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Mineral elements in grasses growing in contrasting environmental conditions in southern Patagonia

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Abstract

The importance of grasses and graminoids for sheep nutrition in Argentinean Patagonia is widely recognized. Focusing on sheep nutrition, we assessed the concentration of mineral elements in grasses growing in three ecological areas of southern Patagonia, representing a vegetation and climate gradient. With the aim of establishing potential relationships, tissue concentrations of several essential and non-essential elements for plants were determined, analyzing also soil properties. Soil and plant tissue mineral element concentrations varied between ecological areas and also depending on the particular element. The results obtained provide novel information about the nutritional characteristics of the main feeding source for sheep in southern Patagonia, but further trials will be required for improving our understanding of mineral element nutrition in relation to sheep production.

Key words: grasses; mineral elements; *Nothofagus antarctica*; sheep nutrition; silvopastoral systems; soil properties; steppe

Introduction

Grazing with domestic livestock is a widespread activity around the world (Gillson and Hoffman 2007, Järveranta et al. 2014) covering more than 60 million km^2 of the earth`s surface (Reid et al. 2008) and playing a very important role for the human feeding. For successful livestock production, animals should have access to herbage of acceptable nutritional value throughout the grazing season (Bailey and Ulyatt 1970). In Argentina, the stock of cattle is around 52 million heads with more than 65 % concentrated in the Pampa Region, while sheep is estimated in around 15 million heads where more than 60 % is produced in the Patagonian Region (SENASA 2015). In southern Santa Cruz province, (51°00' – 52°20' LS) extensive sheep production occupies broad land extensions in an area of approximately 3.5 million ha (Sturzenbaum 2012) that expands from the mountains to the sea in a West-East (W-E) gradient with distinct environmental conditions. This W-E gradient implies that in the east (near the Atlantic Ocean coast) rainfall does not exceed 150 mm yr^{-1} while at the Andes Mountains (west) precipitations may be higher than 1000 mm yr^{-1} (Hijmans et al. 2005). Livestock activities in the western limit are performed in a relatively narrow strip of *Nothofagus antarctica* (ñire) forests under silvopastoral use (Peri et al. 2015) with sheep and cattle, mostly grazing in the steppe dominated by grasses and shrubs (Oliva et al. 2001). Despite livestock production in Patagonia dates back to the end of the XIX century (around 1880), no significant management improvements have been made for decades (Quargnolo et al. 2007, Ormaechea et al. 2009), being continuous grazing with fixed stocking rates in large paddocks (1000 to 5000 ha) the most common practice (Ormaechea and Peri 2015).

A crucial aspect for the success of livestock activities, particularly lamb production, is the nutritional status of the animals (Smith and Cornforth 1982). The nutritive value of a pasture grass species in respect to ruminant production is a function of voluntary intake and feed nutrient utilization (Stone, 1994). The period prior to mating (i.e., May) is considered highly sensitive for sheep nutrition, because it is necessary to ensure pregnancy and cover the higher nutritional demand of the cold winter season (i.e., June-September) which is coincident with the lower offer of food in natural conditions (Ormaechea and Peri 2015). The importance of grasses and graminoids for sheep nutrition in different environments of Patagonia (forest, wetlands and steppe) is widely recognized, since such species represent the highest proportion (approximately 40 to 70 % of the total) of sheep diet (Bahamonde 2011, Ormaechea and Peri 2015). However, information about the nutritional value of grasses in Patagonia is certainly limited. There are only few reports about the crude protein and dry matter digestibility percentage of grasses in silvopastoral systems occurring in *Nothofagus antarctica* forests (Bahamonde et al. 2012, Peri and Bahamonde 2012); nutrient storage and digestibility of grasses and graminoids in steppe and wetlands (Peri and Lasagno 2010, Ormaechea and Peri 2015). Moreover, the existing information about mineral (macro- and micro-elements) nutrient concentrations of forage for extensive livestock production in Patagonia and Argentina is extremely scarce (Peri et al. 2015).

Patagonia is characterized by a distinct longitudinal gradient of precipitation and soil types and a latitudinal gradient of temperature. Forests dominate the more humid, western Patagonian Region, predominantly on Andisols (i.e., volcanic soils), while a steady transition from grasslands to scattered grass and shrub steppes extends towards the east on Aridisols. In the transitional zone between both regions, vegetation consists of shrubs and grasses on xeric Mollisols or Alfisols. (Mazzarino et al. 1998). There is limited information on the mineral element concentration of Patagonian soils which is largely focused on macro-nutrients (Diehl et al., 2008; Satti et al., 2007). Soils of volcanic origin may be limiting for some elements such as phosphorus (P) (Dielh et al. 2008) but some others like, Al, As, Rb, Cd, Co, Cu, Mn or Zn (Kiliç et al., 2015;) may reach toxic levels for plants and be transferred to the food chain (Dudka and Miller 1999).

