Eur J Nutr (2006) 45:335–341
DOI 10.1007/s00394-006-0603-1

ORIGINAL CONTRIBUTION

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Lupin kernel fiber consumption modifies fecal microbiota in healthy men as determined by rRNA gene fluorescent *in situ* hybridization

Received: 28 October 2005
Accepted: 10 May 2006
Published online: 8 June 2006

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Abstract *Background* Changes in the composition of gastrointestinal microbiota by dietary interventions using pro- and prebiotics provide opportunity for improving health and preventing disease. However, the capacity of lupin kernel fiber (LKFibre), a novel legume-derived food ingredient, to act as a prebiotic and modulate the colonic microbiota in humans needed investigation. *Aim of the study* The present study aimed to determine the effect of LKFibre on human intestinal microbiota by quantitative fluorescent *in situ* hybridization (FISH) analysis. *Design* A total of 18 free-living healthy males between the ages of 24 and 64 years consumed a control diet and a LKFibre diet (containing an additional 17–30 g/day fiber beyond that of the control—incorporated into daily food items) for 28 days with a 28-day washout period in a single-blind, randomized, crossover dietary intervention design. *Methods* Fecal samples were collected for 3 days towards the end of each diet and microbial populations analyzed by FISH analysis using 16S rRNA gene-based oligonucleotide probes targeting total and predominant microbial populations. *Results* Significantly higher levels of *Bifidobacterium* spp. ($P = 0.001$) and significantly lower levels of the clostridia group of *C. ramosum*, *C. spiroforme* and *C. cocleatum* ($P = 0.039$) were observed on the LKFibre diet compared with the control. No significant differences between the LKFibre and the control diet were observed for total bacteria, *Lactobacillus* spp., the *Eubacterium* spp., the *C. histolyticum*/*C. lituseburense* group and the *Bacteroides–Prevotella* group. *Conclusions* Ingestion of LKFibre stimulated colonic bifidobacteria growth, which suggests that this dietary fiber may be considered as a prebiotic and may beneficially contribute to colon health.

Key words fecal microbiota – lupin kernel fiber – human diet – prebiotic – FISH

Introduction

Dietary fiber consumption is considered to have a large impact on compositional diversity and metabolic activity of the microbiota population in the human gastrointestinal (GI) tract [1]. However, our knowledge of (i) specific effects of dietary components on the composition of the microbiota and (ii) how changes in an individual's colonic microbiota contribute to human health and disease prevention is limited.

Of particular interest to gut health and well-being are dietary oligo- and polysaccharides claimed to be

EJN 603

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"prebiotics". The criteria for fulfilling the role of a prebiotic include "non-digestible food ingredients" that resist gastric acid, survive undigested in the upper GI tract and reach the colon where they are fermented and selectively stimulate the growth and/or activity of intestinal bacteria associated with health promoting activity and well-being [2, 3]. Several human dietary intervention studies have shown that intake of certain types of dietary fibers can significantly modify the gut surface-associated microbiota. Typically, diets containing highly fermentable oligofructose and fructans (inulin) result in enhanced growth of bifidobacteria [4–6] with a concomitant reduction in potentially pathogenic bacteria such as the *Clostridium coccoides–Eubacterium rectale* group [6]. The efficacy of dietary fibers deemed prebiotics, e.g. highly fermentable oligosaccharides [1, 4, 6], stems from their ability to be fermented selectively by *Bifidobacterium* spp. and *Lactobacillus* spp. and is dependent on the level of dietary intake. Several oligosaccharides meet the criteria for effective prebiotics involving selective promotion of beneficial bacteria. However, evidence for other dietary fibers such as soluble, non-starch polysaccharides (e.g. oat bran, psyllium), insoluble non-fermentable cellulose-based polysaccharides, and slowly fermentable fibers (e.g. wheat or barley bran) [2] is currently lacking.

Obtaining evidence of the efficacy of potential prebiotics requires dependable and quantitative microbiological analyses. One such method is a molecular-based approach using 16S rRNA gene sequence information to perform fluorescent in situ hybridization (FISH) using specific oligonucleotide probes. This method has already been successfully used to examine changes in human infant microbiota, the effects of pro- and prebiotics on the microbiota composition in healthy individuals, and in dietary interventions in animals and is deemed the "gold standard" for such analysis [5–8].

