



## Changes in methanogenic populations residing in the rumen of dairy cows in response to a sainfoin (*Onobrychis viciifolia* Scop.) based diet

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### Introduction

Tannins, mainly condensed tannins, present in sainfoin (*Onobrychis viciifolia* Scop.) can inhibit the methanogenic activity in the rumen (Waghorn et al., 2002) and the methane emission by reducing protozoa number and changing the rumen fermentation parameters. A reduction in methane production could be associated with a change in the number and/or in the diversity of the bacterial community in the rumen.

The current trial involving dairy cows was conducted to study the effect of sainfoin tannins on rumen fermentation and on methanogens functioning, and to assess adaptive behaviour of rumen microbiota when exposed to sainfoin tannins.

### Material and Methods

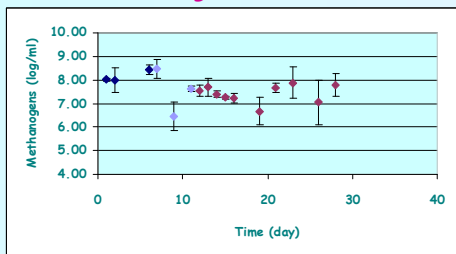
Three rumen fistulated Holstein Friesian dairy cows were placed on a lucerne based 'uniformity diet' for a two-week period, then the lucerne was exchanged for sainfoin. During the first five days of the sainfoin feeding polyethylene glycol (PEG4000) was administered to block condensed tannins present and after this treatment the animals remained on their sainfoin based diet for 7 more weeks. Samples of rumen liquid were collected and prepared for analyses on:

- ciliate protozoa microscopically enumerated (Dehority, 1984);
- methanogenic Archea determined by qPCR assay (Denman et al., 2007);
- determination of diversity in the rumen methanogen population by DGGE (Skillman et al., 2006);
- volatile fatty acids (VFA) by GC;
- ammonia (NH<sub>3</sub>) by a colorimetric method.

Data was analysed using the GLM procedure of SAS 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

### Results

#### Trend of methanogens

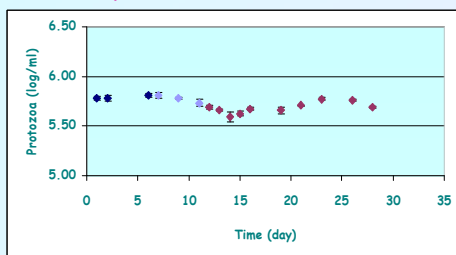


#### Dietary treatment effects on rumen fluid parameters and microbial cell count

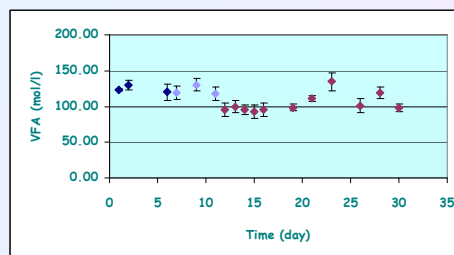
Treatment	Protozoa (log <sub>10</sub> /mL)	Number of Archea (log <sub>10</sub> /mL)	Total VFA (mmol/L)	NH <sub>3</sub> (mg/L)
Lucerne	5.79 <sup>a</sup>	8.14	124.5 <sup>a</sup>	138.2 <sup>a</sup>
Sainfoin+PEG	5.78 <sup>a</sup>	7.69	122.5 <sup>a</sup>	85.4 <sup>bc</sup>
Sainfoin 1 <sup>st</sup> week	5.65 <sup>c</sup>	7.46	95.7 <sup>b</sup>	54.4 <sup>b</sup>
Sainfoin 2 <sup>nd</sup> week	5.72 <sup>b</sup>	7.57	114.7	109.1 <sup>ac</sup>
Sainfoin 3 <sup>rd</sup> week	5.73 <sup>b</sup>	7.41	105.9	94.4 <sup>c</sup>
MSE	0.0006	0.45	152.1	439.5
P-value	0.0002	0.69	0.08	0.01

Means within columns followed by different superscripts are significantly different at P<0.05. MSE: mean square error.

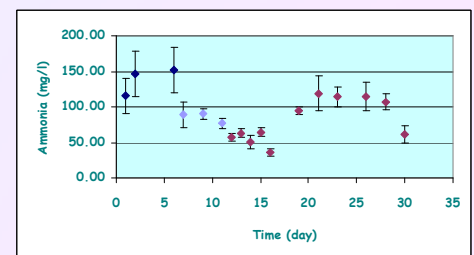
#### Trend of protozoa



#### Trend of VFAs



#### Trend of ammonia



### Conclusions

The tannin content in *Onobrychis viciifolia* Scop. resulted in the inhibition of protozoa and number of methanogens. The tendency of the different parameters suggests that the microbial population (protozoa, Archea, other bacteria) respond in different ways to the sainfoin diet over time, suggesting that rumen microbiota adapt to the dietary conditions. Further work is required to evaluate the relation between the microbial inhibition and the methane production.

### References

- Dehority, B.A., 1984. Applied Environ. Microbiol.: 182-185.  
Denman, S.E., N.W. Tompkins and C.S. McSweeney, 2007. Microbiol Ecol.:62(3): 313-22.  
Skillman, L.C., P.N. Evans, C. Strompl and K.N. Joblin, 2006. Letters Applied Microbiol.: 222-228.  
Waghorn, G.C., M.H. Tavendale and D.R. Woodfield, 2002. Proc. New Zealand Grass. Assoc. 64: 167-171.