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Complementarity between microhistological analysis and PCR-capillary electrophoresis in diet analysis of goats and cattle using faecal samples

J. Pareja [a,*], C. Espunya [b], E. Baraza [c], J. Bartolomé [a]

[a] Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain
[b] Independent researcher, 08290 Cerdanyola del Vallès, Spain
[c] Department of Biology, Universidad de las Islas Baleares, 07122 Palma de Mallorca, Mallorca, Spain

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ABSTRACT

An evaluation is made of the complementarity between two non-invasive techniques, cuticle microhistological analysis (**CMA**) and PCR-capillary electrophoresis (**PCR-CE**) DNA-based analysis, for the determination of herbivore diet composition from faecal samples. Cuticle microhistological analysis is based on the different microanatomical characteristics of the epidermal fragments remaining in the faeces. The PCR-CE technique combines PCR amplification of a *trnL*(UAA) genomic DNA region with amplicon length determination by CE, with this length being characteristic for each species or taxon. A total of 37 fresh stool samples were analyzed, including 16 from feral goats (*Capra hircus*) from the Tramuntana mountain range (Mallorca, Baleares) and 11 from *Bruna dels Pirineus* cattle breed (*Bos taurus*) from the surrounding Montserrat mountain range (Barcelona, Spain). All the animals were in a free grazing Mediterranean pine habitat, dominated by Aleppo pine (*Pinus halepensis*). The results showed that both techniques detected a similar number of plant components in the faeces of goats and cows. In the case of goats, a positive correlation was obtained between the percentage of samples in which a particular taxon is detected by CMA and the percentage of samples in which that taxon is detected by PCR-CE. This correlation was not observed in the case of cows. It is concluded that PCR-CE is a fast and reliable method to detect the different plant components in the faeces of herbivores. However, it cannot be considered as an alternative to CMA, but as a complementary method, since both techniques can detect some taxa that are not detected by the other technique. In addition, CMA detected the presence of the different taxa in a greater number of samples, and at the same time, it enables quantitative data to be obtained for plant diet composition. The species of herbivore also seems to influence the results obtained by PCR-CE, so more studies are required to address this aspect.

Implications

Diet analysis of animals is a relevant aspect in environmental management. In this study, two techniques were compared, the cuticle microhistological analysis and polymerase chain reaction-capillary electrophoresis DNA-based analysis, in feral cows and feral goats in a Mediterranean environment. We found that both techniques had different results in both species, demonstrating a complementary effect of both techniques in the analysis of diet.

Introduction

Many analysis techniques have been used in the past years to quantitatively or qualitatively evaluate the composition of the herbivore diet, the most relevant being: the direct observation of the animal (Shrestha and Wegge, 2006), including the bite or time counting methods (Pinto et al., 2014), near IR reflectance spectroscopy (Coates and Dixon, 2008), quantification of n-alkanes in faeces (Ferreira et al., 2007), isotope stability analysis (Sponheimer et al., 2003; Codron and Brink, 2007), esophageal fistula sampling (Bautista et al., 1996), rumen content sampling (Norbury and Sanson, 1992; Bertolino et al., 2009), microhistological analysis of faeces (Stewart, 1967; Bartolome et al., 1995) and, more recently, molecular analysis based on DNA (Pegard et al., 2009; Soininen et al., 2009). Each method has a number of advantages and limitations when interpreting eating habits (Henley et al., 2001). Among these techniques, cuticle microhistological analysis (**CMA**) is the most used method to study the diet in herbivores, although it requires a considerable training effort (Holechek and Gross, 1982), and there could be errors due to epidermal degradation and the microhistological similarity of some plant epidermis (Henley et al., 2001). For this reason, it is important to carry out a correct processing of the samples and the personnel must first know the microhistological fragments of reference, such as the shape and cell arrangement,

* Corresponding author.
E-mail address: javimev@gmail.com (J. Pareja).

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stomata, trichomes and glands (Chetri, 2006). On the other hand, molecular analyses based on the presence of genomic DNA remnants of ingested plants in faecal samples have been shown to be a useful and rapid tool to determine the components of the herbivore diet (Taberlet et al., 2007; Valentini et al., 2009). Within the molecular techniques, the **PCR-CE** technique (PCR-Capillary Electrophoresis), based on the PCR amplification of a *trnL*(UAA) gene fragment followed by the determination of the length of the amplicon by CE, has been used to qualitatively estimate the presence of a small number of species in a controlled diet for chamois (Espunyes et al., 2019). However, this technique has not yet been used to determine the nature of the plants consumed by herbivores in free ranging situations.

