Eur J Nutr (2005) 44 : 371–376
DOI 10.1007/s00394-004-0537-4

ORIGINAL CONTRIBUTION

Khalid M. Khan
Christine A. Edwards

In vitro fermentation characteristics of a mixture of Raftilose and guar gum by human faecal bacteria

Summary *Background* The therapeutic effects of indigestible carbohydrates in the human colon are well known. Most fermentation studies have examined only single carbohydrates. Considering the idiosyncratic actions of individual carbohydrates and the dose required, it is unlikely that any one carbohydrate will provide ideal physiological actions without having some undesirable effects. Little is known of how mixing non-digestible carbohydrates affects their fermentation. This necessitates fermentation studies using different carbohydrates in mixtures. *Aim of the study* The aim of this study was to test the effect of mixing Raftilose™ (R) and guar gum (G) on short chain fatty acid (SCFA) production in *in vitro* cultures of human faecal bacteria. *Method* The fermentation of the individual carbohydrates (10 mg/ml) was compared with that of a 50:50 mixture in anaerobic *in vitro* cultures of human faeces. *Results* Cultures of R/G mixtures produced significantly more n-butyrate than 100 mg G alone at 8 and 24 hours ($p < 0.02$). There was no significant difference in the production of n-butyrate between cultures of 100 mg R and the R/G mixture at 8 and 24 hours. R (100 mg) produced a propionic/butyric (p/b) acid ratio of 1.18 compared with 3.88 for 100 mg G, whereas R/G mixture produced a p/b ratio of 2.01. *Conclusion* The fermentation of R/G mixture was different compared with 100 mg of R or G alone. There was no loss of n-butyrate in the culture containing R/G (50 mg of each) mixture compared with the 100 mg R culture.

Key words fermentation – short chain fatty acids – raftilose – guar gum – fructooligosaccharides

Received: 6 October 2003
Accepted: 4 August 2004
Published online: 5 November 2004

K. M. Khan · Dr. C. A. Edwards (✉)
Human Nutrition Section
Division of Developmental Medicine
University of Glasgow, Yorkhill Hospitals
Glasgow, G3 8SJ, UK
Tel.: +44-141/201-0709
Fax: +44-141/201-9275
E-Mail: cae1n@clinmed.gla.ac.uk

Introduction

The physiological actions of individual dietary fibres or indigestible carbohydrates are determined mainly by their physiochemical properties. Soluble dietary fibres tend to be more fermentable and have been shown to decrease the rate of absorption in the small intestine whereas insoluble fibres increase stool output [1]. Dietary fibres may be fermented to short chain fatty acids (SCFA) by the microflora in the large intestine. The extent of fermentation and proportions of the different SCFA produced may be important determinants of their health benefits [2]. SCFA are potentially protective against colon diseases such as colon cancer [3, 4]. Butyric acid, in particular, has been shown to induce apoptosis and differentiation in colon carcinoma cells [5, 6]. Propionic acid influences peripheral metabolism and has been proposed to inhibit the hepatic lipogenesis [7–10]. The SCFA profiles depend on the physicochemical characteristics of the carbohydrate. For example G has been shown to have a SCFA profile with increased propionate, whereas, R produced more butyrate compared with propionate [11].

Most fermentation studies have tested a single carbohydrate [12] and did not take into account potential synergistic or antagonistic interactions of different carbohydrates. Considering such interactions is important as the human diet contains many different sources of non-digestible carbohydrate and a mixed composition may determine the role of fermentation of carbohydrate in the colon. It is likely that carbohydrates with diffe-

rent fermentative properties will interact when combined together and will affect the SCFA production profile.

Several mixtures of non-digestible carbohydrates have now been incorporated into foods or dietary supplements [13–19]. In a recent study Henningsson and colleagues [13] reported significantly higher butyrate production from the fermentation of a mixture of G and pectin compared with their SCFA profiles and concentrations when fermented individually. This shows that the extrapolation of results from fermentation of individual carbohydrates may not be the best approach to predict the fermentation of mixtures. The fermentation of a mixture may take a different route to the fermentation of its individual ingredients. This necessitates the testing of each carbohydrate mixture.

