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Mineral composition and nutritive value of *Festuca* ecotypes originated from the highland region of Bolivia and cultivars from Argentina

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Abstract

Native grasses constitute the most important source of feed for camelids, sheep and cattle in the highlands of Bolivia, where the genus *Festuca* is one of the major feed components. This study was carried out to investigate the nutritional value of 11 *Festuca* ecotypes from the highlands of Bolivia and two cultivars from Argentina (*Festuca arundinacea* Schreb. cv. 'Taita' and Festulolium). All ecotypes were grown in the same experimental field and their protein, ash, cellulose, moisture and micronutrients (Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Si and Zn) content was determined. Principal component analysis and unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, based on all nutrients, clearly defined the two Argentinian cultivars as outliers. This differentiation was mainly explained by their cellulose, ash, Mn and Al contents. Analysis of variance based on the origin of the accessions revealed highly significant ($P < 0.001$) differences for their cellulose, ash, Mn and Mg contents, while they differ significantly ($P < 0.05$) in Ni, Fe, Na and Al contents. There was a highly significant positive correlation between several pairs of minerals including Mg–Ca (r = 0.94) and Mg–Zn (r = 0.92). Negatively correlated pairs of minerals include B–Fe (r = - 0.65) and B–Ni (r = - 0.58). This study provides useful information about the nutritive quality of Bolivian *Festuca* ecotypes for their use in breeding programs of this forage grass in the Andean highlands of South America.

Keywords: Fescue, forage, micronutrient, principal component analysis.
Abbreviations: ICP-OES_Inductively Coupled Plasma Optic Emission Spectrometry; PCA_Principal Component Analysis; UPGMA_ unweighted pair group method with arithmetic mean.

Introduction

The Andean region of Bolivia comprises about 200 000 km^2 with an altitudinal range between 3600 and 4300 m above sea level (masl) and is characterized by climatic stress including drought and frost as well as unfavorable soil conditions (Geerts et al., 2006). The main activity of farmers is livestock production, where llamas (*Lama glama*) are the most common, constituting around 63% of the South American llama population (Markemann et al., 2009). This animal is generally kept together with sheep in the farming systems playing an important role in the sustainability as it is less susceptible to widespread climatic risks than crops (Camino and Sumar, 2000; Campero, 2004).

The most important feed sources for camelids and ruminant species are native grasslands (Genin and Alzérreca, 2006) that include several unique plant communities adapted to the adverse climate conditions in the area. Among these, "pajonales" occupy extensive areas and are represented by tall and coarse bunchgrasses of the *Stipa, Festuca* and *Callamagrostis* genera (Genin et al., 1994; Sumar, 2010). *Festuca orthophylla* Pilg., known as "paja brava" or "iru ichu" is the most widespread grass in the highlands of Bolivia, occupying almost 30% of the central area (Alzérreca and Lara, 1988; Monteiro et al., 2011). It is considered as the major component of feed preferred by camelids, especially llamas, despite being a poor forage due to its roughness and very low nutritive value (Genin et al., 1994; Zapata, 2005; Monteiro and Körner, 2013).

Despite the fact that the majority of the grass species in the native grasslands are considered as having low nutritive value, farmers believe that natural grasslands of *Festuca* *dolichophylla* J. Presl. also known as "chilliwar" rangelands are a very important source of feed, especially for cattle, llamas and sheep (Mercado et al., 2013). The presence of various associated plant species such as layu (*Trifolium amabile*), sillu sillu (*Lachemilla pinnata*), siqui (*Hypochaeris spp*.), cebadilla (*Bromus catharticus*), cola de ratón (*Hordeum muticum*), chiji blanco (*Distichlis humilis*), kemallu (*Eleocharis spp*.), poita (*Poa annua*) and chiji negro (*Muhlenbergia fastigiata*) improves the forage value of these rangelands (Martínez et al., 2004; Genin and Alzérreca, 2006). However, the rangelands are suffering from a continuous degradation of the soil, narrowing of its range due to the advancing agricultural frontiers and overgrazing (Alzérreca, 2004).

Improvement of livestock outputs requires an efficient use of available feed resources. The nutritive value of forage species is however affected by genetic variation, climate, agronomic practices and feed processing technologies (Khan et al., 2007). Besides nutritive values that can be obtained by measuring crude protein content, acid detergent fiber, neutral detergent fiber and ash, forages are an important source of minerals for ruminants and camelids. In some cases, forages provide adequate quantities of all essential minerals, but in others, they may be deficient in one or more minerals, thus supplementation is required for optimal performance and health of the animals (Spears, 1994; Givens et al., 2000).