Despite it is difficult to establish precise values and thresholds of mineral nutrients to cover the requirements for livestock production, due to the interaction between animal factors (race, age, physiological and nutritional status) and consumed forage (nutrient concentrations, chemical form of minerals in the food) (Suttle 2010), there is abundant information about the key role of nutrients for livestock production. For instance, different nutrients (e.g., P, Ca, Mg, I, Mn, Cu or Se, among others) have been identified as crucial for the successful reproduction of dairy cattle (Wilde 2006). Micro-elements such as Cu, Zn or Mo have also been identified as important for vital processes (e.g. cellular respiration, DNA and RNA replication, sequestration of free radicals, etc.) (Chan et al. 1998). On the other hand, there are some trace-elements which at high levels may result toxic for livestock (e.g., As, Cd, Fl or Pb; Suttle 2010).

Therefore, trace mineral elements should be provided to livestock in a range of concentration that leads to neither deficiency nor excess of nutrients, although sometimes the minimum level required for overcoming deficiency symptoms is not enough for improving cattle productive performance (López-Alonso 2012).

Given the limited information available concerning the mineral element status of Patagonian grazing species and their potential effect on sheep production, the aim of this work is to evaluate the concentration of macro-, micro- and trace-elements in leaves of grasses growing under contrasting environmental conditions in Southern Patagonia, focusing in sheep nutrition. For this purpose, three different ecological areas of southern Patagonia representing a vegetation and climate gradient were selected and the following main questions were addressed: (i) Are soils analyses different among zones and can such results be somehow related to plant tissue mineral element concentrations?, and (ii) in light of mineral element determinations performed in the different experimental sites, is the quality of pastures sufficient to ensure sheep nutrition and which elements may be limiting or toxic?

Material and Methods

Experimental locations

This study was conducted in three different ecological areas under extensive sheep grazing representing a vegetation and climate gradient in Santa Cruz province, southern Patagonia, Argentina. The three locations selected were: First, a *Nothofagus antarctica* forest, silvopastoral system (SP) located in the ecological area called "Complejo andino" (46º 0`14.4” – 50º 53`49.2” LS; 71º 8`46.3” – 72º 51`14.4” LW), with understory vegetation dominated by grasses and graminoids of the genus *Agrostis spp*., *Bromus spp*., *Carex spp*., *Dactilys spp*., *Deschampsia spp*. and *Festuca spp*. (Bahamonde et al. 2012). In this area, the mean annual precipitation (MAP) is 390 mm year^{-1} with a mean annual temperature (MAT) of 4.9 ºC. According to the USDA classification, SP soils are mollisols-haploxerolls.of silty loam texture. The pH is slightly acid. Second, a thicket zone in the ecological area "Matorral de Mata negra" (MT) (49º 38`22.6” –51º 33`25.2” LS; 68º 29`0.6” – 72º 23`26.9” LW), where the shrub *Mulguraea tridens* covers an important proportion of the surface (35 %), which is mostly associated with grasses such as *Jarava chrysophylla, J. ibari, Bromus setifolius* and *Festuca pyrogea* (Peri et al. 2015). The MAP is 155 mm yr^{-1} with a MAT of 6.5 ºC. The predominant soils are mollisols (USDA soil classification) with silty loam texture. Third, a grass steppe (GS) located in the "Estepa magallánica seca" area (51º 2`36.6” – 52º 7`18.1” LS; 68º 42`21.2” –71º 38`21.8” LW), being the dominant grass species *Jarava chrysophylla*, which is associated with *Poa spiciformis*, *Carex andina* and *Rytidopserma virescens*. This zone has a MAP of 235 mm yr^{-1} and 7.1 ºC MAT. Similar the MT area, soils are mollisols (USDA soil classification) with silty loam texture.