In the present study, Raftilose™ (R), a butyrate predominant, and guar gum (G), a propionate predominant fermenting carbohydrate, were fermented *in vitro* separately and in mixture using human faecal bacteria. SCFA production was measured in the culture supernatant at different time points.

Materials and methods

Substrates

Raftilose™ P95 (an oligofructose from Raffinerie Tirlemontoise SA Brussels Belgium) was 93.2 to 95.8 % oligofructose, being composed of 64 % Glucosyl (fructosyl) $_{n-1}$ fructose (GF_n)? GF_m and 36 % homooligomers of fructose (F_n) with a mean degree of polymerisation (m or n) of 4.8. The fructose, glucose and sucrose, fructose units are linked together by β [2–1] linkages, terminated with a glucose unit [20]. Low molecular weight guar gum (Meyprogat 30 MR 4812 Meyhall Chemical AG, Kreuzlingen, Zaandam, Netherlands), a storage polysaccharide derived from seeds of the cluster bean (Cyamopsis tetragonoloba) was used in this study. The molecular weight of the guar gum was 370,000 and it was composed of 83 % galactomannan with a total nonstarch polysaccharide content of 87.3 % (Dr P Ellis – unpublished data).

In vitro model

An *in vitro* fermentation model using human feces in a simple medium of minerals and tryptone [21] was adopted. Raftilose™ and guar gum were used as the sole extraneous sources of carbohydrate. The two carbohydrates were combined, using 50 mg each to make a total 100 mg of substrate in 10 ml culture. These carbohydrates were also incubated *in vitro* in separate portions of 50 mg and 100 mg. A control culture, without added carbohydrate, was incubated for each faecal slurry to allow for fermentation of endogenous carbohydrate.

The fermentation procedure was repeated eight times with individual fresh human faecal samples from eight different healthy subjects (age 26–57 years, four males and four females). Faecal samples were processed within the shortest possible time not exceeding, at any time, one hour after defecation. A faecal slurry (32 %) was made in phosphate buffer (pH 7.0) and 1 ml of this slurry was added to 9 ml pre-reduced medium in McCartney bottles (Merck Ltd., Lutterworth, UK) containing the test substrate or no carbohydrate in controls. Each McCartney bottle was flushed with oxygen free nitrogen (OFN) before incubation. The incubation was carried out at 37 °C in a shaking water bath at 50 strokes per minute. Cultures were stopped at 0, 4, 8 and 24 hours and centrifuged (1300 g, 4 °C) for 30 minutes. The supernatants were stored at –20 °C for determination of SCFA by gas liquid chromatograph [22].

Cultures from three subjects were incubated for an extended period of 48 hours to allow more time for complete fermentation of any residual substrate, as the cultures from the first five subjects showed continuing fermentation up to 24 hours. TLC was used to follow the disappearance of R in these cultures from 4–24 hours [23]. The presence of R was determined by comparison with a standard solution containing mixtures of raftilose, lactulose, sucrose and fructose.

Calculations and statistical analysis

In vitro net total SCFA were calculated by subtracting the values of control cultures from the values with the respective test substrate. A predictive value for the SCFA produced from the mixture was calculated by addition of the SCFA values from the fermentation of 50 mg individual substrates. One-way ANOVA, paired and unpaired t-test were performed for the comparison of means where appropriate.

Results

In vitro fermentation

pH of cultures

The pH of all cultures decreased by 8 h ($p < 0.0001$). The pH in 100 mg R alone cultures was significantly lower than in 100 mg G alone and R/G mixtures at 8 and 24 h ($p < 0.05$). None of these cultures showed any further reduction in pH after 8 h. There was no significant difference in final pH (24 h) in cultures containing 100 mg G alone or R/G mixtures (Table 1).

