56.14 (31.8 – 98.9)	19.67 (0 – 67.8)
SEM	15.168	7.209	238.950	4.929	21.261

TP - total phenols (g/kg DM); PPP - protein-precipitable phenolics (g/kg DM); α-tocopherol (mg/kg DM); SEM - standard error of the mean.
[1] Bahiagrass did not present protein-precipitable phenolics.

Although there was variation of tocopherol concentration throughout the year (Table 4), the multivariate PCA analysis (Table 5 and Figure 2) showed that the α-tocopherol content, mainly in *P. notatum*, is poorly explained by the variations observed by climatic conditions and by the chemical properties of pastures.



TP - total phenols (g/kg dry matter); PPP - protein-precipitable phenolics (bioreactive condensed tannins - g/kg dry matter); CP - crude protein (% dry matter); NDF - neutral detergent fiber (% dry matter); ADF - acid detergent fiber (% dry matter); α-tocopherol (mg/kg dry matter); Tmean (°C) - average temperature; Tmin (°C) - minimum temperature; Tmax (°C) - maximum temperature; precipitation (mm); humidity (%) and radiation (kJ/m^2). The regions: Aceguá (Ac), Alegrete (Al), Eldorado do Sul (El), Encruzilhada do Sul (En), Lavras (Lv), Jaquirana (Jq), Tavares (Tv) and Vacaria (Vc) in five periods May-June (1), July-August (2), September-October (3), November-December (4) and January-February (5).

Figure 2 - Biplots between the studied attributes under the influence of regions and periods for (A) *Desmodium incanum* and (B) *Paspalum notatum*. Dendrogram using the average Euclidean distance by the UPGMA method (Average Linkage) of the variables studied for leaves of (C) *D. incanum* and (D) *P. notatum.*

Table 5 - Correlations (PC1 and PC2) and representation quality (Cos2) between original variables and principal components, considering plant biochemical characteristics of *Desmodium incanum* and *Paspalum notatum* leaves and climatic variables at different sites of native grasslands of southern Brazil

Variable	*Desmodium incanum*			*Paspalum notatum*		
	PC1	PC2	Cos2	PC1	PC2	Cos2
TP	0.64**	0.65**	0.83	0.20	−0.16	0.07
PPP	0.56**	0.72**	0.84	-	-	-
CP	−0.18	−0.85**	0.76	−0.65**	0.44**	0.61
NDF	−0.47*	0.04	0.22	0.42*	−0.73**	0.71
ADF	−0.45*	0.22	0.25	0.66**	−0.64**	0.84
Tocopherol	0.09	0.61**	0.38	0.24	0.04	0.06
Tmean	0.95**	−0.25	0.96	0.85**	0.23	0.78
Tmin	0.87**	−0.05	0.77	0.83**	0.34*	0.80
Tmax	0.74**	−0.50**	0.80	0.95**	0.24	0.95
Precipitation	−0.47**	−0.53**	0.50	−0.23	0.64**	0.46
Humidity	−0.68**	0.45*	0.66	−0.82**	−0.22	0.73
Radiation	0.83**	−0.25	0.76	0.85**	0.35*	0.85
Eigenvalues	4.78	2.94	-	4.88	1.96	-
Accumulated variance (%)	39.79	64.30	-	44.40	62.23	-

TP - total phenols; PPP - protein-precipitable phenolics; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; Tmean - average temperature; Tmin - minimum temperature; Tmax - maximum temperature.
** Significant at P<0.01.
* Significant at P<0.05.

The PCA of *D. incanum* shows that the first component (PC1) accounted for 39.79% of total data variability (Table 5 and Figure 2A) and was highly and positively correlated with six (TP, PPP, Tmean, Tmin, Tmax, and radiation) of the 12 variables, but was highly and negatively correlated with NDF, ADF, precipitation, and humidity. The second principal component (PC2) of *D. incanum* accounted for 24.51% of the variability, with a high positive correlation with TP, PPP, tocopherol and humidity, and a high negative correlation with CP, Tmax, and precipitation.

For *P. notatum*, the first component (PC1) accounted for 44.40% of the total data variability (Table 5 and Figure 2B) and was highly and positively correlated with six (NDF, ADF, Tmean, Tmin, Tmax, and radiation) of the 12 variables, but was highly and negatively correlated with CP and humidity. The second principal component (PC2) of *P. notatum* accounted for 17.83% of the variability, with a high and positive correlation with CP and precipitation, and a high and negative with NDF and ADF. High Cos2 values are associated with the color scale and correlations (Table 5 and Figure 2). The warmer the color (red), the greater the importance of these variables.

Cluster analysis shows the dissimilarity between the different regions and periods (Figure 2C and 2D). Two groups were formed for both *D. incanum* (Figure 2C) and *P. notatum* (Figure 2D). In *D. incanum* (Figure 2C), the first group (G1 - red) stood out for the highest values of temperature (Tmean, Tmax, Tmin), precipitation and radiation, and for the lowest values of TP, PPP, and tocopherol; in the second group (G2 - green), on the other hand, the climate variables had the lowest values, and the highest values were for TP, PPP, and α-tocopherol. In this group, the mean α-tocopherol level was four times higher than in the first group (Table 6). The minimum level was 1295.53 mg/kg DM, and the maximum level was 2066.12 mg/kg DM, while in group G1 the minimum level was 10.19 mg/kg DM, and the maximum level was 979.64 mg/kg DM.

