Intestinal Effects of Mannanoligosaccharides, Transgalactooligosaccharides, Lactose and Lactulose in Dogs¹

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EXPANDED ABSTRACT

Carbohydrates that are indigestible by mammalian enzymes can influence the composition and metabolic activity of the intestinal microflora and are therefore of interest for the formulation of pet food and specific veterinary diets. Mannanoligosaccharides are isolated from yeast cell walls (1). They are not hydrolyzed by digestive enzymes but by different lactobacilli and some bifidobacteria (2,3) and seem to be less fermentable by intestinal bacteria than are fructooligosaccharides (4–7). Galactooligosaccharides consist of β-1,6-linked galactopyranosyl units and an α-glycosidic bonding to a terminal glycopyranosyl residue and have been shown to be fermentable by canine intestinal microflora (6). Transgalactooligosaccharides are produced by the β-galactosidase from Aspergillus oryzae (8). Lactose can be regarded as a facultative fermentable carbohydrate in dogs. The activity of intestinal lactase decreases age dependently with concomitant compensatory fermentation by small intestinal and colonic bacteria (9). Lactulose is an isomeric form of lactose with one galactose molecule linked to fructose by β-1,4-linkage. In vitro investigations demonstrated that lactulose is readily fermented by bifidobacteria and lactobacilli, but also by Clostridium perfringens, Escherichia coli and Bacteroides sp. (10–12). A decrease of colonic pH and blood ammonia concentrations in dogs was found after ingestion of lactulose (13,14).

In the present study mannanoligosaccharides, transgalactooligosaccharides, lactose and lactulose were added to a mixed diet for dogs and investigated for their effects on the fecal quality, nutrient and mineral digestibilities and on some products of intestinal microbial metabolism.

MATERIALS AND METHODS

Animals

Four adult female beagles with an average body weight of 11.7 ± 2.2 kg. Care and treatment of the animals was approved by governmental commission according to the procedures of the animals’ protection law. The dogs were vaccinated and dewormed as usual and housed individually.

Diet

The basal diet⁴ (17 g/kg BW/d) was fed without added carbohydrates in the control periods I and II. Mannanoligosaccharides (MOS, Bio Mos; Alltech, Bad Segeberg, Germany), transgalactooligosaccharides (TGOS, Lactift; Borculo Whey Products, Borculo, The Netherlands), lactose (Variolac 99; Biolac GmbH, Harbarnsen, Germany) or lactulose (Lactuverlan; Verla-Pharm, Tutzing, Germany) were dosed individually for each dog (1 g/kg BW/d) and mixed with the basal diet during four supplementation periods. The experiment was designed as a 4 × 4 Latin square and the dogs received the basal diet without additives before and after the four diets were supplemented with the fermentable carbohydrates. The adaptation periods lasted at least 10 d before collection of the samples.

Variables

The apparent digestibility of crude nutrients [methods in Nau- mann and Bassler (15) and macrominerals, by wet ashing in a mixture of perchloric and nitric acid, atomic absorption spectrophotometry for calcium and magnesium (16), flame photometry for sodium and potassium (17), vanadate molybdate method for phosphorus (18)] was measured after 5-d collection periods in a metabolism cage. Additional variables were a daily scoring of fecal consistency, determination of fecal dry matter (oven drying to weight constancy) and of

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³ Abbreviations used: BW, body weight; IU, international units; MOS, basal diet with mannanoligosaccharides; TGOS, basal diet with transgalactooligosaccharides.

⁴ Ingredients: dry greaves (35%), pressure-cooked rice (35%), fish meal (5%), soya oil (20%), cellulose 3%, and vitamin and mineral supplement (2%); Vitakalk: 21% calcium, 8% phosphorus, 6% sodium, 1% magnesium; per kg: 500,000 IU vitamin A, 40,000 IU vitamin D3, 1000 mg vitamin E, 700 mg copper; Marienfelde GmbH, Roth, Germany. Composition of the mixed diet (g/kg): dry matter, 945; crude ash, 39.8; crude protein, 366; crude fat, 244; crude fiber, 49.5; calcium, 6.76; magnesium, 0.53; phosphorus, 5.06; sodium, 4.23; potassium, 3.06; chloride, 5.31.
RESULTS AND DISCUSSION

The apparent digestibilities of dry matter, crude protein and N-free extracts were lower with the mannanoligosaccharides (Table 1) than those with the other dietary periods.

Apparent absorption rates of calcium (58.9%), magnesium (93.8%), potassium (90.9%), and phosphorus (62.1%) were not in

Statistics

Data were processed by EXCEL 5.0 and SAS 6.04 (20). ANOVA and Tukey test were used for comparison of the Latin square with the supplemented diets. Comparison of supplemented and basal diets I and II was done by t-test. Probability values of <0.05 were taken as significant.

unbound water by centrifugation (weight of fluid after centrifugation of 2 g of feces for 30 min, 30,000 x g; Sorvall Superspeed RC2-B, DuPont, Bad Homburg, Germany) of pH (Knick-pH-Meter; Knick, Berlin, Germany) and volatile fatty acids (VFA) (capillary chromatograph PU 4550; Pye Unicam, Offenbach, Germany) and of pH (Knick-pH-Meter; Knick, Berlin, Germany), and volatile fatty acids (VFA) (capillary chromatograph PU 4550; Pye Unicam, Offenbach, Germany) and urinary nitrogen, indican (19) and urea excretion (Urea Kit, bio Merieux, Nürtingen, Germany). Fecal suspensions (1:10 in pre-reduced physiological saline) were incubated under nitrogen atmosphere for 24 h with measurement of total gas formation and production of VFA.

