British Journal of Nutrition (2003), **90**, 1007–1014
DOI: 10.1079/BJN2003988

Prebiotics affect nutrient digestibility but not faecal ammonia in dogs fed increased dietary protein levels

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(Received 2 January 2003 – Revised 12 June 2003 – Accepted 28 July 2003)

An increased protein content and less digestible protein sources in the diet can induce bad faecal odour. The present study investigated the effect of adding prebiotics to dog diets enriched with animal-derived protein sources on apparent digestibilities and faecal ammonia concentration. In three subsequent periods eight healthy beagle dogs were fed a commercial dog diet that was gradually supplemented by up to 50 % with meat and bone meal (MBM), greaves meal (GM) or poultry meal (PM) respectively. Afterwards, 3 % fructo-oligosaccharides or 3 % isomalto-oligosaccharides were substituted for 3 % of the total diet. Supplementation with animal-derived protein sources did not decrease the apparent N digestibility significantly but oligosaccharides did. On the other hand the bacterial N content (% DM) in the faeces was highest in the oligosaccharide groups followed by the protein-supplemented groups and lowest in the control groups. When the apparent N digestibility was corrected for bacterial N no significant differences were noted anymore except for the GM group where the corrected N digestibility was still lower after oligosaccharide supplementation. The amount of faecal ammonia was significantly increased by supplementing with protein or oligosaccharides in the MBM and GM groups but not in the PM group. When apparent N digestibility is interpreted, a correction for bacterial N should be taken into account, especially when prebiotics are added to the diet. Oligosaccharides did not reduce the faecal ammonia concentrations as expected.

Prebiotics: Dogs: Digestibility: Ammonia

Prebiotics are non-digestible feed components that benefit the host's health by selectively stimulating the growth of one or a limited number of bacteria in the large intestine. Non-digestible oligosaccharides in general and fructo-oligosaccharides (FOS) in particular are commonly accepted as prebiotics (Gibson & Roberfroid, 1995). Flickinger *et al.* (2000) indicated that α-gluco-oligosaccharides resisted hydrolytic digestion in dogs and were fermented in the large intestine. The distinction between oligofructose and inulin is made on the basis of their chain length, which can be defined by the degree of polymerisation. The average degree of polymerisation of oligofructose is 4·8, whereas inulin has an average degree of polymerisation of 12. Inulin consists mainly or exclusively of β-2-1 fructosyl-fructose units (Roberfroid & Delzenne, 1998). Inulin is extracted from chicory roots by using a hot-water process and oligofructose is formed by the controlled enzymic hydrolysis of inulin.

Isomalto-oligosaccharides (IMO), α-1-6-linked glucosyl residues, are not completely indigestible since they are digested very slowly in man and strictly speaking they are only partially prebiotic (Rastall *et al.* 2000). In most species, amino acids and small peptides are derived from ingested feed protein by enzymic digestion in the small intestine and are absorbed in that part of gastrointestinal tract. Especially in the large intestine, absorbed N is mostly derived from ammonia produced by microbes from N entering the large intestine. This N is not utilised for protein synthesis but is excreted as urea in the urine. With the addition of fermentable carbohydrates to the diet, microbial mass is produced during fermentation similar to the increase of microbial mass in the rumen. As a consequence, the addition of fermentable fibre to a diet may induce a lower apparent digestibility of protein. Silvio *et al.* (2000) suggested that this was not due to a lower digestion of feed protein, but rather the consequence of higher bacterial protein excretion. Adding 8 % of oligofructose and 2 % of beet pulp to the diet of dogs slightly reduced the apparent protein digestibility (Diez *et al.* 1997).

During putrefaction in the colon, several components can be produced from endogenous and undigested amino acids by deamination, deamination–decarboxylation or carboxylation (Macfarlane & Cummings, 1991). The major groups are ammonia, aliphatic amines, branched-chain fatty acids,

Abbreviations: ADC, apparent digestibility coefficient; C_{GM}, control diet after greaves-meal diet; C_{MB}, control diet after meat-and-bone-meal diet; C_{PM}, control diet after poultry-meal diet; FOS, fructo-oligosaccharides; GM, greaves meal; IMO, isomalto-oligosaccharides; MBM, meat and bone meal; PM, poultry meal.

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indoles, phenols and volatile S-containing compounds (Macfarlane & Macfarlane, 1995 cited in Hussein & Sunvold, 2000). Adding lactosucrose to a dog food decreased the faecal concentrations of ammonia and other odour components (Terada *et al.* 1992). On the other hand, high-protein diets, especially with a poor-quality protein source, can induce a negative effect on faecal odour (Griess & Enjalbert, 1992). High-protein diets will induce higher numbers of *Clostridium perfringens* whereas fermentable fibres will increase *Bifidobacteria* and *Lactobacilli* concentration (Zentek, 2000).