For *P. notatum* (Figure 2D), the first group (G1 - red) stood out for the lowest values of temperature (Tmean, Tmax, Tmin), precipitation, radiation, and the lowest values of TP and tocopherol; in the second group (G2 - green), on the other hand, the climate variables had the highest values, and the highest values were for TP and α-tocopherol (Table 6). The values in G1 were almost exclusively from the autumn and winter periods (with the exception of Jaquirana in spring – Jq_3), and the second group was exclusive to the summer and spring periods.

Table 6 - Mean values of cluster for regions and periods for *Desmodium incanum* and *Paspalum notatum* of native grasslands of southern Brazil

	TP	PPP	CP	NDF	ADF	Tocop	Tmean	Tmin	Tmax	Prec	Hum	Rad
						Desmodium incanum						
G1	174.05	117.99	12.77	56.97	43.05	361.11	19.15	8.40	32.40	136.68	74.57	862.59
G2	229.34	154.49	9.88	57.19	44.72	1680.12	16.32	6.28	28.92	112.00	77.70	644.88
						Paspalum notatum						
G1	48.72	-	9.03	65.15	26.97	11.73	2.33	28.24	14.21	127.21	80.12	553.42
G2	51.21	-	7.96	65.55	28.57	43.80	9.66	33.85	20.83	146.32	72.02	1004.79

TP - total phenols (g/kg dry matter); PPP - protein-precipitable phenolics (bioreactive condensed tannins - g/kg dry matter); CP - crude protein (% dry matter); NDF - neutral detergent fiber (% dry matter); ADF - acid detergent fiber (% dry matter); Tocop - α-tocopherol (mg/kg dry matter); Tmean (°C) - average temperature; Tmin (°C) - minimum temperature; Tmax (°C) - maximum temperature; Prec - precipitation (mm); Hum - air relative humidity (%); Rad - solar radiation (kJ/m^2).

The subsequent results of TP and PPP will be presented solely for *D. incanum* (Figure 2A) since *P. notatum* exhibited low concentrations of TP and there were no bioreactive condensed tannins (PPP; Figure 2B). The PCA, the PC1 (Figure 2A) indicates a positive correlation between mean temperature and radiation with TP and PPP, and a negative correlation between these plant variables and precipitation and humidity. Higher mean temperature and lower levels of precipitation and humidity were associated with an increase in TP and PPP concentrations in the *D. incanum* leaves. However, when considering different sites in the cluster analysis (Figure 2C), it becomes evident that PC2 (Figure 2A) which provides a good explanation for the separation of the clusters. The positive portion of PC2 displays a positive relationship between the cluster formed by Tavares in autumn, winter, and spring (from May to October), and by Lavras do Sul and Encruzilhada do Sul in autumn (May/June). In this PC2 (Figure 2A), it was observed a negative relationship between this cluster and maximum temperature and crude protein, and a positive relationship with TP and PPP.

The NDF and ADF leaf concentration alterations in *P. notatum* are greater than in *D. incanum* (Figure 2B). The greater the radiation and temperature (minimum and maximum), the higher the fiber content (NDF and ADF) in *P. notatum*. In this species, the ADF content contributed more to the observed variation than the NDF. Both NDF and ADF percentages show, similarly, a negative correlation with CP.

Cluster analysis (Figure 2D), including the effects of location and period on the characteristics of *P. notatum*, showed that the difference between the two groups was determined mainly by the season. This cluster analysis showed a clear distinction between autumn-winter and spring-summer. Only in Jaquirana was spring in the same cluster as autumn-winter. This result may be due to the location being an area with milder spring temperatures when compared to the other sites.

The *D. incanum* antioxidant concentrations were positively correlated with TP and with PPP and the climatic variables minimum and maximum temperatures and solar radiation (Table 7). However, TP and PPP were negatively correlated with CP and precipitation. Tocopherol concentration of *D. incanum* did not show correlations with the climatic variables. The correlations of *P. notatum* antioxidant concentrations (Table 8) indicate that there was only a positive correlation between TP and minimum temperature, and a negative correlation between α-tocopherol and CP.