| TABLE 1 |
| Apparent digestibilities (means ± sd) of dry matter and crude nutrients (% of intake; n = 4)1 |

<table>
<thead>
<tr>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Nitrogen-free extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet I</td>
<td>85.0 ± 1.9†</td>
<td>82.5 ± 3.2†</td>
<td>96.2 ± 0.2</td>
<td>61.8 ± 8.2</td>
</tr>
<tr>
<td>+ MOS2</td>
<td>81.9 ± 0.66†</td>
<td>79.8 ± 1.96†</td>
<td>96.6 ± 0.5a</td>
<td>69.1 ± 2.1a†</td>
</tr>
<tr>
<td>+ TGOs3</td>
<td>87.2 ± 2.4a</td>
<td>85.9 ± 3.6a</td>
<td>96.9 ± 1.0a</td>
<td>67.9 ± 1.7a†</td>
</tr>
<tr>
<td>+ Lactose</td>
<td>87.3 ± 2.8a</td>
<td>86.2 ± 3.7a</td>
<td>97.1 ± 1.0a</td>
<td>67.4 ± 7.5a</td>
</tr>
<tr>
<td>+ Lactulose</td>
<td>86.5 ± 1.9a</td>
<td>84.4 ± 1.0a</td>
<td>96.7 ± 0.7a</td>
<td>71.0 ± 6.1at</td>
</tr>
<tr>
<td>Basal diet II</td>
<td>89.5 ± 0.7a</td>
<td>91.3 ± 0.5a</td>
<td>78.6 ± 0.5</td>
<td>57.7 ± 2.6</td>
</tr>
</tbody>
</table>

1 Means within a column not sharing a common superscript are significantly different at P < 0.05.
2 Basal diet with mannanoligosaccharides.
3 Basal diet with transgalactooligosaccharides.
* Significant difference from basal diets, period I (*) or II (†) (ANOVA and Tukey test for the supplemented periods, t-test for comparison of supplemented and basal diets).

In conclusion, lactose, lactulose or transgalactooligosaccharides had no negative effects on stool quality. The lowest unbound water (5.5%) was found with mannanoligosaccharides (Table 2), accompanied by a lower fecal pH (6.6%). Fecal ammonia concentration and excretion were higher with basal diet I than with basal diet II. The ammonia concentrations were lower after the addition of mannanoligosaccharides than that in basal diet I and the lactulose period.

The fecal VFA concentrations ranged from 139 to 209 mmol/L with no differences attributed to diet (data not shown). Dietary alterations did not influence the renal nitrogen (45–60 mmol/kg BW/d), urea (18–27 mmol/kg) or indocan (10–16 μmol/kg) excretion. Ammonia yield from anaerobic in vitro incubation of fecal suspensions for 24 h in dogs fed lactulose was higher than that when either basal diet was fed. There was a similar trend when the other three supplemented diets were fed (Table 3).

Total gas production was lowest in the initial control period (basal diet I) compared with that of the other experimental diets. The concentrations of VFA (Table 3), mainly of acetic acid, increased during the incubation, whereas the proportion of propionic acid and n-butyric acid decreased. There were no obvious dietary influences on in vitro fermentation.

In conclusion, lactose, lactulose or transgalactooligosaccharides did not alter the measures of microbial metabolism, compared to those of the control periods. Higher intakes might have induced clearer changes, but according to our own preliminary experiments a dosage of 2 g lactulose/kg BW/d induced diarrhea, which shows comparatively narrow limits

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Fecal consistency, dry matter, unbound water, pH and ammonia (means ± sd; n = 4)1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency2</td>
<td>Dry matter, %</td>
</tr>
<tr>
<td>Basal diet I</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>+ MOS2</td>
<td>3.6 ± 0.4a</td>
</tr>
<tr>
<td>+ TGOs3</td>
<td>3.5 ± 0.4a</td>
</tr>
<tr>
<td>+ Lactose</td>
<td>3.5 ± 0.4a</td>
</tr>
<tr>
<td>+ Lactulose</td>
<td>2.9 ± 0.5a†</td>
</tr>
<tr>
<td>Basal diet II</td>
<td>3.7 ± 0.1</td>
</tr>
</tbody>
</table>

1 Means within a column not sharing a common superscript are significantly different at P < 0.05.
2 Consistency: 1 = diarrhea (liquid); 2 = unformed, wet; 3 = formed, but smearable; 4 = formed (optimum consistency); 5 = firm (hard, crumbly).
* Significant difference from basal diets, period I (*) or II (†) (ANOVA and Tukey test for the supplemented periods, t-test for comparison of supplemented and basal diets).
between tolerance and intolerance in dogs. Mannanooligosaccharides resulted in a lower fecal pH, ammonia excretion and apparent digestibilities of crude protein, nitrogen-free extracts and dry matter, compared to those of the control periods and the other carbohydrates. The percentage of total fecal water increased and the unbound water decreased substantially during this period, which can be interpreted as a change in the physical properties of the intestinal chyme. This higher water binding could have influenced the solubility of nutrients, which might explain the lower digestibilities and also the activity of the intestinal microflora. Further studies are warranted to confirm these effects and the underlying mechanisms.

**LITERATURE CITED**


