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Diet Composition of Two Sheep Breeds Grazing in Chiloe Archipelago, Chile

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Abstract: Chilota and Suffolk Down are the main sheep breeds used for productive purposes in Chiloe Archipelago. Compared to Suffolk Down, Chilota shows a better performance in the harsh environment of the islands as a result of its adaptative process since the introduction 500 years ago. Differences in diet selection between breeds have been described before but an accurate tool to quantify between breed differences in diet selection is needed, the n-alkane technique in combination with microhistological procedures was used. The results show that there are differences in forage selection between Chilota and Suffolk Down adult sheep grazing in naturalized grasslands of Chiloe Archipelago and that the n-alkane method in combination with microhistology can be successfully used to estimate diet selection in different sheep breeds.

Key words: Sheep, diet composition, n-alkanes, microhistology, Chilota sheep breed

INTRODUCTION

Chilota and Suffolk Down are the most used sheep breeds in Chiloe Archipelago (Southern Chile). Chilota is a local breed derived from Iberic sheep populations (De la Barra *et al*., 2012a) which has been submitted for 500 years to the harsh agroecological conditions of the Archipelago (De la Barra *et al*., 2010) undergoing a co-evolutive and adaptative process to this environment. Suffolk Down sheep breed has been recently introduced to the islands (Mujica *et al*., 2012). There are evidences of a better performance of Chilota breed in Chiloe compared to other breeds. Chilota have a high maternal ability given its high milk production (Martinez *et al*., 2011) which allow to produce a finished lamb in shorter times (De la Barra *et al*., 2012a, b). It has been also detected some degree of hardiness related toresistance to parasitism, food damages and food shortages (Martinez *et al*., 2012) when Chilota is compared to Suffolk Down. Variation in diet selection between sheep breeds has been previously documented (Brand, 2000; McCloskey and McAdam, 2010; Wilkes *et al*., 2012). Grazing sheep show dietary preferences consuming a diet that varies in terms of plant species from the available forage (Mayes and Dove, 2000; Cosgrove and Edwards, 2007). This is a relevant fact in extensive production, since performance of grazing sheep is mainly determined by the nutrient intake. The most widespread pastures in Chiloe Archipelago are the naturalized grasslands (Ruiz, 1996) which contain grasses, legume and shrub species showing higly variable nutritive values. Since, naturalized grasslands are the only available source of food for most of free-ranging sheep in the Chiloe Archipelago, the diet consumed by grazing and browsing sheep in this area will be heterogeneous in terms of plant species and plant parts consumed. The diet of Chilota and Suffolk Down adult sheep grazing on naturalized grasslands on the Chiloe Archipelago could show between-breed variability in terms of diet composition owing to their different degree of agroecological adaptation to the agroecological conditions but despite one study carried out in lambs (Gallardo *et al*., 2014) to date there are no data regarding to diet composition in these two sheep breeds grazing in a typical production system in Chiloe Archipelago.

Gathering of these data can be carried out if the botanical composition of the consumed diet is estimated with relative precision. The use of n-alkanes has been spread around the world as a non-invasive, animal-based analytic tool for estimating diet composition in small ruminants in different production systems (Mayes *et al*., 1986; Valiente *et al*., 2003; Dove and Mayes, 2005; Keli *et al*., 2008) and there are evidences abut tha fact that the combination of this technique with microhistology can improve the reliability of the results. Given that quantifying the between-breed differences in the nutritional adaptative response of Chilota and Suffolk Down breeds by means of an an accurate tool is needed, the aim of this study was to estimate diet composition of two sheep breeds grazing on a typical vegetation community of Chiloe Archipelago using n-alcane markers in combination with microhistological procedures.

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MATERIALS AND METHODS

The experiment was conducted at INIA Butalcura Research Center (Chiloe Archipelago, Chile) (latitude 42°15'S, longitude 73°39'W) during November and December 2012.