Lupin kernel fiber (LKFibre) is a novel food ingredient obtained from the kernel of Australian sweet lupin (*Lupinus angustifolius*) (ASL). ASL is a legume with a large production in Australia. ASL is widely used as animal feed but underutilized as a human food source [9, 10]. Dietary fiber represents 40% of the kernel weight of ASL, which is a higher level than most other legumes. LKFibre has potential as a human food ingredient as it has been used in the production of palatable fiber-enriched baked goods and pasta [9, 10]. This dietary fiber has a rhamnogalacturonan backbone [11] with galactose and arabinose side chains and may represent a new prebiotic, although evidence of its functionality as a prebiotic is lacking.

The present study investigated the effect of LKFibre on fecal microbiota after 28 days of LKFibre consumption by healthy humans in a single blind randomized crossover dietary intervention study. Fecal microbiota was quantified by FISH using target-specific 16S rRNA-based probes including one recognizing the health-promoting bifidobacteria in order to determine if LKFibre acts as a bifidogenic factor and therefore could be classed as a prebiotic.

Subjects and methods

Subjects

This study involved 18 healthy men randomly selected from the 38 who completed a major physiological study [10]. Volunteers were recruited by newspaper articles, radio announcements, posted notices and direct personal communication in Melbourne, Australia. Exclusion criteria were smoking, food allergy, history of cardiovascular disease or diabetes, and any use of antibiotics for 2 months prior to commencement of the study. The subjects in the present study were aged (mean ± standard error of the mean, SE) 42.8 ± 2.8 years (range 25–64 years) with a body mass index (mean ± SE) of 26.8 ± 0.6 kg/m^2 (range 20.9–31.2 kg/m^2).

Study design

This study formed part of a larger study investigating the physiological response of LKFibre in the human diet [10]. The study was approved by Deakin University Ethics Committee and complied with the Helsinki Declaration of 1975, as revised in 2000. In brief, the study design involved free-living subjects consuming two semi-controlled diets, one a high-fiber diet, and the other a lower fiber control diet of otherwise equivalent nutritional profile but containing no LKFibre. Each diet was consumed for 28 days each in a single-blind, randomized, crossover dietary intervention design with a minimum of a 28 days washout period between diets.

Experimental diets and dietary assessment

The experimental diets and dietary assessment have been described in detail elsewhere [10]. In brief, the two diets had the same self-managed background menus, each with the addition of seven experimental foods supplied by the researchers (bread, muffin, chocolate brownie, chocolate milk drink, toasted muesli, pasta and instant mashed potato), which either contained LKFibre or did not. The two experimental diets were designed to have the same macronutrient content except for dietary fiber. Depending on energy intake, the LKFibre diet was designed to contain an additional 17–30 g/day fiber

beyond that of the control diet. The LKFibre and control experimental foods were manufactured by George Weston Foods (Enfield, New South Wales).

Subjects were instructed to avoid legumes, high-fat foods, known lipid-modifying foods, such as phytosterol-containing spreads and fermented foods such as yoghurt.

Each subject completed a 4-day weighed food record of their habitual diet and during the second week of each intervention. The food records were analyzed by FoodWorks version 3.01, build 472 (Xyris Software, Brisbane, Australia), which incorporates the AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia).

Fecal collection

All feces passed during days 24, 25 and 26 of each intervention were collected by the subject into containers that were initially stored in insulated boxes containing solid carbon dioxide ('dry-ice'). Subjects recorded the time and date of each defecation event. Collected fecal samples were transported to Deakin University each day by the researchers, weighed and then stored at −20°C.

