Scientific Paper

Forage yield and nutritional quality of varieties of *Elymus scabrifolius* (Döll) J. H. Hunz and *Thinopyron ponticum* (Podp.) Bartworth et Dewey

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Abstract

Objective: To evaluate forage yield and nutritional quality of two varieties of *Elymus scabrifolius* (Döll) J. H. Hunz and four varieties of *Thinopyron ponticum* (Podp.) Bartworth et Dewey on a soil without salinity and alkalinity problems, and on an alkaline saline soil in the central semiarid region of Argentina.

Materials and Methods: *T. ponticum* (elongated wheatgrass, AA; varieties Pucará, Hulk, Barpiro and a local variety, seed identified) and *E. scabrifolius* (Creole wheatgrass, AC; varieties FA UNLPam and Don Alberto INTA) were evaluated on two types of soils: without limitation by salinity or alkalinity and sodic saline soil. Two trials were conducted at the Anguil National Institute of Agricultural Technology, according to a split-plot design, the main plot being the soil type and the sub-plots, the varieties. Forage production was evaluated per cut, per year and over a three-year period. Forage nutritional quality was determined at two times of the year (autumn and spring).

Results: There was no interaction between soil type and variety or differences between varieties in total accumulated forage production during the period. The total accumulated forage production during the evaluated period in the saline-alkaline soil was reduced between 40,0 and 50,0 % compared with that of the soil without salinity-alkalinity limitations. In the two varieties of *E. scabrifolius*, crude protein in the alkaline saline soil was 38,0 %, lower with regards to the soil without limitation by salinity or alkalinity. Meanwhile, in *T. ponticum*, crude protein was 48,0 % lower in the sodic saline soil, this being the reason for the interaction. In both varieties of *E. scabrifolius*, 9,0 more neutral detergent fiber (NDF) was observed in the autumn compared with spring in the soil without limitations, and the NDF was significantly higher in the saline alkaline soil with regards to the non-saline one.

Conclusions: The results showed the ability to implant and produce forage of both species in saline and alkaline environments. Forage nutritional quality was affected by salinity-alkalinity. In general, fiber content increased and crude protein decreased.

Keywords: fibres, pastures, proteins, salinity

Introduction

In Argentina, animal husbandry has shifted towards soils with greater edaphic limitations, including salinity or alkalinity, mainly due to the shifting of the agricultural frontier. In recent years, the use of pure pastures of *Thinopyron ponticum* (Podp.) Bartworth et Dewey (elongated wheatgrass) and *Elymus scabrifolius* (Döll) J. H. Hunz (creole wheatgrass) has increased in the semiarid Pampean region (Ruiz *et al*., 2018; Kent and Ruiz, 2019). These pastures can be used as combustion feedstock on arable land under marginal conditions (Ciria *et al*., 2020) and in phytoremediation of areas with significant salinity-alkalinity problems (Clay *et al*., 2023).

E. scabrifolius and *T. ponticum* are perennial grasses of spring autumn-winter growth, which stand out for their great hardiness and adaptation to extreme conditions of humidity (Iturralde-Elortegui *et al*., 2020) and drought (Borrajo *et al*., 2018, Tian *et al*., 2022, Zhang *et al*., 2022). These species are tolerant to saline as well as alkaline-sodic, poorly drained soils (Andrioli, 2023). Therefore, they can be cultivated almost everywhere in Argentina, especially where other forage resources do not thrive or are scarce (Temel *et al*., 2015, Pesqueira *et al*., 2017, Lavandera and Andres, 2018). Among the various forage species adapted to extreme conditions are *T. ponticum* and *E. scabrifolius* (Kent and Ruiz, 2019). In turn, in both species, it has been documented that there are differences among populations with regards to their performance in situations of saline or water stress, and both (Acuña, 2019; Melani *et al*., 2020; Zabala *et al*., 2020; Borrajo *et al*., 2021; 2022).

From the point of view of pasture utilization, not only production is important, but also nutritional quality, a characteristic that is commonly evaluated

Received: July 29, 2024
Accepted: November 15, 2024

How to cite a paper: Ruiz, María de los Ángeles; Blain, Gabriel; Ernst, Ricardo Daniel & Villagra, Diana Elizabeth. Forage production and nutritive quality of varieties of *Elymus scabrifolius* (Döll) J. H. Hunz and *Thinopyron ponticum* (Podp.) Bartworth et Dewey. *Pastures and Forages*. 47:e19, 2024.

through determinations of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The chemical composition of biomass can vary according to forage plant (species and variety), climate, phenological stage of development, soil conditions, shading, and cutting or grazing management, which influences the leaf/stem ratio and senescent material (Jones and Moseley, 1993). NDF and ADF values are not static, they change as pasture height is modified and controlled (Avila *et al*., 2014).

