Effects of the type and level of dietary fibre supplements on nitrogen retention and excretion patterns

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The hypothesis was tested that fermentable dietary fibre (DF) sources elevate faecal N excretion at the expense of urinary N without affecting N retention. DF that substantially increase fermentation (pectin, sugarbeet and soya bran) or are poorly fermented (crystalline cellulose and maize bran) were fed as supplements to a basal DF-free diet at three dose levels: 0, 50 and 100 g supplement/kg basal diet. The diets were fed to juvenile male Wistar rats for 2 weeks before a 7 d period when faeces and urine were collected. Faecal excretion of N was significantly increased, dose-dependently, by all DF supplements and was positively correlated to faecal bulking. Urinary excretion of N was lower at the high doses of the DF supplements but reached significance only with the highly fermentable (0·68) sugarbeet-supplemented diets. Regression analysis showed that the major part (0·75) of the increase in faecal N excretion due to DF supplementation was balanced by a reduction in urinary excretion; N retention was therefore, at the dose levels used, only affected to a small extent. Only in the maize-bran-supplemented diets were the reductions in N retention significant. The shift in N excretion from urine to faeces can be explained largely by the degree of microbial fermentation in the large intestine caused by the addition of DF supplements and emphasizes the modifying role that certain DF supplements may have on the enterohepatic cycle of N. Possible implications of these findings for patients with liver or renal failure or for conditions when the intake of dietary protein is marginal are discussed.

Nitrogen retention: Nitrogen excretion: Dietary fibre

The evaluation of the quality of dietary protein has been of research interest almost since the beginning of nutritional science (Gallaher & Schneeman, 1986). Protein quality is generally determined through measurements of N balance. In recent years particular interest has been shown in the influence on protein utilization of dietary components other than protein. The rapid expansion occurring in the number of high-dietary-fibre (DF) products or supplements available for consumers has increased the need for studies on the effects of these products on protein metabolism, including effects on digestibility and utilization.

Earlier studies with rats (Mason & Palmer, 1973) demonstrated different effects on faecal N excretion after intake of various starches. Studies in pigs with infusion of maize starch into the terminal ileum (Misir & Sauer, 1981) or infusion of maize and potato starches into the caecum (Just *et al.* 1981) showed that easily fermentable substrates reduce apparent N digestibility. Effects of easily fermentable carbohydrates may be different from those of less fermentable DF.

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That DF has physiological actions along the gastrointestinal (GI) tract has also been well established (Eastwood & Morris, 1992). In the small intestine DF may modify, and usually decreases, the digestibility of N (Kritchevsky, 1988) while in the large intestine DF may alter considerably the metabolism of the bacteria residing in this part of the GI tract, which may lead to a profound change in N excretion patterns and possibly affect the overall N balance (British Nutrition Foundation, 1990). Generally it is found that DF has a bulking effect.

In a recent comparative study it was concluded that some differences might exist between man and the rat in the ability to digest N and to ferment dietary fibre but that the ranking of nutrient digestibility was the same in the two species (Bach Knudsen *et al.* 1994). Given the difficulties with methods for analysis of DF and the wide variations in digestibility of DF observed between laboratories, the similarity in DF digestibility between man and rat is remarkable (Livesey, 1992).

In the present study we have measured the effects on N excretion patterns and N retention in rats of feeding DF sources of varying solubility and fermentability. The effect of dose levels of DF supplements on excretion and retention of N was determined. Additionally, the correlation between faecal excretion of N and of DM was examined.

METHODS

The observations were made on animals from one laboratory in a study originally designed as a European Union interlaboratory study to determine energy conversion factors for DF supplements and fermentabilities of NSP contained in the supplements investigated, the results of which are published elsewhere (Livesey *et al.* 1995).

Diets

The design involved dietary treatments in which five DF supplements were each added at three doses (0, 50 or 100 g/kg; Table 1).

