Fermentative activity of horse gut contents assessed in vitro

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Introduction

There is little published information concerning microbial diversity in the horse gut. However, microbial instability can influence gut lumen pH and lactate accumulation, both of which have been implicated in the occurrence of laminitis and colic. In this study three substrates, hay (H), oats (O) and a 50:50 hay:oats (DM basis) mixture (HO) were used to evaluate in vitro microbial fermentative activity in four sections of the equine gut.

Materials and methods

Digesta samples (stomach, caecum, ventral colon and rectum), obtained from 4 animals that were euthanased, were used as the inocula in the in vitro technique of *Theodorou* et al. (1994). Samples were taken within 2 minutes of euthanasia. Samples were kept continually under CO_2. Five replicates from each section were incubated at 38°C with 1 g (DM) of substrate in 90 ml buffer and 10 ml sample. Three replicates were measured for gas production (GP) and two for dry matter degradation (DMD) kinetics.

DMD kinetics were estimated by recovering fermentation residues from two replicates at 7 intervals throughout the 72 h incubation period.

HO (HOA) values for GP and DMD were measured for all four sections. Predicted HO (HOP) values were derived by calculating the mean of the measured O and H replicate values.

A curve fitting model (*France* et al. 1993) was applied to provide mathematical descriptions of the resulting GP profiles. Data was analysed by the General Linear Model and the Tukey test of SPSS 12.0 for Windows. Significance was accepted at $P<0.05$.

Results

For all sections DMD of the 3 substrates differed significantly ($P<0.001$, Table 1). A small trend towards less actual DMD than predicted was evident and occasionally significant ($P<0.05$, Table 3) for all sections excluding the stomach. Total GP was significantly different ($P<0.001$) for each feed within each section and was consistently in the order O> HOA>H for all sections. For all sections, H and O, and HOA and O were significantly different ($P<0.01$). However, excluding the stomach which showed significance for all interactions ($P<0.01$), no significant difference existed between H and HOA (Table 1).

Table 1 Total gas produced (ml/g DM), gas produced at 4 h (ml/g DM) and DMD.

	H	O	HOA	s.e.d.
Stomach				
Total GP (ml/g DM)	27.73 ^{a}	67.77 ^{b}	48.24 ^{c}	0.245
GP at 4 h (ml/g DM)	8.14 ^{a}	17.25 ^{a}	8.15 ^{a}	0.545
Total DMD	0.25 ^{a}	0.76 ^{b}	0.52 ^{c}	0.036
Caecum				
Total GP (ml/g DM)	58.95 ^{a}	129.13 ^{b}	72.82 ^{a}	0.149
GP at 4 h (ml/g DM)	0.71 ^{a}	0.71 ^{a}	0.97 ^{a}	0.443
Total DMD	0.51 ^{a}	0.74 ^{b}	0.56 ^{c}	0.006
Colon				
Total GP (ml/g DM)	83.24 ^{a}	131.19 ^{b}	88.67 ^{a}	0.074
GP at 4 h (ml/g DM)	1.04 ^{a}	0.23 ^{a}	0.83 ^{a}	0.080
Total DMD	0.57 ^{a}	0.78 ^{b}	0.58 ^{c}	0.007
Faeces				
Total GP (ml/g DM)	84.75 ^{a}	126.28 ^{b}	102.22 ^{a}	0.061
GP at 4 h (ml/g DM)	0.0 ^{b}	1.62 ^{a}	0.9 ^{ab}	0.052
Total DMD	0.59 ^{a}	0.72 ^{b}	0.64 ^{c}	0.008

Values with letters of differing superscripts within rows differ significantly ($P<0.01$).

Up to 12h of incubation, GP for HOP tended to be lower than for HOA, suggesting a positive associative effect, although this was rarely significant. From 12h onwards, HOP was higher than HOA describing a trend towards a negative associative effect which was more frequently significant (Table 2). Incubations with stomach inocula were not continued beyond 12 h as it was not considered representative of in vivo.

Significant differences were observed for cumulative gas production at 4 h only for faecal inocula between H and O ($P<0.01$, Table 1). HOA produced more gas than for H or O when incubated with caecal inocula, but not significantly.

The curve fitting model of *France* et al. (1993) was applied for incubation with O and HOA. However, data for incubation with H for all sections could not be modelled possibly due to the non-sigmoidal nature of the data.