The bioavailability of major and minor essential elements, as well as trace elements constitute the cornerstone for increasing the performance and health of grazing livestock (National Research Council, 2007). The mineral content of forages depends on the interaction of a number of factors including soil, forage species, stage of maturity and pasture management. In addition, climate affects plant growth rate and plant maturity, thereby altering mineral content (Givens et al., 2000). Hence, the bioavailability of minerals is also an important consideration when evaluating dietary adequacy (Lewis and Bayley, 1995).

There is scant research on botanical composition and nutritional value of native grasslands in the highland region of Bolivia (Alzérreca and Cardozo, 1991; Mamani-Linares et al., 2013). Thus, a comprehensive study on the nutritional value of Bolivian *Festuca* species, which are the most important native forage grasses in Bolivia, is essential. The main goal of this research was to assess different *Festuca* ecotypes for protein, cellulose, ash and mineral composition in order to identify promising candidates for future use in breeding programs.

Results

Nutritional content and mineral composition

Significant differences in ash and cellulose content were found among *Festuca* ecotypes from Bolivia and cultivars from Argentina (Table 1). Ash content ranged from 7.3% (Accession 27) to 16.5% (Accession 44). The two Argentinian cultivars, *F. arundinacea* cv. 'Taita' and Festulolium had the highest ash content with 16.5% and 14.3% respectively, followed by the Bolivian accessions 19 (9.7%) and 38 (9.5%). Cellulose content ranged from 18.7 % (accession 44) to 33.6 % (accession 27). Accessions 9 and 19 also showed quite high cellulose content, 33.4% and 33.3%, respectively. The protein content obtained with the organic elemental analyzer (OEA) ranged from 6.7 % (accessions 38 and 27) to 12.2 % (accession 29) and protein content obtained with the Kjeldahl method ranged from 6.1% (accession 38) to 12.4% (accession 45). Both methods showed that accessions 29 and 45 were the top two in protein content among the accessions studied. When a comparison between origins was performed, ash and cellulose content showed highly significant differences ($P < 0.001$), but the differences for both protein and moisture were not-significant ($P > 0.05$). Results were very similar when comparing the averages of the micronutrients of Bolivian ecotypes and Argentinian cultivars (Table 2).

The Argentinian *F. arundinacea* cv. 'Taita' (accession 44) had the highest Mg, Fe, Mn and Al content, while Festulolium (accession 45) had the highest Ca, P, Na, K, S, and Zn content (Table 2). Among the Bolivian ecotypes, accessions 32, 21 and 10 were the top three in terms of Ca (≥ 2795mg kg^{-1}) and Mg (≥ 1590 mg kg^{-1}) content, while accessions 38, 29 and 23 were at the top in terms of their P content (≥ 1550mg kg^{-1}). Interestingly, accessions 41 and 43 showed more than a two-fold higher Na content (≥ 160 mg kg^{-1}) than other Bolivian ecotypes, whereas accession 21 and 29 had the highest S content (≥ 1600 mg kg^{-1}) and accession 23 and 29 the highest K (12000 mg kg^{-1}) content. Accession 27 showed the lowest Ca (1890 mg kg^{-1}), P (886 mg kg^{-1}), K (6400 mg kg^{-1}), S (800 mg kg^{-1}), Zn (11 mg kg^{-1}) and Mn (29 mg kg^{-1}) content (Table 2).

PCA analysis and UPGMA clustering of the nutritional values by origin

The Principal Component Analysis (PCA) based on mineral and nutrient content data showed good reproducibility between technical laboratory replicates per accession.

The two outliers were the Argentinian cultivars *F. arundinacea* cv. 'Taita' (accession 44) and Festulolium (Accession 45), which were clearly separated from the Bolivian ecotypes (Figure 1). The ecotypes from Cochabamba and La Paz were clustered together, which could be explained by their lower content of protein (except for accession 21), Ca, Mg and Cu (except for accession 43). Similarly, the clustering among ecotypes from Oruro and Potosí could be explained by their higher content of protein, Ca, Mg and Zn as compared with the other Bolivian ecotypes (Figure 1).

According to the PCA (Table 3), the first two PCs together explained 69.9% of all the nutrients variability among the accessions. The first PC explained 48% of the total variation, mainly due to differences in Mg, Ca, Zn, Mn, cellulose, S, ash, K, Fe, P, moisture and Al contents. The traits that contributed more to differentiation at the second PC, which accounted for 21.9% of the total variation, were Cu, Cd and protein content. These results indicate that Mg, Ca, Zn, Mn, cellulose, S, ash, K, Fe, P, moisture, Al, Cu, Cd and protein are of primary importance in differentiating accessions.

