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Functional classification by NIRS of plant parts selected by sheep on a shrubby rangeland

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Abstract. The diet of livestock foraging on rangelands is highly diversified and changes daily, seasonally and annually. Thus, a classical assessment of diet nutritive value with forage sampling followed by chemical analysis would be too challenging. We explore an alternative method to characterize the diversity of food offered based upon the classification of food items into a limited number of "functional" nutritive classes. The area of study was a Mediterranean *garrigue* rangeland, grazed by sheep in spring. Based on sheep foraging behaviour, 103 food items were identified as "bite categories" (BCs), from 60 plant species; 245 samples were taken. They were oven dried and grinded before Near-infrared spectra (NIRS) collection. Hierarchical classification of NIRS spectra produced "functional" classes of BCs. A few classes were homogeneous while others gathered plants from diverse botanical origins, but displaying similar characteristics such as chemical composition. Other Mediterranean rangelands and other grazing periods need to be studied in order to validate the functional nutritive classes identified. This method paves the way to better characterize the potential nutritive value of rangelands by taking into account foraging selection at bite level.

Keywords. Rangeland – Nutritive value – Classification – Sheep – Near infrared spectroscopy.

Classification fonctionnelle par SPIR des parties de plantes sélectionnées par des brebis sur un parcours embroussaillé

Résumé. *Le régime alimentaire des troupeaux sur parcours est très diversifié et change selon les jours, les saisons, les années. Les méthodes classiques d'appréciation de la valeur nutritive du régime basées sur l'échantillonnage et les analyses chimiques seraient trop compliquées et coûteuses à mettre en place. Nous explorons ici une méthode alternative, basée sur une classification comportant un nombre limité de « classes nutritives fonctionnelles ». La zone d'étude était un parcours de garrigue méditerranéenne pâturé par des brebis au printemps. Sur la base du comportement alimentaire, 103 aliments ont été identifiés en tant que « plantes x prises » (PP), sur 60 espèces végétales, et ont donné lieu à 245 échantillons. Ceux-ci ont été séchés puis broyés et passés au spectromètre de proche infrarouge. La classification hiérarchique des spectres a donné des classes « fonctionnelles » de PP. Certaines classes étaient homogènes, d'autres regroupaient des plantes d'origines botaniques variées, mais avec une composition chimique similaire. Pour valider les classes fonctionnelles, il sera nécessaire d'étudier d'autres parcours méditerranéens et d'autres saisons de pâturage. Cette méthode ouvre la voie vers une meilleure caractérisation de la valeur nutritive potentielle des parcours, en prenant en compte la sélection alimentaire à l'échelle de la bouchée.*

Mots-clés. *Parcours – Valeur nutritive – Classification – Ovins – Spectrométrie dans le proche infrarouge.*

I – Introduction

In the Mediterranean area, large parts of landscape are covered by heterogeneous vegetation made of contrasting mosaics of semi-natural swards, scrublands and woodlands. Such rangelands have been increasingly disregarded by farmers who often do not consider them as a proper feed resource, especially for animals with "high nutritional requirements" (Jouven *et al.*, 2010). Rangelands from southern Europe are still largely marginalized and under-utilized, mainly because they cannot be evaluated and managed with the same knowledge and criteria as for cultivated grasslands. The feeding value of mixed rangeland results from the selection by the grazing animals of feeding patches, plant species and plant parts. Selection of plant parts

varies according to vegetation communities' structure, diversity and characteristics of the full range of reachable food items, conditions and daily grazing schedules, feeding requirements, as well as previous feeding experience and habits of the animals (Meuret and Provenza, 2015).

The objective of our study was to test an original mixed approach to characterize the nutritive value of Mediterranean rangelands. Based on previous studies (Bonnet *et al.,* 2015), we hypothesize that rangeland vegetation can be described with functional nutritive categories applied to the different plant parts potentially ingested. Besides, since animals select plant parts based not only on protein and energy content but also on multiple primary and secondary compounds (Meuret and Provenza, 2015), we based our classes on the NIRS spectra, which contain the whole range of information available about sample chemical composition.

II – Material and methods

1. Study area

The study area was a mixed grassy, scrubby and wooded rangeland, dominated by stretches of live oak (*Quercus ilex* L.) coppice, located 35 km North from Montpellier, which represented a typical Mediterranean rangeland on calcareous and dry soils. The area is usually grazed in spring (April-June) and late autumn (November-December) by a flock of 150 ewes of *Raïole* breed and their lambs. For our study, we chose to work on sites that had not been grazed yet. Three sites within a radius of 10 km were chosen for the collection of food samples: 1) a live oak coppice and its edges, with trees, undergrowth shrubs and creepers; 2) clearings with many trees and shrubs regrowths after logging operations that occurred 4-5 years earlier; 3) swards dominated by annual plants and comprising short bushes.

2. Sample collection and preparation

The samples (245 samples of 103 BCs) were collected by hand-plucking between the 27th of April and the 5th of May 2015, as a reproduction of the bites taken by sheep in the same grazing sites and within the same season. To cope with the diversity of ingestive bites, we used the "bite categories" (BC) method, initially developed for continuous bite monitoring (Agreil and Meuret, 2004; Bonnet *et al*., 2015). The samples collected were made of plant parts, defined as possible bites taken by the grazing sheep. Each sample was related to a given plant species and a distinctive BC, then stored in a paper bag identified with a code and then quickly frozen at -21°C. Later, the samples were dried (55°C) and ground in a cutter mill with a 1 mm sieve for further analysis and spectra collection.

