Hydrogen and methane exhalation after ingestion of different carbohydrates (starch, inulin, pectin and cellulose) in healthy horses

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Introduction

Determination of hydrogen and methane concentration in exhalated breath can be used to get indirect information on intestinal microbial activity. Although this technique was used in horses for several times (*Tappeiner* 1881, *Nyari* 1992, *Murphy* et al. 1994, *Bracher* et al. 1995a,b, *Meyer* et al. 1997, *Sasaki* et al. 1999) the information about the influence of different carbohydrates are limited. Of special interest are those carbohydrates which can hardly modify the intestinal microbial community and are involved in the pathogenesis of laminitis (starch, fructans). Commonly it is assumed that microbial activity is focussed on the hindgut. That is true concerning the partitioning of cellulose or pectin digestion, but it is an incomplete view if the number of microbes in the different parts of the digestive tract are taken into account (*de Fombelle* et al. 2003). Investigation in dogs show that not exclusively the microbes in the hindgut are sensitive against fermentable substrates (*Zentek* 1992). Assuming that hydrogen exhalation reflects a part of the microbial activity it should be possible to get in addition to the microbial response in general, a further information about the localisation of microbial activity by measuring the postprandial hydrogen exhalation.

The feedstuffs used in this study (oats, Jerusalem artichoke, sugar beet pulp and grass meal) were chosen to investigate the effects of starch, inulin, pectin and cellulose on intestinal microbial activity. Special emphasis was laid on inulin as an example for fructans because it is supposed that rapid fermentation of fructans in the hindgut of horses has a key role in the development of laminitis (*Pollitt* and *van Eps* 2002).

Material and methods

Six Standardbred geldings aged from 3 to 22 years (mean body weight 473 kg) were fed in a randomised order oats (starch), Jerusalem artichoke (inulin), sugar beet pulp (pectin) and grass meal (cellulose) over a period of ten days once a day. All diets contained 1.5g hydrolysable carbohydrates/kg bodyweight (BW) per day, except for Jerusalem artichoke (1.5g inulin/kg BW/d). In addition the horse were fed hay three times a day. Between the different test runs a wash-out-period was carried out by feeding exclusively hay for at least ten days. On test days the Jerusalem artichoke was given per nasogastric tube, to realise a standardised intake, while the other test meals were given to the horses for oral intake. Samples were taken on day one, three, eight and ten of each feeding period. On these days the horses were given no hay and feed intake was inhibited twelve hours before starting the test. Breath samples were taken before and after feeding in intervals of 30 minutes over ten hours at the end of exhalation by using a tight fitting facemask. Hydrogen and methane concentrations were analysed by gas chromatography (GC 14 A, Fa. Shimadzu). Once in each testing period the horses were given 500 g of a 50 % glucose and 50 % grass meal mixture additionally to the test meal. Glucose served as a time marker for absorption from the small intestine. On those days blood samples were taken simultaneously to the breath samples to measure glucose using a commercial test kit (Gluco-quant, Roche).

Results

Feeding Jerusalem artichoke and oats caused a distinct and early rise of hydrogen in exhalated breath with a significant effect of time and treatment whereas grass meal and sugar beet pulp caused nearly constant hydrogen concentrations without any effect of time after feeding (Figure 1). Methane exhalation stayed nearly constant after feeding of oats and Jerusalem artichoke but rose in the third part of the testing period for grass meal and sugar beet pulp.

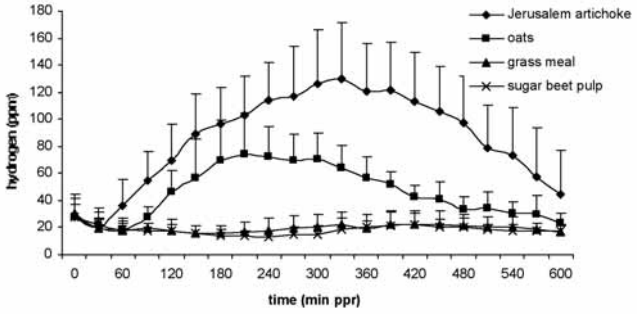

Fig 1 Mean hydrogen concentration (ppm) after feeding different test meals (N=6, mean of all days).

The mean AUC of hydrogen and methane exhalation - as a summary of the response - was 4.9 fold higher for Jerusalem artichoke and 2.6 fold higher for oats in comparison to the AUC observed after feeding of grass meal, whereas the methane exhalation was slightly lower (0.86 resp. 0.84). For sugar beet pulp the mean AUCs resembled to grass meal (0.97 of hydrogen and 1.02 of methane).

Table 1 Maximum values of hydrogen and methane concentrations (ppm), time reaching maximum values (min) and area under the curve (AUC).