The five DF supplements were selected to have negligible or minimal starch content and to have a wide range of fermentability. The DF comprised: apple pectin, a high-methoxyl pectin from Sanofi Bio-Industries (Paris, France); maize bran from Honeyville Grain Inc. (Salt Lake City, Utah 84120, USA); cellulose ('Solca Floc'), grade BW 2030, from Jurgenson and Wettre Ltd, Wokingham, Berks.); sugarbeet DF ('Betafibre') from British Sugar plc (Peterborough, Cambs.) and soya bran ('Fibrim 2000 (red)') from Protein Technologies International (Corby, Northants).

Animals

Male Wistar rats (*n* 120), with a mean body weight of 88·4 (SD 5·9) g, were obtained from a local supplier (Molgaards Breeding Centre, Lille Skensved, Denmark). Throughout the study each rat was kept individually in a plexiglass metabolism cage with a stainless-steel mesh bottom and in a controlled environment (temperatures of 22–24°, relative humidity 50%, light–dark period cycles of 12 h: 06.00–18.00 hours).

Experimental design

The study comprised five consecutive N balance studies. On each occasion twenty-four rats were allocated randomly to one of the dietary treatments, two rats per DF source at each dose level and four rats per basal diet. In total this made 120 observations (5 occasions × (5 DF sources × 2 doses × 2 rats + 4 basal-diet-fed rats)). The rats were fed for a total of 21 d, divided into an adaptation period of 14 d and a balance period of 7 d.

Table 1. *Composition of basal and experimental diets*

Composition of basal diet	
Maize starch (100 g moisture/kg)*	330 g
Sucrose (10 g moisture/kg)	360 g
Casein (50 g moisture/kg)†	200 g
DL-Methionine	2 g
Maize oil‡	80 g
Vitamin mixture§	20 g
Mineral mixture‖	40 g
Total weight	1032 g
Composition of experimental diets	
	Basal diet¶ Fibre supplement¶
Basal diet	1000 g + 0 g
Lower dose dietary fibre supplement	1000 g + 50 g
Higher dose dietary fibre supplement	1000 g + 100 g

* 'Snowflake' maize starch from Corn Products Ltd, Manchester.

† Edible casein, mesh 30, from G. Fiske and Co. Ltd, Richmond, Surrey.

‡ Mazola from CPC International, Esher, Surrey.

§ Produced the following amounts in the basal diet (mg/kg): nicotinic acid 60, cyanocobalamin in mannitol 50, calcium D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pteroylmonoglutamic acid 10, pyridoxine 10, D-biotin 1, phylloquinone 2, Rovimix E-50 (containing 7·5 mg RRR-tocopherol acetate, Roche, Welwyn Garden City, Herts.), Rovimix A-500 (containing 3·75 mg retinol, Roche) 25, Rovimix D_3-500 (containing 0·19 mg cholecalciferol, Roche) 15, choline bitartrate 1800, maize starch carrier ('Snowflake') 17800.

‖ Produced the following amounts in the basal diet (g/kg): $CaHPO_4$ 13, $CaCO_3$ 8·2, KCl 7·04, Na_2HPO_4 7·4, $MnSO_4$ 0·18, $MgSO_4.H_2O$ 0·18, $FeSO_4$ 0·144, $CuSO_4$ 0·023, KIO_3 0·001.

¶ Nitrogen content (g N/kg DM): basal diet 30·7; pectin 6·6; maize bran 9·4; cellulose 0·8; sugarbeet 15·5; soya bran 17·9; For further details of supplements, see p. 462.

Each day the rats received, between 09.00 and 10.00 hours, 15 g basal diet or 15·8 g lower dose DF-supplemented diet or 16·5 g higher dose DF-supplemented diet. Any spillage from the previous day was added to the daily ration. Water was available *ad lib*. The body weight of each animal was recorded in a prefed state on days 1, 15 and 22 of the study.

During the 7 d balance period faeces were collected daily before feeding. The faecal collections from each animal were pooled and kept in the freezer at −18° until dry weight and N measurements were made. Urine was collected quantitatively in a glass vessel throughout the 7 d balance period. The urine samples were preserved with dilute H_2SO_4 (0·5 M) until N determination.