4. Discussion

This study shows that both *P. notatum* and *D. incanum* can be important sources of antioxidants such as phenolic compounds and α-tocopherol. According to Salami et al. (2023), plant antioxidant concentrations are largely influenced by plant genetic characteristics, but the environment can also greatly influence their synthesis and concentration. Recent studies have reinforced that interspecific variation in antioxidant capacity is a key component of plant adaptive strategies, particularly in grasses exposed to heterogeneous and stressful environments such as natural grasslands (Bautista

Table 7 - Spearman correlations between the concentrations in *Desmodium incanum* leaves of secondary compounds, chemical composition and climatic variables of native grassland of subtropical regions of Brazil during the different seasons of 2019

	TP	PPP	CP	NDF	ADF	Tocoph	Tmin	Tmax	Tmean	Precip	Rel Hum	Rad
TP	1.0	0.96263 (<0.0001)	−0.56282 (0.0015)	−0.30834 (0.1037)	−0.16851 (0.3822)	0.36145 (0.0540)	0.52360 (0.0036)	0.11079 (0.5672)	0.42008 (0.0233)	−0.49124 (0.0068)	−0.04900 (0.8007)	0.40934 (0.0275)
PPP		1.0	−0.64126 (0.0002)	−0.16633 (0.3976)	0.03786 (0.8483)	0.37871 (0.0469)	0.46633 (0.0124)	0.00043 (0.9983)	0.34060 (0.0761)	−0.49913 (0.0069)	−0.01727 (0.9305)	0.35505 (0.0637)
CP			1.0	−0.13681 (0.4792)	−0.26511 (0.1646)	−0.45780 (0.0125)	−0.10606 (0.5840)	0.20296 (0.2910)	−0.00484 (0.9801)	0.59236 (0.0007)	−0.18114 (0.3470)	−0.01143 (0.9531)
NDF				1.0	0.96844 (<0.0001)	−0.03709 (0.8485)	−0.22420 (0.2423)	−0.17683 (0.3588)	−0.35214 (0.0610)	0.37983 (0.0421)	0.21625 (0.2599)	−0.08520 (0.6604)
ADF					1.0	0.04986 (0.7973)	−0.20761 (0.2799)	−0.26468 (0.1653)	−0.37398 (0.0457)	0.29628 (0.1186)	0.31930 (0.0913)	−0.09982 (0.6064)
Tocoph						1.0	0.02501 (0.8975)	−0.14941 (0.4392)	−0.06425 (0.7406)	−0.23315 (0.2235)	0.06030 (0.7560)	−0.12029 (0.5342)

TP - total phenols; PPP - protein-precipitable phenolics; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; Tocoph - α-tocopherol; Tmin - minimum temperature ; Tmax - maximum temperature; Tmean - average temperature; Precip - precipitation; Rel Hum - relative humidity; Rad - solar radiation.

Table 8 - Spearman correlations between the concentrations in *Paspalum notatum* leaves of secondary compounds, chemical composition and climatic variables of native grassland of subtropical regions of Brazil during the different seasons of 2019

	TP	CP	NDF	ADF	Tocoph	Tmin	Tmax	Tmean	Precip	Rel Hum	Rad
TP	1.0	−0.01285 (0.9416)	0.14000 (0.4225)	0.15461 (0.3752)	−0.02510 (0.8862)	0.34624 (0.0416)	0.10411 (0.5517)	0.20408 (0.2396)	−0.17391 (0.3177)	−0.01116 (0.9493)	−0.01184 (0.9462)
CP		1.0	−0.44743 (0.0070)	−0.61095 (<0.0001)	−0.36760 (0.0298)	−0.41022 (0.0144)	−0.30470 (0.0751)	−0.46921 (0.0045)	0.41660 (0.0128)	0.40573 (0.0156)	−0.40908 (0.0147)
NDF			1.0	0.83704 (<0.0001)	−0.07999 (0.6479)	0.08881 (0.6119)	0.19556 (0.2602)	0.20288 (0.2425)	−0.26927 (0.1178)	−0.21087 (0.2240)	0.13231 (0.4487)
ADF				1.0	0.03564 (0.8389)	0.39411 (0.0191)	0.29590 (0.0844)	0.44217 (0.0078)	−0.38177 (0.0236)	−0.42549 (0.0108)	0.41091 (0.0142)
Tocoph					1.0	0.29095 (0.0899)	0.01201 (0.9454)	0.22374 (0.1963)	−0.09203 (0.5990)	0.02794 (0.8734)	0.27610 (0.1084)

TP - total phenols; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; Tocoph - α-tocopherol; Tmin - minimum temperature; Tmax - maximum temperature; Tmean - average temperature; Precip - precipitation; Rel Hum - relative humidity; Rad - solar radiation.

et al., 2016; Qaderi et al., 2023). There are plant species that are more likely than others to tolerate stress through increase of the antioxidant concentrations (Peter Constabel et al., 2014) while others depend more on growth and structural mechanisms (Briske, 1996). Our study shows that *D. incanum* had greater TP and PPP concentrations in the leaves that might protect against herbivory (Tedeschi et al., 2014). On the other hand, *P. notatum* may rely more on structural defenses rather than biochemical compounds to deter herbivory, likely due to its prostrate growth habit and high variability in NDF and ADF leaf concentrations. Structural components such as lignin, cellulose, and hemicellulose play a crucial role in plant defense by reducing digestibility and palatability for herbivores. Among these, lignin is the primary factor limiting cell-wall degradation (Jung and Allen, 1995), as it cross-links with polysaccharides and proteins, making fiber less accessible to microbial fermentation in the rumen. Since voluntary forage intake is a key determinant of ruminant performance, a higher cell-wall concentration, particularly with increased lignification, can negatively impact nutrient availability and overall animal productivity. This suggests that *P. notatum* may adopt a structural defense strategy to regulate grazing pressure and persistence in grassland ecosystems.

