Bacterial colonisation of fresh and dried perennial ryegrass in the rumen

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Introduction

The first step of degradation of plant material within the rumen involves rapid colonisation of the material by a complex bacterial community[1]. Previously, colonisation of conserved hay stems by celluloytic bacteria (*Fibrobacter succinogenes* (Fs), *Ruminococcus albus* (Ra) and *R. flavefaciens* (Rf)) was shown to occur at equal rates[2]. However, how this compares to fresh grass is unclear.

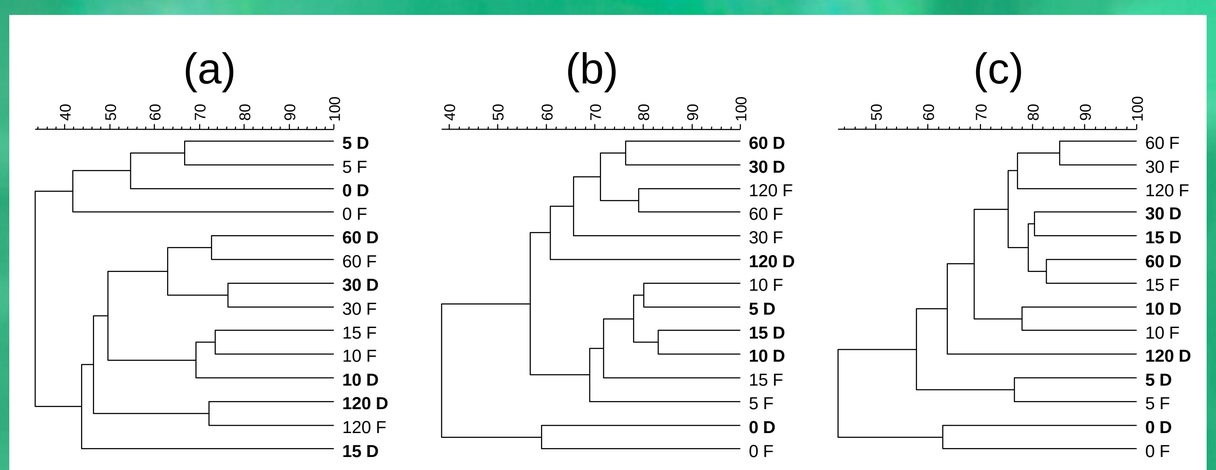
Aim

To characterise early (<2 h) populations of rumen eubacteria and cellulolytic bacteria colonising fresh and dried perennial ryegrass (PRG), and determine the corresponding dry matter (DM) loss.

Method

- Fresh or dried PRG (mechanically processed to mimic mastication) was incubated *in sacco* in the rumen of three rumen fistulated, non-lactating dairy cows grazing a ryegrass sward.
- For each cow, duplicate polyester bags of each forage type were incubated per time point (5, 10, 15, 30, 60 and 120 min) with 0 min bags processed directly. Bag residues were hand washed and snap-frozen in liquid N. Rumen contents were also sampled (0, 60 and 120 min) and snap-frozen.
- DNA was extracted from the residual DM (RDM), and the colonising bacteria analysed by eubacterial 16S ribosomal DNA based denaturing gradient gel electrophoresis (DGGE)[1] and quantitative PCR (eubacteria[1], Fs[2], Ra[2] and Rf[2]). Rumen contents were analysed similarly.

Fig 1. Cluster analysis (% similarity) of DGGE profiles of the eubacteria colonising fresh (F) and dried (**D**) PRG incubated in the rumen of three different cows (a-c). Branch labels denote incubation time (min) and PRG preparation (e.g. 120 F).



Results

- PRG preparation and incubation time did not affect the composition of the colonising rumen eubacterial populations consistently (Fig 1).
- Colonising eubacteria, Fs, Ra and Rf increased over time (P <0.01), and were greater with fresh PRG than dried (P <0.001) (Fig 2).
- Relative abundance of the celluloytic bacteria in rumen content was Rf>Fs>Ra for all cows, but for colonising cellulolytic bacteria the relative species abundance differed by cow (data not shown).
- Initial DM loss (0 min) was greater with dried PRG than with fresh (18.4 *v* 5.5 %; P <0.01).
- A linear interaction (P <0.001) between forage and time in terms of DM loss (relative to 0 min) was observed (Fig 3), with dried PRG showing greater apparent DM loss after 2 h than fresh PRG (25.0 *v* 7.3 %; P <0.05).

Fig 2. Eubacterial DNA (a) and cellulolytic bacterial DNA (b-d) on fresh (red) and dried (black) PRG incubated *in sacco* in the rumen. Data points represent the mean of duplicate bags for each cow.