The present study investigated the effect of adding prebiotics to a maintenance dog diet enriched with animal protein sources on the apparent digestibility of nutrients. The animal protein sources were added to a balanced ration in order to increase the faecal N output and to stimulate faecal odour, generated by volatile N components, especially ammonia. In the present study the influence of two different prebiotics on faecal odour generated by the addition of three different protein sources to a basal diet was measured in dogs.

Materials and methods

In three consecutive periods, eight healthy adult beagle dogs were fed a commercial dog diet (RCCI size Medium Adult 1 M25; Royal Canin, Brussels, Belgium). This was supplemented with an equal amount (w/w) of meat and bone meal (MBM; Quartes, Deinze, Belgium) (period I), greaves meal (GM; Quartes, Deinze, Belgium) (period II) and poultry meal (PM; Quartes, Deinze, Belgium) (period III) (Fig. 1; Table 1). Because only eight dogs were available, the three protein sources were tested in three different periods. The protein sources were thus confounded by time in this design, but a control diet was inserted in every period after a washout period. Showing differences in protein sources was not the aim of the present study. The purpose of the trial was to investigate the effect of prebiotics on high-protein diets.

During the adaptation period, these protein sources were added progressively, starting with 20 % on the first day and increasing with another 10 % every 3 d in order to reach 50 % of total diet (w/w) at the 10th day. The 50 % supplemented diet was fed during 12 d; a 7 d pre-collection period followed by a 5 d faecal collection period (collection period A). The protein supplements were introduced gradually to decrease the risk of diarrhoea. After the first collection period (A) the dogs were randomly assigned to two groups. Four dogs (group I) received the supplemented diet in which 3 % of the total ration was replaced by FOS (Raftilose P95; Orafti s.a., Oreye, Belgium). In the diet of the other four dogs (group II) 3 % IMO (CO19T0; Cerestar, Vilvoorde, Belgium) were substituted for 3 % of the total diet. Both groups received the oligosaccharides-supplemented diet for 14 d; a 9 d pre-collection period followed by a 5 d collection period (collection period B). This was followed by a 16 d washout period with the control diet (all eight dogs) and a subsequent third collection period of 5 d (collection period C). The whole protocol was first completed with the MBM (period I), then with the GM (period II) and last with the PM (period III). The same dogs were allocated to receive the same oligosaccharide source throughout the whole study. At 5 d before each collection period, 1·5 % Celite (VWR International, Leuven, Belgium), a non-digestible mineral, was added to the diet as a marker for faecal production as described by Kotb & Luckey (1972). At that time, the dogs were moved to digestibility cages. During the whole study, except for the collection periods and the preceding 5 d, the dogs were housed by two in a kennel with free outdoor access.

Table 1. Diet analysis (as-fed basis)

	Control diet*	C + MBM†	C + GM†	C + PM†
Moisture (%)	8·7	7·2	6·5	6·8
Crude protein (%)	27	45	50	45
Crude fat (%)	7·5	5·7	10·2	10·4
Crude ash (%)	3·9	15·5	6·5	8·6

C + MBM, control diet + meat and bone meal (50:50); C + GM, control diet + greaves meal (50:50); C + PM, control diet + poultry meal (50:50).
* The control diet is a commercial dog diet (RCCI size Medium Adult 1 M25; Royal Canin, Brussets, Belgium).
† The protein supplements (meat and bone meal, greaves meal and poultry meal) are supplied by Quartes (Deinze, Belgium).

The dry control diet was ground to allow thorough homogenisation with the protein sources, prebiotics and Celite. The food was mixed with an equal amount of lukewarm water to increase palatability. The diets were given to meet the daily energy requirements estimated at 415 kJ metabolisable energy/kg$^{0·75}$. During the experiment, body weights were checked and one dog was losing weight. Therefore, the amount of food for this dog was increased by 10 % during all periods except for the collection periods and the preceding 5 d. The ratios of the basal diet to the different supplements were always respected. During the washout period, all dogs were fed *ad libitum* (Fig. 1).

Food and water intake and faecal productions were registered daily during the collection periods. A code for

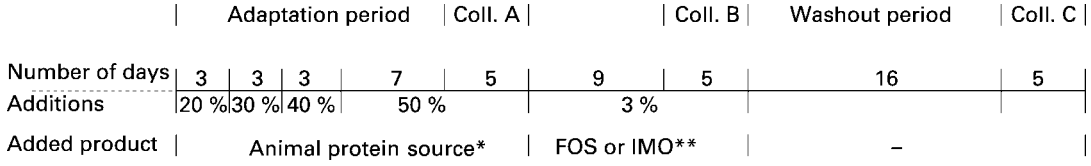

Fig. 1. Testing schedule. The study was divided into three testing periods, during which a different animal-protein source was added to the basal diet. Each testing period consisted of 56 d, as shown in the schedule, and included three collection periods (COLL. A, B and C) of 5 d. *During the first period (testing Period I) the added animal-protein source was meat and bone meal, during testing Period II greaves meal was used, and during the last testing period (Period III) poultry by-product meal was added to the basal diet. **3 % of the protein supplemented diet was replaced by fructo-oligosaccharides (FOS) or isomalto-oligosaccharides (IMO).

faecal consistency (3 being normal, 4 being constipation, 1 being watery diarrhoea) was noted daily for each defecation. Every morning, faeces were collected and frozen. Between 08.00 and 16.30 hours, the dogs were checked hourly for defecation. Faecal samples were collected as freshly as possible and immediately frozen until the measurement of faecal ammonia and pH. If no defecation had occurred by 16.30 hours, a rectal faecal sample was taken carefully.