Cluster analysis based on UPGMA method resulted in a clear grouping of the accessions into two major clusters, with the first cluster further separated into two sub-clusters (Figure 2). Sub-cluster IA included ecotypes from La Paz and Cochabamba (accessions 27, 38, 41, 9 and 19), which are characterized by a

Table 1. Nutrient content and moisture of *Festuca* Bolivian ecotypes and Argentinian cultivars

Accession	Origin	Ash (%)	Cellulose (%)	Protein-1 (%)	Protein-2 (%)	Moisture (%)
9	Cochabamba	7.5	33.4	7.8	8.1	53.1
23	Cochabamba	7.8	31.8	8.1	8.7	58.9
38	Cochabamba	9.5	30.6	6.1	6.7	44.6
41	Cochabamba	8.9	30.9	6.8	7.2	50.9
43	Cochabamba	8.4	29.8	9.6	9.9	50.6
19	La Paz	9.7	33.3	7.8	8.2	53.8
21	La Paz	8.8	29.3	10.3	10.2	56.7
27	La Paz	7.3	33.6	6.3	6.7	35.5
10	Oruro	9.2	32.8	11.5	11.5	55.6
29	Potosi	8.1	28.3	12.1	12.2	58.4
32	Potosi	9.3	27.6	10.0	10.1	51.3
44	Argentina	16.5	18.7	6.8	7.7	55.4
45	Argentina	14.3	20.3	12.4	11.8	66.1
Comparison between origins (*P*-value)		< 0.001	< 0.001	n. s.	n. s.	n. s.
Bolivian ecotypes, average		8.6 b	31.0 a	8.8 a	9.0 a	51.8 a
Cultivars from Argentina, average		15.4 a	19.5 b	9.6 a	9.8 a	60.8 a

Protein–1: corresponds to the concentration obtained with the Kjeldahl method. Protein–2 corresponds to the concentration obtained using the organic elemental analyzer.

The probability (*P*) values are for the comparison among accessions within their origin. Different letters within each column indicate a significant difference at *P* = 0.05 level, when comparing accessions from Bolivia and cultivars from Argentina.

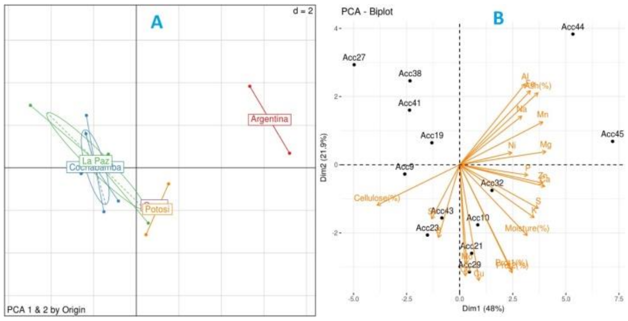

Fig. 1: A) Principal component analysis (PCA) scores plot on aggregated data of ash, cellulose, protein, moisture and micronutrients in *Festuca* ecotypes and cultivars from Argentina showing how accessions are clustered according to their origin. B) Loadings showing the relationships among variables and with PCs using the average of the two replicates per accession. Dim1 and Dim 2 represent the first and the second principal components, which explain 48% and 21.9% of the total variance among the accessions.

Table 2. Mineral element contents of 11 ecotypes from the highlands of Bolivia and two Argentinian cultivars of *Festuca*