		Grass meal	Oats	Jerusalem artichoke	Sugar beet pulp
H_2	Max	32.6 ± 19.7 ^{a}	106 ± 43 ^{b}	154 ± 68,6 ^{c}	30.8 ± 18.5 ^{a}
	Time max	226 ± 158 ^{a}	301 ± 102 ^{a}	339 ± 85 ^{a}	259 ± 183 ^{a}
	AUC	10765 ± 4914 ^{a}	27734 ± 9262 ^{b}	52434 ± 24460 ^{c}	10359 ± 4717 ^{a}
CH_4	Max	733 ± 200 ^{a}	615 ± 154 ^{b}	622 ± 140 ^{b}	760 ± 141 ^{a}
	Time max	440 ± 95 ^{a}	399 ± 193 ^{a}	201 ± 192 ^{b}	389 ± 177 ^{a}
	AUC	325987 ± 106993 ^{ab}	281038 ± 81959 ^{bc}	275223 ± 63762 ^{c}	331457 ± 77781 ^{a}

Means with varying small letter superscripts are different (P<0.05)

Blood glucose levels showed a typical postprandial pattern, but did not differ after feeding the different feedstuffs inclu-

ding glucose. In Figure 2 the plasma glucose curve after feeding Jerusalem artichoke plus glucose and hydrogen exhalation after feeding of Jerusalem artichoke are shown. Within 2 h ppr a rapid and parallel increase in blood glucose and hydrogen occured. The hydrogen concentration in breath continued to increase up to 360 min ppr, when plasma glucose levels had already decreased clearly.

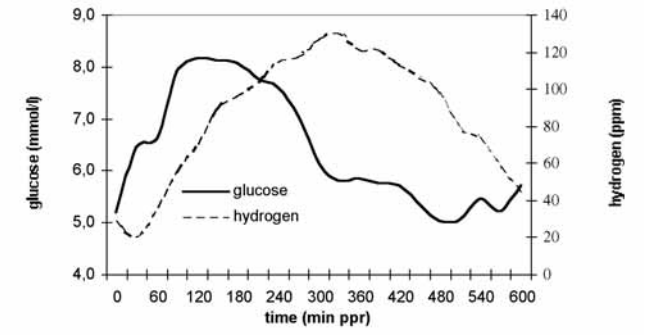

Fig 2 Mean glucose concentration (mmol/l) in plasma after application and mean hydrogen concentration (ppm) in exhalated breath of Jerusalem artichoke and glucose (mean of all days) after application of Jerusalem artichoke.

Discussion

The results suggest that inulin and starch are quickly fermented. Inulin and oats are fermented in the fore- and hindgut in comparison to the pattern of blood glucose level and breath hydrogen concentrations (Fig. 2). In healthy humans fructans are exclusively fermented in hindgut and therefore used as praebiotics. Although pectin is fast fermentable no differences in pattern of hydrogen and methane exhalation of pectin and cellulose were observed in this study, even those differences could be seen in in-vitro fermentation (*Plumhoff* 2004).

Hydrogen seems to be produced mainly, but not exclusively in the foregut of healthy horses while methane seems to be produced almost exclusively in the hindgut. The fact that - dependent on the type of feed - hydrogen is produced in the foregut of healthy horses leads to a different pattern of hydrogen excretion in comparison to humans. This finding is caused by the great differences in counts of micro organism in small intestine in humans and horses. In healthy humans there are only very low counts of bacteria in small intestine, while in foregut of healthy horses up to 10^9 colony forming units of anaerobic micro organism per ml ingesta (*de Fombelle* et al. 2003) are found. Therefore no difference in total number of bacteria can be observed comparing small and large intestine of horses. It is concluded that the hydrogen breath test cannot be transferred without modifications from human to the horse.

Conclusions

In conclusions, the results of this study indicate that rapid fermentation of oats and Jerusalem artichoke occurs in healthy horses and needs to be considered in the use of those feeds for safe feeding strategies. The mechanism in developing of laminitis is yet not known completely. Since laminitis was released by a single dose of fructan (7.5 g / kg bw) by *Pollitt* and *van Eps* (2002) a lot of studies were performed to proof the role of fructan in this disease. It was suggested that – like in humans- fructan is not digested in the foregut of horses and therefore reaching the hindgut where a rapid fermentation occurs. We have shown that rapid fermentation of inulin is already beginning in foregut. Because of this finding it should be assumed that rapid fermentation of fructan and starch in praecaecal parts of the gut could be involved in triggering laminitis. Up to now a lot of work was done to investigate the fermentative processes and microbial changes in the hindgut of horses with laminitis, but information on microbial changes in the foregut in case of laminitis are rare. This aspect should be regarded in further investigations.

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