The objectives of this work were to determine if the technique of the PCR-CE is able to identify the taxa of the diet in field grazing animals and to compare the results obtained by PCR-CE with those obtained by CMA, by evaluating the complementarity between both techniques. For this evaluation, faecal samples of animals that graze in Mediterranean environments were used, such as cows (*Bos taurus*) of the *Bruna dels Pirineus* breed in the surroundings of the Montserrat mountain, Barcelona, and feral goats (*Capra hircus*) on the island of Mallorca.

Material and methods

Study area

This study was conducted with faecal samples of goats (*Capra hircus*) and cows (*Bos taurus*) that were fed in the undergrowth of Mediterranean pine forests. Samples of goats were collected in the Tramuntana mountain range, on the island of Mallorca (39°48′28″N2°47′37″E), where there is a population of feral animals (Vives and Baraza, 2010). The mean annual temperature and precipitation in the mountains are 18 °C and 650 mm, respectively. The cows were located around the Montserrat Mountain Natural Park in the province of Barcelona (41°40′28"N1°46′33″E), where the mean annual temperature is of 13 °C, and rainfall of 610 mm. In this case, the animals spend the winter months and part of the spring in the area, then they transhumance to the Pyrenees. The vegetation of both areas is dominated by trees of Aleppo pine (*Pinus halepensis* M.) with a Mediterranean scrub undergrowth with abundance of *Erica multiflora* L., *Pistacia lentiscus* L. and *Rosmarinus officinalis* L. The herbaceous stratum is dominated by the poaceae of the genus *Brachypodium* and, in the case of Mallorca, also by *Ampelodesmos mauritanica* (Poiret) T. Durand et Schinz.

Sample collection

Faecal samples were collected between the winter of 2016 and spring of 2017. Goat faeces were obtained directly from the rectum, from the animals killed during the culls made by the local administration. Cow faeces were collected right after their defecation. In order to prepare a reference collection for microhistological and molecular analysis, 100 g of leaves of the most abundant plant species in each study area was collected. A total of 27 faecal samples were collected, 11 from cows and 16 from feral goats. The samples were labelled according to the ear tag number of each cow. To prevent DNA degradation, samples were kept refrigerated during the transport from the field to the laboratory and then stored at −20 °C until analysis.

Cuticle microhistological analysis

To determine the botanical composition of the diet in faeces by CMA, the procedure used by Stewart (1967) was followed. The faecal samples were crushed in a mortar, and then 0.5 g of sample was placed in a test tube, where the content was digested with 3 ml of 65% concentrated nitric acid in a thermal bath at 80 °C for 2 min. This content was diluted in 250 ml of distilled water and successively filtered through two sieves of 1 and 0.125 mm pore size. The material obtained in the second sieve was homogeneously distributed onto three slides with 50% glycerine and was sealed with DPX resin (Herter Instruments, Barcelona, Spain). To obtain the reference epidermis collection, the leaf samples were subjected to a scalpel scrape until the epidermis of both faces (abaxial and adaxial) was obtained. This epidermis was mounted onto slides with glycerine. The most recalcitrant species, in which epidermal tissue could not be obtained by this method, were subjected to acid digestion, following the same protocol as in the case of faecal samples. Preparations were observed under an optical microscope (Motic BA210, MoticEurope SLU, Barcelona, Spain) at 100 and 400 magnifications, equipped with a Moticam 2300 camera. By using the Motic Images Plus 2.0 software, images of cellular forms, trichomes, and stomata were obtained from each plant sample, as a reference shown in Supplementary Figure S1. Subsequently, the faecal samples were then observed under the same microscope, making linear transects until a total of 200 epidermal fragments per sample were identified. The microhistological analysis was performed at the animal production laboratory of the Veterinary Faculty of the Universitat Autònoma de Barcelona (**UAB**). This laboratory is part of the ASFAC-LAB quality programme consisting of an exercise of intercomparison of laboratories performing analysis of feed and raw materials for animal feed (http://www.asfac-lab.com/en/).