Acc	Origin	Ca^a	P^a	Na^a	Mg^a	K^a	S^a	Fe^b	Cu^b	Zn^b	Mn^b	Mo^b	Al^c	B^c	Ni^c	Si^c	Cd^d
9	Cbba	2505	1270	60	1320	10000	1000	860	5.4	13	33.5	1.7*	464	22.5	1.8*	510	<0.13
23	Cbba	2580	1550	54	1295	12000	1550	785	5.2	12	41.0	2.2*	414	26.0	1.9*	596	<0.14
38	Cbba	2425	1720	58	1105	8300	1450	1100	4.4	12	38.0	0.8*	600	21.5	1.9*	562	<0.11
41	Cbba	2135	1420	160	1265	10000	1100	995	4.4	12	36.5	1.0*	543	26.0	2.0*	513	<0.12
43	Cbba	2355	1275	205	1310	11000	1500	1000	7.0	14	37.5	1.8*	520	28.5	1.4*	488	<0.13
19	LP	2590	1350	61	1365	9250	1200	1200	5.3	14	35.5	1.4*	647	21.5	2.2*	499	<0.13
21	LP	3045	1545	75	1600	11000	1600	1100	5.9	14	45.5	2.5*	596	27.5	2.3*	602	<0.14
27	LP	1890	886	63	1200	6400	800	1100	4.4	11	29.0	1.4*	590	21.0	2.1*	496	<0.11
10	Oru	2795	1360	58	1590	11000	1550	1350	5.4	14	38.5	2.7*	686	18.5	3.9*	570	<0.14
29	Pot	2755	1600	75	1555	12000	1700	825	6.9	14	38.0	1.4*	404	25.0	1.8*	514	<0.14
32	Pot	3060	1415	78	1840	9450	1400	1600	6.5	16	41.5	1.6*	830	23.5	3.2*	518	<0.15
44	Arg	3350	1585	235	2235	12000	1700	2200	4.6	15	74.0	1.4*	1130	21.0	3.0*	514	<0.13
45	Arg	3375	2125	250	2080	15000	2100	1700	5.5	19	65.0	1.3*	830	20.5	2.9*	436	<0.11
Comparison between origins (*P*-value)		n.s.	n.s.	0.03	<0.001	n.s	n.s.	0.02	n.s.	n.s.	<0.001	n.s.	0.04	n.s.	0.01	n.s.	n.s.
Bolivian ecotypes, average		2558b	1399a	86b	1404b	10036a	1350a	1083b	5.5a	13b	37.7b	1.7a	572b	23.8a	2.2a	534a	0.1a
Cultivars from Argentina, average		3362a	1855a	242a	2158a	13500a	1900a	1950a	5.0a	17a	69.5a	1.3a	980a	20.8a	3.0a	474a	0.1a

Micronutrients were recorded in mg kg^{-1}. Acc (accession), Cbba (Cochabamba), LP (La Paz), Oru (Oruro), Pot (Potosí), Arg (Argentina), ^{a} = macromineral, ^{b} = micromineral, ^{c} = trace element, ^{d} = toxic element, < = the content is below the limit of detection, * = the content is close to the level of detection. The probability (*P*) values are for the comparison among accessions within their origin. Different letters within each column indicate a significant difference at *P* = 0.05 when comparing accessions from Bolivia and cultivars from Argentina.

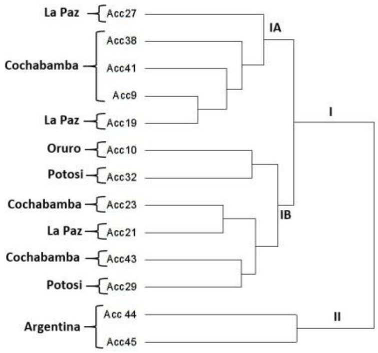

Fig 2. Dendrogram from cluster analysis (UPGMA) of the 11 Bolivian ecotypes and two Argentinian cultivars based on protein, ash, cellulose, moisture and micronutrients content.

Table 3. Principal component (PC) analysis of nutritional value according to the origin of the accessions revealing eigenvalue, total variance, eigenvectors and contribution to total variation explained by the first two PC axes. Ash, cellulose, protein and moisture were recorded in percentage and micronutrients were recorded in mg kg^{-1}.

	Total variance			Eigenvectors					
	Eigenvalue	Indiv (%)	Cum (%)	Nutrient	PC-1	PC-2	Nutrient	PC-1	PC-2
PC-1	10.1	48.0	48.0	Mg	0.94	0.09	Al	0.71	0.55
PC-2	4.6	21.9	69.9	Ca	0.93	-0.15	Na	0.67	0.33
				Zn	0.90	-0.12	Ni	0.57	0.08
				Mn	0.90	0.29	Cu	0.21	-0.78
				Cell	-0.90	-0.27	Cd	0.06	-0.75
				S	0.85	-0.29	Pro–2	0.57	-0.73
				Ash	0.85	0.49	Pro–1	0.56	-0.71
				K	0.81	-0.36	Mo	0.08	-0.67
				Fe	0.77	0.50	B	-0.23	-0.49
				P	0.74	-0.07	Si	-0.31	-0.37
				Mois	0.73	-0.48			

Indiv = individual, Cum = cumulative, Cell = cellulose, Mois = moisture, Pro–2 = protein–2, Pro–1 = protein–1.