Animals and diets: Twenty adult healthy non-pregnant, non-lactating sheep (ten Chilota and ten Suffolk Down) with an initial live weight of 50.0±5.23 kg (Chilota) and 53.5±5.26 kg (Suffolk Down) all in body condition score of 3 were used for the study. One paddock of 2, 5 ha of naturalized grassland was used for the study. Samples for botanical composition of the grassland were taken by means of 0.5×1 m^2 randomly allocated and the whole sample was separated on its different plant species. A sample of each vegetal species found on the paddock was taken and frozen (-20°) for further analysis.

A 19 days confinement period was performed in order to estimate the individual recovery of n-alkanes. During this period, sheep were daily offered 10 kg of freshly cut grass from the experimental paddock (5 kg at 07:00 h and 5 kg at 19:00 h). The first 7 days were used for the habituation of sheep to the experimental conditions and diets and the last 12 days were used for sample collection. Inmediatly after the confinement period, a 12 days grazing period was carried out. During this period, sheep were allowed to free ranging on the same experimental paddock with no access to the previously cutted area. The first 7 days were used for the habituation of sheep to the experimental conditions and diets and sample collection was carried out on the last 5 days.

Animals were handled according to criteria from the European Union for care and use of laboratory animals in research and the experimental protocol was approved by the Ethical Commitee for Animal research of INIA and Fondecyt (Chile).

Experimental: During the confinement period, sheep were housed in individual pens (2 m length×1.2 m width) with slotted floor while in the grazing period sheep were allowed to free-ranging. During the last 12 days of confinement period, the amount of feed and refusals were daily recorded and feed and refusals samples when occurred were taken, frozen at -20°C and pooled for the whole period. After thawing for 24 h at 5°C, a representative sample of the pool was dried at 60°C for 72 h in a forced-air oven for DM determination, ground through a 1 mm screen and analyzed for n-alkane analysis in the feed quality laboratory at INIA Remehue (Osorno, Chile). The last five days of confinement at 08:00 h, total fecal production was collected by hand, directly from the non-slotted floor, weighed, hand a subsample (5% on weight basis) was taken, pooled by sheep and stored frozen at -20°C until the end of the collection period. After thawing for 24 h at 5°C, pooled samples were dried at 60°C for 72 h in a forced-air oven for DM determination, ground through a 1 mm screen and analyzed for n-alkanes. During the grazing period, sheep were allowed to free-ranging. From days 8-12, a spot sample of feces was taken daily at 8:00 h, pooled by sheep and kept frozen at -20°C for further n-alkane and microhystological analysis.

Analytical procedures

n-alkane methodology: n-alkanes in samples of grass, refusals and feces were extracted following the technique proposed by Mayes *et al.* (1986) with the modifications described by Keli *et al.* (2008) and those concerning to laboratorial conditions in the framework. Gas chromatography analyses of the purified extracts were carried out on a Shimadzu 2010 Plus Gas Chromatograph fitted with an automatic injector and a flame ionization detector, a capillary column RTX-1 (Restek) (30 m lenght×0.53 mm i.d., 1.50 μm film thickness) and a SGE syringe (10 and 4 μL inyection) with He as carrier and make up gas to the detector. Detector response factors for individual n-alkanes were determined by injecting onto the chromatograph a standard n-alkane mixture (C_{21}-C_{36} inclusive) after every eight sample extracts. Alkanes C_{22} and C_{34} were used as internal standards. Oven conditions were 230°C×0.2 min; 300°C×18 min at a speed of 6°C min^{-1}. Inyector and detector temperatures were 233°C and 350°C, respectively. Air and H flows were 420 and 42 mL min^{-1}, respectively. Make up was 30 mL He min^{-1} and column flow was 10 mL min^{-1} with a split of 1 mL min^{-1}. Heptane was used in the washing vials, thrice before and after simple inyection.

Microhystological analysis: A bank of micro-histological images of the different species identified pictures was made for further identification of cuticular structures on sheep's feces. Plant species and feces samples were analyzed following the protocol.