Molecular analysis (PCR-capillary electrophoresis)

Genomic DNA (gDNA) from the most abundant plant species in each area studied and from faeces samples was extracted with the DNeasy Plant Mini Kit and the QIAamp DNA faeces Kit (QIAGEN, Hilden, Germany), respectively, following manufacturer's instructions. Genomic DNA of plants and faeces was diluted in 100 μl of Milli Q water and stored at −20 °C. DNA concentration was measured with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, USA), and the quality of the gDNA was analyzed on a 0.8% agarose gel electrophoresis in 1 × TAE buffer (40 mM Tris pH 7.6, 20 mM acetic acid, 1 mM EDTA). The sequences corresponding to the chloroplast gene *trnL*(UAA) were amplified by PCR from the oligonucleotides *trnL*-G (5'gggcaatcctgagccaaatc3') and *trnL*-D (5'ggggatagagggacttgaac3') (Taberlet et al., 2007), using plant and faeces gDNA as a template. The PCRs contained 1 × MyTaq reaction buffer (Bioline Reagents Ltd., London, United Kingdom), oligonucleotides at a concentration of 0.4 μM each (Stab Vida, Caparica, Portugal), 1 U MyTaq DNA polymerase and 20 ng of gDNA, in a final reaction volume of 20 μl. The *trnL*-D oligonucleotide was labelled with 6-FAM (carboxyfluorescein) fluorochrome to allow fluorescent detection by CE. The amplification reaction comprised different steps: initial denaturation of the gDNA at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 15 s and elongation at 72 °C for 45 s, ending with a final elongation step at 72 °C for 3 min. The amplifications were performed on a MJ Research PTC-100 Thermal Cycler (MJ Research Inc., Mississauga, Canada). The amplification results were visualized on a 1.5% agarose gel electrophoresis in 1× TAE.

The length of the amplicons was determined by CE using a Genetic Analyzer 3130xl system (Applied Biosystems, Waltham, USA), using the LizS600 marker as standard length. The results were obtained as electropherograms where the amplified DNA fragment appeared as a fluorescence peak in the corresponding length. The electropherograms were analyzed using the Peak Scanner version 2.0 software (Applied Biosystems, Waltham, USA). The components of the diet were identified by comparing the lengths of the different amplicons obtained from the analysis of the faeces of cows and goats, with the reference collection established from the taxa considered in Table 1.

The molecular analysis was performed at the Genomics and Bioinformatics service of the UAB, which has a quality management system based on the ISO9001: 2015 standard and complies with the UAB code of good practice in research (https://sct.uab.cat/genomica-bioinformatica/en).

Table 1
Length of the amplicons, in base pairs, of the plant species used as a reference to know the diet in cows and feral goats using the PCR-Capillary electrophoresis technique.

Species	Amplicon length (bp)
Aphyllanthes monspeliensis	647
Carex sp.	598
Smilax aspera	540
Lolium perenne	524
Dorycnium sp.	520
Brachypodium sp.	515
Osyris alba	504
Ampelodesmos mauritanica	496
Cneorum tricoccon	495
Rhamnus alaternus	494
Arbutus unedo	492
Cistus sp.	489
Rubia peregrina	485
Chamaerops humilis	484
Psoralea bituminosa	483
Buxus balearica/Genista scorpius	479
Erica sp.	477
Bupleurum rigidum	472
Rosmarinus officinalis	461
Rubus ulmifolius	460
Genista lucida/Quercus sp./*Sanguisorba minor*	458
Pinus halepensis	456
Globularia alypum	445
Olea europaea/Phillyrea angustifolia	444
Pistacia lentiscus	425
Hieracium pilosella	418
Ononis sp.	395
Juniperus oxycedrus	265
Ephedra fragilis	260

bp = Base pairs.

Statistical data processing

The analyses were performed separately for each animal species. For CMA analyses, the abundance of each taxon in the diet was determined as a percentage of epidermal fragments identified in the faeces. A generalized linear mixed model of binomial distribution was performed in order to determine the relationship between % of epidermal fragments identify in each sample and the probability of being detected through PCR-CE analysis. The percentage of fragments quantified was considered as a fixed predictive variable, the probability of being detected by PCR-CE as a response variable, and each species as a random factor (B (nt/x) = $\alpha + \beta 1$ percentage of epidermal + γ specie + ε). The glmer function from the lme4 (Version 1.1–8) package of R version 3.6.2 (Bates et al., 2015) was used.

Because of the lack of normality, a nonparametric Spearman correlation was used to analyze the relationship between the percentage of samples which detected each taxon by PCR-CE and the percentage of sample which was detected by CMA. In addition, the overall composition of the analyzed diet was compared with each of the techniques using non-metric multidimensional scaling. In this analysis, each sample was considered separately and the distance matrix was calculated based on the binomial distance of Jaccard. The monoMDS function from the vegan package (Version 2.5-2) of R version 3.6.2 (Oksanen et al., 2018) was used. The script used for the statistical analysis is available as Supplementary Material S1.

Results

By means of the molecular technique of PCR-CE applied to the 39 selected taxa, it was possible to amplify a fragment corresponding to the gene *trnL*(UAA) in all of them, with lengths varying between 647 and 260 base pairs (**bp**) (Table 1). These data were used as a reference, shown in Supplementary Figure S2, for comparison with the size of amplicons detected in feces.