Shikimate, derived from erythrose 4-phosphate and phosphoenolpyruvate, forms a secondary metabolic pathway responsible for the synthesis of tannins and α-tocopherol in plants (Hermann and Weaver, 1999). This pathway occurs when there is a deviation from the primary metabolic pathway, glycolysis. Glycolysis and the pentose phosphate pathway are connected by many intermediate compounds, not only to generate energy-rich cofactors, but also to generate the carbon skeleton required for biosynthetic reactions involved in the formation of TP, PPP, and α-tocopherol.

Therefore, as plants grow, there is an internal competition between primary and secondary metabolite production. Plant N compounds, such as nucleic acids, amino acids, peptides, and proteins, are examples of primary metabolites that compete with secondary compound pathways (Ramawat and Goyal, 2020), including PPP and α-tocopherol. This indicates that, when there was greater N availability, demonstrated by higher concentration of CP, there is a decrease in secondary metabolite biosynthesis, as shown in this study.

Both plant species analyzed here, *D. incanum* and *P. notatum*, showed greater biosynthesis of phenolic compounds when they were subjected to stress. The balance between primary and secondary metabolism has been recognized as a central mechanism underlying plant responses to environmental stress, particularly in forages growing under variable climatic conditions (Ramawat and Goyal, 2020). There was, for example, a negative correlation of TP and PPP with precipitation. We hypothesize that, as the plant grows, the biosynthesis of primary compounds increases (via glycolysis), while the production of phenolic compounds decreases (via the pentose phosphate pathway) to maintain plant homeostasis. However, when there is stress, plants reduce growth and increase the production of phenolic compounds. These secondary metabolites are directly linked to hydrocarbon structures only secondarily associated with lipophilic plant structures (Bryant et al., 1983; Bautista et al., 2016). Thus, plants with more lignified structures, instead of accumulating CP, invest in phenolic compounds when they are more susceptible to damage (herbivory, solar radiation, excess or lack of water) and when there is development of reproductive organs.

The greater and more prolonged the plant exposure to high temperature and solar radiation, the more damage can occur to proteins and nucleic acids. Plants protect themselves from exposure to ultraviolet rays (Verdaguer et al., 2017) and tend to synthesize more condensed tannin when there is greater exposure to sunlight. These compounds are concentrated mainly in plant tissues able to repair damage and stimulate enzyme production, thereby preventing the production of free radicals (Hemingway and Karchesy, 1989; Heil et al., 2002). Therefore, as observed in *D. incanum* leaves, greater average air temperature favors the accumulation of TP and PPP, while greater precipitation and CP have a negative effect on these secondary compound concentrations. The positive correlations verified in this study between TP and PPP with Tmin, Tmean, and solar radiation for *D. incanum* leaves may be due to the increase in photosynthetic reactions and, consequently, to an increase in secondary compound formation. In summer in subtropical regions, the solar incidence can be very intense, and phenolic compounds contribute to the control of leaf development and photoprotection.

During our study, there were negative correlations between TP and PPP with precipitation. These strong negative correlations might be related to the fact that, when there was rain, plants invested more in growth than in plant secondary compounds, as discussed before. Precipitation contributes to water availability for the plant, which favors physiological processes, such as stomata opening and closing. This change interferes with plant nutrient reserve mobilization, development, and, consequently, photosynthesis (Isah, 2019). In contrast to plant phenolic compound accumulation, α-tocopherol concentration did not respond to precipitation, indicating that its concentration in leaves was more stable than TP and PPP, and did not show great variation according to climatic conditions.

We also observed greater PPP concentration in months (from November to May) with greater development of D. incanum inflorescences and seed production. Peter Constabel et al. (2014) explain that, when exposed to herbivores, some plants can protect their reproductive structures by increasing tannin content, thereby attenuating herbivory.

The α-tocopherol, as a lipid-soluble antioxidant, can protect photosynthetic membranes from oxidative stresses (Maeda and DellaPenna, 2007). α-tocopherol leaf content was less affected by climatic variables than TP and PPP, indicating that there were fewer changes in α-tocopherol caused by changes in temperature, precipitation, relative humidity, and solar radiation. However, Lushchak and Semchuk (2012) explain that α-tocopherol is mainly found in plant plasmatic membranes to protect them against free radicals. Therefore, plant α-tocopherol concentration can increase under stress conditions, such as high-intensity light, drought, high salinity, heavy metals, and chilling conditions. This could indicate that environmental conditions present in native subtropical grasslands are not sufficiently extreme to elicit measurable variation in plant α-tocopherol concentration.

Although greater amounts of TP and PPP were found when there was greater temperature and radiation, similar to plant cell wall components, the influence of plant fiber content on TP, PPP, and α-tocopherol was low. TP and PPP did not have a strong relationship with plant structure, despite, as mentioned by Khoddami et al. (2013), condensed tannin and lignin being phenolic compounds that can have a common function of protecting the plant against herbivory.