Calculation procedures and analysis: The fecal recovery of n-alkanes may be affected by diet by individual variability (Hendricksen *et al.*, 2002). This fact is relevant when the n-alkane technique is to be used in grazing animals in which the estimation of individual recoveries is difficult in this sense, values obtained from confined animals offer a better performance of the technique (Dove and Mayes, 1996). The individual recovery of each n-alkanes for each sheep was calculated from the confinement period data by means of multiplying the fecal concentration by a recovery correction factor, calculated as the ratio between the amount excreted in feces and the amount ingested which is the sume of the amount ingested and dosified as follows:

$$\text{Recovery} = \frac{(C_{Hi} \times FP)}{(Di + (DMI \times C_{li}))}$$

Where:

C_{Hi} = Fecal concentration of alkane i
FP = DM Fecal Prodution
Di = External dose of alkane i
DMI = Dry Matter Intake
C_{li} = Concentration of alkane i on the consumed diet

Discriminant analysis was performed with SPSS Statistical Package (V.22) in order to obtain the most discriminant n-alkane patterns for each sheep and as a result, only the most discriminant n-alkanes were used for calculations of diet composition. It was estimated by minimisation of the sum of squared discrepancies between the measured fecal proportions of individual n-alkanes (recovery-corrected and expressed relative to the total fecal n-alkanes) in pooled samples from the grazing period and kane proportions (of the total n-alkanes) calculated from the most discriminant alkane profiles of dietary components (previously detected by fecal microhistology) as follows:

$$\sum [R\text{-}E]^2_{alk:1...n} = \sum \left[\frac{H_i}{H_t} - \frac{xA_i + yB_i + zC_i}{xA_t + yB_t + zC_t} \right]^2_{alk:1...n}$$

Where:

x, y and z = Proportions of the componentes A, B and C in the diet
H_i, A_i, B_i and C_i = Concentrations of the alkane i in the feces (recovery-corrected) and components A, B and C
H_t, A_t, B_t and C_t = Total n-alkane concentrations

The Excel Solver Method has been used in numerous studies to estimate diet composition of grazing sheep, validating the results in confinement trials (Dove and Moore, 1995; Valiente *et al*., 2003; Keli *et al*., 2008). Found that it is better to use only the most discriminating alkanes in the calculations instead of all available, corroborating the results by Valiente *et al*. (2003) and Keli *et al*. (2008). Therefore, the Solver routine of Microsoft© Excel without negative restrictions was used for above calculations.

Data were submitted to a Shapiro-Wilk normality test. Afterwards, two statistical treatments were defined. Mean percentages of each species in the diet of each breed were analysed by means of a one-way ANOVA. Presence/absence of vegetal species in fecal samples in both breeds was compared to presence/absence obtained with the n-alkane technique by means of the Kolmogorov-Smirnof test. The Microsoft© Excel XLSAT Pro V.2014 atatictical package was used for the analysis.

RESULTS

The naturalized grassland used for this study was composed by grasses (66.06%) and legumes (15.59%) with other dicotyledosn as graminoids (5.67%) or shrubs (1.45%) to a lesser exent. From all the available species, only ten were detected in fecal microhistology samples (Table 1). When n-alkane calculations were performed, *Juncus procerus* and *Berberis microphylla*, previously tagged as consumed in feces were not detected at all. Nevertheless, signifficant differences between methods of estimation were only found for one species (*Agrostis capillaris*) both in Chilota (p = 0.007) and Suffolk Down (p = 0.001) breeds. This species was found in almost all fecal samples but n-alkane estimations only showed percentages of this species in four of the twenty sheep's

Table 1: Presence of plant species in sheep's diet detected by means of fecal microhistology and percentage of each species in the diet of sheep estimated by the n-alkane technique