Some species that belong to the same genus had the same amplicon length, and therefore, they were grouped into genus: *Dorycnium pentaphyllum* and *Dorycnium hirsutum* (*Dorycnium* sp.); *Brachypodium retusum* and *Brachypodium phoenicoides* (*Brachypodium* sp.); *Cistus albidus*, *Cistus monspeliensis* and *Cistus salviifolius* (*Cistus* sp.); *Erica multiflora* and *Erica arborea* (*Erica* sp.); *Quercus coccifera* and *Quercus ilex* (*Quercus* sp). Taxa in which the species could not be determined at the time of collection were also considered at the genus level, as was the case of *Carex* sp. and *Ononis* sp. Finally, species of a different genus or family but with identical amplicons were considered in the same group (*Buxus balearica* and *Genista scorpius*; *Genista lucida*, *Quercus* sp. and *Sanguisorba minor*; *Olea europaea* and *Phillyrea angustifolia*). The mean number of taxa identified per sample was higher when CMA was employed in both species, cows and goats (Table 2).

Table 3 shows the percentages of epidermal fragments identified in the faeces of cows together with the percentages of individuals in which a specific taxon analyzed by CMA and/or PCR-CE appears. Cuticle microhistological analysis succeeded in identifying 19 taxa, while 20 were identified by PCR-CE. Some taxa were only detected with only one of the techniques. These were *Aphyllanthes monspeliensis*, *Carex* sp. and *Rosmarinus officinalis* by CMA and *Ephedra fragilis*, *Genista scorpius*, *Hieracium pilosella* and *Osyris alba* by PCR-CE. Eight taxa appeared in a greater proportion using CMA technique, and six using PCR-CE. The dominant taxon in the diet of cows was *Brachypodium* sp., which was more than a quarter of the diet. The PCR-CE technique forced to group different species that has the same number of amplicon bps, as is the case of *Genista lucida*, *Quercus* sp. and *Sanguisorba minor*, and in the case of *Olea europaea* and *Phillyrea angustifolia*. In both cases, CMA only detected fragments of one of the taxa, specifically *Quercus* sp. and *Olea europaea*. In addition, considering that *Genista lucida* is not in the Montserrat study area, this species would no longer be part of the first group, in the case of cows.

Table 4 shows the percentages of epidermal fragments identified in the faeces of goats, together with the percentages of individuals in which a specific taxon analyzed by CMA and/or PCR-CE appears. In goat faeces, a total of 17 taxa were identified by CMA and 15 by PCR-CE. *Smilax aspera* was only detected by CMA, and *Genista lucida* only by PCR-CE. Most taxa appeared in a greater proportion of individuals using CMA, and only *Arbutus unedo* using PCR-CE. As in the case of cows, from the group formed by *Genista lucida*, *Quercus* sp. and *Sanguisorba minor* by PCR-CE, only *Quercus* sp., were identified by CMA. On the contrary, from the group of *Olea europaea* and *Phillyrea angustifolia*, CMA distinguished fragments of both.

No significant correlation was found between the percentage of samples in which a particular taxon is detected by CMA with the percentage of samples that the taxon is detected by PCR-CE no for cows ($\rho = 0.17$; $P = 0.44$ Spearman correlation test) neither for goats ($\rho = 0.43$; $P = 0.06$ Spearman correlation test). Similarly, the percentage of fragments of a taxon detected by CMA does not appear to have a significant effect on the probability that taxon is detected by PCR-CE in cow faeces ($P = 0.4$ GLMM-binomial, Fig. 1). But contrary, in the case of goats, this effect is significant ($P = 0.006$ GLMM-binomial, Fig. 1).

Table 2
Number of taxa identified per faecal sample in cows and feral goats (maximum, minimum and mean) by each technique (CMA and PCR-CE).

Taxon/sample	Cows		Feral goats	
	CMA	PCR-CE	CMA	PCR-CE
Maximum	16	13	14	8
Minimum	11	3	10	2
Mean	13	9	12	6
SD	2	4	1	2

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis.

Table 3
Percentage (%) of epidermal fragments in cow faeces determined by CMA and percentages of individuals in which a particular taxon appears by CMA and PCR-CE techniques.