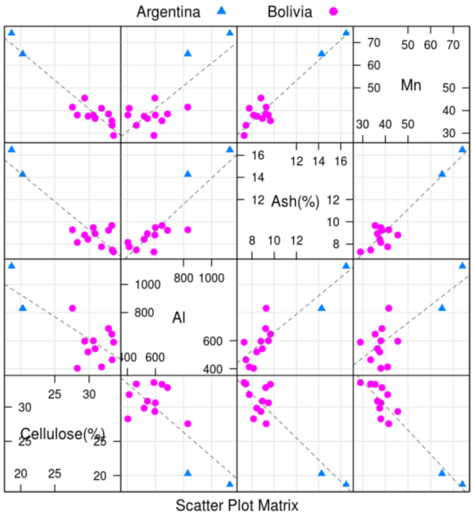

Fig 3. Scatter plot matrix of the four variables (cellulose, ash, manganese (Mn) and aluminum (Al)) explaining the separation of the Bolivian ecotypes from the two cultivars from Argentina.

Table 4. Geographic positions and altitudes of seed collection sites for *Festuca* ecotypes from Bolivia

Accession	Origin	Locality	LAT (S)	LON (W)	ALT
9	Cochabamba	Tiraque	17˚28'50.3''	65˚37'08.9''	3673
23	Cochabamba	Melga	17˚25'33.2''	65˚54'57.4''	3338
38	Cochabamba	Vacas	17˚31'03.0''	65˚36'48.2''	3790
41	Cochabamba	Sunjani	17˚10'12.6''	66˚21'08.4''	3974
43	Cochabamba	Parque Tunari	17˚16'15.9''	66˚19'49.7''	3809
19	La Paz	Orkojipina	16˚31'44.2''	68˚ 20'53.7''	3862
21	La Paz	Sica Sica	17˚ 19'05.1''	67˚46'02.7''	3906
27	La Paz	Konchamarca	17˚ 22'29.8''	67˚27'27.5''	3987
10	Oruro	Chilliwani	17˚37'51.6''	67˚13'45.6''	3799
29	Potosi	Janco Huaje	19˚52'52.3''	69˚40'58.9''	3659
32	Potosi	Cerro Potosi	19˚37'25.2''	65˚44'02.2''	4316

LAT (S) = latitude (south of Equator), LON (W) = longitude (West of Meridian), ALT = Altitude above sea level in meters.

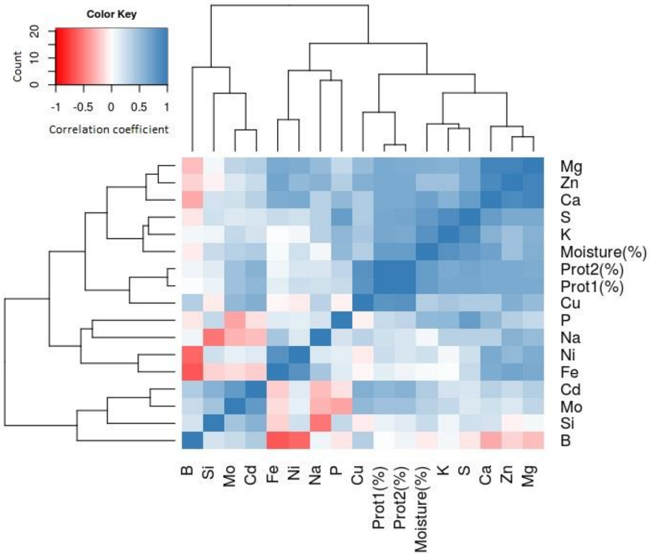

Fig 4. Heatmap showing the correlation between any of two variables measured in this study. Individual values contained in the matrix are represented as colors. The color intensity indicates the magnitude of the Spearman correlation coefficient either as positive (blue) or as negative (red).

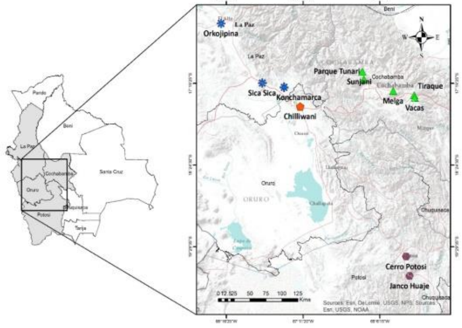

Fig 5. Map of the Plurinational state of Bolivia showing its nine departments (left) and collecting sites of seeds of *Festuca* ecotypes (right). (See Table 4 for further details).

relatively low protein content. Sub-cluster IB comprised six ecotypes (accessions 10, 32, 23, 21, 43 and 29) having a relatively high content of protein and was further divided into three subgroups. The first subgroup included ecotypes of Oruro and Potosi (accession 10 and 32), the second subgroup included ecotypes from Cochabamba and La Paz (accessions 23 and 21) and the third subgroup included ecotypes from Cochabamba and Potosi (Accessions 43 and 29). Cluster II comprised of two Argentinian cultivars (Accessions 44 and 45) (Figure 2).

