Hydrogen Production in Dogs Adapts to Addition of Lactulose and to a Meat and Rice Diet$^{1,2}$

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EXPANDED ABSTRACT

The hindgut fermentation of undigestible carbohydrate macromolecules yields short-chain fatty acids such as acetate, propionate and butyrate, and gases (Bergman 1990). The last mentioned are mainly hydrogen and methane. These metabolites are readily absorbed from the colon and transported through the blood circulation. Hydrogen and methane pass through the pulmonary alveoles where they are both rapidly eliminated in breath. In human studies, the hydrogen breath test is currently used to assess qualitatively the fermentation process of undigestible substrates (Christl et al. 1992, Rumes- sen 1992). The aim of this study was to apply this approach in dogs fed a control meat-based diet to clarify whether colonic microflora could adapt and ferment an undigestible carbohydrate (lactulose).

Materials and methods. Six adult Beagle dogs (14.3 ± 1.2 kg, mean ± SEM) of both sexes were supplied by the kennels of the National Veterinary School of Nantes. They were studied according to the French Ministry of Agriculture and Fisheries regulatory rules for animal welfare.

Protocol. Dogs were fed once daily the control diet [two thirds beef (S.E.R., Cuiseaux, France, 20% protein, 0% carbohydrate and 15% lipids) plus one-third extruded rice and a vitamin and mineral addition] on the basis of 555 kJ/(kg⁰.⁷⁵ d) of energy requirement for 3 wk. During wk 2, 10 g of lactulose (100% fermentable disaccharide, Duphar, Villeurbanne, France) was added to the control diet. Meals were eaten within 20 min.

On four occasions, breath samples were collected at regular times for 10 h after food intake. The first breath sampling (Experiment A) was performed on d 7, after 1 wk of consuming the control diet. The next one (Experiment B) was done the following day, d 8, the first day lactulose was added. After 1 wk of lactulose adaptation, breath samples were collected on d 14 (Experiment C). A final breath sampling (Experiment D) was performed on d 21, after a final week consuming the control diet.

Breath sampling technique. The day of the study, dogs were placed in individual boxes. The breath sampling was performed with a permeable bag filled through a dual-inlet valve system connected to a mask that was put on the dog's face. During the inhalation, the bag valve automatically closed, whereas during the exhalation, breath was allowed to enter the bag through the open valve. The bag was filled within 2–4 min. On d 7 and 21, samples were collected every 30 min and on d 8 and 14, they were collected every 20 min. Each breath sample (30 mL) was kept in an airtight syringe at 4°C until analyzed within 24 h.

Analysis of gases. Hydrogen and methane concentrations in breath samples were measured on a Microlyzer DP gas chromatograph (Quintron instrument, Milwaukee, WI). Results were expressed as parts per million (1 ppm ~ 0.05 μmol/L).

Statistics. All results are expressed as means ± SEM (n = 6 dogs). Paired t tests were performed between the peak and the initial level of hydrogen breath response for different experiments with Instat statistical software (GraphPad, San Diego, CA). Experiments were compared by using ANOVA with Instat software.

Results. The weight of the dogs was unchanged during the 3 wk of the study. The methane level in the dogs' breath was insignificant throughout all experiments (CH₄ < 2 ppm). Hydrogen level increased 5 h after the meal in all four experiments. In Experiment A (Fig. 1), during the first 5 h, hydrogen level was 2 ± 1 ppm; it increased rapidly and significantly up to 24 ± 10 ppm from t = 5–8 h and returned to near basal level at 10 h (8 ± 1 ppm). The first day of lactulose addition (Experiment B), hydrogen excretion increased progressively from 4 ± 2 ppm at 2 h to 21 ± 2 at 7 h without any return to the initial level after 10 h (Fig. 2). In contrast, after 7 d of lactulose ingestion (Experiment C), the hydrogen breath concentration was low from time 0 to 5 h 20 min (4 ± 1 ppm) and, after a slight increase, remained at a low level from 5 h 40 min to 10 h (7 ± 1 ppm). This plateau was significantly lower

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than that in the other 3 experiments (P < 0.05, peak vs. peak, Fig. 2). After 1 wk of again consuming the control diet (Experiment D), the hydrogen stabilized throughout the first 5 h and increased rapidly from 4 ± 1 ppm (t = 5 h) to 19 ± 8 ppm (t = 9 h, Fig. 3).