Taxon	Faecal epidermal fragments (CMA)	Individuals with the presence of the taxon (CMA)	Individuals with the presence of the taxon (PCR-CE)
Aphyllanthes monspeliensis[1]	5.5	100.0	0.0
Arbutus unedo	1.7	81.8	54.5
Brachypodium sp.	27.6	100.0	63.6
Bupleurum rigidum	0.6	9.1	36.4
Carex sp. [1]	6.1	90.9	0.0
Cistus sp.	0.7	63.6	54.5
Dorycnium sp.	4.1	100.0	27.3
Ephedra fragilis[2]	0.0	0.0	18.2
Erica sp.	4.8	90.9	72.7
Sanguisorba minor	0.0	0.0	(81.8)
Quercus sp.	3.5	72.7	
Genista scorpius[2]	0.0	0.0	9.1
Globularia alypum	0.3	18.2	18.2
Hieracium pilosella[2]	0.0	0.0	9.1
Juniperus oxycedrus	1.3	81.8	72.7
Olea europaea	6.5	100.0	(90.9)
Phillyrea angustifolia	0.0	0.0	
Ononis sp.	0.05	9.1	27.3
Osyris alba[2]	0.0	0.0	9.1
Pinus halepensis	6.0	100.0	54.5
Pistacia lentiscus	4.1	90.9	100.0
Rhamnus alaternus	1.5	54.5	54.5
Rosmarinus officinalis[1]	9.0	100.0	0.0
Rubus ulmifolius	3.7	90.9	27.3
Smilax aspera	1.3	54.5	72.7
Other taxa	11.65	100.0	–

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis.
[1] Taxa detected only by CMA.
[2] Taxa detected only by PCR-CE.

Considering the overall diet composition of each sample in terms of the presence/absence of each taxon, the diets of both cows and goats are more similar to each other using the CMA method than using the PCR-CE, as shown by the results of the non-metric multidimensional scaling analysis (Fig. 2). In both cases, the stress function was minimal, 0.05 in the case of cows, and 0.1 in the case of goats, ensuring that the two-dimensional representation adequately collects the multidimensional information of all taxa.

Discussion

The results obtained in this work show that the two analysis techniques, CMA and PCR-CE, are able to detect a similar number of plant components in the faeces of cows and goats. Similar results have been obtained recently in the study of the chamois diet (*Rupicapra rupicapra*) (Espunyes et al., 2019), although in this case, with controlled diets and using two amplicons in the molecular analysis. The use of a single amplicon in the PCR-CE technique simplifies the method. Our results validate the PCR-CE method previously developed showing that it can also be applied to studies of diet composition of field grazing herbivores. Thus, the PCR-CE analysis would be a good alternative to CMA, since the latter requires a long training process (Holechek and Gross, 1982), and can be affected by the subjectivity of the observer and the differential digestibility of each plant species (Leslie et al., 1983; Bartolome et al., 1995). For this reason, molecular analysis to determine the composition of the diet is becoming widespreadly used, although it is not exempt

Table 4
Percentage (%) of epidermal fragments in goat faeces determined by CMA and percentages of individuals in which a particular taxon appears by CMA or by PCR-CE techniques.

Taxon	Faecal epidermal fragments (CMA)	Individuals with the presence of the taxon (CMA)	Individuals with the presence of the taxon (PCR-CE)
Ampelodesmos mauritanica	12.2	100.0	25.0
Cneorum tricoccon	2.1	62.5	6.3
Arbutus unedo	0.5	25.0	68.8
Brachypodium sp.	9.3	100.0	31.3
Chamaerops humilis	6.1	87.5	31.3
Cistus sp.	19.2	93.8	93.8
Ephedra fragilis	1.0	50.0	6.3
Erica sp.	4.6	93.8	56.3
Genista lucida	0.0	0.0	(43.8)
Quercus sp.	2.1	62.5	
Sanguisorba minor	0.0	0.0	
Globularia alypum	1.2	62.5	12.5
Juniperus oxycedrus	1.0	56.3	6.3
Olea europaea	8.7	100.0	(68.8)
Phillyrea angustifolia	0.9	43.8	
Pinus halepensis	3.7	93.8	6.3
Pistacia lentiscus	5.7	93.8	93.8
Rosmarinus officinalis	5.1	100.0	18.8
Smilax aspera[1]	1.8	56.3	0.0
Others	14.8	100.0	–

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis.
[1] Taxa detected only by CMA.