Morning faeces were used to determine the apparent digestibility of nutrients. Digestibility parameters were calculated using the indicator method. Crude protein was calculated from Kjeldahl N values (N × 6·25) and fat content was determined by acid hydrolysis followed by diethyl ether extraction (Association of Official Analytical Chemists, 1984). In the morning faeces, bacterial N was estimated as soluble N according to the method of Mason (1969), using SDS to dissolve the bacterial material. The basal diet contained 10·07 % (as-fed basis) SDS-soluble protein (N × 6·25). The three mixed diets with 50 % MBM, GM, or PM all contained similar percentages of SDS-soluble protein (8·84–10·47 %). The Mason (1969) method was described for use in sheep. After analysis of the pure animal protein sources, it was concluded that the SDS-soluble protein not only contained protein of bacterial origin but also of animal origin. Because of the high levels of SDS-soluble protein in the diets, the Mason (1969) method was adapted for diets rich in animal protein. The samples were divided into two samples: one was analysed after adding SDS (Mason (1969) method); one was analysed without adding SDS. In the last sample, the bacterial N fraction is included in the insoluble fraction together with the protein of vegetable origin. The soluble fraction only contains protein of animal origin. Bacterial N was estimated by difference after analysis with and without SDS. The percentages of bacterial protein estimated by this method were between 0·48 and 2·75 % in all diets. This might be attributed to the addition of animal protein sources where parts of the gastrointestinal tract with their bacterial flora are included. For the faecal samples the adapted Mason (1969) method was also used. Ammonia (as the sum of NH_3 and NH_4^+) was determined by steam distillation.

The experimental procedures and housing were approved by the Ethical Committee of the Faculty of Veterinary Medicine at the Ghent University (02/02/01). All statistical analyses were performed using SPSS 10·0 (SPSS, Chicago, IL, USA). The univariate general linear model with treatment, period, animal and all two-way interactions as fixed factors was used for statistical evaluation. The model did not exclude confounding between protein source and time but the aim was not to compare the different protein sources as such but to test the effect of prebiotics in several protein-supplemented diets.

Results

Except for the faecal DM and moisture content (%), no differences were noticed between supplementation with FOS and IMO. Faecal DM content was slightly but significantly higher in the FOS-supplemented dogs (42·7 %) compared with the IMO-supplemented dogs (40·5 %) ($P = 0·014$). Because no other significant differences between FOS and IMO were noted, the two oligosaccharide sources were statistically processed as one group.

Every day, the total ration was eaten within 5 min by all dogs during all collection periods. Adding MBM to the basal diet significantly increased the daily faecal amount compared with the control diet (control diet after MBM diet; C_{MB}) (Table 2). GM also increased faecal weight, though not statistically significantly so, whereas PM did not cause any increase. Adding oligosaccharides further increased daily faecal production in all three groups, although statistical significance was only reached for the GM diet. The moisture content (%) of the faeces was highest in the control diet and was significantly decreased after adding the different protein sources. The oligosaccharides did not influence the faecal moisture content. Although the Pearson correlation coefficient was low (11·4 %), there was a significant ($P = 0·039$) correlation between faecal DM content (%) and the code for faecal consistency (results not shown). The addition of the different protein

Table 2. Faecal characteristics*†
(Mean values and standard errors of the mean)

	Meat and bone meal			Greaves meal			Poultry meal			
	C+MBM	C+MB+O	C_{MB}	C+GM	C+GM+O	C_{GM}	C+PM	C+PM+O	C_{PM}	SEM
Faecal production (g/d)	116·3^{bcd}	122·6^{d}	94·3^{a}	100·5^{ab}	119·2^{cd}	93·04^{a}	104·1^{abc}	115·4^{bcd}	102·7^{abc}	2·12
Faecal moisture (%)	58·2^{ab}	56·7^{a}	62·4^{c}	58·6^{ab}	60·1^{bc}	62·6^{c}	59·2^{ab}	58·3^{ab}	65·9^{d}	0·42
Faecal DM (g/d)	48·5^{c}	52·9^{c}	35·1^{a}	41·3^{b}	47·5^{c}	34·7^{a}	42·1^{b}	47·7^{c}	34·8^{a}	1·36
Water intake (g/d)	326·6^{ab}	357·8^{b}	244·4^{a}	308·9^{ab}	368·7^{b}	294·0^{ab}	307·8^{ab}	350·8^{ab}	264·5^{ab}	11·4

C+MBM, control diet + meat and bone meal (50:50); C+MBM + O, C+MBM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{MB}, control diet after washout period; C+GM, control diet + greaves meal (50:50); C+GM+O, C+GM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{GM}, control diet after washout period; C+PM, control diet + poultry meal (50:50); C+PM+O, C+PM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{PM}, control diet after washout period.