Comparison of accessions based on their origin

The results of the multivariate matrix of nutrient content and mineral data at $\log_2$ scale together with the Empirical Bayes and shrinkage methods favored a stable analysis under small sample size scenarios. These results were further confirmed after the analysis of variance showing significant differences based on the origin of the accessions, for cellulose ($P < 0.001$), ash ($P < 0.001$), Mn ($P < 0.001$), Mg ($P < 0.001$), Ni ($P = 0.01$), Fe ($P = 0.02$), Na ($P = 0.03$) and Al ($P = 0.04$) (Table 1, Table 2).

Principal component plot (PC) score-plots

In order to determine the variables contributing to the clustering of accessions, a PC biplot on aggregated data was generated (Figure 1B). The interpretation of the results was made according to the direction of the vectors in the biplot. Accession 27, 38, 41 and 19 had a low content of protein, P, Ca, K, S, Zn and Cu. Accessions 21 and 29 were located almost at the end of the projected vectors corresponding to content of Cu, Mo and Cd, suggesting high content of these minerals. Accessions 44 and 45 corresponded to higher content of Na, Mg, Fe, Mn, Al, Ni and ash content than the other accessions but had the lowest cellulose content. Accession 27 exhibited low protein and micronutrients content, while accession 43 had the highest B content. Protein content data obtained with the Kjeldahl method and the organic elemental analyzer were highly correlated as their vectors ended almost at the same point, indicating that both methods consistently measured the protein content.

A PCA on aggregated data was conducted to avoid overestimation of the significance since the two outliers (the Argentinian accessions 44 and 45) could influence the correlation between the nutrients. The results showed that cellulose, ash, Mn and Al account for the separation of the two Argentinian cultivars as outliers from the Bolivian ecotypes (Figure 3). Once these four variables were excluded in a new PCA, Spearman's correlation analyses were performed among the mineral data, nutritional content and moisture (Figure 4). The observed correlation coefficients were Mg–Ca ($r = 0.94$; $P < 0.0001$), Mg–Zn ($r = 0.92$; $P < 0.0001$), Zn–Ca ($r = 0.90$; $P < 0.0001$), K–S ($r = 0.86$; $P = 0.0001$), K–moisture ($r = 0.83$; $P = 0.0004$), Ni–Fe ($r = 0.83$; $P = 0.0005$), Cu–protein content ($r = 0.81$; $P = 0.0006$) and S–moisture ($r = 0.77$; $P = 0.002$). Boron was negatively correlated to Fe ($r = -0.65$; $P = 0.0170$) and Ni ($r = - 0.58$; $P = 0.0373$), while Na was negatively correlated with Si ($r = - 0.53$; $P = 0.059$). Molybdenum, Ni and Cd were either under the limit of detection or close to the limit of detection in all the analysed accessions, and hence it is difficult to make a conclusion due to their low values.

Discussion

Native forages in the highlands of Bolivia are important for the survival of Andean livestock producers (Genin and Alzérreca, 2006). Hence, information regarding the nutritional quality of the most important forages is essential to promote the recovery and development of grasslands. A few research reports on the nutritive value of *Festuca dolichophylla* and *Festuca orthophylla* in the highlands of Bolivia are available (Alzérreca and Lara, 1988; Genin et al., 1994; Alzérreca, 2004; Genin and Alzérreca, 2006; Condori, 2014). To the best of our knowledge, the present study may be, however, regarded as the most thoroughly undertaken analysis for this forage in Bolivia.

Genin et al. (1994) noticed 6.6% crude protein (in the rainy season) and 1.4% (in the dry season) for *F. orthophylla*. In addition, Genin and Alzérreca (2006) found 7.7% crude protein content in *F. dolichophylla* and 3.9% in *F. orthophylla*, while Mamani (2003) noted 3.7% protein in *F. dolichophylla* and 2.7% in *F. orthophylla*. A review presented by Alzérreca and Cardozo (1991) reported an average of 6.8% in *F. dolichophylla* and 4.2% in *F. orthophylla*. In the present study, the protein content of the ecotypes ranged from 6.1% to 12.4%, which is slightly higher than in most previous reports.

According to the National Research Council (2007), the species and the maturity stage of the plants are among the major factors affecting the protein content in forages. Hence, these factors might have contributed to the discrepancies between the present and previous results, in addition to differences in the methods used.