Sampling and measurements

At each ecological area (never subjected to agricultural activities and fertilization) ten composite samples (from five sub-samples each, using a rectangular frame of 1 x 0.2 m) of grasses and graminoids (selecting fully-expanded leaves) were taken during the period between 20th February and 10th March 2015. Sampling dates were selected since they are prior to mating, a period considered key for the proper nutrition of animals as preparation for reproduction. It has been shown that nutrition during the weeks before mating has an important effect on ovulation and lambing rates (González et al. 1997). The specific collection of grasses and graminoids in sample areas was based on preliminary trials in which we observed that the majority of such species as described above are consumed by sheep. Andrade et al. (2015) evaluated sheep diet in MT and GS areas and found that above 90% of the grass and graminoid species were fed by sheep. Similarly, Ormaechea and Peri (2015) reported that grass and graminoid species were the major sheep diet components in the SP zone. Plant tissue samples were collected as transects with the following coordinates: (i) . SP Area: from 51º 13' 21" LS - 72º 15' 34" LW to 51°57'22.7"LS - 71°31'40.7" LW; (ii) GS Area: from 51°17'49.8"LS - 69°14'56.5" LW to 51°56´59.0”LS – 70°25´10.22”LW; and MT Area: from 50°57'36.4"LS - 70°27'56.2" LW to 51°05'38.1" LS - 70°40'53.4" LW. Plant tissues were taken to the lab in closed plastic bags and kept at 3 ºC until processing. Thereafter, samples were kept at 3 ºC in the refrigerator, before discarding the senescent material. Tissue to be analyzed was carefully washed with 0.1 % detergent (CIF, Unilever) for removing surface contaminants. Plant tissues were rinsed with abundant tap and then distilled water. They were subsequently oven-dried at 70 ºC for two days, weighed and ground prior to mineral element determination after dry-washing. Nitrogen was measured with an elemental analyzer (TruSpec, Leco Corporation, Michigan, USA). The remaining elements were determined by inductively coupled plasma (ICP, Perkin-Elmer, Optima 3000) following the UNE-EN ISO/IEC 17025 standards for calibration and testing laboratories (CEBAS-CSIC Analysis Service, Murcia, Spain).

Additionally, three composite samples (from five sub samples) of first 20 cm of soils were collected per each ecological area, in places where plant tissues were also sampled. Soil pH was determined for saturated soil pastes (30 ml deionised water per 100 g soil). Organic matter (OM) content was measured according to the Loss of Ignition method. Samples were dried to eliminate water, were subsequently heated for 2 h at 600 C and the weight loss was recorded (Nadal et al., 2004). Soil nutrient concentrations were determined as follows: total N was measured by spectrophotometry, available P by the Olsen method, ammonium acetate extracted Mg, Ca, K and Na concentrations were determined by Atomic Absorption Spectroscopy (AAS) while the concentrations of DTPA extractable Fe and Mn were also determined by AAS (AQM Laboratories, Valladolid, Spain). For having a threshold with respect to sheep nutrient (macro- and micro-elements) requirements and potentially toxic element levels reference values found in the literature were included (e.g., Freer et al. 2007, Suttle 2010). For this purpose we used the term "requirements" referring to the nutrient concentrations in forage that sheep need for their normal development according to their age.

Data analysis

Exploratory testings were carried out to verify the compliance with the assumptions of normality, homoscedasticity and independence of data for each evaluated condition. While the Shapiro-Wilk test was performed to verify the normality of the data, the Levene test was used to verify homoscedasticity. Data independence was verified by analysing residuals from graphs. Mineral element concentrations were analysed by analysis of variance (ANOVA) with ecological area as a factor. Tukey tests were performed to test differences among ecological areas when F-values were significant ($P<0.05$). Pearson correlation analyses were evaluated between plant tissue and soil mineral element concentrations to check for potential relationships between them.

Results

The N, P and S concentrations measured followed a similar trend, with values being significantly higher in the silvopastoral area (SP) compared to the thicket zone of "matorral" (MT) and the grass steppe (GS) (Table 1). According to the literature, P (except in SP area), N and S concentrations were under the minimal values to satisfy sheep requirements in all areas. Calcium concentration in MT zone resulted lower than two others areas, but all values were above the sheep minimal requirements threshold (Table 1). Potassium and Mg concentrations were observed to follow a gradient from higher values in SP, to intermediate in GS and lower in MT. For these elements, the level of cover requirements depended of the used reference to compare (Table 1).