Although TP, PPP and α-tocopherol content varied due to environmental variables, these antioxidant levels varied in different regions of the southern grasslands of Brazil. According to *D. incanum* cluster analysis (Table 6), cluster G1 showed lower concentrations of TP, PPP, and α-tocopherol than G2, but, on average, the environment in this group presented greater temperature, precipitation, and solar radiation. Our hypothesis is that sites with higher temperature, precipitation, and solar radiation favor greater plant growth (formation of primary compounds) to the detriment of secondary compound production. On the other hand, we also observed that in *P. notatum*, G2 was characterized by greater temperature, while precipitation and solar radiation were correlated with greater TP and tocopherol levels. Our hypothesis, therefore, is that plants that genetically produce less secondary compounds exhibit lower competition between primary and secondary compound production. In this case, the greater the growth, the greater the production of secondary compounds. However, further studies should be carried out to elucidate these relationships.

Recent studies across different crop and forage species have shown that secondary metabolites, particularly polyphenols and antioxidant compounds, are strongly influenced by both genetic variability and environmental stress conditions. Variations in moisture availability, especially drought stress, have been consistently associated with changes in antioxidant profiles, suggesting that these compounds are integral components of plant stress-response strategies (Salami et al., 2022). Moreover, substantial genetic variability in secondary metabolite production has been reported among genotypes and ecotypes, indicating a significant potential for selection and adaptation under contrasting environments (Riasat et al., 2018). In forage grasses, recent research has further demonstrated that genetic differences among ecotypes influence physiological, forage, and phytochemical traits under water deficit conditions, reinforcing the relevance of genotype × environment interactions in shaping stress tolerance (Shariatipour et al., 2022, 2023). Collectively, these findings highlight the importance of integrating antioxidant-mediated stress responses and genetic variability when evaluating forage

species, particularly in the context of climate variability and the development of resilient grassland systems.

Taken together, the patterns observed in this study reinforce the relevance of antioxidant compounds and other secondary metabolites as functionally important traits in native forage species, linking plant physiological responses to abiotic factors in grazing systems. Beyond their role in plant defense and stress tolerance, secondary metabolites such as condensed tannins and tocopherols may exert beneficial effects at the animal and system levels. Condensed tannins can improve nitrogen use efficiency by reducing ruminal protein degradation, mitigate methane emissions, and contribute to the control of gastrointestinal parasites, thereby enhancing sustainability in ruminant production systems (Mueller-Harvey et al., 2019; Poli et al., 2025). Similarly, tocopherols act as key antioxidants in animal tissues, improving oxidative stability and shelf life of meat and milk products, while also contributing to animal health and providing potential benefits for human consumers (Jacondino et al., 2024). In this context, native grassland species that naturally express these compounds represent valuable biological resources, as their phytochemical attributes may simultaneously influence pasture resilience, forage quality, animal performance, and ecosystem functioning. Thus, understanding the dynamics of secondary metabolites in native forage species contributes to a more integrated view of grassland management under environmental variability.

5. Conclusions

Our study demonstrated that the legume *D. incanum* is an important source of bioactive compounds, such as TP, PPP, and α-tocopherol, with higher concentrations than the grass *P. notatum* in the subtropical grasslands of southern Brazil. The concentrations of these compounds varied seasonally, with the highest TP levels observed in spring–summer (280% higher than in winter), PPP peaking in summer (167% higher than in winter), and α-tocopherol showing a 108% increase in autumn compared to spring.

The α-tocopherol concentration remained relatively stable and was minimally influenced by environmental factors, suggesting that temperature and precipitation variations did not significantly impact this antioxidant. In contrast, higher average temperatures and greater solar radiation favored TP and PPP accumulation. In addition, environments and seasons with lower minimum temperatures also had higher concentrations of PPP and TP.

These findings highlight the influence of seasonality and climatic conditions on the biochemical composition of forage species. Further studies are necessary to explore the interactions between environmental factors and the production of bioactive compounds in plants.

Data availability

All data were presented in the manuscript. Additional information can be provided by the corresponding author.

Author contributions

Conceptualization: Poli, C. H. E. C. and Tontini, J. F. **Formal analysis:** Poli, C. H. E. C.; Tontini, J. F. and Oda-Souza, M. **Funding acquisition:** Poli, C. H. E. C. **Investigation:** Tontini, J. F.; Sgierz, A. P.; Mello, R. O.; Motta, J. H. and Muir, J. P. **Methodology:** Poli, C. H. E. C.; Tontini, J. F.; Sgierz, A. P.; Motta, J. H. and Muir, J. P. **Project administration:** Poli, C. H. E. C. **Resources:** Poli, C. H. E. C.; Tontini, J. F.; Sgierz, A. P.; Mello, R. O.; Motta, J. H. and Muir, J. P. **Supervision:** Poli, C. H. E. C.; Tontini, J. F. and Oda-Souza, M. **Validation:** Poli, C. H. E. C. **Visualization:** Poli, C. H. E. C. **Writing – original draft:** Poli, C. H. E. C. and Sgierz, A. P. **Writing – review & editing:** Poli, C. H. E. C.; Tontini, J. F.; Oda-Souza, M.; Mello, R. O.; Motta, J. H.; Campos, F. M. and Muir, J. P.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank all the producers who allowed this work to be carried out on their properties. We also thank everyone who helped collect data in the field, especially the team from the following UFRGS research groups: Laboratory of Quantitative Ecology (ECOQUA), Teaching and Research Center for Sheep Production (CEPOV), and Research Group on Pasture Ecology (GPEP). The authors Cesar Henrique Espírito Candal Poli and Jalise Fabíola Tontini are members of the SISPEC (Intelligent and Sustainable Livestock Systems Network) funded by CYTED (ref. 125RT0167). Dr. Tontini, J. F. was supported by a research fellowship from the CNPq-Brazil: no. 32/2023 PDJ - 172370/2023-0.