Table 1 Changes in pH (mean ± SEM) of human faecal cultures containing Raftilose™, guar gum and a mixture of the two over 24 h (n = 8) and 48 h (n = 3) after *in vitro* anaerobic incubation

	Initial pH	pH at 8 h	pH at 24 h	pH at 48 h
R (100 mg)	7.8 ± 0.18[a]	5.7 ± 0.19*[a]	5.9 ± 0.11	5.8 ± 0.10
G (100 mg)	7.7 ± 0.18[b]	6.5 ± 0.30[b]*	6.2 ± 0.07	6.4 ± 0.17
R/G mixture	7.8 ± 0.18[c]	5.9 ± 0.18[c]	6.1 ± 0.09	6.1 ± 0.11

R Raftilose; *G* Guar gum
pH was compared (ANOVA) between types of culture at 8 and 24 h. Values carrying similar symbols are significantly different from each other. * $p < 0.05$
pH was compared with paired t test t between 0 and 8 hours for the same type of culture [a, b, c] $p < 0.0001$

Total SCFA

The R/G mixture produced significantly higher net total SCFA than G alone ($p < 0.002$), but not significantly less SCFA than 100 mg R alone at 8 h. There was no significant difference in SCFA production between the different types of cultures at 24 h. The SCFA production rate declined in cultures containing either 100 mg R or G alone, whereas SCFA production in the cultures containing the R/G mixture continued to increase after 24 h. SCFA concentrations in the cultures containing the R/G mixture were as predicted from the addition of SCFA produced in cultures containing 50 mg single substrates at 8 and 24 h (Fig. 1).

Acetic acid

Cultures containing 100 mg R alone produced significantly higher acetate concentration, compared with the cultures containing 100 mg G alone at 8 and 24 h ($p < 0.05$). The R/G mixture produced significantly higher acetate concentrations than 100 mg G alone at 8 h ($p < 0.02$), with no significant difference between the two types of cultures at 24 h. Acetate concentrations were significantly lower in actual mixtures than the predicted values from the 50 mg of two individual cultures at 8 and 24 hours ($p < 0.02$; Fig. 1).

When the acetic acid concentration was compared as a molar proportion of total SCFA, there was no significant difference between the three types of cultures at 8 h. However, at 24 h the proportion of acetate was significantly higher in cultures containing 100 mg R than in those containing 100 mg G alone ($p < 0.05$). R/G did not show significantly different acetate ratios compared with the cultures containing 100 mg G alone at 24 hours; (Fig. 2).

Propionic acid

G cultures (100 mg) produced the highest propionate concentrations (Fig. 1). At 24 h, 100 mg G alone cultures produced a significantly higher proportion of propi-