FISH analysis

The 3-day fecal collections of each diet were pooled per subject by thawing rapidly in a warm water bath. Representative aliquots ($n = 3$) of fecal sample (~0.5 g) were suspended in 4.5 ml of phosphate-buffered saline (PBS) homogenized, fixed and processed according to the FISH assay protocol [12]. Essentially, fecal samples were homogenized by vortexing with 3–4 glass beads (4 mm diameter) for 5 min and the fecal debris removed by centrifuging at 700*g* for 1 min. Cells were then fixed in 3% paraformaldehyde in PBS overnight at 4°C and dilutions of the samples in PBS were applied to 0.1% gelatin-coated slides and fixed to the slides with 96% ethanol [12] and hybridized with 5 ng/μl of the oligonucleotide probes. The following fluorescent-labeled probes were used: the Bact338 probe for total bacteria [12], the Bif164 probe for the genus Bifidobacteria [12], the Lab158 probe for the genera lactobacilli and enterococci [12], the Bac303 probe for genera *Bacteroides* and *Prevotella* [12], the Erec482 probe specific for most eubacteria and clostridia belonging to the *Clostridium coccoides–Eubacterium rectale* group (*Clostridium* clusters XIVa and XIVb) [12, 13], the EC1531 probe for a selection of enterobacteria including *E. coli* from within *Enterobacteriaceae* [14], the combined Clit0135/Chis150 probes specific for most species of the *Clostridium lituseburense* group (*Clostridium* cluster XI) and most species of the *Clostridium histolyticum* group (*Clostridium* clusters I and II) [12, 15] and the Cspiro222 probe for the group of *Clostridium ramosum*, *C. spiroforme* and *C. cocleatum* [16]. The hybridized samples were then covered with Vectashield containing the DNA-binding reagent (DAPI) (Vector Laboratories Inc., Burlingham, CA) for enumerating total cells and the fluorescent cells enumerated automatically (DAPI, Bact 338, Bac303 and Erec 482) by Quantimet (Leica DM/RXA ultraviolet microscope and Leica 550HR image analysis) or visually by an epifluorescence Olympus BH2 microscope or Olympus IX-70 microscope (EC1531, Lab158, Clit0135/Chis150, Bif 164 and Cspiro222). For image analysis, a white tophat and binary mode filter were used and fluorescent areas of five pixels or more were measured. The operator was blind to the identity of the diet from which each fecal sample originated. Duplicate slides with six wells per slide were used. A minimum of 25 fields of view per well were counted. Appropriate dilutions were used to give between 30 and 200 bacteria per field of view. Water content of individual fecal samples was measured and this factor was used to convert the number of cells/g wet weight of feces to number of cells/g dry weight. In addition, the percentages of each bacterial group were calculated compared with total bacteria enumerated by DAPI-staining and Bact338 probe.

Statistical analysis

All results are expressed as means ± Standard Error (SE). The normality of the study data was evaluated using a Kolmogorov–Smirnov test. A paired samples *t*-test or 2-related sample non-parametric (Wilcoxon-Signed ranks) test for non-normal data was used to compare the effect of dietary intervention on bacterial

Table 1 Daily dietary intakes from weighed food records during intervention periods[a]

	Diet	
	Control	LKFibre[b]
Energy (kJ/d)	10749 ± 595	10817 ± 530
Total protein (g/d)	104.1 ± 6.1	106.6 ± 5.6
Available carbohydrate (g/d)	334.8 ± 22.1	314.9 ± 17.8
Total dietary fiber (g/d)	23.2 ± 1.3	45.4 ± 2.3[c]
Sugar (g/d)	152.8 ± 12.7	162.3 ± 11.5
Starch (g/d)	180.7 ± 10.8	151.2 ± 7.7[c]
Total fat (g/d)	76.8 ± 3.9	78.6 ± 4.3
Saturated fatty acids (g/d)	25.0 ± 1.4	26.7 ± 1.7
Monounsaturated fatty acids (g/d)	27.8 ± 1.3	27.9 ± 1.5
Polyunsaturated fatty acids (g/d)	17.7 ± 1.2	17.3 ± 1.1
Dietary cholesterol (mg/d)	208.2 ± 16.4	217.1 ± 16.9
Alcohol (g/d)	9.0 ± 2.5	13.0 ± 4.5

[a]Mean ± SE; $n = 18$
[b]Australian sweet lupin (*Lupinus angustifolius*) kernel fiber
[c]Significantly different from the control diet, $P < 0.05$

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numbers using SPSS software, version 11.5 (SPSS Inc, Chicago, IL, USA). For all analyses, $P < 0.05$ was considered significant.