Reduced growth of species adapted to saline environments is generally associated with water deficiency conditions, accumulation of toxic ions in tissues and ion imbalance, due to difficulties in the absorption of essential nutrients (Borrajo *et al*., 2018; Jha *et al*., 2019; Borrajo *et al*., 2021; Kozłowska *et al*., 2021; Hu and Schmidhalter, 2023; Taher *et al*., 2023).

The objective of this work was to evaluate forage production and nutritive quality of two *E. scabrifolius* and four *T. ponticum* varieties, on a soil without salinity and alkalinity problems and on an alkaline saline one, in the central semiarid region of Argentina.

Materials and Methods

Location. The trials were conducted at the Anguil Ing. Agr. Guillermo Covas Agricultural Research Station, INTA, located in the La Pampa province, National Route No. 5 km 580. It is located at -36.542 latitude, -63.991 longitude and 165 m above sea level.

Edaphoclimatic characteristics. The study was carried out on a loam soil (pH 6,0; EC 2,1 DS/m), without alkalinity or salinity problems (NS) and on a sandy loam soil, saline and alkaline (SS; pH 8,6, EC 5,4 DS/m) (Willadino and Camara, 2003), eventually waterlogged, both belonging to the biogeographic area of the thorn forest, comprised by the Caldén district. Figure 1 shows the total monthly rainfall occurred during the course of the trial (2014-2016) and the historical total monthly average rainfall (1973-2016).

Treatments and experimental design. Twelve treatments were evaluated, resulting from the combination of soil (two sites) and species/varieties (six). The field design was a split-plot design, being the soil the main plot, with two sites: saline-alkaline (SS) and non-saline-alkaline (NS) soil. The subplots were the species with their varieties: two varieties of *E. scabrifolius* and four varieties of *T. ponticum*, with four replicas at each site.

Two populations of *E. scabrifolius* from the La Pampa province (Argentina) were evaluated. One population came from INTA EEA Anguil, Don Alberto (DA), and the other came from School of Agronomy (FA) UNLPam. Regarding *T. ponticum*, the commercial varieties used were Pucará (P), Hulk (H), Barpiro (B) and one widespread in the area as identified seed (C).

Experimental procedure. Sowing was done on April 21, 2014, manually; at 1,5 cm depth, in furrows spaced 20 cm from each other. The experimental units were plots of each variety, 1,4 m × 3,0 m (4,2 m^2). The trial was conducted by cutting for three years.

Measurements. Forage weight was determined by cutting the plots with a motor mower at 5 cm above the ground. Fresh forage was obtained from an area of 3 m^2. It was weighed in the field (fresh weight: FW) and then a 200-g sample was taken

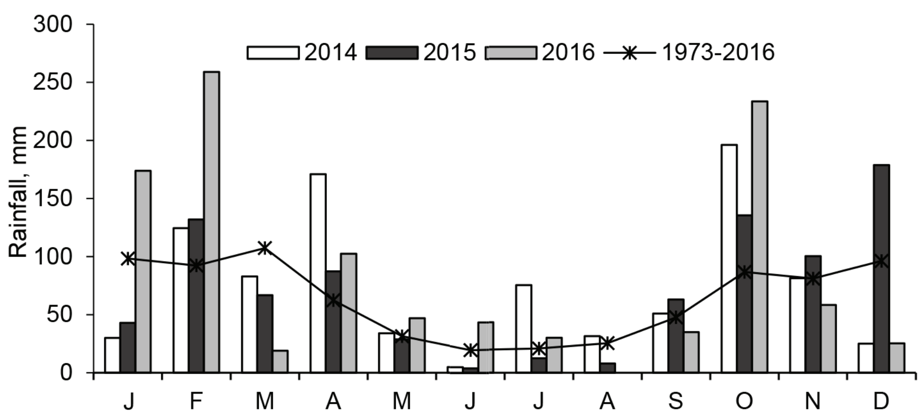


Figure 1. Monthly rainfall in 2014-2016, and historical average for a 44-year period at EEA. INTA Anguil

to the oven (60 °C) to obtain the percentage of dry matter (% DM). With this value, the dry forage weight was determined (DW= FW x % DM). Cuttings were performed on November 5, 2014, April 23, 2015, October 9, 2015, November 23, 2015, June 6, 2016 and November 9, 2016.