Financial support

This study was financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

AOAC - Association of Official Analytical Chemists. 1995. Official methods of analysis. 15th ed. AOAC, Washington, DC.

Bautista, I.; Boscaiu, M.; Lidón, A.; Llinares, J. V.; Lull, C.; Donat, M. P.; Mayoral, O. and Vicente, O. 2016. Environmentally induced changes in antioxidant phenolic compounds levels in wild plants. Acta Physiologiae Plantarum 38:9. https://doi.org/10.1007/s11738-015-2025-2

Boldrini, I. I. 2009. A flora dos campos do Rio Grande do Sul. p.63-77. In: Campos Sulinos. Pillar, V. D. P.; Müller, S. C.; Castilhos, Z. M. S. and Jacques, A. V. A., eds. MMA, Brasília.

Bryant, J. P.; Chapin III, F. S. and Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368. https://doi.org/10.2307/3544308

Briske, D. D. 1996. Strategies of plant survival in grazed system: a functional interpretation. p.37-67. In: The ecology and management of grazing systems. Hodgson, J. and Illius, A. W., eds. CAB International, Wallingford, UK.

Caumo, M.; Freitas, E. M.; Silva, V. L.; Toldi, M.; Silva Alves, L. and Orlandi, C. R. and Fior, C. S. 2021. Grassland community structure in Permanent Preservation Areas associated with forestry and livestock in the Pampa biome, Southern Brazil. South African Journal of Botany 139:442-448. https://doi.org/10.1016/j.sajb.2021.03.001

Cooper, C. E.; Naumann, H. D.; Lambert, B. D.; Muir, J. P. and Kattes, D. H. 2014. Legume protein precipitable phenolic and nutrient concentration responses to defoliation and ontogeny. Journal of Plant Interactions 9:468-477. https://doi.org/10.1080/17429145.2013.860561

Descalzo, A. M. and Sancho, A. M. 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Science 79:423-436. https://doi.org/10.1016/j.meatsci.2007.12.006

Devincenzi, T.; Nabinger, C.; Cardoso, F. F.; Nalério, E. S.; Carassai, I. J.; Fedrigo, J. K.; Tarouco, J. U. and Cardoso, L. L. 2012. Carcass characteristics and meat quality of Aberdeen Angus steers finished on different pastures. Revista Brasileira de Zootecnia 41:1051-1059. https://doi.org/10.1590/S1516-35982012000400030

Foucher, A.; Tassano, M.; Chaboche, P. A.; Chalar, G.; Cabrera, M.; Gonzalez, J.; Cabral, P.; Simon, A. C.; Agelou, M.; Ramon, R.; Tiecher, T. and Evrard, O. 2023. Inexorable land degradation due to agriculture expansion in South American Pampa. Nature Sustainability 6:662-670. https://doi.org/10.1038/s41893-023-01074-z

Gebrehiwot, L.; Beuselinck, P. R. and Roberts, C. A. 2002. Seasonal variations in condensed tannin concentration of three *Lotus* species. Agronomy Journal 94:1059-1065. https://doi.org/10.2134/agronj2002.1059

Goering, H. K. and Van Soest, P. J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agricultural Handbook No. 379. US Agricultural Research Service, Washington, DC.

Górnas, P.; Siger, A.; Czubinski, J.; Dwiecki, K.; Seglina, D. and Nogala-Kalucka, M. 2014. An alternative RP-HPLC method for the separation and determination of tocopherol and tocotrienol homologues as butter authenticity markers: A comparative study between two European countries. European Journal of Lipid Science and Technology 116:895-903. https://doi.org/10.1002/ejlt.201300319

Hagerman, A. E. and Butler, L. G. 1978. Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural and Food Chemistry 26:809-812.

Haring, D. A.; Suter, D.; Amrhein, N. and Lüscher, A. 2007. Biomass allocation is an important determinant of the tannin concentration in growing plants. Annals of Botany 99:111-120. https://doi.org/10.1093/aob/mcl227

Heil, M.; Baumann, B.; Andary, C.; Linsenmair, E. K. and McKey, D. 2002. Extraction and quantification of "condensed tannins" as a measure of plant anti-herbivore defence? Revisiting an old problem. Naturwissenschaften 89:519-524. https://doi.org/10.1007/s00114-002-0366-3

Hemingway, R. W. and Karchesy, J. J. 1989. Chemistry and significance of condensed tannins. Springer Science and Business Media, NY, USA.