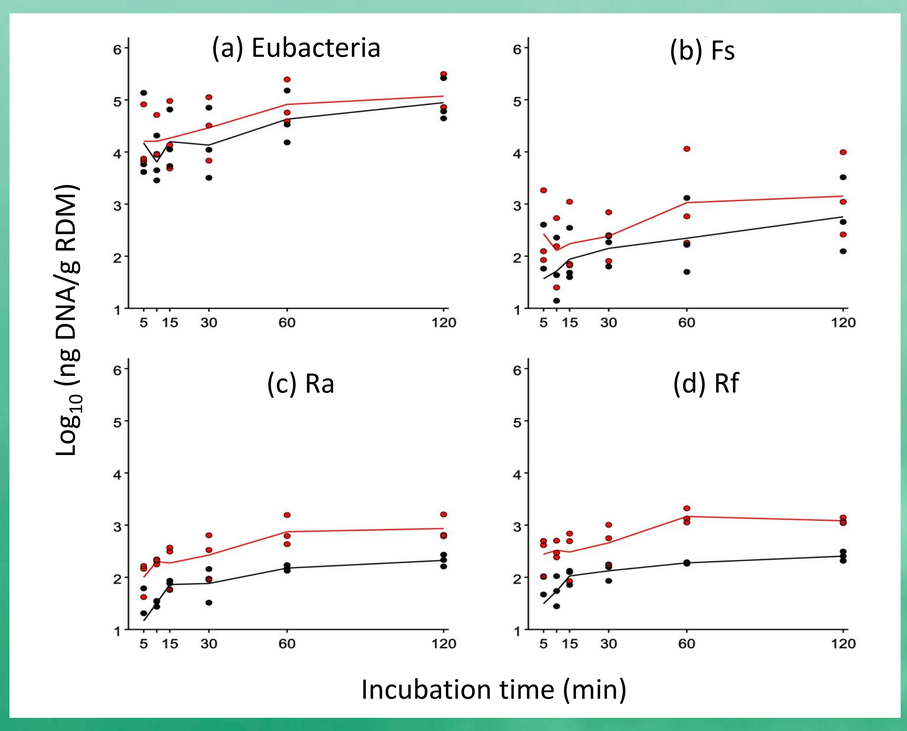
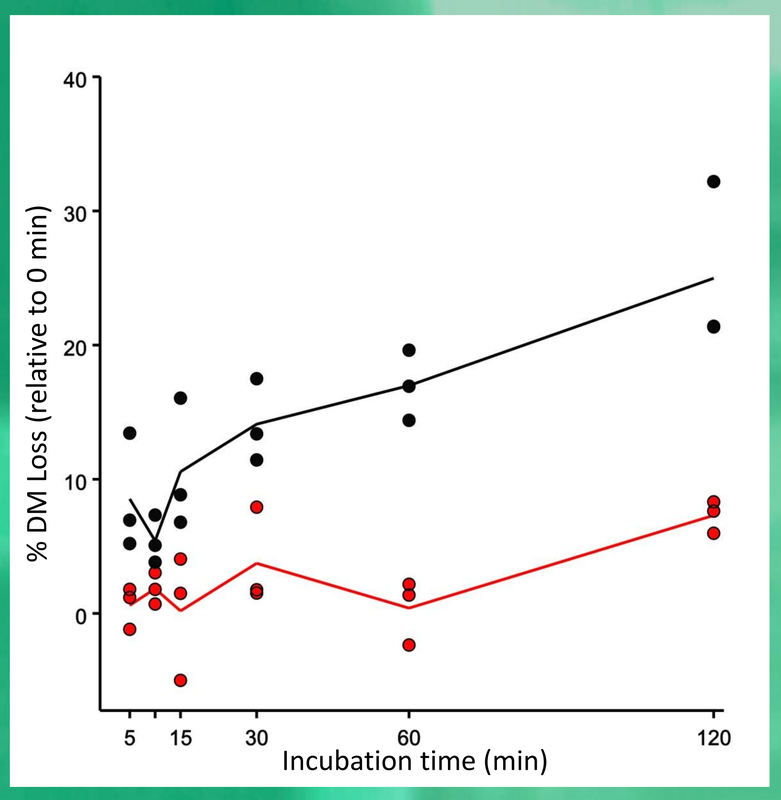


Fig 3. DM loss from fresh (red) and dried (black) PRG incubated *in sacco* in the rumen. Data points represent the mean of duplicate bags for each cow.



Conclusion

- Colonising rumen bacterial populations were larger with fresh rather than dried PRG, but this was not reflected in DM loss.
- Animal differences in relative abundances of colonising cellulolytic bacteria were more apparent than any forage associated effect on total population composition.
- Clarification as to whether the observed differences in initial and ruminal DM loss may have resulted from differing responses to the mechanical processing (to mimic mastication) is required.

References

1. Edwards JE, Huws SA, Kim EJ & Kingston-Smith AH (2007) *FEMS Microbiol. Ecol.* 62, 323-335.
2. Koike S, Pan J, Kobayashi Y & Tanaka K (2003) *J. Dairy Sci.* 86, 1429-1435.

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