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

Methanogenesis in the rumen is a natural metabolic process which keeps the fermentation on by continuously utilising the hydrogen (H2) released by fermentation of carbohydrates. Methane emissions by ruminants have implications for (1) animal productivity because they constitute a significant loss of dietary energy, and (2) the environment because methane is considered to be one of the more powerful greenhouses gases involved in global warming. Several plant secondary metabolites can be used for selective inhibition against the methanogens in the rumen. An antimethanogen activity has been attributed to tannins, mainly condensed tannins, present in sainfoin (*Onobrychis viciifolia* Scop.) (Waghorn *et al.*, 2002). These polyphenolic compounds can reduce 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. Polyethylene glycol (PEG) has a strong affinity with condensed tannins present in sainfoin and it is commonly used to block their action. The current trial involving dairy cows was conducted to study the effect of sainfoin tannins on rumen fermentation and on methanogen functioning, and to assess adaptive behaviour of rumen microbiota when exposed to sainfoin tannins for a prolonged period.

Material and methods

Prior to receiving the experimental diet three rumen fistulated Holstein Friesian dairy cows (DIM = 226±64 d; milk production = 29.4±6.1 kg/ d; mean ± se) were placed on a lucerne based 'uniformity diet' for a 2-wk period to allow animals to adapt to a tannin free legume-based diet. After 2 wk, the lucerne was exchanged for sainfoin. During the first 5 d of the sainfoin feeding polyethylene glycol (PEG4000; 975 g/cow/d) was administered in three portions per day through the fistula. After the PEG treatment the animals remained on their sainfoin based diet for 7 more weeks. Rumen content samples were taken three times per week during the experimental period. Samples were collected before the morning feeding and were prepared for analyses on fermentation end-products: volatile fatty acids (VFA) by gas chromatography and ammonia (NH_3) by a colorimetric method. Ciliate protozoa were microscopically enumerated in rumen fluid samples using a Sedgewick Rafter counting chamber (Dehority, 1984). DNA was extracted from rumen fluid collected and the number of methanogenic Archea were determined in the sample using the SYBR green qPCR assay; *Methanobrevibacter smithii* was used as the reference strain. The PCR conditions and primers used were as reported by Denman *et al.* (2007). In addition, samples were taken to determine the diversity of the rumen methanogen population by denaturing gradient gel electrophoresis (DGGE, Skillman *et al.*, 2006). Data were analysed using the GLM procedure of SAS® 9.1 (SAS Institute Inc., Cary, NC, USA). Differences between dietary treatments (lucerne, sainfoin+PEG, sainfoin) with time were tested using a repeated measures statement.

Results and discussion

The results show a significant (P<0.05) decrease in the number of protozoa, and a non- significant decrease in the total number of Archea in the first week after changing to the sainfoin diet (Table 1). The Archea followed a similar tendency as the protozoa but animal variation seemed to be considerably higher. The decrease in Archea numbers already started during the PEG administration period. This suggests that PEG may not have been fully successful in completely blocking the effect of tannins. Total VFA and NH_3 followed a pattern similar to the protozoa numbers. The VFA profile shows some significant differences among diet treatments. The results show no differences in total VFA between the uniformity diet and the sainfoin + PEG diet, but a distinct and significant (P<0.05) drop in VFA when PEG treatment was stopped (122.5 vs. 95.67 mmol/l). In contrast, NH_3 shows a significant (P<0.05) decrease when animals changed from uniformity diet to the sainfoin + PEG diet, followed by a further drop in the first few days after stopping the PEG treatment; further supporting our observation that PEG was not fully effective. The difference in the DGGE animals profile through time involving all methanogenic groups shows an inhibition in the whole methanogen community.

Table1. Dietary treatment effects on rumen fluid parameters and microbial cell count.

Treatment	Protozoa (log_{10}/ml)	Number of Archea (log_{10}/ml)	Total VFA (mmol/l)	NH_3 (mg/l)
Lucerne	5.79^{a}	8.14	124.5^{a}	138.2^{a}
Sainfoin+PEG	5.78^{a}	7.69	122.5^{a}	85.4^{bc}
Sainfoin 1st wk	5.65^{c}	7.46	95.7^{b}	54.4^{b}
Sainfoin 2nd wk	5.72^{b}	7.57	114.7	109.1^{ac}
Sainfoin 3rd wk	5.73^{b}	7.41	105.9	94.4^{c}
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.

Conclusion

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) responds 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 methane production.

References

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