Samples were collected for forage nutritional quality analysis on October 9, 2015, and June 6, 2016. Samples of dry material were ground in a cyclone mill with 1 mm mesh and saved for further analysis. The percentage of CP, NDF and ADF were determined by NIRS technology (FOSS NIRSystems 6500 equipment in reflectance mode, range 400 to 2 500 nm) with duplicate analysis (Juan *et al*., 2016).

Mathematical analysis. Analysis of variance was performed, according to factorial design, soil type (main plot) x species/variety (subplot) x season and the respective interactions. Cutting dates were considered as repeated measures in time. Means were separated by the least significant difference (LSD) test ($p < 0.05$). The normality of the variables was previously tested by the Shapiro-Wilks test and the homoscedasticity of variances by Levene. The statistical package Infostat® (2018) was used.

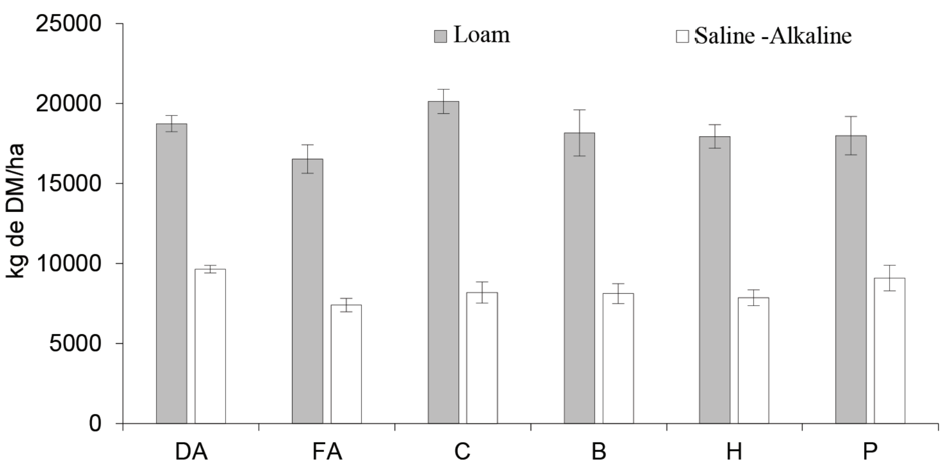
Results and Discussion

Forage production. In the variable total cumulative forage production of the period, there was no interaction between soil type and variety ($p > 0,05$) and there were no differences between varieties ($p > 0,05$). Only differences between soil types were found ($p < 0,05$). The total cumulative forage production of the evaluated period in the saline-alkaline soil (figure 2) was reduced between 40,0 and 50,0 % compared with that of the soil without salinity-alkalinity limitations.

In the variable total annual accumulated forage production, soil type, year and soil x year interaction effects were significant ($p < 0,05$). The variety effect was not significant. In the loam soil, the total annual accumulated forage in 2016 was significantly higher ($p < 0,05$) with regards to 2014 and 2015 (figure 3). These differences were not evident in the saline soil.

For the analysis by cut, a significant soil x cut interaction was found ($p < 0,05$). Soil, cutting date and variety effects were also significant ($p < 0,05$). In all cutting dates, the average forage production of *E. scabrifolius* and *T. ponticum* varieties was significantly ($p < 0,05$) higher in the loam soil than in the saline-alkaline soil. The differences were more noticeable in five of the six dates and exceeded by more than 30 % the forage production obtained in the loam soil with regards to that of the saline-alkaline soil. In the cut made in November, 2015, the difference was less than 15,0 % (figure 4).

Salinity affects leaf biomass, stem, plant height and photosynthesis of forage species (Borrajo *et al*., 2022, Li *et al*., 2023a, Li *et al*., 2023b). Although *T. ponticum* has the ability to establish and survive in salt marsh environments, total, annual and cut forage production (figures 2, 3 and 4) was affected



E. scabrifolius- DA: Don Alberto; EEA Anguil and FA: School of Agronomy, UNLPam
T. ponticum- P: Pucará PV INTA, H: Hulk Gentos, B: Barpiro Barenbrug and C: variety widespread in the area

Figure 2. Cumulative forage production over a three-year period of two varieties of *E. scabrifolius* and four varieties of *T. ponticum* on two soil types (kg DM/ha).