Results

In this study, the influence of dietary ingestion of an additional 17–30 g/day of dietary fiber in the form of LKFibre for 28 days on the fecal microbiota of 18 healthy male volunteers was investigated. Self-reported dietary intakes for subjects during the control and LKFibre diets are given in Table 1. Comparison of the LKFibre diet to the control diet demonstrated that the diets were generally well-balanced with no significant difference between the protein and available carbohydrate levels in the diets ($P > 0.05$) although there was a small but significant reduction in starch intake during the LKFibre diet. Total dietary fiber intake (g/day) during the LKFibre diet was significantly higher (approximately 20 g/day) than the control diet.

Fecal analysis using FISH

Mean fecal bacterial cell numbers for the control and LKFibre diets and the treatment effect are given in Table 2. An independent samples *t*-test determined that there was no diet order effect ($P > 0.05$).

Significant differences between the two diets ($P < 0.05$) were observed in the numbers of *Bifidobacterium* spp. and the *Clostridium ramosum*, *C. cocleatum, C. spiroforme* group (Table 2). There was a very strong trend, almost reaching significance, of decreasing numbers of the *Bacteroides–Prevotella* group bacteria on the LKFibre diet using the Bac303 probe ($P = 0.053$). No significant differences were observed between the two diets ($P > 0.05$) for total bacterial cells enumerated using DAPI-staining or Bac338 probe, for *E. rectale–C. coccoides* group, for Bacteroides–Prevotella group, for lactic acid bacteria, for *Clostridium histolyticum–Clostridium litusebu-rense* group (*Clostridium* clusters I, II, XI) and the *Enterobacteriaceae* group.

The proportion of microbiota detected by each specific probe can be expressed as a percentage of the DAPI stain and the Bact 338 probe for the control and LKFibre diets. The total percentage microbiota detected by the combination of specific probes in the control diet was 57% compared to the DAPI stain and approximately 78% compared to the Bact 338 probe. However, for the LKFibre diet, only 43% of bacteria were detected by the group specific probes compared to the DAPI stain and 52% compared to the Bact 338 probe. Bifidobacteria numbers as a percentage of total

Table 2 Mean bacterial numbers (cells/g dry weight feces) of 18 volunteers determined by FISH or DAPI staining for control and LKFibre[a] diets

Population	Probe	Cells/g dry weight			Mean difference (LKFibre[a]-control)	95% confidence interval of treatment effect (lower, upper)[b]	% Total microbiota control diet		% Total microbiota LKFibre[a] diet	
		Control diet Mean (SE)	LKFibre[a] diet Mean (SE)	*P*			DAPI	Bact338	DAPI	Bact338
Total cells	DAPI	6.7×10^{10} (1.2)	6.0×10^{10} (0.8)	0.514	-0.7×10^{10}	-2.9×10^{10}, 1.5×10^{10}	100.00		100.00	
Total bacteria	Bact338	5.1×10^{10} (0.8)	5.0×10^{10} (0.6)	0.869	-0.1×10^{10}	-2.0×10^{10}, 1.7×10^{10}	76.11	100.00	81.96	100.00
E. rectale–C. coccoides	Erec482	1.1×10^{10} (0.2)	1.0×10^{10} (0.2)	0.522	-0.1×10^{10}	-5.6×10^{9}, 3.0×10^{9}	16.42	21.57	15.90	19.40
Bacteroides–Prevotella	Bac303	2.5×10^{10} (0.7)	1.3×10^{10} (0.3)	0.053[c]	-1.2×10^{10}	–	37.31	49.02	21.31	26.00
Enterobacteriaceae	EC 1531	1.4×10^{9} (0.8)	0.9×10^{9} (0.04)	0.327[c]	-0.5×10^{9}	–	2.09	2.75	1.48	1.80
*C. histolyticum/C. litusebur*ense group	Clit0135/Chis150	3.5×10^{8} (0.6)	4.2×10^{8} (1.2)	0.306[c]	0.7×10^{8}	–	0.52	0.68	0.68	0.84
Lactobacillus–Enterococci	Lab158	1.4×10^{7} (0.5)	1.8×10^{7} (0.4)	0.248[c]	0.4×10^{7}	–	0.02	0.03	0.03	0.04
Bifidobacterium	Bif 164	0.8×10^{9} (0.2)	1.7×10^{9} (0.5)	0.001[c,d]	0.9×10^{9}	–	1.21	1.59	2.78	3.40
C. ramosum, C. spiroforme and C. cocleatum	Cspiro222	7.9×10^{7} (1.1)	6.0×10^{7} (0.6)	0.039[c,d]	-1.9×10^{7}	–	0.12	0.15	0.10	0.12