Discussion

Karlsson et al. (2000) reported that in vivo, the inclusion of a small amount of oats in a hay ration (80:20 hay:oats DM basis) increased microbial activity, but a higher inclusion rate (60:40 upwards) had a negative associative effect. In the current study, an increase in DMD and GP were observed when O were incubated with H compared with H alone. A negative associative effect was observed for DMD and GP in the current study when H and O were incubated together at a level of 50:50 DM basis. Prior to 12h of incubation, a predominantly positive effect was observed for GP. However, a trend towards a negative associative effect throughout the incubation was observed for DMD. Reasons for these differences are not clear but may be due to changes in the populations of the starch and/or fibre utilising bacteria. Further analysis of VFA's and microbial DNA (data not shown) may help to explain these effects. *Karlsson* et al. (2000) suggested a decreased digestibility of fibre as the main cause of the

GP	2	4	8	12	16	20	24	30	36	48	72	significance
Stomach												
HOP	4.91	7.82	14.23	20.83								NS
HOA	2.90	5.25	16.74	23.34								
Caecum												
HOP	0.00	0.71	5.61	15.65	22.28 --	14.00 --	9.00	4.40	3.48++	7.41	11.51	P<0.01
HOA	0.00	0.96	7.64	12.13	13.22	6.02	5.47	5.88	5.29	6.47	9.7	
Colon												
HOP	0.45 --	0.28	7.15	16.17	18.09 --	14.80 --	8.66 --	8.61	7.03 --	8.58 --	17.49 --	P<0.05
HOA	0.66°	0.22	9.49	18.36	13.44	9.93	6.88	7.59	5.95	5.98	10.22	
Faeces												
HOP	1.22 --	0.94	9.55++	14.75++	19.29 --	13.57 --	9.34 --	9.63 --	6.89 --	12.22 --	14.75	P<0.05
HOA	0.00	1.02	13.66	18.15	12.45	9.21	7.09	8.02	6.15	9.98	16.01	

Contrasting HOA and HOP values within sections with ++ or -- symbols are positively or negatively significantly associated respectively.

Table 2 Association of incubating H and O: HOA DMD (12 h for stomach and 72 h for all other sections) and HOP.

DMD	4	8	12	16	20	30	72	significance
Stomach								
HOP	0.31	0.41	0.51					NS
HOA	0.31	0.43	0.52					
Caecum								
HOP	0.38 --	0.39	0.42	0.43	0.46	0.56 --	0.62 --	P<0.05
HOA	0.30	0.35	0.40	0.43	0.43	0.47	0.56	
Colon								
HOP	0.34 --	0.39	0.47	0.50	0.52	0.60	0.67 --	P<0.05
HOA	0.25	0.38	0.47	0.50	0.54	0.55	0.58	
Faeces								
HOP	0.35 --	0.36	0.42 --	0.48 --	0.51	0.57 --	0.65	P<0.05
HOA	0.30	0.38	0.40	0.40	0.46	0.48	0.64	

Contrasting HOA and HOP values within sections with ++ or -- symbols are positively or negatively significantly associated respectively.

Table 3 Association of incubating H and O: HOA GP (ml/g DM) (12 h for stomach and 72 h for all other sections) and HOP.

negative effect observed and that at a ration of H:O 40:60, NDF and ADF were significantly lower than for H alone. Decreased cellulolytic activity, during negative associative effects, is often due to a drop in pH. However in this study pH did not drop below 7.9 at any point other than at 0h for stomach inocula (data not shown).

Feed was not pre-digested as this was not a feed evaluation study but an investigation of the function and dynamics of microbial populations in the four sections sampled. In vitro there was no complicating effect of feeding the concentrate part of ration before or after hay as in vivo allowing a clearer description of the function of the microbial communities. However, similar DMD values were found in comparison with in vivo (*Karlsson* et al. 2000), therefore a pre-digest may not be necessary for DMD evaluation of H and O.

Conclusion

When H and O are fed together in vitro, prior to 12 h a positive effect occurs on GP, whilst a negative effect predominates for DMD at all times and from 12 h onwards for GP. Further analysis of VFA concentrations may help to explain these effects. DNA analysis would allow a much greater understanding of the microflora and associated changes with time and substrate.

References

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