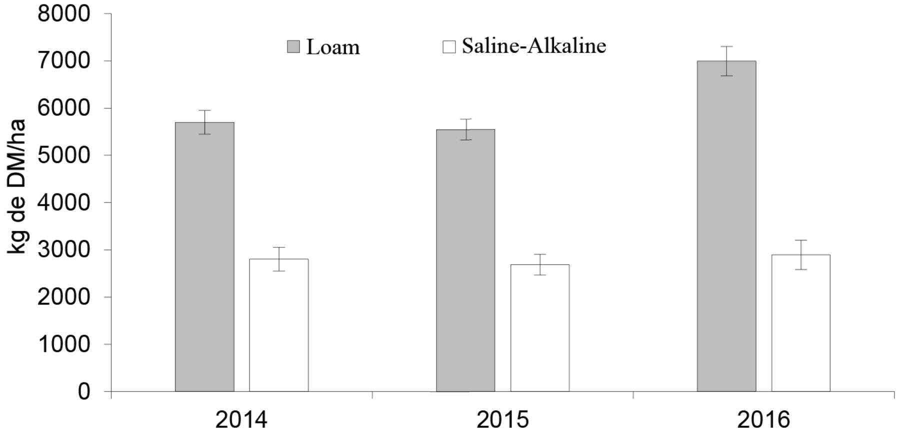


Figure 3. Average annual cumulative forage production of *E. scabrifolius* and *T. ponticum* varieties on two soil types.

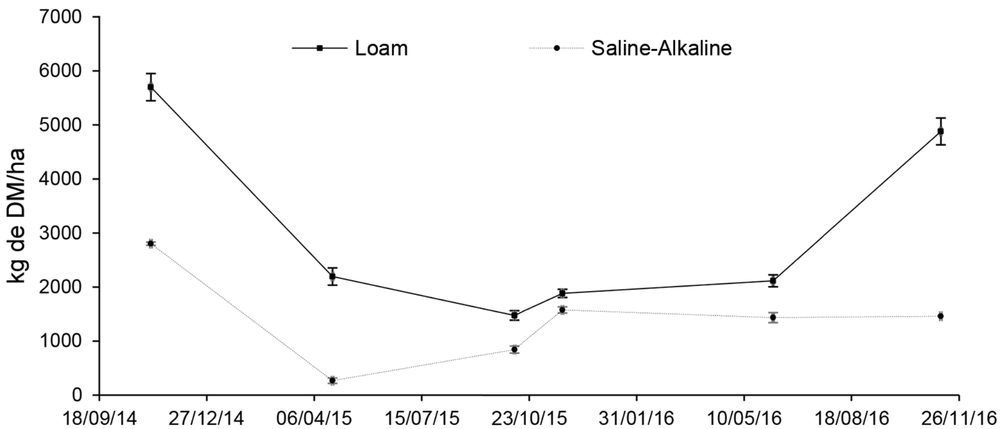


Figure 4. Average forage yield per cutting date of *E. scabrifolius* and *T. ponticum* varieties on two soil types (kg DM/ha).

by salinity-alkalinity, as well as forage nutritional quality. The same occurred with *E. scabrifolius*, which showed good performance in this saline-alkaline environment. Temel *et al*. (2015) and Borrajo *et al*. (2018) observed that under stress situations *E. scabrifolius* and *T. ponticum* varieties manifested reduction in dry matter production, lower leaf and tillers size, lower tillers number and lower leaf area index. In addition, Borrajo *et al*. (2018) found variation in stability for forage production, which was linked to number of tillers.