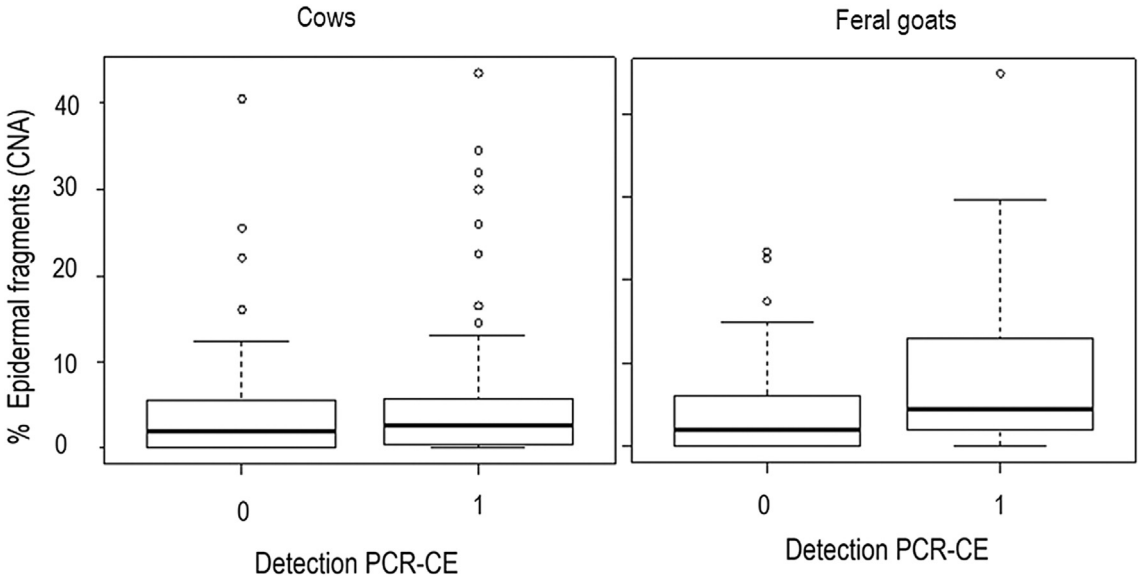

Fig. 1. Box diagram showing the median (thick line) and distribution of the percentage of fragments found in faecal samples of cows and goats by Cuticle microhistological analysis (CMA) for taxa not detected (0) and detected (1) by PCR-Capillary electrophoresis (PCR-CE).

from limitations, such as their extreme dependence on the degradation of the gDNA caused by the animal's own digestion or by environmental factors (Deagle et al., 2006; Espunyes et al., 2019). However, the results obtained here suggest that both techniques can be complemented rather than substituted. This is based on the fact that some species are only detected by one of the two methods.

In this work, differences between individuals have also been detected when both techniques are used. Thus, in the case of cows, no correlation was observed between techniques, indicating that if a taxon appears in many individuals applying one technique, the same does not have to happen when applying the other. In contrast, a positive correlation was observed in goats, which suggested that both techniques will detect a greater or lesser percentage of individuals with a given taxon. In addition, the fact that taxa with higher percentages in the average diet of goats appear in more individuals, and that this does not occur in the case of cows, reinforces the idea that the results of both techniques vary depending on the animal species. This difference is difficult to explain and may be based on the particularities of each digestive tract (Garnick et al., 2018; Sugimoto et al., 2018), or on the amplification bias due to DNA degradation (Valentini et al., 2009). It is also worth noting the fact that, as regards the presence or absence of a certain taxon, the samples of both animals are more similar to each other when the CMA technique is applied. In this sense, CMA would provide more information than the PCR-CE in the analysis of the components of the diet. These results agree with those obtained by Murphree (2012), who, when working with controlled diets, obtained a mean of 89% of correct identifications using CMA, and only 50% using molecular analysis. Also, Valentini et al. (2009) indicated that by using the molecular method, 50% of the ingested taxa could be identified.

Finally, it should be noted that the main difference between the two methods is the possibility of quantifying the components of the diet. Thus, proportions of the different fragments detected in the faeces can be obtained by CMA and, in general, it is assumed that these proportions correspond to the animal's intake. On the other hand, PCR-CE shows