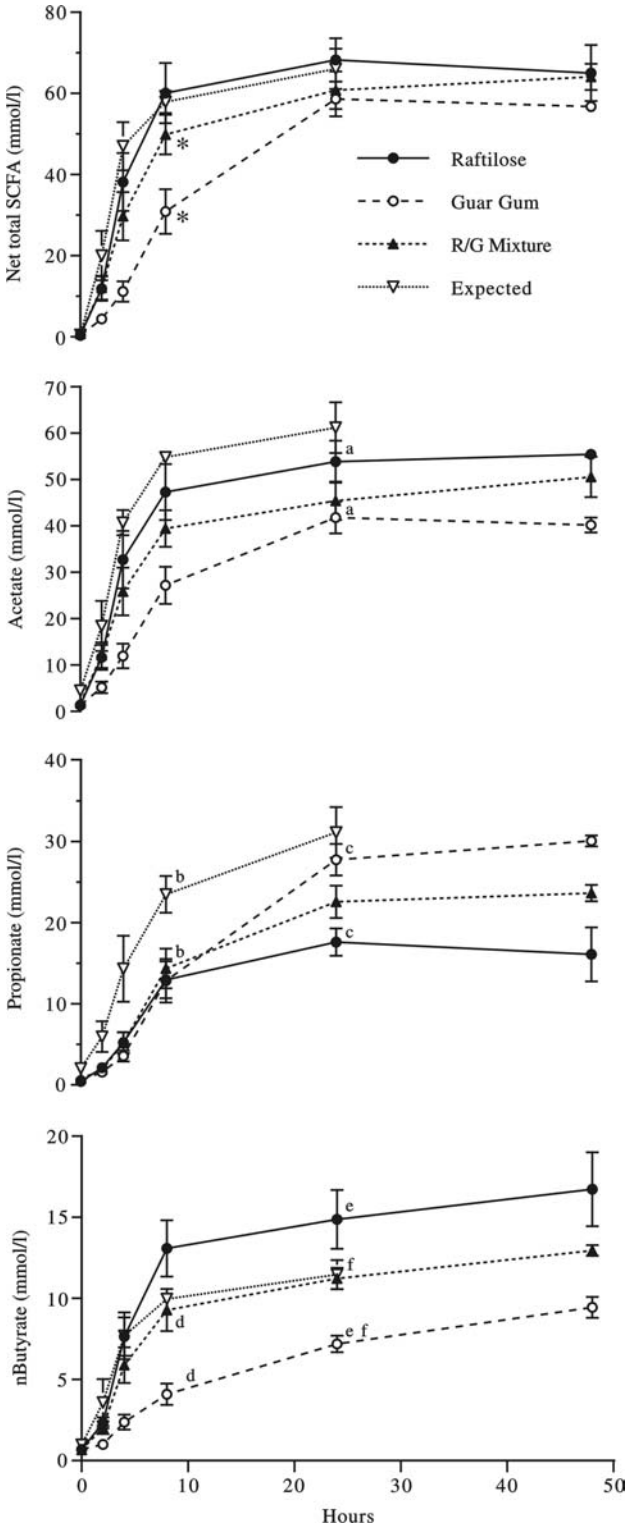

Fig. 1 Concentrations of short chain fatty acids (mmol/l; mean ± SEM) produced by the fermentation of Raftilose™, guar gum and a mixture of the two with human faecal bacteria over 24 h (n = 8) and 48 h (n = 3) incubation *in vitro*. Time points with the same symbols are significantly different from each other ($p < 0.05$). Expected is the value calculated by addition of the amounts produced with 50 mg of the individual carbohydrates