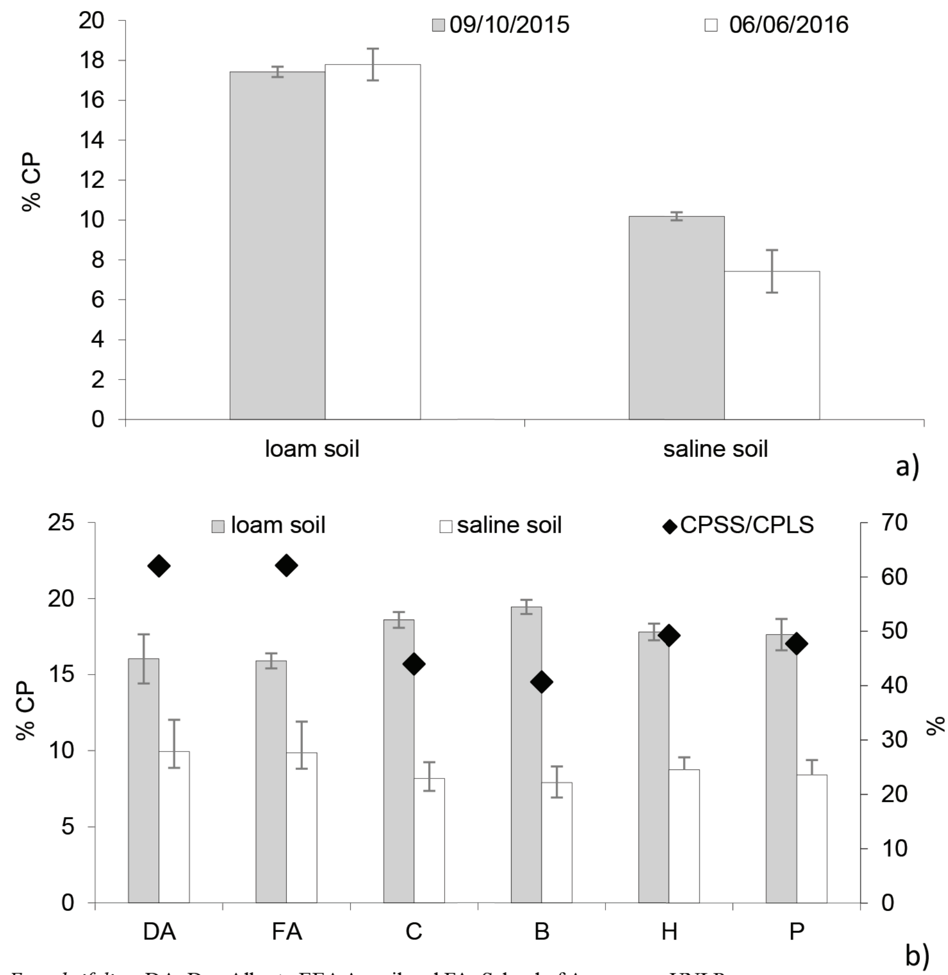
Overall, in this work, no differences were found between species and varieties of *E. scabrifolius* and *T. ponticum* in terms of cumulative forage production (figure 2). In coincidence, Lavandera *et al*. (2021) in a sodic soil observed similar forage production between varieties of *T. ponticum*. Ruiz *et al*. (2018) recorded significant differences in two-year cumulative forage production, being higher in *T. ponticum* compared with criollo and crestado in a non-saline-alkaline sandy loam soil.

In salinity-alkalinity limiting environments, plants are under stress pressure, which leads to a reduction in growth and, consequently, in yield, which can be higher or lower, depending on the species. In other cases, for those species that are unable to tolerate this condition, plant death may occur. In this trial, plant survival was not affected during the evaluated period. This could be explained by specific mechanisms of *E. scabrifolius* and *T. ponticum*, such as osmoregulation through the accumulation of some compounds such as proline and specific proteins (El-Moukhtari *et al*., 2020), in addition to increased water use efficiency, processes that help to overcome stress (Borrajo *et al*., 2018; 2021) or sodium exclusion (Zabala *et al*., 2020; Zhou *et al*., 2020).

A difference in favor of T. ponticum varieties over *E. scabrifolius* varieties was expected, due to the latter being less drought tolerant. The results showed that both species showed good establishment, plant survival and forage production similar or higher than that of the native vegetation. The good performance of the *E. scabrifolius* varieties could be explained by the abundant rainfall in those three years. However, due to the fact that this is a semi-arid region, the water balance is negative during January, June and July, even under circumstances of rainfall close to the historical average. The trial's forage production peaks and, therefore, cuts, occurred in autumn and spring, which coincides with the peak forage production stage of temperate species (Kent and Ruiz 2019).

Forage nutritive quality

Crude protein. For CP percentage of harvested forage, soil type*cutting date and soil type*variety interactions were significant (p < 0,01). Regardless of variety, only in the saline soil, the percentage of CP at the spring cutting date was higher with respect to that in the autumn (p < 0,05) (figure 5a). The CP percentage of the varieties was lower in the saline soil with regards to the loam (figure 5b). In the two populations of *E. scabrifolius*, the CP in the saline



E. scabrifolius- DA: Don Alberto EEA Anguil and FA: School of Agronomy, UNLPam.
T. ponticum- P: Pucará PV INTA, H: Hulk Gentos, B: Barpiro Barenbrug and C: variety widespread in the area.
CPSS/CPLS: ratio between the percentage of crude protein of forage harvested on the saline soil and the percentage of crude protein of forage harvested on the loam soil.

Figure 5 a) Average CP percentage of forage harvested in spring (gray bars) and in autumn (white bars) on two soil types.
b) Percentage of crude protein of forage harvested from two varieties of *E. scabrifolius* and four varieties of *T. ponticum* on two soil types.

soil was 38,0 % lower compared with the loam soil. While in *T. ponticum*, CP was 48,0 % lower in the alkaline saline soil, this being the reason for the observed interaction. This is due to the fact that in the loam soil the crude protein of *T. ponticum* varieties was higher than that of *E. scabrifolius* varieties.

