Fermentative Gases in Breath Indicate that Inulin and Starch Start to Be Degraded by Microbial Fermentation in the Stomach and Small Intestine of the Horse in Contrast to Pectin and Cellulose¹,²

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EXPANDED ABSTRACT

KEY WORDS: • horse • inulin • laminitis • fermentation • hydrogen exhalation

Hydrogen (H₂) and methane (CH₄) concentrations in exhaled breath can be used to obtain indirect information on intestinal microbial activity in horses (1–7) and other species (8–9). H₂ can be produced anywhere in the gastrointestinal tract, whereas CH₄ is mostly produced in the large intestine except in pigs and rabbits (8). The objective of the present study was to measure exhaled gases after feeding of different carbohydrates (starch, inulin, pectin, cellulose) to healthy horses. Rapid fermentation of carbohydrate in the large intestine is regarded as a risk factor for laminitis in horses (10,11). Thus, knowledge of the site of fermentation of different carbohydrates, particularly those such as inulin that are fermented in the same way as fructan, could be useful in terms of producing preventive strategies.

MATERIAL AND METHODS

Six trotter geldings aged from 3 to 22 y with mean body mass (M)⁴ 473 ± 44 kg were fed hay; 15.3 ± 1.4 g kg⁻¹ dry matter (DM) in 3 equal meals/d. In addition, oats (O), Jerusalem artichoke (JA), sugar beet pulp (SBP), and grass meal (GM) were each fed separately in a randomized order of 4 periods (10 d each) for each horse once a day. The supplementary feeds provided 1.5 g carbohydrate kg⁻¹ DM, except for JA, which was fed to provide 1.5 g inulin kg⁻¹ DM. Between each 10-d feeding of supplementary feed, hay was fed exclusively for 10 d as a “washout” period. On breath-sampling days (d 1, 3, 8, and 10 of each feeding period), hay was withheld, and carbohydrate intake during the preceding 10 d was counted. Blood glucose (Glucoquant, Roche) was measured by blood sampling. Breath samples were taken before and after feeding at intervals of 30 min over a 10-h period at the end of an exhalation using a tight-fitting face mask. Hydrogen and methane concentrations were analyzed by gas chromatography (GC 14 A, Shimadzu). Once during each feeding period (on d 3), horses were given 500 g of a 50% glucose and 50% grass meal mixture in addition to the test meal. On these days, blood samples were taken at the same time as breath samples to measure blood glucose (Glucquant, Roche). All animal experiments were conducted after approval by the animal welfare committee of the University of Veterinary Medicine and the local government of Lower Saxony.

A multifactor ANOVA for repeated measures was performed (Statistica, version 5.1) with test day, time after feeding, and type of diet as independent and breath H₂ or CH₄ as dependent variables; the LSD test was used as a post hoc test for significance. Results are presented as means ± SD.

RESULTS

Feeding JA and O resulted in a marked, early rise in the H₂ concentration of exhaled breath (Fig. 1); there were significant effects of time and treatment. H₂ concentrations remained fairly constant when GM and SBP were fed (Fig. 1). The time taken to reach maximum breath H₂ concentrations was not significantly different between O and JA (Table 1). CH₄ exhalation was less affected by feeding of O and JA, but it increased to significant levels at ~400 min after ingestion of GM and SBP (Table 1).

The AUC for hydrogen exhalation when horses were fed GM was taken to equal 1; the other feeds were indexed against this base. The response to JA was 4.9, and that for O was 2.6. When the same approach was used for CH₄ exhalation, JA was 0.86, and O was 0.84 in comparison to GM. SBP AUCs were similar to those for GM for both gases (0.97 H₂, 1.02 CH₄).

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⁴ Abbreviations used: GM, grass meal; JA, Jerusalem artichoke; M, body mass; O, oats; SBP, sugar beet pulp.
Plasma glucose levels showed typical postprandial changes (Fig. 2) after feeding of the test meals; there were no apparent differences between feeds when glucose was included. There was a parallel increase in plasma glucose and \( \text{H}_2 \) exhalation (Fig. 2) after feeding, although \( \text{H}_2 \) concentration of exhaled air showed a lag time of 30–60 min. Maximum breath hydrogen values occurred after peak plasma glucose levels had been achieved.

**DISCUSSION**

In healthy horses a lot of hydrogen seems to be produced in the stomach and small intestine, whereas methane seems to be produced almost exclusively in the hindgut; this is a different pattern of hydrogen excretion from that found in humans and is probably a result of differences between humans and horses in terms of their intestinal microbial profile. In healthy humans there are only low counts of bacteria in the small intestine, whereas there are up to \( 10^9 \) cfu/mL of anaerobic microorganisms in the digesta in the foregut of healthy horses (13). The total number of bacteria/mL is similar in both the small and large intestines of the horse. The hydrogen breath test produces a very different result in humans and horses.

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>GM</th>
<th>SBP</th>
<th>O</th>
<th>JA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2 ), mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t_0 )</td>
<td>28.2 ± 18.3(^a)</td>
<td>27.6 ± 17.4(^a)</td>
<td>27.9 ± 13.3(^a)</td>
<td>28.1 ± 21.1(^a)</td>
</tr>
<tr>
<td>Max</td>
<td>38.3 ± 19.9(^a)</td>
<td>33.8 ± 18.8(^a)</td>
<td>105 ± 43.2(^b)</td>
<td>150 ± 69.7(^c)</td>
</tr>
<tr>
<td>Time max, min</td>
<td>198 ± 220(^a)</td>
<td>216 ± 246(^a)</td>
<td>301 ± 117(^ac)</td>
<td>339 ± 92(^bc)</td>
</tr>
<tr>
<td>AUC</td>
<td>11,096 ± 6137(^b)</td>
<td>10,772 ± 5805(^b)</td>
<td>28,152 ± 9539(^b)</td>
<td>52,854 ± 23,594(^c)</td>
</tr>
<tr>
<td>( \text{CH}_4 ), mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t_0 )</td>
<td>565 ± 165(^a)</td>
<td>519 ± 181(^ac)</td>
<td>464 ± 106(^bc)</td>
<td>537 ± 116(^bc)</td>
</tr>
<tr>
<td>Max</td>
<td>736 ± 200(^a)</td>
<td>767 ± 142(^a)</td>
<td>635 ± 173(^b)</td>
<td>625 ± 135(^b)</td>
</tr>
<tr>
<td>Time max, min</td>
<td>391 ± 150(^a)</td>
<td>389 ± 177(^a)</td>
<td>386 ± 211(^b)</td>
<td>466 ± 200(^b)</td>
</tr>
<tr>
<td>AUC</td>
<td>343,345 ± 105,244(^a)</td>
<td>339,240 ± 81,486(^a)</td>
<td>286,001 ± 84,903(^b)</td>
<td>280,390 ± 61,306(^b)</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD, \( n = 6 \), tested on 4 d for each diet during 10 h after eating a meal of oats (O), Jerusalem artichoke (JA), sugar beet pulp (SBP), or grass meal (GM). Each value represents the mean of 6 horses measured using 3-factorial ANOVA and LSD test as post hoc procedure. Means in a row without a common superscript letter differ, \( P < 0.05 \).
tract must be involved in some way, perhaps by modifying prececal fermentation patterns. Until now, work has been focused on the fermentative processes and microbial changes in the hindgut of horses with laminitis, but perhaps now, microbial changes in the foregut of horses should be elucidated.

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LITERATURE CITED