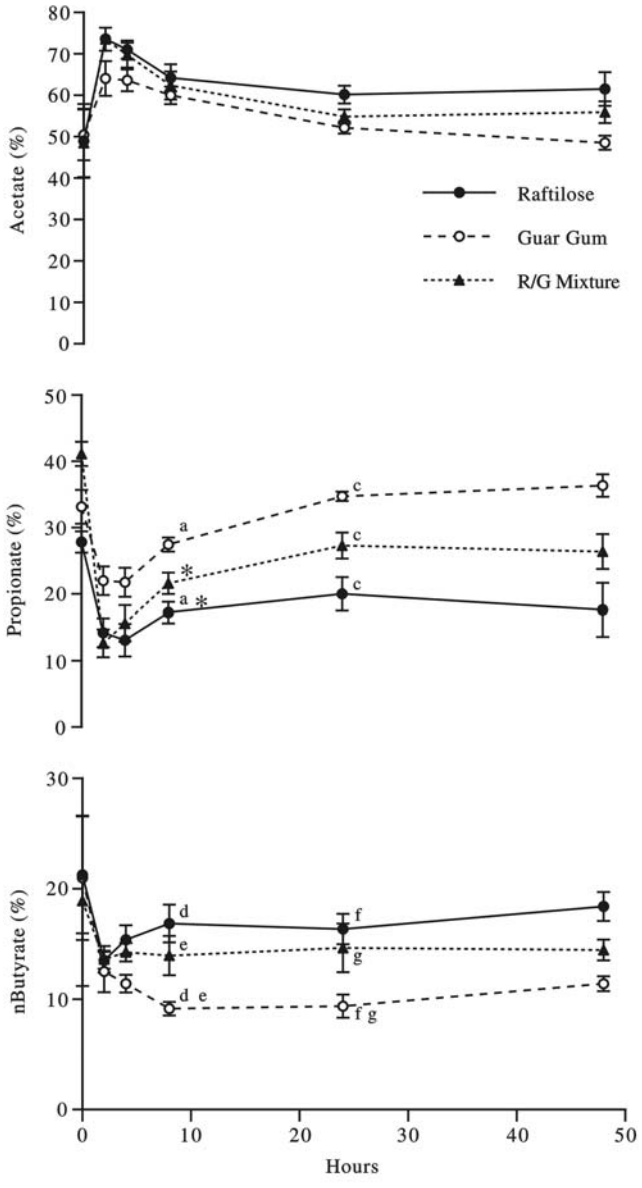

Fig. 2 Individual short chain fatty acids as a percentage of total short chain fatty acids produced by the fermentation of Raftilose™, guar gum and a mixture of the two with human faecal bacteria over 24 h (n = 8) and 48 h (n = 3) incubation *in vitro*. Time points with the same symbols are significantly different from each other ($p < 0.05$)

onate than cultures containing 100 mg R alone ($p < 0.01$). The R/G mixture did not show any significant difference in the production of propionate from the two individual carbohydrates at 24 hours and this production, in contrast to cultures of 100 mg R alone, showed a continuous increase in propionate production after 24 h. Propionate concentrations in the R/G mixture were significantly lower than predicted values from the addition of the 50 mg single substrate cultures at 8 and 24 h ($p < 0.05$; Fig. 1).

R/G mixtures did not show a significantly different proportion of propionate from 100 mg G or 100 mg R alone cultures. Cultures containing 100 mg G alone produced a significantly higher proportion than 100 mg R alone at 8 h ($p < 0.02$). The proportions of propionate were intermediate in the R/G mixture and significantly different from cultures of 100 mg of individual constituent carbohydrates at 24 h ($p < 0.01$; Fig. 2).

n-Butyric acid

R (100 mg) and R/G mixtures produced significantly higher n-butyrate concentrations than 100 mg G cultures at 8 and 24 h ($p < 0.01$). There was no significant difference between the cultures containing 100 mg R alone and R/G mixture at 8 and 24 h. n-Butyrate concentration for the R/G mixture was not significantly different from the predicted value calculated by the addition of the amounts produced by the 50 mg of individual substrate cultures (Fig. 1).

R (100 mg) and R/G mixtures produced significantly higher proportions of n-butyrate than 100 mg G alone at 8 and 24 h ($p < 0.05$). The proportion of n-butyrate was not different in 100 mg R alone cultures and those containing the R/G mixture at 8 and 24 h (Fig. 2).

Residual Raftilose™

There was no detectable residual R in cultures at 8 and 24 h.

Discussion

In this study, the colonic fermentation of a mixture of two carbohydrates with different chemical composition and physical properties was evaluated *in vitro* to determine whether the SCFA profiles and/or rate of fermentation were different from that of the individual carbohydrates.

R is a fructo-oligosaccharide of low molecular weight and may cause osmotic diarrhoea if used in large amounts. On the other hand, it is considered to be a prebiotic, stimulating growth of bifidobacteria in the colon [24]. G, a soluble and less rapidly fermenting polysaccharide, can reduce postprandial glycaemia and plasma cholesterol [25–27]. The two carbohydrates in the present study were chosen because of their different physicochemical properties but also because previous studies have indicated a difference in their SCFA profile when they were fermented individually. R has been shown to promote butyric acid production whereas G is known for increasing propionic acid concentration *in vitro* [2, 11].

In the present study cultures containing 100 mg G alone produced p/b acid ratio 3.14 at 8 h and 3.88 at 24 h

whereas when 100 mg R alone was fermented the ratio was 0.99 at 8 h and 1.18 at 24 h. These ratios were slightly higher than the p/b ratios (2.03 and 0.85 at 24 h for G and R respectively) that were reported in an *in vitro* recent study, though the SCFA production patterns were similar [11]. The fermentation of the R/G mixture *in vitro* in the present study with a p/b ratio of 1.55 at 8 h and 2.01 at 24 h was different compared with 100 mg of its constituent carbohydrates. The R/G mixture also produced significantly higher butyric acid concentration than the 100 mg G alone ($p < 0.02$) but not significantly less than the 100 mg R alone cultures. Although the concentration of propionate for the mixture fell half way between the two, the level of acetate and butyrate production was skewed toward that of 100 mg R alone. Acetate and propionate concentrations were less than that predicted by addition of the values from fermentation cultures of 50 mg of the individual carbohydrates, whereas butyrate did not show any significant difference from predicted values.