Research by Villca and Genin (1995) regarding the feeding behavior of llamas and sheep when grazing concluded that both types of animals preferred *F. dolichophylla* (chilliwa) in the dry and rainy season. *F. orthophylla* (iru ichu) was, however, preferred by llamas throughout the year. Interestingly, accession 27 in the present study, which has been identified as *F. orthophylla,* had the lowest ash (7.3%) and protein (about 6%) contents, both of which were higher than those (2.3% and 2.3%) reported by Mamani-Linares et al. (2013). The difference might be due to differences in the genetic background of the studied material, weather conditions and soil fertility, as reported in previous research (National Research Council, 2007; Merlo et al., 2018). According to Alzérreca and Cardozo (1991), the average value reported for ash content in *F. orthophylla* was 7.4%, which is similar to our results for *F. orthophylla* (accession 27). Mamani-Linares et al. (2013) reported 7.2% of ash content in *F. dolichophylla* whereas it was somewhat higher (8.7%) in the Bolivian ecotypes (excluding accession 27) in the present study (Table 1).

Acid detergent fiber and neutral detergent fiber measurements are generally taken because both are based on the digestibility of the plant material, thus allowing the determination of how much energy animals receive from the consumed feed. Fiber that has higher cellulose, lignin and hemicellulose will typically take up more space in the stomach providing smaller amounts of energy to the animal. According to our results, the highest cellulose content was found in accessions 27, 9 and 19, which suggest a provision of less energy to animals.

Festuca arundinacea Schreb. and Festulolium constitute important sources of forage due to their nutritional value (Barnes, 2018; Macleod et al., 2013), thereby providing efficient forage production if climatic, edaphic and geographical factors are considered to support a sustainable agricultural practice.

The National Research Council (2007) reported 15% protein content for *Festuca arundinacea* cv. KY 31 while Kaplan et al. (2017) reported 5.6% for cv. Olympus. These results are different from the ones presented in the present study, which were 7.7% and 6.8% of protein content, respectively, indicating substantial variation between cultivars.

The ash content reported for *Festuca arundinacea* cv. KY 3 by the National Research Council (2007) was 9%, while Kaplan et al. (2017) reported 12.0% for cv. Olympus. The present study reported 16.5% for cv. 'Taita' again indicating variation between cultivars.

Meneses et al. (2017) reported 13.3% protein and 11.3% ash content in Festulolium while our results were 11.8% protein and 14.3% ash content, which might be explained by different soils and weather.

Minerals are separated into macrominerals, microminerals and trace elements according to the amount required by the animals. Hence, information on forage mineral supply is the starting point for an appropriate balanced mineral supplement ensuring optimal production, health and fertility in ruminants and camelids (National Research Council, 2007). Micronutrient content of forages depends on soil pH and fertility, forage species and maturity stage, season and climate, irrigation, and atmospheric inputs (McDowell et al., 1996; Givens et al., 2000). The variability of the micronutrients observed in the present study may be attributed not only to genetic differences but also to the interaction of all the factors just mentioned above. Eventhough the soil analysis was not performed in this study (due to financial limitations), previous reports showed that only a small fraction of micronutrients from soils are available to plants (Gupta et al., 2008). It is important to highlight that an acid soil (pH < 6.0) may, however, limit absorption of P, K, S, Ca, Mg and Se while increasing absorption of Fe, Mn, B, Cu and Zn. In contrast, excesses of Mo and Se and deficiencies in Fe, Cu, Zn, B and Mn can be found in forages grown in alkaline soils (National Research Council, 2007).

The macrominerals Ca, P and Na are required for normal life processes (Hays and Swenson, 1985). Hence, their levels in forage grasses are important attributes to be considered. The previously reported amounts of Ca in forage grasses varies from 1890 to 3380 mg kg^{-1}. Our results fit well into this range with the highest content of Ca in Festulolium (3375 mg kg^{-1}) and the lowest in accession 27 (1890 mg kg^{-1}). Phosphorus is required by ruminal microorganisms for digestion of cellulose (Burroughs et al., 1951) and synthesis of microbial protein (Breves and Schröder, 1991). Phosphorus content varies from 883 to 2140 mg kg^{-1} depending on the type of forage grass. Our results are within this range with the highest content noted in accession 45 (2125 mg kg^{-1}) and the lowest in accession 27 (886 mg kg^{-1}).

Sodium along with Cl and K, when found in proper concentration and proportion, are indispensable for a number of important functions in animals. The commonly reported range of Na in forage grasses is from 54 to 250 mg kg^{-1}. In the present study, the Na content ranged from 54 mg kg^{-1} (accession 23) to 250 mg kg^{-1} (accession 45), and hence accessions that combine higher values for Na and other important mineral elements need to be considered for use in the *Festuca* breeding programs.