Chemical analyses

DM in DF supplements, diets and faeces was determined on duplicate samples by oven-drying at 105° for 20 h. N was determined on duplicate samples of DF supplements, diets, freeze-dried faeces and wet urine by an automatic Kjeldahl procedure (KjellFoss 16200; Foss Electric A/S, Denmark).

Calculations and statistical analyses

N retention was calculated using equation 1:

$$N_{retention} = N_{intake} - (N_{urine} + N_{faeces}). \quad (1)$$

N digestibility was calculated as apparent digestibility using equation 2:

$$\text{Apparent } N_{digestibility} = (N_{intake} - N_{faeces})/N_{intake}. \quad (2)$$

Table 2. *Nitrogen intake, retention and excretion in rats fed on a basal diet or diets supplemented with dietary fibre at low or high dose levels*

(Values are means for ten rats (supplemented diets) or twenty rats (basal diet))

	Dose (g/kg)	N intake (mg/d)	N retention (N retention/ N intake)	N urine (N urine/ N intake)	N faeces (N faeces/ N intake)
Basal diet	—	433·0^{fg}	0·502^{a}	0·450^{abc}	0·050^{g}
Pectin	50	437·9^{def}	0·472^{ab}	0·452^{abc}	0·076^{cd}
Pectin	100	432·7^{fg}	0·471^{ab}	0·439^{abc}	0·092^{ab}
Maize bran	50	438·8^{def}	0·453^{b}	0·480^{a}	0·065^{e}
Maize bran	100	440·4de	0·452^{b}	0·464^{abc}	0·085^{bc}
Cellulose	50	430·4^{g}	0·481^{ab}	0·458^{abc}	0·061^{ef}
Cellulose	100	441·1^{cd}	0·479^{ab}	0·450^{abc}	0·071de
Sugarbeet	50	442·0^{cd}	0·450^{b}	0·470^{ab}	0·082^{bc}
Sugarbeet	100	450·4^{b}	0·477^{ab}	0·423^{c}	0·100^{a}
Soya bran	50	447·7^{bc}	0·477^{ab}	0·442^{abc}	0·080^{cd}
Soya bran	100	457·5^{a}	0·470^{ab}	0·431^{bc}	0·096^{a}
Mean (n 120)		440·4	0·474	0·451	0·076
SE*		7·7	0·050	0·049	0·012
Statistical significance of					
Diet, $P <$			0·055	NS	0·0001
Dose, $P <$			NS	NS	0·0001
Diet × dose, $P <$			NS	NS	NS

$^{a-g}$ Mean values within a column with unlike superscript letters were significantly different ($P < 0·05$) according to Student's least significant difference test (df 108).

* Standard error of the analysis between diets, within dose of dietary fibre supplement.

The results were initially examined in two-way ANOVA (Armitage, 1971):

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk},$$

where X_{ijk} is the dependent variable, μ is the overall mean, α_i is the effect of diet, β_j is the effect of dose of DF supplement and ϵ_{ijk} is the normally distributed random variable. When a factor was not significant either as a main effect or in interactions it was omitted from the statistical model and so included in the error (root mean square error (MSE)) of the statistical test. Standard errors (SE) presented in the tables are derived from the root MSE. All differences between means were tested at $P < 0·05$, using a t test (least significant difference; LSD). Correlation and regression analyses were performed on individual figures. The Statistical Analysis Systems (SAS) statistical software package was used (SAS Release 6.04, SAS Institute Inc., Cary, NC, USA).

RESULTS

Animal performance

All diets (Table 1) were well received by all the animals with very little daily feed refusal. Due to the supplementary feeding of the DF, DM intake was higher in the DF-supplemented groups than in the group receiving the basal diet alone. The total DM intake over the 7 d balance period in the rats receiving the basal diet (n 20) was 98·5 (SD 0·91) g, in the rats (n 50) receiving the lower dose DF-supplemented diets it was 103·6 (SD 0·80) g, and in the rats (n 50) receiving the higher dose DF-supplemented diets it was 108·4 (SD 1·01) g.