[a]Australian sweet lupin (*Lupinus angustifolius*) kernel fiber
[b]Paired *t*-test
[c]Wilcoxon Signed Ranks test for non-normal distribution
[d]Significant difference between LKFibre and control diets ($P < 0.05$)

Table 3 Individual subjects fecal microbiota numbers of *Bifidobacterium* spp. and *Clostridial* spp. (*C. ramosum, C. spiroforme and C. cocleatum*) in the control and LKFibre diets

Subject	Bifidobacteria using Bif164 probe mean (SE) cells/g dry weight		Clostridia using Cspiro222 probe mean (SE) cells/g dry weight	
	Control diet	LKFibre diet[a]	Control diet	LKFibre diet[a]
A	1.8×10^8 (1.1)	**3.7×10^8 (0.2)**	1.2×10^8 (0.2)	**5.2×10^7 (0.6)**
B	2.5×10^8 (0.8)	**2.4×10^9 (0.1)**	6.7×10^7 (1.5)	**5.3×10^7 (0.7)**
C	4.0×10^8 (1.3)	**2.7×10^9 (0.8)**	9.7×10^7 (1.4)	**7.4×10^7 (0.6)**
D	2.9×10^8 (1.1)	**4.0×10^8 (0.1)**	5.9×10^7 (2.1)	6.9×10^7 (0.7)
E	1.6×10^8 (1.2)	**2.8×10^8 (0.2)**	1.6×10^8 (0.2)	**9.5×10^7 (1.1)**
F	1.4×10^8 (0.7)	1.3×10^8 (0.1)	4.3×10^7 (1.7)	**3.9×10^7 (0.5)**
G	5.4×10^8 (0.3)	**8.2×10^8 (0.3)**	2.3×10^8 (0.1)	**7.9×10^7 (0.7)**
H	3.0×10^8 (0.3)	**2.1×10^9 (0.7)**	2.5×10^7 (2.0)	8.6×10^7 (0.9)
I	6.4×10^8 (0.6)	**2.0×10^9 (0.7)**	3.2×10^7 (1.5)	**2.3×10^7 (0.4)**
J	1.3×10^8 (0.3)	1.3×10^8 (0.1)	1.0×10^8 (0.2)	**3.8×10^7 (0.5)**
K	5.0×10^8 (0.5)	**3.3×10^8 (0.2)**	6.8×10^7 (1.3)	1.1×10^8 (0.1)
L	7.9×10^8 (0.5)	**9.7×10^8 (0.5)**	7.9×10^7 (1.0)	**5.0×10^7 (0.4)**
M	9.7×10^8 (0.3)	**1.4×10^9 (0.5)**	7.1×10^7 (0.9)	**6.2×10^7 (0.8)**
N	3.7×10^9 (1.1)	**6.6×10^9 (0.3)**	3.4×10^7 (0.4)	**1.7×10^7 (0.3)**
O	3.4×10^8 (0.2)	**6.7×10^8 (0.2)**	5.3×10^7 (0.6)	5.5×10^7 (0.8)
P	2.7×10^9 (0.8)	**3.1×10^9 (0.4)**	5.5×10^7 (0.7)	**4.4×10^7 (0.5)**
Q	2.1×10^9 (0.4)	**6.7×10^9 (0.1)**	7.7×10^7 (0.7)	**5.3×10^7 (0.8)**
R	4.2×10^8 (0.5)	4.2×10^8 (0.2)	7.0×10^7 (0.7)	7.7×10^7 (1.1)
Mean (SE)	8.1×10^8 (0.2)	**1.7×10^9 (0.5)**	7.9×10^7 (1.1)	**6.0×10^7 (0.6)**

