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HEALTHYHAY
Marie Curie Research Training Network

Methane production of different varieties of sainfoin (*Onobrychis viciifolia*) in dairy cows measured by *in vitro* techniques

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Introduction

Formation of methane by ruminants forms a major environmental concern as it is an important greenhouse gas with a high global warming potential. Methane is being produced by methanogenic bacteria residing in the rumen. Tannins may affect on the growth of proteolytic and cellulolytic rumen bacteria and protozoa, and hence, may alter the type of fermentation and amount of methanogenesis occurring in the rumen. An *in vitro* batch culture essay was used to assess the effect of tannins from sainfoin (*Onobrychis viciifolia*) on cumulative gas and methane production.

Objectives

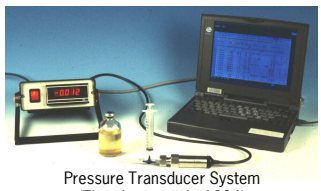
- Compare the gas and methane production from tannin rich sainfoin varieties with forages with a low tannins content like lucerne and rye grass
- Compare how different binding agents that precipitate the tannins, affect the gas and methane production

Materials

1. Plant material: Sainfoin (*Onobrychis viciifolia*) var. Cotswold Common 0-1, 0-3, 0-4; Lucerne (*Medicago sativa*), Rye grass (*Lolium perenne*). Freeze dried 1-mm ground samples.
2. Binding agents: PEG 6000 (Polyethylene Glycol-6000) and PVPP (Polyvynilpolypyrrolidone).
3. Rumen inoculum pooled from two non lactating Holstein dairy cows.
4. All the 5 different plant samples were combined with both the binding agents according the following :
   1. no binding agents (NONE); 2. PEG; 3. PVPP.

Methods

- *In vitro* cumulative gas production test, using a manually operated pressure transducer technique[3]. Gas measurements at fixed time points for a total of 102 hours.
- Methane concentration determined by gas chromatography.
- End point determination of volatile fatty acids (VFA), ammonia (NH_3) and pH.



Pressure Transducer System
(Theodorou et al., 1994)

Results

Table 1. total cumulative gas and methane (CH_4) production kinetics.

Substrate	Additive	pH	OMCV (mL/g OM)	OMCV (CH4) (mL/g OM)	Tmax (h)	Tmax (CH4) (h)	Rmax (mL/ h)	Rmax (CH4) (mL/ h)
Grass	NONE	6.43	298.1	28.13	13.13	23.62	9.66	0.538
Grass	PEG	6.42	304.6	27.47	12.26	21.04	10.99	0.661
Grass	PVPP	6.44	302.2	25.33	11.64	19.89	11.02	0.651
Lucerne	NONE	6.49	317.3	31.23	6.77	23.01	12.64	0.754
Lucerne	PEG	6.46	316.9	35.45	6.79	19.46	14.69	0.886
Lucerne	PVPP	6.45	316.7	33.70	5.95	17.57	15.68	0.914
Sainfoin 0-1	NONE	6.44	267.5	24.57	8.63	21.87	9.03	0.570
Sainfoin 0-1	PEG	6.45	286.7	26.37	8.01	19.99	11.19	0.665
Sainfoin 0-1	PVPP	6.45	277.4	27.60	6.71	19.70	10.94	0.625
Sainfoin 0-3	NONE	6.44	266.9	26.20	8.07	21.65	9.05	0.597
Sainfoin 0-3	PEG	6.44	280.1	27.57	7.14	19.67	10.89	0.642
Sainfoin 0-3	PVPP	6.44	274.7	28.10	6.09	19.81	10.60	0.637
Sainfoin 0-4	NONE	6.43	241.3	22.93	7.61	19.89	8.62	0.486
Sainfoin 0-4	PEG	6.45	266.6	26.70	7.07	20.06	11.06	0.622
Sainfoin 0-4	PVPP	6.46	248.3	23.60	6.44	18.62	10.79	0.555
SEM		0.01	4.9	1.51	0.39	1.33	0.28	0.051
Modelestimates (P-values)								
Substrate	(S)	0.0001	<.0001	<.0001	<.0001	0.4420	<.0001	<.0001
Additive	(A)	0.8090	0.0012	0.1044	<.0001	0.0055	<.0001	0.0052
Interaction	(S x A)	0.0442	0.3655	0.4808	0.8271	0.8472	0.1348	0.9515

OMCV: Organic matter corrected volume; Tmax: Time at which Rmax is reached; Rmax: Maximum rate of gas production; SEM: Standard error of mean

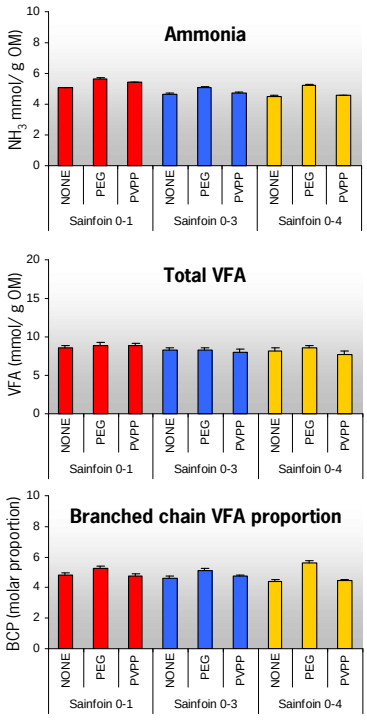


Fig.1. Ammonia, volatile fatty acid concentration and the proportion of branched chain fatty acids for different sainfoin sources (0-1, 0-3, 0-4), without (NONE) or with binding agent (PEG, PVPP) after incubation with rumen fluid from non-lactation dairy cows.

Conclusions

- The different plants considered (rye grass, lucerne and sainfoin) show differences in gas and methane production kinetics
- The two tannins binding agents affect both gas and methane production. PEG6000 results more effective)
- Both the kinetics of methane and gas production as well as fermentation end products (VFA & NH_3) are affected by tannins in sainfoin