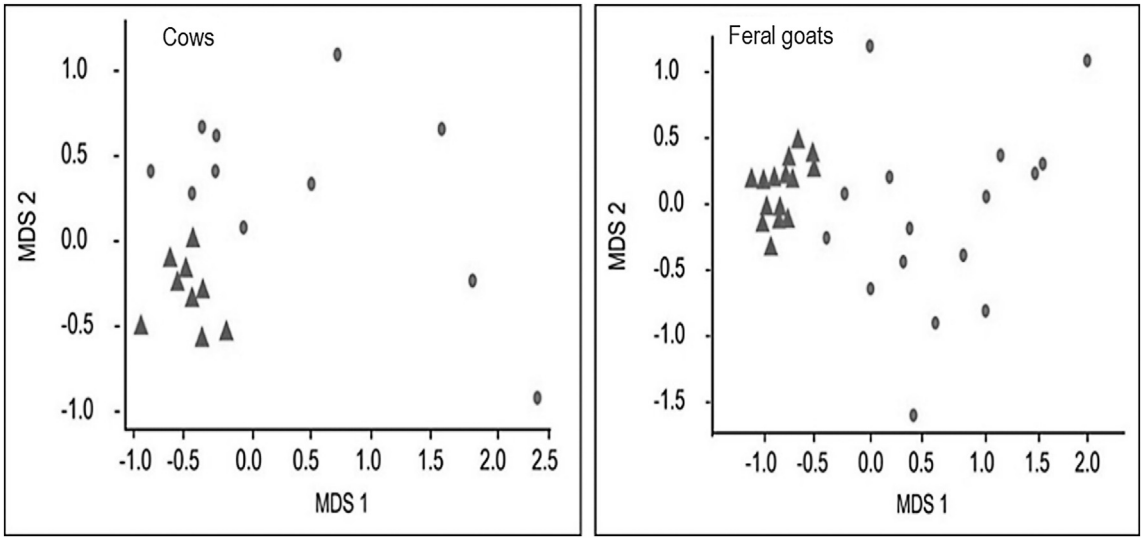

Fig. 2. Non-metric multidimensional scaling (NMDS) representing the distance between faecal samples of cows and goats as a function of the species composition of each sample analyzed by Cuticle microhistological analysis (CMA) in triangle and PCR-Capillary electrophoresis (PCR-CE) in circle, using the Jaccard distance matrix.

severe inaccuracies since the amount of extracted DNA from faeces cannot be related to the amount of food ingested, probably due to the variability in the degradation of the DNA during digestion (Deagle et al., 2009 and 2010; Valentini et al., 2009). Another limitation is the similarity of the amplicon size that can occur between some species. Valentini et al. (2009) recommend, for these "problematic" taxa, to complement with another additional method, such as the CMA, or simply work with larger taxonomical groups, such as genders or families.

It is concluded that the molecular analysis by PCR-CE, based on the length of a single amplicon, is a fast and reliable method to detect the different plant components in the faeces of herbivores. The type of herbivore seems to influence the results obtained with this technique, so more studies are required to address this aspect. There is no doubt that the PCR-CE can complement the CMA by being able to detect some species that leave few epidermal fragments and consequently are not detected by CMA. However, it cannot be considered as an alternative, since CMA detects the presence of the different taxa in a greater number of samples and at the same time is able to obtain percentage data of the diet content.

Supplementary materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2020.100145.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository, but are available upon request only for research use. The model was not deposited in an official repository, but scripts of model are available at Supplementary Material S1.

Author ORCIDs

Javier Pareja: 0000-0001-5759-4215.
Carme Espunya: 0000-0002-4581-5006.
Elena Baraza: 0000-0002-2928-3978.
Jordi Bartolomé: 0000-0002-3784-5248.

Author contributions

Javier Pareja: Validation, Formal analysis, Investigation, Writing – original draft, Visualization and Writing - review & editing. Carme Espunya: Term, Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation and Writing - review & editing. Elena Baraza: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft and Writing - review & editing. Jordi Bartolomé: Term, Conceptualization, Methodology, Investigation, Visualization, Supervision, Project administration and Funding acquisition.

Declaration of interest

None.

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References

Bartolome, J., Franch, J., Gutman, M., Seligman, N.G., 1995. Physical factors that influence fecal analysis estimates of herbivore diets. Journal of Range Management Archives 48, 267–270.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48.

Bautista, J., Medina, J., Mamani, G., 1996. Selectividad y degradabilidad in situ de ingesta de pastizales nativos en alpacas y llamas en puna húmeda. Revista de Investigación de Camélidos Sudamericanos ALLPAK'A 6, 27.

Bertolino, S., Di Montezemolo, N.C., Bassano, B., 2009. Food-niche relationships within a guild of alpine ungulates including an introduced species. Journal of Zoology 277, 63–69.

Chetri, M., 2006. Diet analysis of gaur (*Bos gaurus gaurus*; Smith, 1827) by microhistological analysis of fecal samples in parsa wildlife reserve, Nepal. Our Nature 4, 20–28.

Coates, D.B., Dixon, R.M., 2008. Development of near infrared analysis of faeces to estimate non-grass proportions in diets selected by cattle grazing tropical pastures. Journal of Near Infrared Spectroscopy 16, 471–480.

Codron, D., Brink, J.S., 2007. Trophic ecology of two savanna grazers, blue wildebeest Connochaetes taurinus and black wildebeest Connochaetes gnou. European Journal of Wildlife Research 53, 90–99.

