In vitro fermentation of three species of fresh grass differing in water-soluble carbohydrate content with an equine faecal inoculum

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Introduction

Water-soluble carbohydrates (WSC) comprising glucose, sucrose, fructose and fructan are readily fermented by equine hind-gut micro-organisms. The fructan fraction of WSC is poorly degraded by mammalian enzymes (*Nilsson* et al. 1988) and therefore passes relatively undigested into the hind-gut where it is rapidly fermented. This rapid fermentation can produce an overgrowth of lactate producing bacteria which, in turn, may result in hindgut acidosis. Temperate pasture grasses can accumulate high concentrations (>300g/kg dry matter) of fructan, the amounts varying with plant species and environmental conditions. It is therefore possible that horses grazing such pasture may ingest potentially dangerous amounts of fructan (*Longland* and *Cairns* 2001). Whilst the effects of varying amounts of WSC in dried, ground grass on fermentation kinetics have been studied in vitro (*Longland* and *Murray* 2003), there is no published work to date using fresh grass. In order to better emulate grazing conditions, this study sought to determine the rate and extent of fermentation by an equine hindgut microbial inoculum of three species of fresh grass was investigated in vitro.

Materials and methods

Fresh grasses (equivalent of 1g dry matter [DM]) Timothy (T), Cocksfoot (C) and Perennial ryegrass (P) containing 215, 258 and 349g WSC/kg DM respectively, of which 164, 179 and 185g/kg DM was fructan were harvested onto ice, chopped to 1cm lengths and fermented in vitro at 39°C under anaerobic conditions with an equine faecal inoculum. The evolution of head-space gas was used as an indirect measurement of fermentation and was monitored for 72h. Rate and degradation parameters were determined by modeling gas production profiles.

Results

The fractional rate of gas production was significantly ($P<0.05$) greater for P than C and T, P being fermented at twice the rate of T (0.06973h^{-1} compared to 0.03543h^{-1}). C was intermediate (0.04647h^{-1}) and significantly faster ($P<0.05$) than T. DM loss was significantly greater ($P<0.05$) for P (971g/kg) than C (945g/kg) and T (933g/kg), as was the total gas pool accumulated (252.2ml for T compared to 325.3ml and 359.0ml for C and P respectively, see Table 1).

Table 1 Water-soluble carbohydrate (WSC) content and fermentation parameters of three species of fresh grass when incubated in vitro with an equine faecal inoculum. * FRGP, fractional rate of gas production.

Parameter	Perennial ryegrass	Cocksfoot	Timothy	Sed
Total WSC (g/kg DM)	34.85	25.83	21.54	–
Total Gas pool (ml)	359.0^{b}	325.3^{b}	252.2^{a}	13.61
FRGP (h^{-1})*	0.06973^{c}	0.04647^{b}	0.03543^{a}	0.001731
DM loss (g/kg)	971^{b}	945^{a}	933^{a}	0.440

* FRGP, fractional rate of gas production

Values across rows not bearing the same superscript differ significantly ($P<0.05$)

Discussion

Both the fractional rate (FRGP) and extent (DM loss) of fermentation were significantly greater for P than C and T reflecting differences in the WSC contents of the three grass species. FRGP reflects the proportions of readily or poorly fermentable material within a substrate; substrates with high proportions of readily fermentable material have faster fractional rates of gas production than those with high proportions of poorly fermentable material. P contained the highest proportion of readily fermentable WSC which was reflected in its rapid FRGP, whilst T had the lowest content of WSC and the slowest FRGP.