^{a,b,c,d}Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and Figure 1.

† Fructo-oligosaccharide supplement was Raftilose P95 (Orafti s.a., Oreye, Belgium) and Isomalto-oligosaccharide supplement was CO19TØ (Cerestar, Vilvoorde, Belgium).

sources significantly increased the absolute faecal DM production with MBM having the most pronounced effect. The supplementation with oligosaccharides further increased faecal DM production except for the MBM group, in which faecal DM production was already more prominent. Water intake tended to increase in the MBM-supplemented diet and increased significantly by the addition of oligosaccharides compared with the control diet. Similar trends were noted in the other periods.

The apparent DM digestibility (apparent digestibility coefficient; ADC) was significantly reduced after adding the protein supplements to the control diets (Table 3). The effect was most pronounced in the MBM-supplemented group, as already expected from the absolute faecal DM productions. Adding oligosaccharides further decreased the DM ADC.

The apparent digestibility of the diethyl ether extract was not reduced by adding GM or PM, but was significantly decreased by the addition of MBM (Table 3). The supplementation with oligosaccharides reduced the digestibility of the diethyl ether extract even further in the MBM group whereas it did not in the GM or PM group. Supplementation with the animal-derived protein sources did not decrease the apparent N digestibility; on the contrary, there was a trend for a higher N digestibility compared with the control diet. The apparent N digestibility was the same for the three animal protein-supplemented diets. The addition of oligosaccharides, on the other hand, caused a significant reduction in the apparent N digestibility in the MBM- and GM-supplemented groups, with the greatest reduction in the GM group, and a non-significant decrease in the PM-supplemented group.

Compared with the control diet, the bacterial N content (on a DM basis) was significantly increased by adding the protein sources. However, when the bacterial N was expressed as a percentage of faecal N, significance was only reached in the MBM and PM groups. Adding the protein sources had no effect on bacterial N expressed as a percentage of N intake. Moreover, an unexpected significant difference was observed in both bacterial N on a DM basis or as a percentage of N intake between the control diet in period III (control diet after PM diet; C_{PM}) and in periods I (C_{MB}) and II (control diet after GM diet; C_{GM}). Bacterial N, expressed as a percentage of faecal N, showed a linear increase from C_{MB} to C_{PM}. Bacterial N as a percentage of faecal N was higher in the PM group than in any other group with or without oligosaccharides. The percentage of bacterial N on a DM basis and also as a percentage of faecal N or N intake was significantly increased by adding oligosaccharides to the protein-supplemented diets with the most pronounced effect in GM. The lower apparent N digestibility after supplementation with oligosaccharides disappeared after correction for bacterial N in MBM but not completely in GM. The corrected N digestibility in the MBM group was significantly higher compared with the control diet.

In periods II (GM) and III (PM), the faecal pH (Table 4) was significantly lower in the control diets (C_{GM} and C_{PM}) than in the protein- and oligosaccharide-supplemented diets. A similar but non-significant effect was seen in the MBM group. Again, the response of the control diet after MBM (C_{MB}) was significantly different from C_{PM}. The highest faecal pH in the control diets was measured in the MBM group followed by the GM and PM groups.

The faecal ammonia concentration (on a DM basis) increased significantly by adding MBM and GM to the control diet and tended to increase in the PM-supplemented diet (Table 4). Ammonia as a percentage of faecal N was only increased when MBM was supplemented. It was also significantly lower in the control group during period III

Table 3. Apparent digestibility coefficients (ADC) and bacterial nitrogen content*§
(Mean values and standard errors of the mean)