Deagle, B.E., Eveson, J.P., Jarman, S.N., 2006. Quantification of damage in DNA recovered from highly degraded samples - a case study on DNA in faeces. Frontiers in Zoology 3, 11.

Deagle, B.E., Kirkwood, R., Jarman, S.N., 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Molecular Ecology 18, 2022–2038.

Deagle, B.E., Chiaradia, A., McInnes, J., Jarman, S.N., 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? Conservation Genetics 11, 2039–2048.

Espunyes, J., Espunya, C., Chaves, S., Calleja, J.A., Bartolomé, J., Serrano, E., 2019. Comparing the accuracy of PCR-capillary electrophoresis and cuticle microhistological analysis for assessing diet composition in ungulates: a case study with Pyrenean chamois. PLoS One 14, e0216345.

Ferreira, L.M.M., Garcia, U., Rodrigues, M.A.M., Celaya, R., Dias-da-Silva, A., Osoro, K., 2007. Estimation of feed intake and apparent digestibility of equines and cattle grazing on heathland vegetation communities using the n-alkane markers. Livestock Science 110, 46–56.

Garnick, S., Barboza, P.S., Walker, J.W., 2018. Assessment of animal-based methods used for estimating and monitoring rangeland herbivore diet composition. Rangeland Ecology & Management 71, 449–457.

Henley, S.R., Smith, D.G., Raats, J.G., 2001. Evaluation of 3 techniques for determining diet composition. Rangeland Ecology & Management/Journal of Range Management Archives 54, 582–588.

Holechek, J.L., Gross, B., 1982. Training needed for quantifying simulated diets from fragmented range plants. Rangeland Ecology & Management/Journal of Range Management Archives 35, 644–647.

Leslie, D.M., Vavra, M., Starkey, E.E., Slater, R.C., 1983. Correcting for differential digestibility in microhistological analyses involving common coastal forages of the pacific northwest. Journal of Range Management Archives 36, 730–732.

Murphree, J.J., 2012. Evaluation of the efficacy of DNA sequencing and microhistological analysis for determining diet composition in ungulates. MSc thesis. Arizona State University, Tempe, AZ, USA.

Norbury, G.L., Sanson, G.D., 1992. Problems with measuring diet selection of terrestrial, mammalian herbivores. Australian Journal of Ecology 17, 1–7.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2018. Vegan: community ecology package, R package version 2.5-2. Retrieved on 2 February 2019 from https://CRAN.R-project.org/package=vegan.

Pegard, A., Miquel, C., Valentini, A., Coissac, E., Bouvier, F., François, D., Taberlet, P., Engel, E., Pompanon, F., 2009. Universal DNA-based methods for assessing the diet of grazing livestock and wildlife from feces. Journal of Agricultural and Food Chemistry 57, 5700–5706.

Pinto, R.R., Gómez, C.H., Guevara, H.F., Hernández, S.D., Ruiz, S.B., 2014. Preferencia y conducta ingestiva de ovinos alimentados con frutos arboreos tropicales. Revista Científica 24, 158–163.

Shrestha, R., Wegge, P., 2006. Determining the composition of herbivore diets in the trans-Himalayan rangelands: a comparison of field methods. Rangeland Ecology & Management 59, 512–518.

Soininen, E.M., Valentini, A., Coissac, E., Miquel, C., Gielly, L., Brochmann, C., Brysting, A.K., Sønstebø, J.H., Ims, R.A., Yoccoz, N.G., Taberlet, P., 2009. Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. Frontiers in Zoology 6, 16.

Sponheimer, M., Lee-Thorp, J.A., DeRuiter, D.J., Smith, J.M., Van Der Merwe, N.J., Reed, K., Grant, C.C., Ayliffe, L.K., Robinson, T.F., Heidelberger, C., Marcus, W., 2003. Diets of southern African Bovidae. Journal of Mammalogy 84, 471–479.

Stewart, D.R.M., 1967. Analysis of plant epidermis in faeces: a technique for studying the food preferences of grazing herbivores. Journal of Applied Ecology 4, 83–111.

Sugimoto, T., Ito, T.Y., Taniguchi, T., Lkhagvasuren, B., Oyunsuren, T., Sakamoto, Y., Yamanaka, N., 2018. Diet of sympatric wild and domestic ungulates in southern Mongolia by DNA barcoding analysis. Journal of Mammalogy 99, 450–458.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., Willerslev, E., 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35, e14.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J.E., Taberlet, P., 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. Molecular Ecology Resources 9, 51–60.

Vives, J.A., Baraza, E., 2010. La cabra doméstica asilvestrada (Capra hircus) en Mallorca ¿Una especie a erradicar? Galemys 22, 193–205.