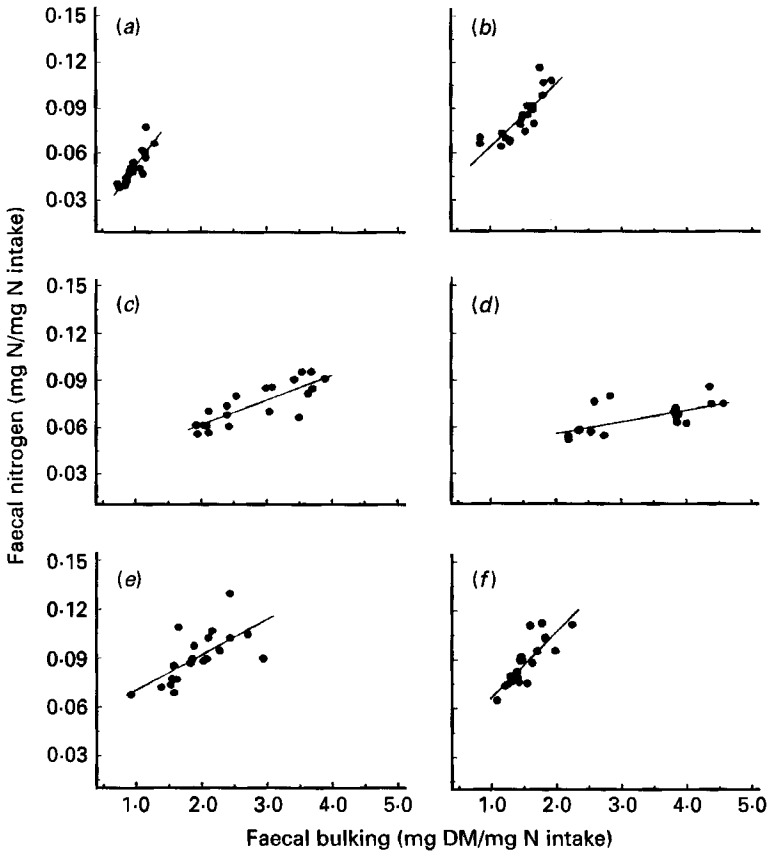

Fig. 1. Regression of faecal bulking (mg DM/mg N intake) *v.* faecal nitrogen (mg faecal N/mg N intake) in rats fed on a basal diet or on the same basal diet supplemented with a dietary fibre source. (a), Basal diet: r 0·87, $P < 0·0001$, SE 0·006; (b), pectin-supplemented diets: r 0·83, $P < 0·0001$, SE 0·010; (c), maize-bran-supplemented diets: r 0·83, $P < 0·0001$, SE 0·008; (d), cellulose-supplemented diets: r 0·66, $P < 0·005$, SE 0·009; (e), sugarbeet-supplemented diets: r 0·67, $P < 0·005$, SE 0·013; (f), soya-bran-supplemented diets: r 0·82, $P < 0·0001$, SE 0·007 (df 19 in each dietary group).

Mean body weight of all 120 rats after the 14 d adaptation period was 181·0 (SD 7·3) g. Body-weight gain during the 7 d balance period was similar in all dietary groups with an average of 33·8 (SD 4·9) g.

Nitrogen intake and retention

The N content of the experimental diets reflected the N dilution that occurred when the basal diet was supplemented with DF supplements containing negligible (pectin, maize bran and cellulose DF supplements) or very low levels (sugarbeet and soya bran DF supplements) of N (footnote, Table 1). Due to the N content of the sugarbeet and soya bran DF supplements the average daily N intake in these dietary groups differed between doses and from the basal diet. All these differences in N intake were small in both absolute and relative terms.