Discussion. This study focused on breath hydrogen excretion as evidence of microflora activity in the intestinal tract of dogs. The beagles fed the control diet (meat and extruded rice) exhaled hydrogen 5 h after feeding. The addition of lactulose to the food induced an increase in the area under the curve of breath hydrogen on d 1, whereas daily supplementation decreased the hydrogen exhalation considerably. This study demonstrates an adaptation of the microflora in dogs after chronic ingestion of lactulose.

Experiment A. Although the rice and meat meal was a low residue diet, part of it escaped digestion and was degraded by microflora, as shown from a net increase of breath hydrogen 5–6 h after eating. Some of the ingested food may have reached the microbial activity in the colon, undergone fermentation and produced hydrogen. Recent studies in dogs offer another explanation; some of the diet can be catabolized by bacteria in the ileum, and these microorganisms are capable of degrading amino acids and of producing a large amount of hydrogen (Zentek 1995a and 1995b).

Experiments B and C. To assess the fermentation process, dogs were fed a basal diet composed of rapidly and highly digestible meat and carbohydrate to which a nondigestible carbohydrate had been added (lactulose). Although the basal diet induced exhalation of hydrogen breath, which is evidence of bacterial activity, lactulose supplementation resulted in a major change in gas excretion. On d 1 of lactulose intake (Experiment B), the transit time has shortened, and the microflora produced a larger amount of hydrogen as represented by the greater area under the curve. Lactulose may have modified the upper gastrointestinal transit rate, thereby spreading the dietary substrate irregularly to the microflora. Thus, undigested substrate could rapidly reach the ileum, resulting in rapid hydrogen exhalation probably produced from amino acid degradation (Zentek 1995a). Lactulose, which is degraded by colonic bacteria in nonruminants (Bergman...
1990), would be a likely source of the hydrogen produced in the large intestine 10 h after meal ingestion, compared with hydrogen exhalation in Experiment A, which returned toward its initial level at the end of the study (10 h).

After 7 d of lactulose supplementation (Experiment C), hydrogen exhalation had dramatically diminished although an increase at 5 h 40 min was still apparent. This decrease in breath hydrogen could have come from a shorter transit time and a quicker flushing of the intestine, although no diarrhea was noticed. The cecal content could be rapidly emptied into the distal colon, resulting in less bacterial activity and hydrogen production. This decrease in hydrogen exhalation was also observed in humans who consumed lactulose daily for 1 wk (Florent et al. 1985). Cecal acetate concentration also increased, showing a change in the microbial behavior and metabolism probably due to lactulose supplementation. The authors concluded that the fermentation process within the microflora evolved to a lower production of hydrogen, with the metabolism of the microflora changing and adapting to a new environment enriched in lactulose. This change may also be the result of a competition between bacteria, resulting in a flora composed of fewer hydrogen producers or more hydrogen consumers. Clearly, an adaptation of the intestinal microflora occurred during the lactulose supplementation. Dogs chronically fed lactulose demonstrate behavior similar to that of humans who have colonic microflora capable of entirely fermenting the lactulose fraction. Our study suggests that dogs adapt and probably ferment lactulose at a low level compared with humans because breath hydrogen test values of humans are three or four times higher than those of dogs when similar amounts of lactulose are ingested (Rumessen 1992).

From this study, it is clear that a meat and extruded rice diet induces hydrogen breath production probably due to partial degradation by bacteria in the intestine; adaptation to lactulose induces a change in the colonic bacterial flora that results in a decrease in hydrogen production. Dogs probably contain microflora in the intestine that spreads from the ileum to the distal colon with different populations capable of adapting quickly to the nondigested substrates. Further microbial studies in the lumen of the alimentary tract would be most beneficial in understanding to what extent dogs are capable of degrading fermentable carbohydrates.

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LITERATURE CITED