In Table 2 the concentration of micro-elements, which followed different trends depending of ecological area are shown. For instance, Zn concentrations were lower in MT, while Fe concentrations were the highest in the same ecological area. About the covering requirements, Zn concentration in the MT area and Cu in the GS area are within the threshold recommended in the literature (Table 2). On the other hand, the Mn concentration in the SP area was recorded to be above the maximal value permitted in FEDNA (Spanish Foundation for the Development of Animal Nutrition). The concentration of Na and trace elements that could be toxic for livestock when present in excess in their diet is shown in Table 3. The levels of Na in all areas are within the recommended thresholds. On the other hand, the concentrations of all the trace elements evaluated are well below of the thresholds reported as dangerous in the literature. However, some differences between ecological areas were observed depending on the elements. For instance, Al concentrations are considerably higher in the MT area compared with the two other experimental zones analysed; Lead, B, Cr, Ni and Ti also were higher in the MT area, but further differences among other areas were also observed. In all experimental areas, plant tissue Arsenic, Cd and V concentrations were low and under the ICP detection limit. Concerning soil mineral element concentrations and remaining properties, only Na and pH were not significantly different between ecological areas, while the remaining variables changed qualitative and quantitatively depending on e.g., the element or ecological zone (Table 4). For example, OM, P and Fe were higher in the SP area, while K was higher in the GS zone. Significant correlations between soil and grasses mineral element concentrations were found for N, P, Ca and Fe, being the first three positive and the last one negative (Table 5).

Discussion

The metabolic and physiological importance of essential nutrients for plant growth is widely recognised, while excess or toxic mineral elements may be detrimental for plant growth (Marschner 2012), and toxic for animal feeding (e.g., Smith and Cornforth 1982, McDowwell 1996). Plant tissue nutrient concentrations may be related to factors such as water availability (da Silva et al. 2011), soil characteristics or interactions between mineral elements (Marschner 2012), and this will ultimately influence the nutritional value of pasture for sheep feeding (García-Ciudad et al. 1997; Järvenranta et al. 2014). Plant tissue samples were collected during the period prior to mating, since nutrition during this lapse of time has been reported to be critical for ovulation and lambing rates (González et al. 1997). Later during the season (i..e, in May and June) the major limiting factor under Southern Patagonian conditions is forage quantity rather than nutritional quality (Hall and Paruelo 2006; Peri 2009). The mineral element concentrations determined are discussed regarding the potential influence of the prevailing water availability regime in the different ecological areas subjected to investigation (namely, a more humid silvopastoral *N. antarctica* forest, versus a drier grass steppe and especially a thicket), nutrient dynamics, soil properties and nutritional value for cattle feeding.

Macro-nutrients

Plants growing in the *Nothofagus* forest (SP) appeared to have the highest N, P and K concentrations (Table 1). Such forest soil has significantly higher organic matter, N and P concentrations compared with steppe (GS) and thicket (MT) soils (Table 4). Similar extractable P values to those obtained for SP areas have been reported for Andean-Patagonian forests which are related to a volcanic origin (Borie and Rubio, 2003; Diehl et al., 2008). In general, all soil macro-nutrient concentrations are within the range described for forest and steppe Sothern Patagonia Soils (e.g., Diehl et al., 2003; 2008; Peri and Lasagno 2010; Satti et al.2007). The higher soil N concentrations recorded for SP soils may be to the highest rates of leaf litter decomposition the more humid silvo-pastoral *N. actarctica* forest compared to steppe zones (Peri et al. 2015). However, the lower precipitation regime in GS and MT may have a strong influence chiefly on soil K but also on Mg and P supply, since in addition to other factors such as soil type, drought is known to limit nutrient availability (da Silva et al 2011, Marschner 2012). Additionally, the N concentrations determined in this study are similar to those reported by Peri and Lasagno (2010) and Bahamonde et al. (2012) for grasses and graminoids in the steppe and the *N. antarctica* forest in southern Patagonia, respectively. Concerning P concentrations, Peri and Lasagno (2010) determined higher values, for grasses sampled in summer in the steppe of southern Patagonia, being these values similar to the ones recorded for the SP ecological area. The P concentrations obtained are also within the range reported by Bertiller et al. (2006) for perennial grasses growing in a broader range of environmental conditions across Patagonia, with an average of 0.15 % P. Compared to our results, Peri and Lasagno (2010) recorded higher concentrations of Ca, lower of K, and similar values of Mg and S. This may be for instance due to interannual variations and/or different environmental conditions and show be assessed in future investigations. Differences between ecological areas at least concerning N, P and Ca concentrations could be explained in part due to the existing soil concentrations of these elements, because in some instances we found positive correlations between soil and plant mineral element concentrations. In the case of N, the higher tissue N levels measured in the SP area may be related to the higher precipitation regime (Peri et al., 2015). It is known that higher water availability improves plant root N absorption (Jungk 2002), a fact which has been corroborated in Patagonia by Austin and Sala (2002) who reported higher N concentrations in dominant plants with increased of mean annual precipitations. Contrary to our results, Bertiller et al. (2005) found that N concentrations in green leaves of perennial grasses growing in Patagonia increased with aridity, while P concentrations did not vary between sites with different precipitation regimes. Concerning the mineral element requirements for sheep, the apparent deficiency in certain instances as derived from the plant tissue values recorded may have different implications depending on the element and ecological zone. Nitrogen deficit in diet could reduce the growth rate especially in young animals (Freer et al. 2007). Nevertheless, additional nutritional components of sheep´ diet in southern Patagonia, such as herbs and legumes (Ormaechea and Peri 201[illegible]) may also be an important source of N and Ca (Suttle 2010). Similarly, temporal variations grass tissue N concentrations have been reported in southern Patagonia, where values were found to decrease significantly from September to April (Bahamonde et al. 2012).. Moreover, is well recognised that ruminants can recycle N (transfer endogenous N to rumen) in periods of N scarcity in the feed (Freer et al. 2007), which could compensate for the N shortage when plat tissue N concentrations may be low during the growing season. Similar to N, various reports showed that P, K, Mg and S concentrations in grasses and graminoids in southern Patagonia steppe during the springtime are higher than in summer, reaching the recommended values to satisfy sheep requirements (Peri and Lasagno 2010). Although Ca concentrations are within the recommended range, it must be noted the importance of vitamin Da as a prerequisite for the proper absorption and metabolism of Ca in animals. Vitamin Da levels are additionally related to skin solar radiation (Suttle 2010) which is serious limiting factor at high latitudes such as southern Patagonia.