[a]Bold indicates subjects with a difference in population numbers of specific microbiota between control and LKFibre diet

microbiota (as measured by comparison to the Bact 338 probe) increased in the LKFibre diet compared to the control diet whereas numbers of the *Clostridium ramosum*, *C. cocleatum*, *C. spiroforme* group decreased. Using the Cspiro222 probe and based upon cell morphology (results not presented), 92% of the clostridia observed were *C. ramosum* in both diets.

Individual subjects were also examined for changes in the numbers of microbiota within the *Bifidobacterium* spp. and the *C. ramosum*/*C. cocleatum*/*C. spiroforme* groups (Table 3). Of the 18 subjects 15 had higher numbers of bifidobacteria during the LKFibre diet than the control diet. Among 18 subjects 13 demonstrated lower numbers of the *C. ramosum*/*C. cocleatum*/*C. spiroforme* group during the LKFibre diet than the control.

Discussion

This study provides the first published data on the influence of LKFibre on human gut microbiota populations using FISH analysis with group-specific 16S rRNA gene-targeted oligonucleotide probes. Since the diets were well-balanced for energy and most macronutrients, changes in the probed bacterial populations can be primarily attributed to the LKFibre in the diet.

In the present study, enumeration of bifidobacteria using FISH analysis demonstrated that LKFibre acts as a bifidogenic factor in a similar way to that previously reported for fructooligosaccharides (FOS) and galactooligosaccharides (GOS), which are proposed as prebiotics [2, 6, 7]. The lack of effect of LKFibre on numbers of *Lactobacillus* spp. and *Enterococcus* spp., known residential microbiota of the small colon, and total bacteria is consistent with studies by Tuohy et al. and Harmsen et al. on inulin [6, 7] indicating the influence of this oligosaccharide on colonic microbiota is restricted primarily to bifidobacterium. In previous studies with human subjects, it was shown that dietary intake of FOS and GOS reduced levels of pathogens such as *Clostridium histolyticum–Clostridium lituseburense* group (*Clostridium* clusters I, II, XI) and *Eubacterium* spp. [6, 13, 15]. No changes in the numbers of these bacteria were observed in other human dietary studies investigating inulin or oligofructose [4, 17] or in the present study using both the Erec482 probe for most eubacteria and clostridia belonging to the *Clostridium coccoides–Eubacterium rectale* group (*Clostridium* clusters XIVa and XIVb) [12] and the combined Clit/Chis150 probes for clostridia [13]. However, the Cspiro 222 probe [16] detected a significant decrease in the *C. ramosum*/*C. spiroforme*/*C. cocleatum* group in the LKFibre diet compared to the control diet. This effect was observed in 13 of the 18 subjects. This is the first time this probe has been used in studies for assessing a dietary fiber as a potential prebiotic and suggests that other species-specific FISH probes should be considered in preference to the more general genus-specific FISH probes. The finding of lower clostridia numbers in subjects on the LKFibre diet is consistent with the previous findings by Wildeboer-Veloo et al. [16] that the prevalence of these bacteria can be influenced by dietary-derived sub-

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strates. Although these clostridia are not considered to be major causes of disease, the group of *C. ramosum*, *C. spiroforme* and *C. cocleatum* appear to play an important role in bilirubin conversion and it has also been hypothesized that they may have a role in stimulating the development of the immune system [16, 18, 19].