N retention was influenced by diet ($P < 0·055$) but not by dose of DF supplements (Table 2). The DF supplements tended to reduce N retention in all diets but the reductions

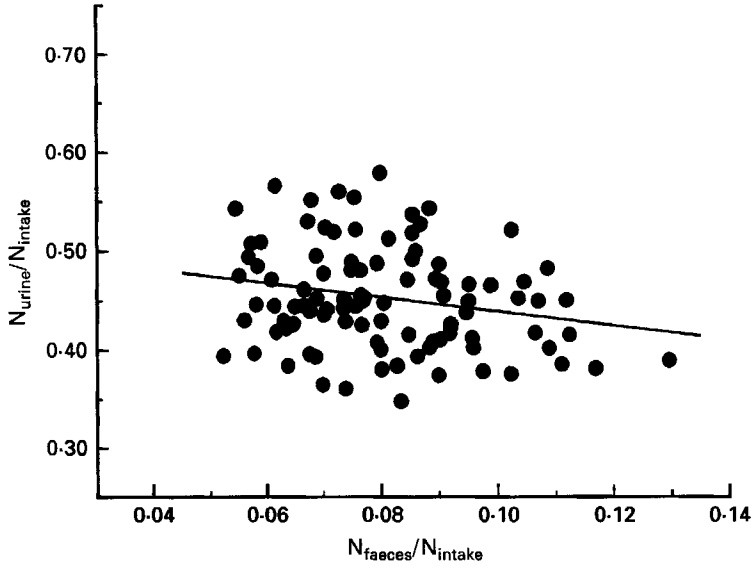

Fig. 2. Regression of nitrogen excretion in faeces *v.* that in urine in rats fed on a basal diet supplemented with various dietary fibre souces. The regression line is described by the equation $Y = 0{\cdot}51 - 0{\cdot}72X$; *n* 100, df 98 ($r$ 0·23, $P < 0{\cdot}02$, SE 0·013).

reached significance only in the two groups, those given maize bran and those given the lower dose sugarbeet DF.

Nitrogen excretion

Faecal N excretion was significantly ($P < 0{\cdot}0001$) influenced by both the diet and the dose of DF supplement (Table 2). Compared with the basal diet all DF supplements caused a significant increase in faecal N. No interaction was found between diet and dose.

In all the DF-supplemented diets a reduction in the urinary N excretion was seen at the higher dose compared with the lower dose. Only in the case of the sugarbeet-supplemented diets did the difference reach significance.

Relation between faecal nitrogen excretion and faecal bulking

Faecal bulking calculated as average DM excretion/N intake per rat per day ranged from an average of 0·981 (SD 0·14) mg DM/g N intake in the basal dietary group to 4·03 (SD 0·27) mg DM/g N intake in the high-dose cellulose-supplemented group. Faecal N excretion was positively correlated to faecal bulking. With the data divided into the different diets the Pearson correlation coefficients ranged from 0·66 in the cellulose-supplemented diets to 0·87 in the basal diet (Fig. 1).

Relation between faecal and urinary nitrogen excretion

The large loss of N to urine compared with that to faeces, together with relatively few observations per diet, made it difficult to observe for each DF supplement whether a decrease in urinary N excretion occurred to compensate for the increase in faecal N. Among all those animals given DF, i.e. excluding those given no fibre, the urinary N excretion decreased with increasing faecal N excretion (Fig. 2). The exchange was 0·72 (SE 0·013) g urinary N for 1 g faecal N ($P < 0{\cdot}02$), suggesting that almost 0·75 of the faecal N was compensated by a reduction in urinary N.

DISCUSSION

The DF supplements chosen for the present experiment were purified fibre sources that are commercially available for human consumption. The supplements were selected for their negligible or low levels of starch and their expected wide range of fermentabilities. The fermentability of the DF sources was determined previously in a pan-European study reported by Livesey *et al.* (1995) where the average fermentabilities of the DF supplements were, in descending order: pectin 0·93, soya bran 0·87, sugarbeet 0·68, maize bran 0·16, and cellulose 0·07. The choice of purified fibre supplements rather than fibre-rich foods had the advantage of negligible or low levels of N or other energy-contributing substances that could otherwise interfere with the variables examined.