Micro-nutrients

Concerning the concentration of micro-nutrients, no significant correlations between soil and grass tissue element concentrations were generally found with the exception of Fe, where a negative significant correlation was determined. In general, the Zn concentration in grasses has been reported to be lower than that of e.g., legumes or herbs (Kabata-Pendias 2011). Nevertheless, the values obtained in this study are lower than those reported for fresh and dry grass tissues in other studies (36 and 33 mg kg^{-1}, respectively; e.g., Givens and Moss 1990, Suttle 2010), and are actually within the range of concentrations found in straw. Zinc concentrations are lower in the MT area and may be insufficient to meet sheep requirements according the thresholds reported, which could among other detrimental effects, decrease male fertility (Martin and White 1992) and appetite (O`Dell and Reeves 1989). The Cu concentrations in the SP and MT areas are within the range reported for other grasses and legumes in a broad range of conditions (e.g., García-Ciudad et al. 1997, Kabata-Pendias 2011), but concentrations in the GS area were lower and insufficient to meet sheep nutritional requirements. Copper plays a fundamental role in the activity of numerous enzymes, cofactors and reactive proteins for plants and animals (Marschner 2012, Suttle 2010), therefore its low availability for animal feeding could be extremely harmful. Nevertheless, preliminary information indicated that the interaction of Cu with others elements (e.g., Mo, S, Zn or Fe) can affect its bioavailability (Howell and Gawthorne 1987). Hence, the Cu concentration of pastures is a poor indicator of real Cu availability for livestock and the recommendations on dietary requirements should be interpreted with caution (Freer et al. 2007).

The Fe concentrations in soils measured in this work are below the range reported for other silty loam soils (Kabata-Pendias 2011). The Fe concentrations determined in grass tissues were within the range of other grasses growing in different countries (e.g., García-Ciudad et al. 1997, Kabata-Pendias 2011, Suttle 2010) and may not be limiting to cover sheep nutritional requirements. The soil Mn concentrations recorded are low compared to those reported for soils of different countries by Kabata-Pendias (2011). However, irrespective of the ecological areas, the leaf Mn concentrations determined in this investigation are within the range measured for grasses in various studies (e.g., Suttle et al. 2003, Kabata-Pendias 2011), which is sufficient to meet sheep Mn requirements. A relationship between increased leaf Mn concentrations and the so-called "carboxylate-releasing P-mobilising strategy" in natural environments where the P availability is low has been suggested (Lambers et al. 2015). The significantly higher grass tissue Mn concentrations recorded in forest species may be, for example related to P acquisition mechanisms (note the significantly higher leaf P concentrations of SP), improved root Fe acquisition strategies or soil-related factors (Marschner, 2012), but further trials will be necessary to explore mineral element absorption and homeostasis in plants native to Patagonia.