	Meat and bone meal			Greaves meal			Poultry meal			
	C+MBM	C+MB+O	C_{MB}	C+GM	C+GM+O	C_{GM}	C+PM	C+PM+O	C_{PM}	SEM
ADC of DM (%)	$74{\cdot}3^{ab}$	$71{\cdot}9^{a}$	$81{\cdot}1^{e}$	$78{\cdot}2^{d}$	$75{\cdot}0^{bc}$	$81{\cdot}3^{e}$	$77{\cdot}7^{cd}$	$74{\cdot}8^{ab}$	$81{\cdot}2^{e}$	0·50
ADC of diethyl ether extract (%)	$84{\cdot}4^{b}$	$81{\cdot}3^{a}$	$88{\cdot}2^{cd}$	$88{\cdot}5^{cd}$	$89{\cdot}3^{d}$	$86{\cdot}4^{bc}$	$90{\cdot}2^{d}$	$88{\cdot}3^{cd}$	$89{\cdot}7^{d}$	0·40
ADC of N (%)	$83{\cdot}1^{c}$	$79{\cdot}7^{b}$	$81{\cdot}0^{bc}$	$83{\cdot}4^{c}$	$76{\cdot}6^{a}$	$81{\cdot}7^{bc}$	$83{\cdot}1^{c}$	$80{\cdot}3^{bc}$	$80{\cdot}9^{bc}$	0·40
Bacterial N† (% faecal DM)	$1{\cdot}62^{b}$	$2{\cdot}04^{c}$	$1{\cdot}3^{a}$	$1{\cdot}95^{c}$	$2{\cdot}84^{e}$	$1{\cdot}34^{a}$	$2{\cdot}16^{c}$	$2{\cdot}47^{d}$	$1{\cdot}62^{b}$	0·52
Bacterial N† (% faecal N)	$31{\cdot}8^{bc}$	$37{\cdot}2^{e}$	$27{\cdot}5^{a}$	$29{\cdot}7^{ab}$	$35{\cdot}7^{de}$	$29{\cdot}0^{ab}$	$37{\cdot}0^{e}$	$41{\cdot}7^{f}$	$33{\cdot}6^{cd}$	0·60
Bacterial N (% N intake)	$5{\cdot}38^{abc}$	$7{\cdot}55^{d}$	$5{\cdot}20^{ab}$	$4{\cdot}95^{a}$	$8{\cdot}39^{d}$	$5{\cdot}3^{abc}$	$6{\cdot}23^{bc}$	$8{\cdot}18^{d}$	$6{\cdot}44^{c}$	0·19
Corrected ADC of N‡ (%)	$88{\cdot}4^{cd}$	$87{\cdot}3^{bcd}$	$86{\cdot}2^{ab}$	$88{\cdot}3^{bcd}$	$85{\cdot}0^{a}$	$87{\cdot}0^{abc}$	$89{\cdot}3^{d}$	$88{\cdot}5^{cd}$	$87{\cdot}3^{bcd}$	0·27

C+MBM, control diet + meat and bone meal (50:50); C+MB+O, C+MB containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{MB}, control diet after washout period; C+GM, control diet + greaves meal (50:50); C+GM+O, C+GM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{GM}, control diet after washout period; C+PM, control diet + poultry meal (50:50); C+PM+O, C+PM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{PM}, control diet after washout period.

[a,b,c,d,e]Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* For details of diets and procedures, see Table 1 and Figure 1.

† Bacterial N was estimated according to Mason (1969) adapted for animal protein sources.

‡ ADC of N corrected for bacterial nitrogen; N digestibility was calculated instead of protein digestibility to avoid the use of conversion factors.

§ Fructo-oligosaccharide supplement was Raftilose P95 (Orafti s.a., Oreye, Belgium) and isomalto-oligosaccharide supplement was CO19TØ (Cerestar, Vilvoorde, Belgium).

Table 4. pH and ammonia content in freshly collected faeces*‡
(Mean values and standard errors of the mean)

	Meat and bone meal			Greaves meal			Poultry meal			
	C+MBM	C+MB+O	C_{MB}	C+GM	C+GM+O	C_{GM}	C+PM	C+PM+O	C_{PM}	SEM
Faecal pH	$7{\cdot}098^{c}$	$7{\cdot}099^{c}$	$6{\cdot}8^{bc}$	$7{\cdot}03^{c}$	$7{\cdot}095^{c}$	$6{\cdot}58^{b}$	$6{\cdot}80^{bc}$	$6{\cdot}83^{bc}$	$5{\cdot}96^{a}$	$5{\cdot}1 \times 10^{-2}$
NH_3 (% DM)	$0{\cdot}98^{d}$	$1{\cdot}03^{d}$	$0{\cdot}67^{c}$	$0{\cdot}94^{d}$	$1{\cdot}099^{d}$	$0{\cdot}61^{bc}$	$0{\cdot}56^{abc}$	$0{\cdot}41^{a}$	$0{\cdot}47^{ab}$	$3{\cdot}4 \times 10^{-2}$
NH_3 (% total faecal N)†	$15{\cdot}5^{c}$	$15{\cdot}6^{c}$	$11{\cdot}7^{b}$	$11{\cdot}9^{b}$	$11{\cdot}4^{b}$	$10{\cdot}99^{b}$	$7{\cdot}87^{a}$	$5{\cdot}68^{a}$	$8{\cdot}18^{a}$	0·46
NH_3 (g/d)	$0{\cdot}48^{c}$	$0{\cdot}54^{c}$	$0{\cdot}24^{a}$	$0{\cdot}38^{b}$	$0{\cdot}53^{c}$	$0{\cdot}21^{a}$	$0{\cdot}23^{a}$	$0{\cdot}20^{a}$	$0{\cdot}16^{a}$	0·17