	Chilota											Suffolk Down										
Species	1	2	3	4	5	6	7	8	9	10	p-values	11	12	13	14	15	16	17	18	19	20	p-values
T. repens	D	D	D	D	D	D	D	D	D	D	1.000	D	D	D	D	D	D	D	D	D	D	1.000
%	0.18	0.44	0.59	0.1	0.33	0.53	0.26	0.59	0.37	0.17		0	0.53	0.45	0.46	0	0.21	0.36	0.49	0.46	0.47	
A. odoratum	D	ND	D	D	D	D	D	D	D	D	**0.007**	D	D	D	D	D	D	D	D	D	0.47	**0.0012**
%	0	-	0	0	0	0.37	0	0	0.25	0		0	0	0	0	0	0.17	0	0.21	0	D	
A. capillaris	D	D	ND	D	D	ND	D	D	ND	D	0.111	D	D	D	D	D	D	D	D	D	0	0.3129
%	0.42	0.11	-	0	0	-	0	0	-	0		0.68	0	0	0.3	0.57	0	0.48	0	0.39	D	
H. Lanatus	ND	D	D	ND	ND	D	D	D	D	D	0.675	D	D	D	D	D	D	D	D	D	0.2	0.3129
%	-	0	0.21	-	-	0	0.41	0	0.15	0.35		0.32	0.47	0.55	0.12	0.43	0.62	0.08	0.3	0.08	D	
L. corniculatus	ND	D	ND	D	ND	D	D	ND	ND	D	1.000	ND	D	ND	D	ND	ND	D	ND	D	0.17	1.000
%	-	0.45	-	0.34	-	0.1	0.33	-	-	0.38		-	0	-	0.12	-	-	0.08	-	0.08	D	
L. campestris	D	ND	ND	ND	D	ND	ND	D	D	ND	1.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.17	1.000
%	0.4	-	-	-	0.67	-	-	0.41	0.23	-		-	-	-	-	-	-	-	-	-	ND	
C. holosteoides	D	ND	ND	D	ND	ND	ND	ND	ND	ND	1.000	ND	ND	D	ND	ND	ND	ND	ND	ND	ND	1.000
%	0	-	-	0.07	-	-	-	-	-	-		-	-	0	-	-	-	-	-	-	-	
T. officinale	ND	ND	D	D	ND	ND	ND	ND	ND	D	1.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.000
%			0.2	0.49						0.1												
J. procerus	D	ND	D	ND	ND	D	ND	ND	D	ND	0.313	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.000
%	0	-	0	-	-	0	-	-	0	-		-	-	-	-	-	-	-	-	-	-	
B. microphylla	ND	ND	D	ND	ND	D	ND	ND	D	D	0.313	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.000
%	-	-	0	-	0	-	-	-	0	0		-	-	-	-	-	-	-	-	-	-	

Bolded p-values indicate significative differences between methods. D: Detected in feces; ND: Not Detected in feces

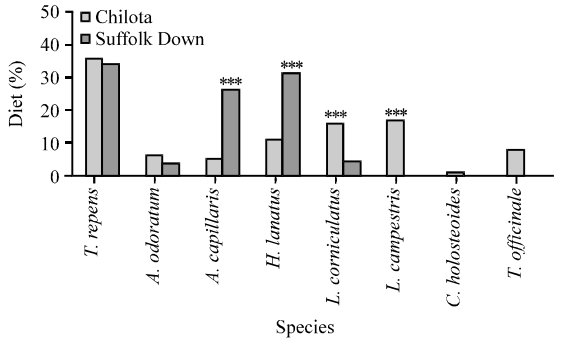

Fig. 1: Paired comparisons for mean percentage of several plant species in CH and SD breed's diet (Fisher LSD). ***p<0.001; Chilota (35.61, 6.20, 5.31, 11.20, 16.07, 17.15, 0.69, 7.87) and Suffolk Down (34.30, 3.84, 26.19, 31.33, 4.35, 0.00, 0.00, 0.00)

diets. Chilota sheep consumed all the species detected in feces in different proportions whereas *Luzula campestris*, *Cerastium holostehoides* and *Taraxacum officinale* appeared in faceces but were not consumed by Suffolk Down.

Trifolium repens was the most consumed species in both breeds (Fig. 1). Suffolk Down consumed higher ($p<0.001$) average amounts of *Agrostis capillaris* and *Holcus lanatus* than Chilota did (0.26 vs. 0.05% and 0.31 vs. 0.11% of total diet, respectively) whereas Chilota sheep consumed significantly ($p<0.001$) higher average amounts of *Lotus corniculatus* and *Luzula campestris* than Suffolk Down (0.15 vs. 0.04% and 0.17 vs. 0%, respectively).