As expected, salinity reduced forage nutritive quality, increased fiber contents (figures 6 and 7) and decreased CP (figures 5a and 5b), possibly due to the acceleration of lamina death (Borrajo *et al*., 2021) and the acceleration of the change from phenological to reproductive stage (hatching). At the same time, the percentage of fiber (NDF and ADF) was higher in autumn. Borrajo and Alonso (2001) explain that the lower nutritive value would be related to the greater proportion of developed stems and, consequently, a greater amount of

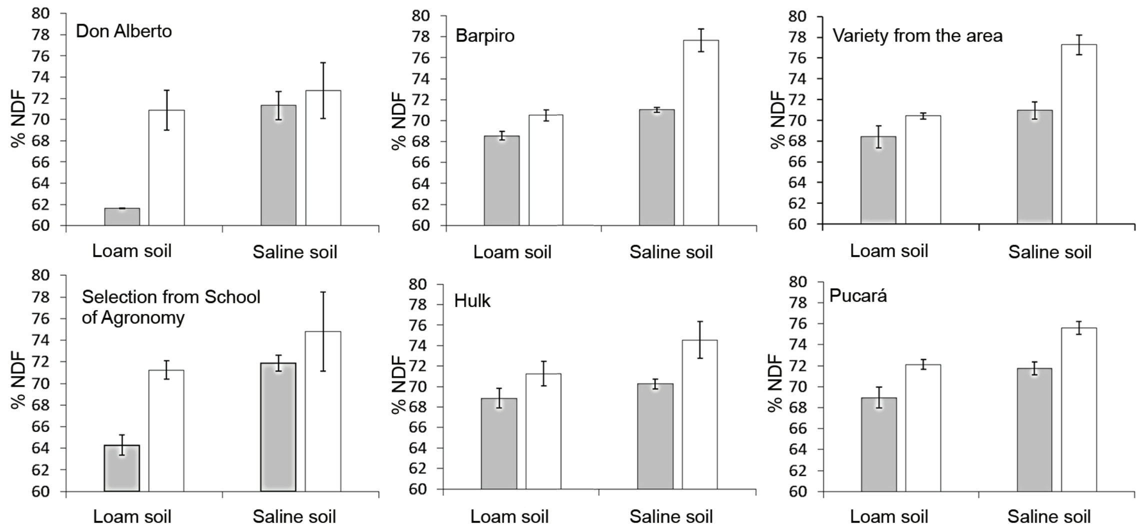


Figure 6. Percentage of NDF of forage harvested in spring (gray bars) and fall (white bars) of *E. scabrifolius* varieties Don Alberto INTA EEA Anguil and School of Agronomy, UNLPam and *T. ponticum* varieties Barpiro, Hulk, a variety widespread in the area, and Pucará on two soil types.