C+MBM, control diet + meat and bone meal (50:50); C+MBM + O, C+MBM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{MB}, control diet after washout period; C+GM, control diet + greaves meal (50:50); C+GM+O, C+GM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{GM}, control diet after washout period; C+PM, control diet + poultry meal (50:50); C+PM+O, C+PM containing 3 % oligosaccharides (fructo-oligosaccharides or isomalto-oligosaccharides); C_{PM}, control diet after washout period.
[a,b,c,d]Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* For details of diets and procedures, see Table 1 and Figure 1.
† N in ammonia ($NH_3 + NH_4^+$)/100 g faecal N.
‡ Fructo-oligosaccharide supplement was Raftilose P95 (Orafti s.a., Oreye, Belgium) and is isomalto-oligosaccharide supplement was CO19TØ (Cerestar, Vilvoorde, Belgium).

(C_{PM}) compared with the same control diet during the first two periods (C_{MB} and C_{GM}). The addition of oligosaccharides did not reduce the ammonia concentration (neither on a DM basis nor as a percentage of faecal protein). In the PM group a non-significant reduction was seen for the ammonia concentration on a DM basis but this effect disappeared when expressed as a percentage of faecal N or as absolute daily production. The absolute ammonia production, on the other hand, was increased by adding oligosaccharides to the GM-supplemented group.

The *P* values for the data shown in Tables 1–4 are given in Table 5.

Discussion

There was no increased faecal moisture content (%) after supplementation with FOS or IMO but within the oligosaccharide group the moisture content in the IMO group was slightly higher compared with the FOS group.

In a previous study with cats, the moisture content was significantly increased with higher supplementation rates (6 and 9 % of oligofructose) but not with 3 % (Hesta *et al.* 2001). The significantly lower moisture content in the protein- and oligosaccharides-supplemented diets, compared with the control diets, was just below the normal range for dogs, i.e. 60–80 % (Guilford & Strombeck, 1996). Perhaps increased moisture content would have been seen if the protein sources were not introduced gradually. When the MBM-supplemented diet was fed and even more frequently when the MBM+oligosaccharide-supplemented food was given, faeces were sometimes dry and crumbly. This was probably due to the high ash (Ca level) content of the MBM which may have caused the decrease in DM digestibility seen when the dogs received MBM-supplemented diets as opposed to when the control diet was supplemented with the other protein sources.

The lower apparent diethyl ether extract digestibility after MBM supplementation could also be the consequence of the higher crude ash content of the MBM (27·1 %) due to the formation of indigestible Ca soaps. The fat digestibility significantly decreased further by adding oligosaccharides to the MBM group. Because

Table 5. *P* values*

	Treatment	Protein source	Animal	Treatment × protein source	Treatment × animal	Protein source × animal
Faecal production (g/d)	0·000	NS	0·001	0·094	0·047	NS
Faecal moisture (%)	0·000	0·009	0·003	0·022	NS	NS
Faecal DM (g/d)	0·000	0·002	0·013	NS	0·093	NS
Water intake (g/d)	0·000	NS	0·000	NS	NS	0·003
ADC of DM (%)	0·000	0·001	0·013	NS	0·091	NS
ADC of diethyl ether extract (%)	0·008	0·000	0·030	0·000	NS	NS
ADC of N (%)	0·000	NS	0·000	0·021	NS	0·045
Bacterial N (% DM)	0·000	0·000	NS	0·000	NS	NS
Bacterial N (% faecal N)	0·000	0·000	NS	NS	NS	NS
Bacterial N (% N intake)	0·000	0·006	NS	NS	NS	NS
Corrected ADC of N (%)	0·000	0·002	0·000	0·014	NS	0·083
Faecal pH	0·000	0·000	0·000	0·037	NS	NS
NH_3 (% DM)	0·000	0·000	NS	0·009	NS	NS
NH_3 (% total faecal N)	NS	0·000	NS	0·075	NS	NS
NH_3 (g/d)	0·000	0·000	NS	0·007	NS	NS

ADC, apparent digestibility coefficient.
* For details of animals and treatments, see Figure 1.

bacteria also contain lipids in their membranes, this could also explain the lesser apparent fat digestibility although this was not seen in a significant way in the other protein-enriched diets. Diez *et al.* (1998) also noted a decreased fat digestibility after supplementation with 7 % of inulin in dogs fed a diet with normal Ca (0·56 %) and P (0·48 %) contents.

The apparent digestibilities of N in the different protein-supplemented groups tended to increase compared with the control. Digestibility of these animal protein sources is indeed slightly higher compared with the protein of the control diet, which also contained vegetable ingredients (maize as the main ingredient). But because the crude protein content of these protein-supplemented diets was almost doubled compared with the control diet, in absolute terms much more undigested protein will have entered the large intestine and will have been available for the large-intestinal flora. Adding oligosaccharides to the protein-supplemented diets decreased apparent N digestibility and increased the bacterial N content of the faeces. When N digestibility was corrected for bacterial N, the differences between the protein- and oligosaccharide-supplemented groups disappeared. This indicates that the lower total-tract N digestibility was not a consequence of a lower small-intestinal digestibility but because of a higher faecal N content originating from bacteria in the large intestine. However, in the GM group a significant difference was still present after the correction for increased bacterial N content although less pronounced than before.