Hermann, K. M. and Weaver, L. M. 1999. The shikimate pathway. Annual review of Plant Physiology and Plant Molecular Biology 50:473-503. https://doi.org/10.1146/annurev.arplant.50.1.473

Isah, T. 2019. Stress and defense responses in plant secondary metabolites production. Biological Research 52:39. https://doi.org/10.1186/s40659-019-0246-3

Jacondino, L.; Poli, C.; Tontini, J.; Correa, G.; Silva, I.; Nigeliskii, A.; Mello, R.; Pereira, A.; Magalhães, D.; Trindade, M.; Carvalho, S. and Muir, J. 2024. Plant bioactive compounds of Brazilian Pampa biome natural grasslands affecting lamb meat quality. Foods 13:2931. https://doi.org/10.3390/foods13182931

Jolliffe, I. T. 1986. Principal component analysis. Springer-Verlag, New York.

Jung, H. G. and Allen, M. S. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. Journal of Animal Science 73:2774-2790. https://doi.org/10.2527/1995.7392774x

Kassambara, A. and Mundt, F. 2020. Package "factoextra": Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7.

Khoddami, A.; Wilkes, M. A. and Roberts, T. H. 2013. Techniques for analysis of plant phenolic compounds. Molecules 18:2328-2375. https://doi.org/10.3390/molecules18022328

Köppen, W. 1948. 'Climatología.' Fondo de Cultura Economica, Mexico, DF. 71p.

Lê, S.; Josse, J. and Husson, F. 2008. FactoMineR: An R package for multivariate analysis. Journal of Statistical Software 25:1-18. https://doi.org/10.18637/jss.v025.i01

Lushchak, V. I. and Semchuk, N. M. 2012. Tocopherol biosynthesis: chemistry, regulation and effects of environmental factors. Acta Physiologiae Plantarum 34:1607-1628. https://doi.org/10.1007/s11738-012-0988-9

Maeda, H. and DellaPenna, D. 2007. Tocopherol functions in photosynthetic organisms. Current Opinion in Plant Biology 10:260-265. https://doi.org/10.1016/j.pbi.2007.04.006

Maxin, G.; Cornu, A.; Andueza, D.; Laverroux, S. and Graulet, B. 2020. Carotenoid, tocopherol, and phenolic compound content and composition in cover crops used as forage. Agricultural and Environmental Chemistry 68:6282-6296.

Mengue, V. P.; Freitas, M. W. D.; Silva, T. S.; Fontana, D. C. and Scottá, F. C. 2020. LAND-USE and land-cover change processes in Pampa biome and relation with environmental and socioeconomic data. Applied Geography 125:102342. https://doi.org/10.1016/j.apgeog.2020.102342

Mueller-Harvey, I.; Bee, G.; Dohme-Meier, F.; Hoste, H.; Karonen, M.; Kölliker, R.; Lüscher, A.; Niderkorn, V.; Pellikaan, W. F.; Salminen, J.-P.; Skøt, L.; Smith, L. M. J.; Thamsborg, S. M.; Totterdell, P.; Wilkinson, I.; Williams, A. R.; Azuhnwi, B. N.; Baert, N.; Brinkhaus, A. G.; Copani, G.; Desrues, O.; Drake, C.; Engström, M.; Fryganas, C.; Girard, M.; Huyen, N. T.; Kempf, K.; Malisch, C.; Mora-Ortiz, M.; Quijada, J.; Ramsay, A.; Ropiak, H. M. and Waghorn, G. C. 2019. Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. Crop Science 59:861-885. https://doi.org/10.2135/cropsci2017.06.0369

Nabinger, C. and Dall'Agnol, M. 2019. Guia para reconhecimento de espécies dos campos sulinos. Ibama, Brasília.

Nabinger, C.; Ferreira, E. T.; Freitas, A. K.; Carvalho, P. C. F. and Sant'Anna, D. M. 2009. Produção animal com base no Campo Nativo: Aplicações de resultados de pesquisa. p.175-197. In: Campos Sulinos. Pillar, V. D. P.; Müller, S. C.; Castilhos, Z. M. S. and Jacques, A. V. A., eds. MMA, Brasília.

Naumann, H. D.; Hagerman, A. E.; Lambert, B. D.; Muir, J. P.; Tedeschi, L. O. and Kothmann, M. M. 2014. Molecular weight and protein-precipitating ability of condensed tannins from warm-season perennial legumes. Journal of Plant Interactions 9:212-219. https://doi.org/10.1080/17429145.2013.811547

Naumann, H. D.; Tedeschi, L. O.; Muir, J. P.; Lambert, B. D.; Kothmann, M. M. 2013. Effect of molecular weight of condensed tannins from warm, season perennial legumes on ruminal methane production in vitro. Biochemical Systematics and Ecology 50:154-162. https://doi.org/10.1016/j.bse.2013.03.050

Peter Constabel, C.; Yoshida, K. and Walker, V. 2014. Diverse ecological roles of plant tannins: plant defense and beyond. p.115-142. In: Recent advances in polyphenol research. Romani, A.; Lattanzio, V. and Quideau, S., eds. https://doi.org/10.1002/9781118329634.ch5