DISCUSSION

The present study was thus aimed at the possibility of appliying the microhistological and n-alkane techniques in order to identify differences in diet composition between two sheep breeds grazing in naturalized grasslands in Chiloe Archipelago, Chile. In order to obtain good n-alkane based estimations of diet composition, it is essential to correctly identify the plant species actually consumed by sheep since, the inclusion in the calculations of not consumed species is a source of error (Dove and Mayes, 2005). In this sense, the combination of fecal microhistological with alkanes can reduce this error. In some cases, the method of n-alkanes did not include dietary plant species that were detected in feces as it has been previously observed. It may be because some of these species were consumed in very small amounts as could be the case of *Berberis microphylla* or *Juncus procerus* and therefore its profile alkanes did not contribute significantly to the total detectable in feces. It is also possible that the microhistological fecal samples for each sheep were not representative of those analyzed for n-alkanes. Furthermore, analytical errors can occur when the n-alkane concentrations are low as was the case in the present experiment in some plant species. A minimum n-alkane concentration of 50 mg kg^{-1} MS has been suggested for the usefulness of the technique (Casson *et al*., 1990; Laredo *et al*., 1991) and this fact can have accounted for part of the observed discrepancies.

The lower consumption of some non-herbaceous species like *Berberis microphylla* or *Juncus procerus* can be attributed to the fact that in December, grass and legume production is very high in Chiloe it has be taken into account that in conditions of high density of forage, ruminants decrease their consumption of non-herbal species (Hessle *et al*., 2008). Similar results have been previously presented by Seleccionada (1998) showing that in Spring, Merino and Damara breeds have lower brosing activities since the availability of grass were high.

It has been previously demonstrated that that the n-alkane technique gives gives good stimations of botanical composition of plant mixtures (Dove, 1992) and there are no reasons for a different assumption here. Despite the individual discrepancies observed, results presented in this study show that both methods were similar in their discrimination capacity for almost all species studied.

Regarding to differences in diet composition between breeds, it have been previously documented that these differences in sheep's diet selection actually occurs. Langlands (1966) made some of the first oservations regarding to differences in diet selection depending on breed, age class and previous grazing history. Seleccionada (1998) shown that Merinos ate more grasses whereas Dorper eat more woody plant components. Brand (2000) shown that Dorpers are less-selective grazers compared to Merino-type sheep and they utilise a larger range of different plant species from those available. McCloskey and McAdam (2010) also found significant differences were in the proportions of plant groups consumed by two sheep breeds (Scottish Blackface and Texel x Scottish Blackface sheep) with Scottish Blackface diet containing a greater proportion of heather. Recently, Gallardo *et al*. (2014) showed that Chilota lambs presented longer browsing times compared to Suffolk Down lambs in naturalized grasslands in Chiloe but these fact was not sustained by a higher proportion of shrub fragments in fecal samples. In addition, it has been stated that young animals have

higher relative nutricional requirements and therefore they select a high-quality diet (more grasses) compared to adults (Mobaek *et al*., 2012). These results reinforces the need for more accurate estimation tools for diet composition estimation.

There are evidences that Chilota is more efficient in the use of the foraging resources in absence of supplementation (Martinez *et al*., 2012) it could be thought that this breed has developed skills to consume and take nutritional advantage of low nutritive value species which are common in a naturalized grassland in Chiloe. In addition, the higher preferente for grasses (*A. Capillaris* and *H. lanatus*) shown by Suffolk Down sheep may be due to their higher need of DE since, grass species found in this study contain more DE relative to species selected by Chilota sheep. Similar results were observed by Els (2000) who stated that Dorper were more selective breeds than Karakul and Merino, who found that Dorper and Karakul are more selective breeds than Damara which shows more diverse foraging habits or (Wilkes *et al*., 2012) when comparing Merino and Damara breeds. Furthermore, the higher the selective behavior, the longer the distance that sheep have to walk to obtain the selected diet (Roux, 1992) therefore, longer walks imply higher amounts of energy to meet maintenance energy requirements.

CONCLUSION

These results of this experiment show that there are differences in forage selection between Chilota and Suffolk Down adult sheep grazing in naturalized grasslands of Chiloe Archipelago. The results also suggest that the n-alkane method in combination with microhistology can be successfully used to estimate diet selection in different sheep breeds.

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