Other studies *in vivo* have also shown interaction of carbohydrates, with the added effect on transit time. A delay in fermentation (breath hydrogen production) was seen when psyllium and lactulose were fed together to human subjects [15]. It is very difficult to access fermentation *in vivo* in humans as most fermentation occurs in the proximal colon, which is not accessible. Faecal SCFA are the net result of production and absorption and, therefore, may not reflect real changes in bacterial fermentation. Thus, *in vitro* studies of human faecal cultures are more useful. Most other studies of the fermentation of carbohydrate mixtures have been carried out in rats. Wheat bran and psyllium appeared to shift the fermentation of resistant starch more distally and increased the faecal excretion of SCFA in rats [13, 17]. Wheat bran was also shown to delay the fermentation of the resistant starch in pigs [16]. Topping et al. [19] achieved more efficient butyrate production by combining gum arabic and cellulose in the rat hindgut. In a recent study a mixture of G and pectin produced significantly higher butyrate compared with SCFA produced by the caecal fermentation of the individual carbohydrates in rats. The SCFA profile by the fermentation of mixture was also different compared with that expected from fermentation of G, a propionate predominant, and pectin, acetate predominant [13]. This is an important finding, which suggests that the extrapolation from one fermentation study to another will not be scientifically valid as carbohydrates in mixture may behave totally differently than when considered alone. Thus the fermentation of a mixture may take a different route to the fermentation of its individual ingredients. There are several possible interactions that can happen when mixtures of different carbohydrates are fermented. For example, individual bacterial species have substrate preferences and may act in synergy for the fermentation of carbohydrates in a mixture. Different substrates in a mixture of carbohydrates may stimulate certain species of bacteria, which may result in more efficient fermentation of the other carbohydrate present in the mixture. Other factors such as falling pH and viscosity may also be important. It is not possible to determine which are important in the present study.

In conclusion, R and G behaved differently during fermentation when combined together in cultures of human faecal bacteria than when fermented as single carbohydrate sources. This resulted in the preservation of butyrate production.

Acknowledgements Financial support: The Charles Wallace Pakistan Trust (The British Council, UK) was partially funded by SHS International Ltd, UK.

References

1. Edwards CA, Read NW (1990) Fibre and small intestinal function. In: Leeds AR (ed) Dietary Fibre Perspectives, Vol 2. London: John Libby, pp 52–75
2. Edwards CA, Rowland IR (1992) Bacterial fermentation in the colon and its measurement. In: Schweizer TF, Edwards CA (eds), Dietary Fiber–A Component of Food. London: Springer-Verlag, pp 119–136
3. Scheppach W, Bartram HP, Richter F (1995) Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer 31A: 1077–1080
4. Mortensen PB, Clausen MR (1996) Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. Scand J Gastroenterol 216(Suppl):132–148
5. Heerdt BG, Houston MA, Augenlicht LH (1994) Potentiation by specific short-chain fatty acids of differentiation and apoptosis in human colonic carcinoma cell lines. Cancer Res 54:3288–3293
6. Hague A, Paraskeva C (1995) The short-chain fatty acid butyrate induces apoptosis in colorectal tumour cell lines. Eur J Cancer Prev 4:359–364
7. Wright RS, Anderson JW, Bridges SR (1990) Propionate inhibits hepatocyte lipid synthesis. Proc Soc Exp Biol Med 195:26–29
8. Chen WL, Anderson JW, Jennings D (1984) Propionate may mediate the hypocholesterolemic effect of certain soluble plant fibres in cholesterol fed rats. Proceedings of the Society for Experimental Biology and Medicine 175: 215–218
9. Venter CS, Vorster HH, Cummings JH (1990) Effects of dietary propionate on carbohydrate and lipid metabolism in healthy volunteers. Am J Gastroenterol 85:549–553
10. Todesco T, Rao AV, Bosello O, Jenkins DJA (1991) Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. Am J Clin Nutr 54: 860–865
11. Ferguson MJ, Jones GP (2000) Production of short chain fatty acids following in vitro fermentation of saccharides, saccharide esters, fructo-oligosaccharides, starches, modified starches and non starch polysaccharides. J Sci Food Agric 80:166–170

