

The DNA based characterization of the diet from digested samples: a reliability study in ruminants



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Introduction

Characterizing diet of grazing ruminants in complex environment

= important methodological gap for research on animal-plants relationships in diversified grasslands (permanent or multispecies temporary grasslands)

❖ Current methods

- Direct observation of foraging behaviour
- Microhistological techniques
- Alkanes presents in plant cuticular wax
- Spectral methods



Time-consuming, not very accurate, or not well adapted to diversified swards

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DNA METABARCODING



- ❖ Technique allowing identification of plant species from complex or degraded matrices based on analysis of DNA fragments from plant residues (e.g digesta from ruminants)
- ❖ Adequate barcode:
 - DNA sequence **variable between species** and **highly conserved in a same species** (discrimination)
 - **Short** fragments (degraded DNA – partial DNA fragmentation during digestion)
 - Large number of copies per cell to use small amount of sample



Analysis of chloroplast *trnL* intron



DNA METABARCODING

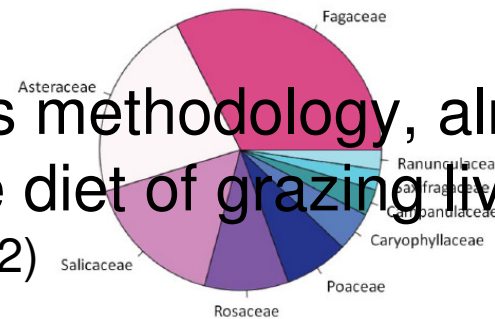
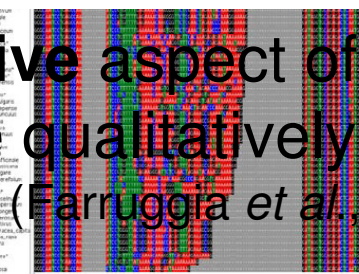


- Sampling (environmental sample)
- DNA amplification with universal primers for plants
- Sequencing of isolated DNA fragment
- Identification of species by comparison to those present in a reference database



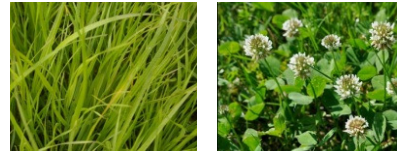
OBJECTIVE

To test the **quantitative** aspect of this methodology, already reliable to characterize qualitatively the diet of grazing livestock