A recent study, by Langlands et al. [20], supplementing the diet with a mixture of 2.5 g inulin and 2.5 g oligofructose three times a day was found to effectively stimulate the growth of mucosal bifidobacteria and lactobacilli in both the proximal and distal colon in 29 healthy subjects. Overall numbers of clostridia were unaffected by the prebiotic diet but species-specific differences were observed including an increase in *C. innocuum* and absence of *C. cocleatum* after prebiotic supplementation. The previously reported loss of the numerically small *C. cocleatum* species is consistent with the observations reported in the present study and highlights similarities in the influence of LKFibre and the prebiotic carbohydrates, inulin and oligofructose, on composition of colonic microbiota [20]. Dietary fibers such as LKFibre may therefore have some beneficial influence on the composition of the fecal microbiota that reflects changes in mucosa associated flora (MAF) in the colon that could affect immune development and responses especially in different age groups. Further dietary studies are required to examine this concept.

The strong trend in decreasing numbers of the Bacteroides–Prevotella group is of interest to health. Bacteroides represent a predominant group within the gut microbiota associated with disease, the bioavailability of vitamins and nutrients and the metabolism of dietary polysaccharides. The trend of decreasing numbers of this group in the present study may suggest that LKFibre is not actively metabolized by these mucosa associated bacteria. Further studies using species-specific probes within the Bacteroides–Prevotella group are required to understand the effect of LKFibre on gut health in more detail.

Previously, Rubio et al. [21] observed that addition of a fiber residue from lupin to the diet significantly decreased the levels of *E. coli* using a rat model as assessed by culture-dependent methods. The results of the present dietary intervention study using the FISH probe EC1531 do not support the findings by Rubio et al. [21], rather they indicate biodiversity of gut microbiota between different mammalian species especially when investigating the influence of diet.

Culture based methods are known to bias towards viable organisms that can be cultured but can never provide analysis of the total population including obligate anaerobes [5]. Molecular analyses such as FISH and PCR-DGGE [22] are limited by the number of probes at species level, however, provide valuable information on dietary influences on microbial population. Using the culture-independent molecular rRNA-based technique the present molecular analyses indicated no significant effects on coliforms, total bacteria, eubacteria, major clostridial groups or lactobacilli, but a trend in decreasing numbers of bacteroides and a significant increase in bifidobacteria in the LKFibre diet. In this study, FISH analysis using the Bif164 probe could not be used to differentiate between the levels of viable and non-viable bacterial numbers within individuals nor between different species of bifidobacteria. The increase in bifidobacteria, however, clearly suggests a selective prebiotic and bifidogenic effect in most subjects (15 out of 18). In this LKFibre study and other studies with inulin [7], partially hydrolyzed guar gum and fructo-oligosaccharides [23], the influence of dietary fibers was evident in most individual responses. This effect along with recently identified beneficial effects of LKFibre on bowel function, in terms of fecal output, transit time and increased levels of short chain fatty acids [24], suggest that this fiber is beneficial for colonic health and potential gut-associated immunomodulation.

Further studies on the effect of LKFibre using FISH probes to other bacterial genera and species in fully nutritionally-balanced dietary studies are now necessary. Examples include probes targeting species within the predominant *Bacteroides* spp. [25] as well as lesser known groups such as *Veilonella* [15]. In addition, 'bacterio-profiling' of resident bacterial communities in subjects by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene amplicons, and sequencing of partial amplified segments of the 16S rDNA gene, would be useful in determining the full effect of dietary LKFibre within individuals and different age groups. Other studies need to be undertaken to investigate dose response of LKFibre. Direct comparison between the effects of LKFibre and established prebiotic carbohydrates would confirm the relative merit of LKFibre for prebiosis. The concept of prebiotics and bifidogenic factors should be carefully considered in terms of both population and individual responses. Further understanding of the relationship between host, diet and population of gut associated microbiota will increase our knowledge of dietary influence, especially that of LKFibre, on colonic health.

Acknowledgements The dietary study was supported by the Grains Research and Development Corporation, the Australian Research Council—Strategic Partnerships with Industry—Research and Training Scheme, the Department of Agriculture Western Australia and Deakin University. The authors are grateful to Food Science Australia for manufacturing the LKFibre, to George Weston Foods for manufacturing the experimental food products and conducting nutritional analyses of these foods and to the study participants for their commitment to the study protocol. The authors also express their thanks to M. Ball for advice on the dietary design, Ms Amynta Baxter and Ms Veronica Chua for their assistance with some parts of this study.

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