The values of soil Na were lower in the experimental zones analysed compared to other temperate zones (e.g., Tisdale et al. 1985), but similar to those reported for other world regions (McGrath and Loveland 1992). Sodium is known to be a beneficial element for plants (Pilon-Smits et al. 2009). The Na values obtained in this study are within the range reported by Kähäri and Nissinen (1978) for timothy in Finland. Sodium (together with Cl) plays a fundamental role in maintaining osmotic pressure, regulating acid-base equilibrium and controlling water metabolism in animals (Suttle 2010).The low Na values found in this study (which will not meet sheep requirements) could hence compromise sheep metabolism if Na shortage is prolonged over time, but additional Na inputs may be obtained from other sources (e.g., from soils and/or water; Suttle 2010).

Antagonistic relationships between several micro-nutrients such Fe, Mn and Zn are well documented in the literature (e.g., Marchner 2012). In the case of Mn, the values recorded will meet sheep requirements, but the concentrations measured in the SP area exceed the permitted thresholds in Europe (150 mg kg^{-1}). However, there is no information available on which were the criteria to establish the maximum limit of 150 mg kg^{-1} Mn shown by FEDNA (Table, 2). Some studies indicate that high levels of Mn in the diet of ruminants may be detrimental for their productive performance due to an anorexic effect and metabolic antagonism with others elements such as Fe or Cu (Freer et al. 2007, Suttle 2010). Nevertheless, these negative effects have been found at Mn concentrations higher than 150 mg kg^{-1}. Grace (1973) observed that sheep grazing in pastures containing 140-200 mg kg^{-1} DM reduced their growth rate when supplementing animals with pellets containing 250 or 500 mg Mn. This supplementation led to lower Fe concentrations in sheep plasma and heart, despite pastures contained 1100-2200 mg Fe kg^{-1} DM. In another study, Black et al. (1985) reported that sheep could tolerate 3000 mg Mn kg^{-1} D.W. during 21 days. On the other hand, Hansen et al. (2006) showed that diets containing 500 mg Mn kg^{-1} D.W. can exacerbate Cu deprivation in heifers.

Potentially toxic effects

In spite of the differences measured between ecological areas, none of the trace elements determined were present at concentrations that could be considered dangerous for sheep and livestock health. In any case, the Al concentrations measured in this study were lower than the ones reported for grasses by Metson et al. (1979) in seven pastures of New Zealand. The authors found mean values of 500 mg Al kg^{-1} D.W. Extremely low As, Cd and V concentrations were determined in plant tissues which subsequently involve no toxicity risk for sheep grazing. These values are within the normal range determined for grasses growing in soils not contaminated with Pb (280-330 µg kg^{-1} D.W.) as described by Kabata-Pendias (2011). It is assumed that root Pb uptake strongly depends on Pb soil concentrations, but it has been observed that this element is generally poorly taken up by plant roots even when appreciable soil Pb concentrations may be present (Longhurst et al. 2004). Therefore, the main Pb toxicity threat for sheep and livestock production could be related to grazing in Pb contaminated land (Abrahams and Steismajer 2003). Despite B, Cr, Ni, Rb and V may potentially be toxic for sheep, these elements can also be considered as beneficial elements because at low concentrations they may be improve animal performance (Suttle 2010). For instance, an association between poor conception and low serum B concentration has been reported for beef cow herds (Small et al. 1997). Similarly, Spears (1984) reported that supplementation of diets having a Ni concentration range from 0.26 to 0.85 mg kg^{-1}, with additional 5 mg Ni as $NiCl_2$, increased the ruminal urease, growth rate and FCE (feed conversion efficiency) of lambs and steers. Kabata-Pendias and Pendias (1992) reported similar concentrations of B and V for grasses and, lower of Cr and Ni and higher values of Ru compared to the values that we obtained. On another hand, the Sr concentrations determined in this study were in the range of 6 to37 mg of Sr kg^{-1} reported by Kabata-Pendias (2011) for aerial parts of grasses. Although titanium (Ti) is not included in the literature as a toxic or beneficial element for sheep (or ruminant in general), we found it interesting to include this information due to the scarcity of plant tissue Ti concentration data. Despite the limited knowledge on plant Ti absorption and metabolism (Carvajal et al. 1998), this element has been considered as relatively scarce for plants and not readily mobile (Kaba-Pendias 2011). Contradictory effects in plants have been attributed to Ti. For example, Wallace et al. (1977) described toxicity symptoms (chlorotic spots on leaves) in bush bean with Ti concentrations of 200 mg kg^{-1}, but also there are studies indicating beneficial effects on yield of several crops (e.g., Pais 1983, Alcázar-López et al. 2003). Kabata-Pendias (2011) indicated that Ti concentration in plants may considerably vary from e.g., 0.15 to 80 mg kg^{-1}.