Flickinger *et al.* (2000) and Silvio *et al.* (2000) also noticed a decreased total-tract digestibility of crude protein by adding 6 % of α-gluco-oligosaccharide or increasing percentages of pectin (10 % maximum) in dogs while the ileal digestibility was unchanged. However total anaerobic and aerobic bacterial concentrations were not increased (Flickinger *et al.* 2000). Faecal bacterial concentrations were expressed as colony-forming units/g faecal DM, and faecal production on a DM basis was not significantly different in the α-gluco-oligosaccharides group compared with the control group. A decreased total-tract digestibility of crude protein by adding fermentable fibre was also confirmed in previous studies (Sunvold *et al.* 1995; Diez *et al.* 1998; Reinhart & Sunvold, 1998).

Howard *et al.* (2000) found no reduction in N digestibility for fermentable fibre-supplemented diets (beet pulp 6 %, FOS 1·5 % and a fibre blend (beet pulp 6 %, gum talha 2 % and FOS 1·5 %)) compared with cellulose (6 %)-supplemented diets in dogs. Strickling *et al.* (2000) also noted no change in ileal, large-intestinal and total-tract N digestibility probably because of the low concentration of the oligosaccharide supplementation (0·5 % FOS, mannanoligosaccharides or xylo-oligosaccharides, XOS).

On the other hand Burkhalter *et al.* (2001) noticed a lower ileal digestibility of crude protein by adding soyabean hulls (7·5 %) containing varying insoluble:soluble fibre ratios, or beet pulp, probably because of a decreased transit time associated with insoluble fibre and a higher viscosity of soluble fibre. Apparent total-tract digestibilities of crude protein were not changed. Zentek *et al.* (2002) noted higher water binding after mannanoligosaccharide addition and explained the lower digestibilities by a lesser solubility of the nutrients. However inulin and oligofructose do not appear to increase viscosity (Schneeman, 1999) and cellulose or other insoluble fibres were not supplemented in large amounts indicating that changes in transit time or solubility are probably not the cause of a lesser digestibility in the present study.

Since the amount of undigested protein entering the large intestine is probably not higher in the oligosaccharide *v*. protein-supplemented groups, another hypothesis for the lower ADC of N in the GM + oligosaccharide group and to a lesser extent in the other two groups can be proposed. This hypothesis is that the urea flux from the blood to the large intestine cannot be completely incorporated into bacterial N due to a relative insufficient energy (non-digestible oligosaccharides) supply in these protein-supplemented diets. The addition of fermentable fibres could have decreased the colonic pH, limiting the diffusion of ammonia. This could be responsible for the significantly lower N digestibility corrected for the bacterial N in the GM group. This is indeed the only protein-supplemented group where the ammonia production increased significantly by adding oligosaccharides. Since the crude protein content of the GM diet was 5 % higher compared with MBM or PM, this could explain the more prominent reaction in the GM group. But even if the ADC of N, corrected for bacterial N, is also adjusted for the faecal N excretion as ammonia, the differences between the GM and the GM + oligosaccharide supplementation remain (data not shown). The bacterial N content (on a DM basis) is highest for the GM + oligosaccharide diet and the ADC of N corrected for bacterial N is lowest for the GM + oligosaccharide diet. This suggests an indirect effect of the bacterial flora on the ADC of N, especially as the ADC corrected for bacterial content is similar in the three protein only-supplemented diets (MBM, GM and PM). The higher bacterial content in the GM + oligosaccharide group might have resulted in a higher faecal metabolic N loss due to the increased proliferation of the intestinal wall. Feeding fermentable fibres (beet pulp, pectin or gum arabica) to dogs increased colonic weight and mucosal surface:volume ratio compared with low-fermentable fibre (cellulose) (Reinhart *et al.* 1994). Dogs fed pectin or gum arabica also showed mucus distension, exfoliation and/or cryptitis.

Bacterial N increased in the protein-supplemented diets and was increased further by the addition of oligosaccharides as expected. Howard *et al.* (2000) also estimated bacterial N, but by measuring purine content and bacterial isolates (using the method of Zinn & Owens, 1986) in dogs after supplementation with different fibre sources. Bacterial N expressed as a percentage of faecal N output increased significantly by adding 1·5 % of FOS to the diet. In the cellulose (6 %)-based diet, 22·8 % of the faecal N was bacterial N compared with 34·4 % after FOS supplementation. In the present study slightly higher percentages were found even in the control diets (27·5–33·6 %). The bacterial N expressed as a percentage of N intake was also slightly higher in the present study (5·2–6·4 % for the control diets and 7·55–8·39 % for the oligosaccharide-supplemented diets) compared with the study of Howard *et al.*