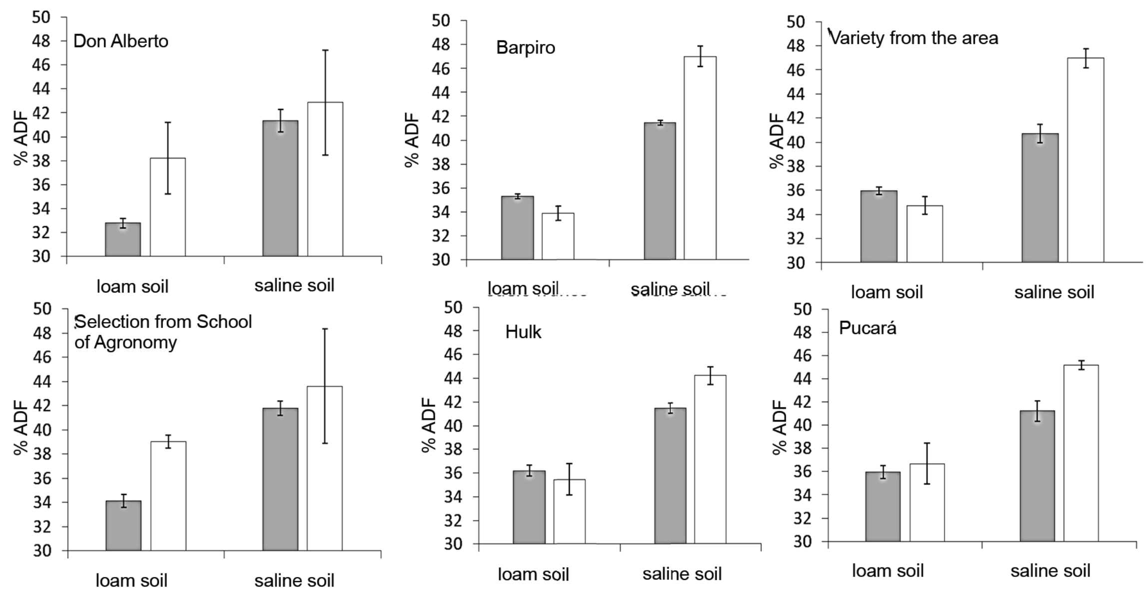


Figure 7. Percentage of acid detergent fiber (% ADF) of forage harvested in spring (gray bars) and fall (white bars) of *E. scabrifolius* Don Alberto and selection of the School of Agronomy, UNLPam; and of *T. ponticum*- Barpiro, Hulk, variety widespread in the area and Pucará on two soil types.

indigestible tissue. Later varieties would have less lignified tissue and a higher concentration of soluble carbohydrates (Borrajo and Alonso, 2001).

Fina *et al*. (2019) observed in *T. ponticum* that the higher the salinity, the higher the forage quality (higher values of CP percentage and lower values of NDF and ADF). This would be associated with lower aerial biomass accumulation, lower growth rates and lower proportion of supporting tissues with respect to metabolic tissues, which is reflected in the higher proportion of lamina in the more saline environments. This difference with the present trial could be associated with a climate with higher water availability.

In agreement with Pesqueira *et al*. (2017), CP values in the saline-alkaline soil were between 8,0 and 10,0 %; while in the non-saline soil it was 18,0 %. Similarly, Temel *et al*. (2015) found in different grass species decrease in protein content, due to salinity and alkalinity condition. In the saline soil, the percentage of CP of the spring cutting was higher than in autumn, which is possibly explained by the sum of salt and water stress in this last season of the year.

The interaction observed in CP in both species was due to the fact that in the saline soil in the two populations of *E. scabrifolius* 38,0 % less protein was recorded compared with the loam soil. In *T. ponticum* it was 48,0 % lower in the alkaline saline soil (figure 5b). This was due to the fact that in the loam soil the crude protein of *T. ponticum* varieties was 4,0 % higher than that of *E. scabrifolius* varieties. This higher CP value of *T. ponticum* varieties may be due to greater tolerance to water stress, which allows these varieties to prioritize vegetative development (tillering) and maintain normal physiological activities (Borrajo *et al*., 2022). In contrast, in another trial conducted on a sandy loam soil, Ruiz *et al*. (2018) referred 5,0 % less CP in *T. ponticum* with regards to *E. scabrifolius* at spring cutting date, and no differences were recorded in autumn. Lauric *et al*. (2022) did not find in *T. ponticum* defined CP performance on soils with different alkalinity limiting factors.

Neutral and acid detergent fiber. In general, regardless of the variety of *E. scabrifolius* and *T. ponticum*, the percentage of NDF of harvested forage was higher in autumn and on saline soil (figure 6).

In NDF, the simple effects soil type, variety, cutting date and variety*soil type*cutting date interaction were significant ($p < 0,05$).

In both populations of *E. scabrifolius*, there was 9,0 % more NDF at the autumn cutting date compared with the spring cutting date in the non-saline soil, as well as significantly higher ($p < 0,05$) NDF percentage in the saline soil compared with the non-saline soil in the spring (figure 6).

In the varieties of *T. ponticum* Pucará and Barpiro there were significant differences ($p < 0,05$) in the percentage of NDF between both types of soil for the same cutting date and significant differences ($p < 0,05$) between both dates on the same soil type (figure 6). The highest values were observed on the saline soil and the cutting date of June six, 2016.

In *T. ponticum* var. Hulk there were no differences in NDF percentage between both soil types for the same cutting date nor between cutting dates for the same soil (figure 6).

In *T. ponticum* spread from the area, there was higher NDF in June compared with October, only in the saline soil ($p < 0,05$). In turn, there was higher NDF in saline soil with regards to non-saline soil, but only at the June cutting date (figure 6).

In general, for *E. scabrifolius* and *T. ponticum* varieties, the percentage of ADF of harvested forage was higher in saline soil than in loam soil (figure 7).

In ADF, the simple effects of soil type, cutting date and the interaction variety*soil type*cutting date were significant ($p < 0,05$).