Concluding remarks

In this study we present novel information about the mineral element concentrations of the main feeding source for sheep in southern Patagonia. However, it is necessary to be cautious when interpreting soil properties in relation to the grass and graminoid mineral element tissue results obtained, since an array of additional biotic and abiotic factors may affect such values. Despite we provided thresholds for either sheep nutritional requirements and toxicity levels gathered from the existing literature, we strongly suggest to consider them only as reference since more detailed information will be required for establishing a nutritional guide for Patagonia.. For instance, mineral element requirements for animal feeding are difficult to be accurately determined, due to the interactions between animal absorption and metabolism in relation to the food eaten, the occurrence of net requirement variations due to the productive capacity of each particular species or breed, the rate of production enabled by other key dietary constituents, the effect of the environment (Suttle 2010) or the occurrence of potential antagonistic effects between mineral elements (e.g., Cu uptake may be inhibited by Mo and S, López-Alonso 2012, Suttle 2010). Moreover, in extensive systems the forage samples collected may not represent the material selected by grazing animals, mainly because the may show preferences for different types and parts of plants. Hence, in future investigations it will be necessary to develop methods for assessing more accurately the mineral element status of animals for spotting potential nutrient deficiencies. For such purpose, blood, urine, saliva and hair (between others) analyses have been carried out to evaluate the mineral status of animals (Suttle 2010) and may be used as tool for analysing nutrient deficiencies in Patagonian sheep. On the other hand, in extensive systems such as in Patagonia, soil ingestion when animals are grazing can significantly contribute to livestock trace mineral exposure (López-Alonso 2012). In this regard some reports indicated that soil ingestion in ruminants can represents up to 18 % of organic matter in cattle (Herlin and Andersson 1996) and more than 50 % in sheep (Thornton and Abrahams 1983). In summary, it is concluded that further trials will be required for improving our understanding of mineral element nutrition as tool for improving sheep production in Patagonia.

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Tables

Table 1 Macro nutrient concentrations (% dry weight, D.W.) in grasses from three different ecological areas in southern Patagonia and range of reference values based on the literature. Data are means ± standard deviations (SD)

Ecological area	Macro-nutrients (% D.W.)					
	N	P	Ca	K	Mg	S
SP	1.5±0.4a	0.17±0.06a	0.19 ±0.06a	1.38±0.33a	0.10±0.02a	0.08± 0.03a
MT	1.0±0.1b	0.05±0.01b	0.14± 0.02b	0.23±0.04c	0.04± 0.01c	0.06±0.01b
GS	0.9±0.2b	0.05±0.01b	0.19±0.04a	0.44± 0.12b	0.08±0.02b	0.06±0.01b
Range of reference values[1#]	1.7 – 3.2	0.09 - 0.3	0.14 - 0.7	0.5	0.09 - 0.12	0.2
Range of reference Values[2#]	nd	0.11 - 0.41	0.14 - 0.7	0.3	0.04 - 0.14	0.09 - 0.21

Different letters in the same columns indicate different levels of significance between ecological areas ($P < 0.05$).

nd: no reference data available

[1]Adapted from Freer et al. (2007).The range represents the concentration in the forage to cover the requirements for sheep of different category and physiological state, from lamb of 20 kg LW (live weight) to sheep of 50 kg LW pregnant or lactating, assuming that the quantity of forage available is not limiting.

[2]Adapted from Suttle (2010).The range represents the concentration in the forage to cover the requirements for sheep of different category and weights, from growing lambs of 20 kg LW (live weight) to pregnant ewe carrying twins of 75 kg LW, assuming that the quantity of forage available is not limiting.

[#]The units of the reference values are the same as are expressed in the plants concentrations.

Table 2 Micro-nutrient and Na concentrations (mg kg^{-1} D.W.) in grasses from three different ecological areas in southern Patagonia and range of reference values for adequate sheep nutrition. Data are means ± SD.

Ecological area	Element concentrations (mg kg^{-1} D.W.)				
	Zn	Cu	Fe	Mn	Na
SP	13.4± 4.9a	8.2±2.5a	39.8±5.6b	218.6±23.3a	68.6±64.9b
MT	6.6±1.6b	8.5± 4.2a	104.0±29.1a	26.5±6.7b	80.0±64.6b
GS	11.6±3.7a	3.5±1.2b	33.7±8.5b	23.5±7.0b	134.0±44.0a
Range of reference values[1#]	9 - 20	4 - 14	40	20 - 25	nd
Range of reference Values[2#]	8.8 - 27	4.3 - 28.4	30 - 50	8 - 20	600 - 1250
Maximum authorized value in Europe according to FEDNA[‡]	150	25	750	150	45000*

Different letters in the same columns indicate different levels of significance between ecological areas ($P < 0.05$).

nd: no reference data available.