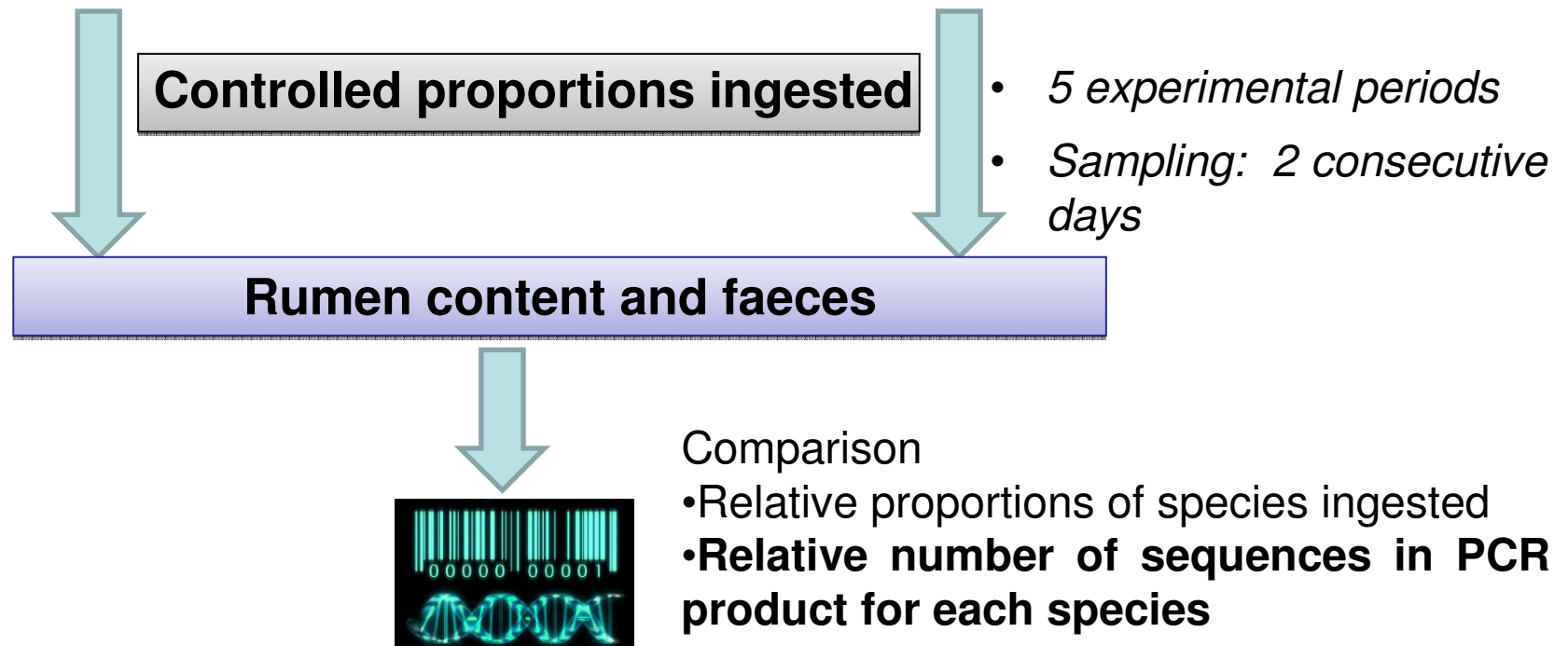


EXPERIMENTAL DESIGN

Perennial ryegrass / white clover



Repeated 5 × 5 Latin square design : 100:0; 75:25; 50:50; 25:75; 0:100 (%)



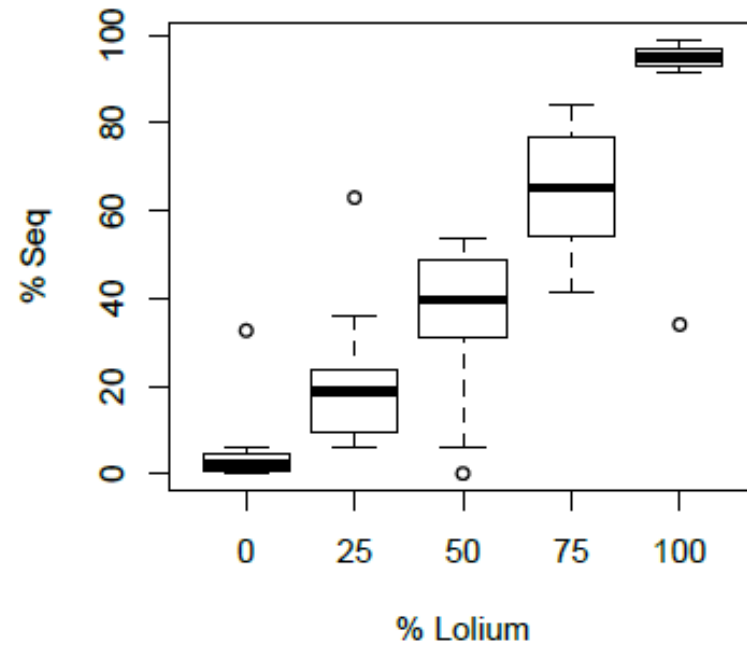
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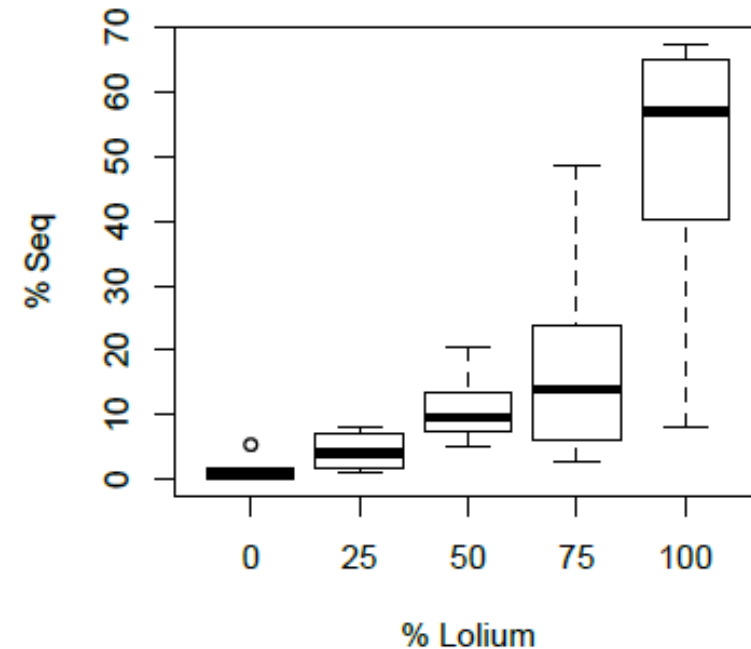
Results



Rumen content



Faeces



Good determination of the proportions ingested

Underestimation % ryegrass

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Conclusion - perspectives

- ❖ **Very promising results were obtained from rumen content:** validates the possibility of determining the proportions of species by DNA metabarcoding for simple mixtures.
- ❖ Need to understand the biases observed between the kind of digestive residues:
 - DNA from feces seems to be less efficiently recovered / rumen content
 - ➡ Bias due to the method of DNA extraction used (intracellular vs. extracellular DNA) ?
 - ➡ **To better adapt the protocol of extraction**
- ❖ Characterization of diet from **multi-species or very diversified grasslands ?**
 - Qualitatively: YES
 - Quantitatively: NO in the next future but rapid progress of sequencing technology



Thank you for your attention