Materials and methods

Germplasm and field experiments

Eleven *Festuca* ecotypes from four Departments of Bolivia (Cochabamba, La Paz, Oruro and Potosi) (Figure 5; Table 4) and two cultivars *F. arundinacea* cv. 'Taita' and Festulolium from Argentina (kindly provided by Oscar Peman y Asociados S.A.) were used in this study. Field trials were conducted from December 2015 to January 2017 at the Centro Experimental Agropecuario Condoriri (CEAC; 17°31'41" S and 67°14'02" W), which is located approximately 50 km from the capital city, Oruro, and 12 km from Caracollo municipality at an altitude of 3830 masl. The average minimum/maximum temperatures registered at CEAC in the study period were -1/19.8°C and the annual precipitation 159 mm with the heaviest rainfall of 59.8 mm registered in February (SENAMHI, 2016). Soil minerals were not measured in this study, however, soils in CEAC have been previously described by Mamani (2003) as alluvial and colluvial origin, with loam, sandy loam and clay loam textures with a pH average of 7.5.

Seeds of each ecotype and cultivar were sowed in pots in May 2015 under greenhouse conditions and seedlings were transplanted to the experimental field (CEAC) in December 2015. The experimental design used was a randomized complete block design (RCBD) with 15 plants per ecotype/cultivar in two replications. The plants were spaced at 0.5 m within the rows and 1 m between rows. Plants were sufficiently watered during transplanting and when necessary during the experimental period. No fertilizer was applied during the experiment and plots were hand-weeded during September to May for two years.

Sampling and nutritional analysis

In January 2017, which is the beginning of the flowering period for fescues, leaves and florets from plants of each accession were cut using a stainless-steel knife simulating grazing height. Plant material of the same accession from the two replicates were mixed, weighed, kept in paper bags and transported to the laboratory of Centro de Alimentos y Productos Naturales (CAPN), Universidad Mayor de San Simón, Bolivia for nutritional analysis.

Water content of the plant material (which will be referred as moisture in this study), was determined in two technical replicates according to the standard air oven method 14.004 (William, 1984). Once the samples were dried and ground to a fine powder, nitrogen content was measured in two technical replicates using the Kjeldahl method 14.026 (William, 1984) and the organic elemental analyzer (Thermo Scientific Flash 2000) where the protein concentration was estimated as: % Protein = % Nitrogen × 6.25. For the determination of ash and cellulose, method 14.006 and Kurschner method were used, respectively (William, 1984).

Mineral elements, Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Si and Zn were analyzed in two replicates using Inductively Coupled Plasma Optic Emission Spectrometry (ICP-OES + SS028311; Perkin-Elmer, OPTIMA 3000 DV) at the ICP laboratory of AB Lennart Mansson International, Helsingborg, Sweden. The content of each element was calculated as absolute content in mg kg^{-1}.

Statistical analysis

Principal component analysis (PCA) was used to verify the reproducibility of the two replicates per accession and the use of their average values for all variables allowed us to characterize the variation among the accessions studied using aggregated data. In addition, clustering based on the unweighted pair group method with arithmetic mean (UPGMA) was used to examine the grouping of the accessions from Bolivia and the two cultivars from Argentina.

Spearman correlation coefficients were calculated between all variables. General linear models (*limma* Package, software R) were used to compare accessions by their region of origin in terms of the multivariate matrix of nutrient content and mineral data at the $\log_2$ scale. Empirical Bayes and shrinkage methods were used to borrow information across nutrients to make the analysis stable under small sample size scenarios, as described by Smyth (2005).

Conclusion

Even though Bolivian ecotypes did not show results comparable to Argentinian cultivars in terms of nutritional value, the results of this study provide important information about the nutritive quality of *Festuca* ecotypes from Bolivia for their further use in breeding for the highlands. It is important to highlight that protein content in accessions 29, 10, 21 and 32 were similar to Festulolium and higher than *Festuca arundinacea*. Additionally, they also showed high Ca and Mg content. On the other hand, accessions 38, 29, 23 and 21 showed high P content. Hence, these accessions could be good candidates for genetic improvement of these traits. Accession 27, which was identified as *Festuca orthophylla* had low protein, ash and micronutrients. Considering the fact that this species is widely distributed in the Bolivian highlands and that it is preferred by llamas, further research needs to be conducted on its genetic resources and those with increased nutritional values need to be incorporated in a breeding program for further improvement.

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