[1]Adapted from Freer et al. (2007). The range represents the concentration in the forage to cover the requirements for sheep of different age and physiological state, from lamb of [illegible]0 kg LW (live weight) to sheep of 50 kg LW pregnant or lactating, assuming that the quantity of forage available is not limiting.

[2]Adapted from Suttle (2010).The range represents the concentration in the forage to cover the requirements for sheep of different category and weights, from growing lambs of 20 kg LW (live weight) to pregnant ewe carrying twins of 75 kg LW, assuming that the quantity of forage available is not limiting.

[‡]FEDNA: Spanish Foundation for the Development of Animal Nutrition. The values are referred to the maximum amounts of trace elements authorized in feed (or its correctors) for various domestic species, according to European Union.

*Value obtained from NRC (2000)

[#]The units of the reference values are the same as are expressed in the plants concentrations.

Table 3 Additional micro-nutrients (B and Ni) and trace element concentrations (mg kg^{-1} D.W.) in grasses from three different ecological areas in southern Patagonia and range of reference values for ruminant toxicity. Data are means ± SD.

Ecological area	Micro-nutrient and trace element concentrations (mg kg^{-1} D.W.)										
	B	Ni	Al	As	Cd	Pb	Cr	Rb	Sr	Ti	V
SP	4.0±1.0c	2.1±0.3b	17.5±4.3b	< 0.1	< 0.1	1.03±0.47a	4.3±0.8b	6.38±4.45a	10.1±1.1b	1.0±0.3c	< 0.5
MT	8.7±1.1a	3.2±1.1a	108.0±34.8a	< 0.1	< 0.1	1.24±0.42a	6.0±2.3a	0.39±0.10b	7.5±1.3c	6.8±2.3a	< 0.5
GS	7.1±1.1b	1.3±0.3c	34.5±12.0b	< 0.1	< 0.1	0.40±0.18b	0.7±0.5c	0.22±0.08b	15.2±3.9a	2.7±1.0b	< 0.5
Range of reference values[1#]	800	100	500 - 8000†	2 -30	1 - 10	5 - 100	1000*	250 - 1000	2000*	nd	200 - 400

Different letters in the same columns indicate different levels of significance between ecological areas ($P < 0.05$).

[1]Adapted from Suttle (2010).The minimum and maximum value of the interval represents the maximum permitted and maximum tolerable, respectively according to the reference. Value intervals are given for ruminants in general (excepting*).

nd: no reference data available

†: According to Jones (2005)

*: According to NRC (2000). Reference values specific for cattle tolerance.

[#]The units of the reference values are the same as are expressed in the plants concentrations.

Table 4 Organic matter (OM), pH and mineral element concentrations in soils from three different ecological areas in southern Patagonia. Data are means ± SD.

Ecological area	OM (%)	N (%)	P (mg kg^{-1})	K (%)	Ca (%)	Mg (%)	Mn (mg kg^{-1})	Na (mg kg^{-1})	Fe (mg kg^{-1})	pH
SP	20.7±12.5a	0.73±0.31a	41.2±4.4a	0.46±0.07b	0.35±0.22ab	0.95±0.54a	13.5±6.5a	538±39a	185± 62a	6.2±0.3a
MT	4.4±1.4b	0.53±0.50ab	23.1±6.8b	0.48±0.22b	0.10±0.04b	0.46±0.18a	6.5±0.3b	634±166a	33±6c	6.3±0.6a
GS	7.9±2.5b	0.28±0.02b	26.2±5.4b	0.86±0.51a	1.00±0.57a	0.08±0.01b	16.0±8a	545±16a	97±31b	5.7±0.3a

Different letters in the same columns indicate different levels of significance between ecological areas ($P < 0.05$).

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Table 5 Coefficient of correlation of Pearson (R) between mineral element concentration in soils (0-20 cm) and plant tissues at three different ecological areas in southern Patagonia.

	N (%)	P (%)	Ca (%)	K (%)	Mg (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Na (mg kg^{-1})
Coefficient of correlation (R)	0.67 (+)	0.86 (+)	0.40 (+)	ns	ns	0.68 (-)	ns	ns

Symbols in parentheses indicate if the relationship is positive or negative.

ns: not significant