3. NIRS-based analysis of samples

Samples were scanned in duplicate on a monochromator spectrometer NIRSystem 5000 (FOSS, Laurel, MD, USA) in reflectance mode from 1100 nm to 2500 nm (with 2 nm steps). A mathematical pre-treatment was applied to spectra (2nd derivative, normalization and de-trending) in order to minimize non-informative content. Unsupervised classification of samples was applied directly on the mathematically pre-treated spectra. Hierarchical ascendant classification (Ward aggregation criterion) was performed with XLSTAT software (Addinsoft, Paris, France). Chemical composition was predicted for each sample on the basis of its NIRS spectrum using existing calibrations (Meuret *et al*., 1993 ; Cirad, unpublished) updated with reference laboratory analyses performed on a selection of 30 samples from this study. The parameters considered were: crude protein (CP, Kjeldahl method); fiber fractions (NDF, ADF, ADL; Van Soest *et al*., 1991); *in vitro* organic matter digestibility (IVOMD; Aufrère *et al.,* 2007).

III – Results and discussion

The hierarchical classification led us to choose seven classes (Table 1), as a compromise between statistical significance (sufficient difference between the classes) and technical applications (classes which can be interpreted in terms of animal foraging and nutrition). Apart for class F, exclusively composed of grass species, the classes were composed of a variety of botanical groups, and a given botanical group could be found in more than one class; for example, *Fabaceae* could be found in classes C, D, E. The seven classes all had a morphological and chemical consistency (Table 1 and Table 2). Tender, digestible BCs poor in fibre such as fruit, flower and soft leaves made up class D. Moderately lignified organs of shrubs and trees, mostly from aromatic plants, made up class C, while class G contained the most fibrous and lignified and the least digestible BCs.

Table 1. General description of the 7 functional classes

Class	Main type	Botanical dominants	Plant parts dominants
A	Developed dicotyledon leaves	Various non fabaceae dicotyledon	Leaves, leaves with stems
B	Young shrub organs	Non Fabaceae dicotyledons, Liliaceae	Shoots and young leaves
C	Lignified shrubs and trees	Dicotyledons including Fabaceae; Gymnosperms; aromatic plants	Leaves; some stems and shoots
D	Fruits, flowers, soft leaves	Liliaceae, Fabaceae, Morus	Fruits, flowers, shoots
E	Shoots	Fabaceae, various dicotyledons	Young leaves, shoots, flowers
F	Grasses and sedges	Poaceae and Carex	Young leaves, panicles
G	Coriaceous organs	Cupressus, Smilax, Ruscus	Leaves and stems

Table 2. Chemical composition of the 7 functional classes (mean), predictions based on the NIRS spectrum. Different letters within a row indicate statistical difference (ANOVA, p<0.05)

Class (No samples)	A (52)	B (37)	C (58)	D (23)	E (47)	F (16)	G (12)
DM (%)	35.7^{b}	26.3^{cd}	38.4^{ab}	20.8^{d}	28.8^{c}	33.7^{bc}	44.9^{a}
CP (% DM)	10.2^{cd}	13.0^{b}	9.2^{d}	15.4^{ab}	17.1^{a}	12.9^{bc}	7.3^{d}
NDF (%DM)	43.8^{c}	42.8^{cd}	43.8^{c}	29.5^{e}	38.7^{d}	65.3^{a}	51.8^{b}
ADF (%DM)	29.6^{b}	28.8^{b}	31.4^{b}	18.2^{d}	23.8^{c}	32.4^{ab}	39.4^{a}
ADL (%DM)	12.4^{b}	12.9^{b}	18.4^{a}	6.3^{c}	10.5^{b}	4.1^{c}	20.9^{a}
Other C* (%DM)	$38.5^{b.c}$	36.8^{c}	$41.6^{a.b}$	46.7^{a}	$37.6^{b.c}$	14.8^{d}	34.8^{c}
IVOMD (%)	59.9^{c}	60.0^{cd}	53.3^{de}	81.8^{a}	68.7^{b}	48.5^{ef}	41.9^{f}

*Other compounds (soluble carbohydrates, lipids and essential oils, polyphenols and other secondary compounds), calculated as: [100 - ash – CP – NDF]

These seven categories were complementary to each other in nutritional terms for the grazing animals: certain categories had intermediate values for chemical compounds (A, B, C) while others contained much fibre (F, G) or crude protein (D, E). Intra-class variability for major compounds remained high, leading to overlapping between classes for a few chemical parameters. However all classes were statistically different from others for 2 or more measured parameters. But they also probably differed in other chemical characteristics (Table 2, “other compounds”: soluble carbohydrates, lipids and essential oils, secondary compounds) that were not measured in this study but which influenced the spectra and thus the classification.

By coupling unsupervised NIRS classification and BCs approaches, we have confirmed that the nutritional quality of most feed items from rangeland is often more related to the type of organ

and the structural attributes of the plant parts than to a botanical group or plant species *per se* (Meuret, 1997; Bonnet *et al*., 2015). Our approach produced a description of a heterogeneous Mediterranean pasture which should make sense in terms of animal feeding behaviour: each functional class provides specific compounds, so different combinations of classes within a mixed diet should enable to meet different nutrient requirements of the grazing animals.

IV – Perspectives

The classification produced will need to be validated and refined by adding new samples, taken in other grazing sites and during other grazing seasons. The final objective is to obtain a stable classification, applicable to other sites and possibly also to a range of conserved feeds which could be distributed as supplements to the grazed diet. Such classification, combined with criteria about the accessibility and abundance of BCs to be given on plant species, as well as experience and other grazing conditions of animals, could be used to characterize more properly the feed profile of mixed rangelands and possibly improve their management and utilization as valuable forage resource.

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