(2000) (2·9 % in the cellulose diet, 4·5 % in FOS). However, neither the analytical methods (both estimations of bacterial protein) nor the diets were equal in the two studies. In cats, similar bacterial protein concentrations (Mason (1969) method) were found (25 % of faecal protein in control to 35·2 % with 6 % inulin) (Hesta *et al.* 2001). The faecal bacterial N content in the control groups increased from C_{MB} to C_{PM}. It is possible that the washout period was not long enough for a complete return to regular situations. Bacterial N content (% DM) was highest in the GM + oligosaccharides group compared with the other supplemented groups. When bacterial N was expressed as a percentage of faecal N, the GM + oligosaccharides group value became the lowest suggesting the bacterial N was diluted in a higher amount of undigested feed N as a consequence of the slightly higher protein intake from the GM diet.

There was no decrease in faecal pH after supplementation with oligosaccharides. A decreased faecal pH was supposed because of the production of short-chain fatty acids and other organic acids (lactic acids) during fermentation. A decreased faecal pH and increased concentration of total short-chain fatty acids was seen after supplementation of oligofructose or inulin to a protein-balanced diet in cats but only when higher concentrations were used and not with a 3 % supplementation (Hesta *et al.* 2001). Possibly, an increased saccharolytic activity is not maintained throughout the large intestine due to the exhaustion of the lower doses of prebiotics. Houdijk (1998) also suggested that the non-digestible oligosaccharides-induced increase of saccharolytic activity was not maintained throughout the hindgut in pigs. However the decrease in pH could also be masked by the higher ammonia production in the protein-enriched diets. Indeed there was a higher pH for the three enriched diets in comparison with the basal diet, undoubtedly due to the higher ammonia concentration (% DM) in these diets. Increased colonic luminal ammonia concentrations can promote tumorigenesis by stimulating cell proliferation (Lupton & Marchant, 1989). However a lower faecal ammonia concentration after oligosaccharide supplementation was not seen in the present study. A study in rats (Lupton & Marchant, 1989) showed decreased ammonia concentrations in the caecum but three times greater concentrations in the colon after supplementing a low- (8 %) as well as a high-protein (24 %) diet with pectin (8 %). This was explained partly by a decreased protein absorption in the small intestine and by an increased number of colonic micro-organisms and consequently increased hydrolysis of urea to NH_3 and NH_4^+. In the present study faecal concentrations were measured and they are not necessarily a good reflection of ammonia production; even within the different parts of the large intestine differences can be noted.

An increase of ammonia following the high-protein diets was not unexpected since a higher quantity of the protein in the diet will increase the amount of undigested protein entering the large intestine. In addition, blood urea-N can be raised in these diets and, after diffusion to the large intestine, urea can be hydrolysed to ammonia.

Faecal ammonia concentrations in the control groups with balanced protein contents were similar compared with previously published data in dogs (Hussein *et al.* 1999; Beynen *et al.* 2001; Martineau & Laflamme, 2002) although lower concentrations of ammonia have also been published (Terada *et al.* 1992; Strickling *et al.* 2000).

Faecal ammonia concentration did not decrease by the addition of oligosaccharides to the protein-supplemented diets, which was confirmed by other experiments where diets with rather high protein content (30·0–36·8 % DM) were supplemented with FOS (0·3–0·9 %) (Strickling *et al.* 2000; Swanson *et al.* 2002) or lactosucrose (1 or 3 g/MJ) (Beynen *et al.* 2001). Probably the rate of ammonia production from undigested N entering the large intestine and possibly from urea diffusion was higher than the rate of bacterial protein synthesis because of the high protein content of the diet and the relative shortage of fermentable energy. With lower dietary protein contents, a decrease of ammonia concentration might be seen as was shown for the addition of lactosucrose (crude protein 27·9 % DM) (Terada *et al.* 1992). Although the protein content of the basal diet was rather high (36·6 %), though lower than in the present study, Zentek *et al.* (2002) noted a decreased ammonia concentration after mannanoligosaccharide supplementation. An increased faecal ammonia concentration was noted after lactulose supplementation (Zentek *et al.* 2002).

The inconsistent influence on ammonia concentration after prebiotic supplementation can be caused by the fact that faecal ammonia concentrations are not a clear reflection of ammonia production since the absorption of ammonia depends on the local pH and the rate of incorporation into bacterial proteins depends on the availability of fermentable energy.

Ammonia is one of the major faecal odour components besides phenolic and S-containing compounds. Due to the versatile metabolism of ammonia in the hindgut, it does not seem a reliable parameter to evaluate the effect of prebiotics on the reduction of faecal odour.

Acknowledgements

The authors would like to thank Royal Canin (Brussels, Belgium), Orafti (Oreye, Belgium), Eridania Béghin-Say (Cerestar, Vilvoorde, Belgium) and Quartes (Deinze, Belgium) for kindly providing the petfood, the oligosaccharides and the protein supplements respectively.

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