Poli, C. H. E. C.; Tontini, J. F.; Jacondino, L. R.; Vilalba, J. J.; Muir, J. P. and Tedeschi, L. O. 2025. Plant tannin for grazing ruminant growth. Animal Frontiers 15:47-58. https://doi.org/10.1093/af/vfaf026

Piluzza, G.; Sulas, L. and Bullitta, S. 2014. Tannins in forage plants and their role in animal husbandry and environmental sustainability: a review. Grass and Forage Science 69:32-48. https://doi.org/10.1111/gfs.12053

Prates, J. A. M.; Quaresma, M. A. G.; Bessa, R. J. B.; Fontes, C. M. G. A. and Alfaia, C. M. P. M. 2006. Simultaneous HPLC quantification of total cholesterol, tocopherols and β-carotene in Barrosã-PDO veal. Food Chemistry 94:469-477. https://doi.org/10.1016/j.foodchem.2005.01.021

Qaderi, M. M.; Martel, A. B. and Strugnell, C. A. 2023. Environmental factors regulate plant secondary metabolites. Plants 12:447. https://doi.org/10.3390/plants12030447

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramawat, K. G. and Goyal, S. 2020. Co-evolution of secondary metabolites during biological competition for survival and advantage: An overview. p.3-17. In: Co-evolution of secondary metabolites. Reference series in phytochemistry. Mérillon, J. M. and Ramawat, K., eds. Springer, Cham. https://doi.org/10.1007/978-3-319-96397-6_45

Riasat, M.; Heidari, B.; Pakniyat, H. and Jafari, A. A. 2018. Assessment of variability in secondary metabolites and expected response to genotype selection in fenugreek (*Trigonella* spp.). Industrial Crops and Products 123:221-231. https://doi.org/10.1016/j.indcrop.2018.06.068

Roesch, L. F. W.; Vieira, F. C. B.; Pereira, V. A.; Schunemann, A. L.; Teixeira, I. F.; Senna, A. J. T. and Stefenon, V. M. 2009. The Brazilian Pampa: a fragile biome. Diversity 1:182-198. https://doi.org/10.3390/d1020182

Salami, M.; Heidari, B. and Tan, H. 2022. Comparative profiling of polyphenols and antioxidants and analysis of antiglycation activities in rapeseed (*Brassica napus* L.) under different moisture regimes. Food Chemistry 399:133946. https://doi.org/10.1016/j.foodchem.2022.133946

Shariatipour, N.; Shams, Z.; Heidari, B. and Richards, C. 2023. Genetic variation and response to selection of photosynthetic and forage characteristics in Kentucky bluegrass (*Poa pratensis* L.) ecotypes under drought conditions. Frontiers in Plant Sciences 14:1239860. https://doi.org/10.3389/fpls.2023.1239860

Shariatipour, N.; Heidari, B.; Shams, Z. and Richards, C. 2022. Assessing the potential of native ecotypes of *Poa pratensis* L. for forage yield and phytochemical compositions under water deficit conditions. Scientific Reports 12:1121. https://doi.org/10.1038/s41598-022-05024-1

Tedeschi, L. O.; Muir, J. P.; Naumann, H. D.; Norris, A. B.; Ramírez-Restrepo, C. A. and Mertens-Talcott, S. U. 2021a. Nutritional aspects of ecologically relevant phytochemicals in ruminant production. Frontiers in Veterinary Science 8:628445. https://doi.org/10.3389/fvets.2021.628445

Tedeschi, L. O.; Greenwood, P. L. and Halachmi, I. 2021b. Advancements in sensor technology and decision support intelligent tools to assist smart livestock farming. Journal of Animal Science 99:skab038. https://doi.org/10.1093/jas/skab038

Tedeschi, L. O.; Ramírez-Restrepo, C. A. and Muir, J. P. 2014. Developing a conceptual model of possible benefits of condensed tannins for ruminant production. Animal 8:1095-1105. https://doi.org/10.1017/S1751731114000974

Tontini, J. F.; Poli, C. H. E. C.; Hampel, V. S.; Minho, A. P. and Muir, J. P. 2019. Nutritional values and chemical composition of tropical pastures as potential sources of α-tocopherol and condensed tannin. African Journal of Range and Forage Science 36:181-189. https://doi.org/10.2989/10220119.2019.1679883

Van Soest, P. J.; Robertson, J. B. and Lewis, B. A. 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Journal of Dairy Science 74:3583-3597.

Verdaguer, D.; Jansen, M. A. K.; Llorens, L.; Morales, L. O. and Neugart, S. 2017. UV-A radiation effects on higher plants: Exploring the known unknown. Plant Science 255:72-81. https://doi.org/10.1016/j.plantsci.2016.11.014

Yang, L.; Wen, K. S.; Ruan, X.; Zhao, Y. X.; Wei, F. and Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. Molecules 23:762. https://doi.org/10.3390/molecules23040762

Wallau, M. O.; Vendramini, J. M. B.; Dubeux Jr., J. C. B. and Blount, A. 2019. Bahiagrass (*Paspalum notatum* Flüggé): Overview and pasture management. EDIS – University of Florida IFAS Extension. https://doi.org/10.32473/edis-ag342-2019