12. Edwards CA, Parrett AM (1999) Colonic fermentation – in vitro and in vivo approaches to measurement. Science des Aliments 19:291–300
13. Henningsson ÅM, Björck IME, Nyman EMGL (2002) Combinations of Indigestible Carbohydrates Affect Short-Chain Fatty Acid Formation in the Hindgut of Rats. J Nutr 132:3098–3104
14. Hazan A, Madar Z (1993) Preparation of a dietary fiber mixture derived from different sources and its metabolic effect in rats. J Am Coll Nutr 12:661–668
15. Washington N, Harris M, Mussellwhite A, Spiller RC (1998) Moderation of lactulose-induced diarrhea by psyllium: effects on motility and fermentation. Am J Clin Nutr 67:317–321
16. Govers MJAP, Gannon NJ, Dunshea FR, Gibson PR, Muir JG (1999) Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. Gut 45:840–847
17. Morita T, Kasaoka S, Hase K, Kiriyama S (1999) Psyllium shifts the fermentation site of high amylose cornstarch toward the distal colon and increases fecal butyrate concentration in rats. J Nutr 129: 2081–2087
18. Campbell JM, Fahey GC Jr., Lichtensteiger CA, Demichele SJ, Garleb KA (1997) An enteral formula containing fish oil, indigestible oligosaccharides, gum Arabic and antioxidants affects plasma and colonic phospholipid fatty acid and prostaglandin profiles in pigs. J Nutr 127:137–145
19. Topping DL, Illman RJ, Trimble RP (1985) Volatile fatty acid concentrations in rats fed diets containing gum arabic and cellulose separately and as a mixture. Nutr Rep Int 32:809–814
20. Dalzenne N, Aertssens J, Verplaetse H, Roccaro M, Roberfroid M (1995) Effect of fermentable fructo-oligosaccharides on mineral, nitrogen and energy digestive balance in the rat. Life Sciences 57: 1579–1587
21. Adiotomre J, Eastwood MA, Edwards CA, Brydon WG (1990) Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. Am J Clin Nutr 52:28–34
22. Spiller GA, Chernoff MC, Hill RA, Gates JE, Nasser JJ, Shipley EA (1980) Effect of purified cellulose, pectin and a low-residue diet on fecal volatile fatty acids, transit time and fecal weight in humans. Am J Clin Nutr 33:754–759
23. Humbel R, Collart M (1975) Oligosaccharides in the urine of patients with glycoprotein storage diseases 1. Rapid detection by thin layer chromatography. Clin Chim Acta 60:143–145
24. Wang X, Gibson GR (1993) Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacteriol 75:373–380
25. Jenkins DJA, Derek T, Hockaday R, Howarth R, Apling EC, Wolever TMS, Leeds AR, Bacon S, Dilawari J (1977) Treatment of diabetes with guar gum. Lancet 2:779–780
26. Blackburn NA, Redfern JS, Jarjis H, Holgate AM, Hanning I, Scarpello JHB, Johnson IT, Read NW (1984) The mechanism of action of guar gum in improving glucose tolerance in man. Clin Sci 66:329–336
27. Blake DE, Hamblett CJ, Frost PG, Judd PA, Ellis PR (1997) Wheat bread supplemented with de-polymerized guar gum reduces the plasma cholesterol concentration in hypercholesterolemic human subjects. Am J Clin Nutr 65:107–113