However, these results may not be entirely due to differences in WSC content. The WSC fraction of grass is composed of many different molecules of differing molecular weight. Fructan molecules exist in a series of increasing size, and the larger molecules may take longer to breakdown and ferment than those of smaller size. Thus, not only WSC content but also WSC composition may be responsible for the observed differences in fermentation kinetics between the 3 grass species. Indeed it is known that Timothy contains high molecular weight fructans and PRG fructans of much lower molecular mass, whilst those of cocksfoot are intermediate (Ince, 2003, unpublished data); these differences would certainly agree with the differences in fermentation rates reported here. However, more work is necessary to clarify whether fermentation rate relies on WSC content only or if it is also affected by the molecular mass of its constituent molecules. This could be achieved by pre-treating the grass by subjecting it to a simulated foregut digestion process which would remove the simple WSC sugars leaving the fructan fraction intact.

Conclusions

Of the three species investigated, T had the lowest WSC content, rate of fermentation and end-point DM disappearance. This suggests that, although T had the lowest nutritive value in terms of DM digestibility, its slower fermentation rate means that, of the three species tested here, it may be the least likely to cause hindgut acidosis.

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Fermentation of selected substrates in vitro using a batch culture with equine faeces as inoculum

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Introduction

Composition and activity of the guts' microbiota contribute essentially to the health of the host. In horses this is impressive highlighted in case of feed-induced laminitis (*Garner* et al. 1975, *Pollitt* and *van Eps* 2002). Thus, the investigation of fermentative processes in the hind gut and particularly the caecum is of special interest, but in vivo and ex vivo in vitro highly complicated. Using faeces as inoculum for in vitro incubation may allow, at least on principle, to study the fermentation of substrates assumed that they enter the terminal gut (*McDaniel* et al. 1993, *Zeyner* 2002, *Longland* and *Murray* 2003, *Moore-Colyer* et al. 2003, *Murray* et al. 2003, *Ince* et al. 2005, *Lattimer* et al. 2005, *Vervuert* et al. 2005). Fermentation characteristics as to dynamics and products may be helpful to value the protective or risk potential of substrates. Therefore, this investigation was conducted to characterise fermentation patterns of individual substrates rich in different carbohydrates (cellulose, pectin, starch, inulin, lactose, lactulose) incubated with equine faeces.

Material and methods

To incubate mixed faeces from two adult horses after four weeks of feeding a forage-based diet, a modified 'Hohenheim Gas Test' was used (*Zeyner* 2002). For this, 250 mg per syringe of cellulose (CL, 995 g cellulose/kg), citrus pectin (PC), Jerusalem artichoke meal (JA, 604 g inulin and 87 g disaccharides/kg), lactose (LA), lactulose syrup (LU, 503 g lactulose and 141 g other disaccharides/kg) and pre-gelatinised wheat starch (ST) were incubated, three times each. In parallel, substrate (SU) and inoculum (IO) alone were repeatedly incubated. The net gas production (NGP) was measured at hrs. 0, 7, 10, 15 and finally 24 of incubation. NGP per hour of the individual time frames of incubation was calculated. After incubation has been finished, the supernatant fluid from each syringe was extracted to measure the pH, short chain fatty acids (SCFA: acetic acid, propionic acid, n- and iso-butyric acid, n- and iso-valeric acid, n-capronic acid) and ammonia as described by Zeyner et al. (2004), D- and L-lactate according to *Noll* (1966; modified by K. D. Markuske, unpublished data) and the total equivalent water-soluble antioxidant capacity (TEAC) by *Miller* et al. (1996). Faecal bacteria groups (total aerobes and anaerobes, Gram-negative aerobes, yeasts, lactobacilli, bifidobacteria, enterococci, C. perfringens) were investigated by cultural studies. SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA) was used to analyse data by means of linear regression and analysis of variance with main factors 'substrate' and 'incubation time' (for NGP only). For post-hoc multiple comparison of means the SNK test was stressed. The level of statistical significance was pre-set at $p < 0.05$.