The method of incorporation of the DF source is of particular interest when studying effects on N utilization. The two methods usually employed in these types of study are substitution and supplementation of a basal diet with the DF sources (Gallaher & Schneeman, 1986). The advantage of the supplementation method is that the N:energy ratio of the diets remains almost constant, a condition that is almost a prerequisite in studying protein utilization, especially in low-protein diets (Delorme *et al.* 1981). However, the energy value of DF is an issue of much recent debate (British Nutrition Foundation, 1990; Livesey, 1990). Using a rat model, the net metabolizable energy values of the DF supplements used in the present study were (kJ/g DF source): soya bran 8·7, pectin 7·3, sugarbeet 6·7, maize bran 2·7, cellulose 0·2 (Livesey *et al.* 1995). With the small contribution of the DF sources to total energy intake, the supplementation method applied in the present study seemed almost ideal.

The most established action of DF along the GI tract is the effect on stool weight (Cummings *et al.* 1992; Eastwood, 1992). Faecal DM consists mainly of bacterial matter with the remainder consisting of unfermented DF and other excreted compounds (Eastwood & Morris, 1992). The pattern of DM excretion in the present study confirmed an inverse relationship between DM excretion and fermentability of the DF supplements.

The faecal N excreted is derived from incomplete digestion of bacterial protein (Mason, 1984), dietary protein, secreted digestive enzymes, and sloughed mucosal cells (Eggum, 1992). In the present study dietary protein was provided mainly by casein, a highly digestible N source, but small amounts of N were further supplied from the sugarbeet and soya bran DF supplements. N bound to the cell walls of these purified DF is less digestible than non-bound N (Donangelo & Eggum, 1985) but due to the relatively high fermentation of these two DF sources (0·68 for sugarbeet and 0·87 for soya bran) the amounts of cell-wall-bound N in faecal matter are considered to be of minor importance in the present study.

The addition of DF to a basal diet increases the flow of substrate to the large intestine, stimulates bacterial growth, and increases N incorporation into the bacterial cell walls (Goodlad & Mathers, 1990). This has also been shown to be the case with certain types of starches whether taken orally (Mason & Palmer, 1973) or infused into the caecum (Just *et al.* 1981). Based on these observations it is possible, therefore, to predict that any fermentable polysaccharide has similar effects on N excretion patterns.

In the present study an association was observed between the degree of fermentation of the DF supplements and the faecal concentration of N. The poorly fermentable DF supplements (cellulose and maize bran) increased faecal bulking and thus led to a dilution of faecal N. In contrast, the highly fermentable DF supplements (pectin, sugarbeet and soya bran) led to an increase in bacterial growth and a reduction in the amount of DF residue resulting in a N enrichment of faecal matter (Fig. 1).

The microbial degradation of complex carbohydrates that occurs in the large intestine

during bacterial fermentation leads to considerable changes in the colonic N metabolism. The total bacterial N requirement which is elevated by the stimulation of bacterial protein synthesis is mainly derived from NH_3 in the large intestine (Wrong & Vince, 1984). The main source of this intestinal NH_3 is endogenous urea (Rémésy & Demigné, 1989).

When data from all DF treatments were considered the reduction in urinary N excretion compensated the major part of the elevated faecal N excretion (Fig. 2). A reduction in protein digestibility may not be a major concern in diets where protein is not a limiting dietary factor for growth and maintenance as was the case in the present study. However, under conditions where dietary protein intake is marginal a reduction in protein digestibility due to high intakes of DF supplements may make a difference between protein adequacy and inadequacy. A reduction of urinary N excretion due to high intakes of DF supplements may be warranted in certain clinical situations, i.e. patients with liver or renal failure.

The elevated excretion of faecal N after eating more DF has long been used to argue that DF will both decrease N retention in the body and impair growth rates. This research shows that this is not the case, rather that urinary N excretion decreases to compensate for most of the increase in faecal N loss. The enterohepatic recycling of N is the probable mechanism, leading to decreased urea excretion, increased microbial urealysis and increased microbial *de novo* amino acid and protein synthesis.

CONCLUSION

The present study confirms our hypothesis that DF added to a basal diet significantly affects N excretion in growing rats, increasing faecal N losses and decreasing urinary N, due to greater fermentation in the hind gut. A major part of the increase in faecal N is compensated by the decrease in urinary N.

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