In the varieties of *T. ponticum* Barpiro, widespread variety in the area, and Pucará, the percentage of ADF of the forage was higher ($p < 0,05$) in saline soil. In turn, differences between cutting dates in these varieties were only observed in the saline soil, the percentage of ADF in June being significantly higher ($p < 0,05$) compared with October (figure 7).

Although there was a tendency for theADF percentage in June to be higher than in October, in both varieties of *E. scabrifolius* no significant differences ($p = 0,12$) were observed between the two cutting dates analyzed on any of the soil types considered.

In *T. ponticum* var. Hulk only significant differences ($p < 0,05$) were observed in the ADF percentage of forage between soil types, with the saline soil having the highest value (figure 7). There were no differences in ADF percentage between cutting dates on any of the soil types.

The cuts were carried out in parallel in SS and NS, so the differences found correspond to pasture stages due to the osmotic and hydric effect of salts and alkalinity. The NDF and ADF values in the saline-alkaline soil showed a range of variation of 60,0-78,0 % and 32,0-48,0 %, respectively (figures 6 and 7). Such minimum values of NDF and ADF coincided with those obtained by Pesqueira *et al*. (2017) in the Salado basin. The higher values of NDF and ADF found in this work could be due to the combination of saline-alkaline stresses plus water deficit.

The higher percentage of NDF observed in *E. scabrifolius* in autumn compared with spring in non-saline soil as well as the higher value in saline soil compared with NS soil in spring (figure 6) could be explained by the greater sensitivity of this species to water deficit (Ferrari, 2001). Regarding *T. ponticum* varieties, the greatest differences between cutting dates were detected in the saline soil, the NDF percentage being higher in autumn. Temel *et al*. (2015) found in different grass species that NDF content increased with alkalinity and decreased with salinity.

Similar to what was observed in the variable NDF with regards to the ADF percentage, in each variety of *T. ponticum* the greatest differences between cutting dates were found in saline soil, such value being higher in autumn (figure 7). In both varieties of *E. scabrifolius*, no differences were found in the ADF percentage of the harvested forage between the two analyzed cutting dates and the considered soil types. This could be due to the high variability in the data.

In this work, it was observed that *E. scabrifolius* varieties showed lower NDF (63,0 %) compared with *T. ponticum* (68,0 %) in spring on loam soil (figure 6). In ADF, the difference between *E. scabrifolius* (33,0 %) and *T. ponticum* (36,0 %) was similar to that found for NDF, but of lesser magnitude (figure 7). These results coincide with those found on a loam soil by Ruiz *et al*. (2018), where in both cutting dates (autumn and spring) *T. ponticum* showed higher percentages of NDF with regards to *E. scabrifolius*, and only higher percentage of ADF in spring.

It should be noted that in *T. ponticum*, Hulk variety, the percentage of NDF did not change between soil types for the same cutting date or between cutting dates for the same soil type. Neither was the percentage of NDF modified between cutting dates on any of the soil types. This nutritional stability in NDF and ADF percentage would be related to mechanisms that allow it to overcome salt and/or water stress, and both (Borrajo *et al*., 2022).

Conclusions

Under the climate conditions in which the trial was conducted, the varieties of *E. scabrifolius* and *T. ponticum* showed forage production higher than 2 800 kg DM/ha/year. However, the average annual total production in the saline-alkaline environment was 54,0 % lower than in the soil without limiting factors.

Soil salinity-alkalinity adversely impacts forage nutritional quality, as evidenced by an increase in fiber content and a decrease in crude protein. In addition, the combined stress of saline-alkaline and water conditions intensifies disparities in forage production and quality among soil types.

The trials showed the ability of *E. scabrifolius* and *T. ponticum* varieties to establish and produce forage in saline and alkaline environments. Further evaluation of the performance of new varieties on a wider range of soil conditions, including waterlogging, would be necessary.

Acknowledgements

MSc. Karina Frigerio is thanked for her critical contributions to this work. The work was funded by INTA (National Institute of Agricultural Technology) projects: PD-L01-I098 Eco-efficient forage production in pastures and rangelands and PE-L01-I008: Technologies for the improvement of productivity and efficiency of mixed systems in La Pampa and San Luis.

Conflict of interest

The authors declare that there is no conflict of interests among them.

Authors' contribution

- María de los Ángeles Ruiz. Generation of the idea, search for bibliographic information, drafting and revision of the manuscript.
- Gabriel Blain. Contribution to the idea, search for bibliographic information, drafting and revision of the manuscript.
- Ricardo Daniel Ernst. Contribution to the idea, search for bibliographic information, drafting of the manuscript.
- Elizabeth Villagra. Contribution to the idea, search for bibliography and manuscript writing.

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