Results

There were no substantial differences between counts of faecal bacteria of the two horses. C. perfringens were found to be below 10^3 colony forming units/g. Because results were similar, data from all substrates incubated without faeces were summed up. With IO, SU and CL, NGP was not different from zero and the pH value was particularly high ($P < 0.05$). LA and ST caused a rapid NGP during early incubation (Fig. 1). With PC, the final NGP was highly elevated. LU induced

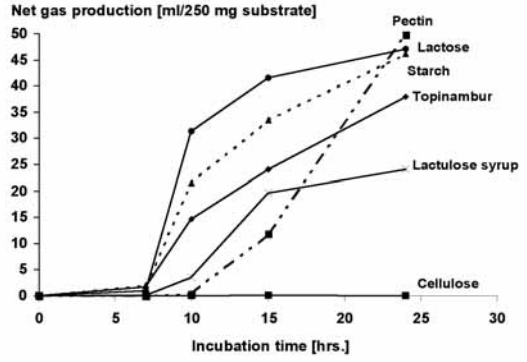

Fig 1 Net gas production (NGP) after incubation of different substrates with equine faeces.

moderate fermentation patterns. IO, but not SU, caused a measurable formation of SCFA, comparably to that with CL. Highest SCFA ($P < 0.05$; Fig. 2) and lowest pH ($P < 0.05$) were measured with PC, JA, LA and ST. The acetate- propionate quotient (A:P) was especially elevated with PC ($P < 0.05$), moderate with LA ($P < 0.05$) and lowest with JA and ST ($P < 0.05$; Fig. 2). With LU and IO, pH was settled between LA and PC. Between final SCFA and pH a close relation existed ($R=-0.865$, $P<0.01$). L- and D-lactate and their quotient were not different ($P>0.05$) between substrates incubated with faeces. Ammonia was found to be highest ($P<0.05$) with CL and further higher ($P<0.05$) with JA and LU than with ST, LA and PC. TEAC after incubation of faeces alone and faeces plus any substrate was about 16-fold higher than with SU ($P<0.05$).

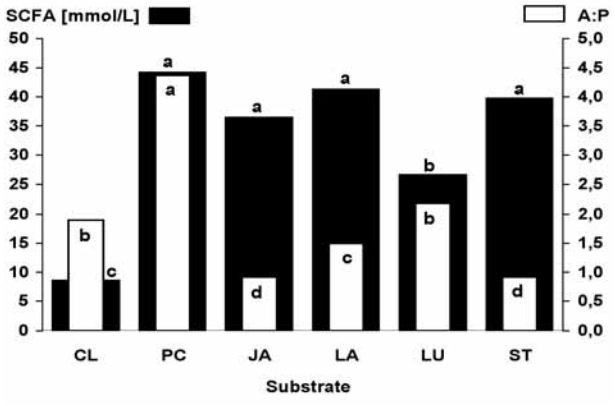

Fig 2 Short chain fatty acids (SCFA) and acetate-propionate quotient (A:P) in the supernatant after incubation of different substrates with equine faeces.

Discussion

The different substrates used in this study caused highly individual fermentation characteristics as to process dynamics and products while incubated with equine faeces. For interpretations, the respective substrate must be regarded as a totality and it's individual effect can not be reduced to any interesting ingredient because other contained substances may have an additional effect. Different fermentation patterns (e.g. NGP, SCFA, pH, ammonia) interact physiologically plausible. In this way, PC, LA and ST caused especially high NGP and SCFA while pH and ammonia were particularly low. It can be speculated that nitrogen has extensively been incorporated into microbes. Because the environment was buffered within a relatively strict frame it had obviously not enough been acidified to promote lactic acid producing microbes as it is expected to be in vivo. Whether this may principally work in a batch culture system needs to be studied. A more sophisticated graduation of highly fermentable substrates was provided by NGP dynamics and SCFA patterns, e.g. A:P, indicating different proliferation rates of individual microbial species and thus substrate valuation regarding the gut's health. Most surprising was the finding that pure cellulose caused obviously a very few fermentative activity.

Conclusions

In vitro fermentation characteristics from a batch culture with equine faeces allows a sophisticated differentiation between substrates as to the potential stimulation of the microbiota and probable changes of the microbial community.

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