British Journal of Nutrition (2010), **104**, 1514–1522
doi:10.1017/S0007114510002461

Effect of multifibre mixture with prebiotic components on bifidobacteria and stool pH in tube-fed children

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(Received 27 August 2009 – Revised 24 May 2010 – Accepted 25 May 2010 – First published online 6 August 2010)

The objective of the present study was to evaluate the effect of a paediatric tube feed supplemented with a multifibre mixture on the gut microbiota and nutritional and micronutrient status of children on long-term enteral nutrition (EN). A randomised, controlled, double-blind, cross-over trial (2 × 3 months) with a washout period of 1 month was carried out. Twenty-seven children (80 % neurologically impaired) aged 11·9 (SD 3·9) years, on long-term EN (4·8 (SD 3·9) years) were recruited. The analyses of the children's faecal pH, microbiota along with anthropometric measures, bowel movements and markers of blood micronutrient status were made. Twenty children completed the study. A significant increase in the proportion of stool bifidobacteria (+16·6 %, $P<0·05$) was observed during the multifibre period than during the fibre-free period, together with a significant reduction in stool pH ($P<0·001$). Stool frequency and consistency as well as growth did not differ between the two periods. There was a significant increase ($P<0·05$) in plasma ferritin at the end of the fibre-free period, but plasma ferritin levels remained within normal ranges during both periods. No diet effects on other blood parameters were observed. In conclusion, addition of a multifibre mixture with prebiotic components to paediatric EN is well tolerated, promotes bifidobacteria and reduces stool pH, indicating an improved gut health.

Enteral nutrition: Fibre: Children: Gut microbiota

Paediatric indications for enteral nutrition (EN) have increased along with the increasing recognition of the clinical efficacy of nutritional support in treating the most severe and chronic diseases in childhood[1]. The long duration of EN, which is explained by the increased life expectancy of children with chronic diseases (e.g. cystic fibrosis and neurological disabilities) requiring home EN, reinforces the need to use EN products closely adapted to the children's needs. EN is intended to provide the individual with all the nutrients necessary for maintaining the optimal growth and development. Depending on the child's age, anthropometry, growth rate and pathology, EN formulas can have energy densities ranging from 3·1 kJ/ml (0·75 kcal/ml) to 6·3 kJ/ml (1·5 kcal/ml). Enteral formulas for paediatric patients also vary in the proportion of nutrients they contain to address patient-specific requirements. Although most of the paediatric EN are nutritionally complete, many do not contain fibre.

The health benefits of dietary fibre have been well described and have formed the basis of dietary recommendations for children and adults[2,3]. Dietary fibre is important to maintain gastrointestinal (GI) health and may, in addition, reduce the risk of CHD[2,4,5]. Recommendations for daily fibre intake in healthy children are primarily based on the extrapolation of data from adults[3,6], and are mostly expressed on a body weight, age or energy intake basis[2–4].

Because dietary fibre has important GI health benefits in childhood, especially in normalising bowel function, fibre-supplemented feeds may be beneficial for children with diarrhoea and constipation[2,4,5,7]. Diarrhoea and constipation, representing the two extremes of bowel function, remain the most frequent problems associated with EN in children. Diarrhoea is regularly reported in critically ill children[8,9], whereas constipation is more common in the chronic-care setting, especially in children with neurodisabilities. About 60–75 % of children with neurological impairments have been reported to suffer from constipation[10,11]. However, there are only limited data available on the role, tolerability and efficacy of fibre-supplemented enteral formulas in children[12–15], and the effect of fibre supplementation on gut microbiota has not been investigated as yet.

Abbreviations: BIA, bioelectrical impedance absorptiometry; EN, enteral nutrition; FFM, fat-free mass; FM, fat mass; GI, gastrointestinal; TEN, Tentrini; TMF, Tentrini multifibre.

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Therefore, the objective of the present study was to evaluate the effect of a paediatric tube feed supplemented with a multifibre mixture on the gut microbiota, bowel function, GI tolerance and nutritional and micronutrient status of children over 7 years of age on long-term EN.

Subjects and methods

Subjects

The present study was a randomised, controlled, double-blind, cross-over trial comparing a standard, fibre-free paediatric tube feed to a multifibre-enriched feed in enterally fed children. Children fed enterally for at least 2 weeks, with a stable condition and nutritional intake, and requiring EN for a minimum of 8 months were recruited at the Centre Hospitalier Régional Universitaire de Lille (France) and affiliated healthcare centre of Vendin Le-Viel (France) between September 2003 and December 2005.

Children were deemed eligible for inclusion in the study if they were at least 7 years old, had a body weight >21 kg, had a stable medical condition and a stable intake of EN contributing at least 50 % of their total energy intake, and had not received a fibre-supplemented enteral formula for at least 2 weeks before inclusion. Eligible children also had to consume less than two servings of yoghurts or fermented products per day, and to have an oral fibre intake <1 g/d. Exclusion criteria were cow's milk allergy, inflammatory bowel disease, bowel resection, other diseases associated with GI disorders and dyslipoproteinaemia. Further exclusion criteria included antibiotic therapy or use of laxatives other than polyethylene glycol or paraffin oil during the 2 weeks preceding the study, acute diarrhoea during the 2 weeks preceding the study and supplementation with Fe and/or any of the other monitored micronutrients during the month before inclusion. Written informed consent was obtained from both parents of every eligible child. The protocol was approved by the ethics committee (Comité Consultatif de Protection des Personnes se prêtant à la Recherche Biomédicale, Lille, France).

Study design

The study products used were manufactured and distributed by Nutricia N.V., Zoetermeer, The Netherlands. Tentrini® and Tentrini® Energy (also known as NutriniMax® and NutriniMax® Energy; both referred to as TEN in the present study) are nutritionally complete, fibre-free paediatric tube feeds for children aged 7–12 years or 21–45 kg in weight, providing 4·2 kJ/ml (1 kcal/ml) and 6·3 kJ/ml (1·5 kcal/ml) of feed, respectively (Table 1). Tentrini® Multi Fibre and Tentrini® Energy Multi Fibre (also known as NutriniMax® Multi Fibre and NutriniMax® Energy Multi Fibre; both referred to as TMF) are similar in composition to Tentrini and Tentrini Energy, but provide 1·1 g/100 ml of a mixed fibre source (Table 1). The Multi Fibre mixture (MF6™) consists of fructo-oligosaccharides (10·3 %), inulin (22·2 %), soya polysaccharides (30·1 %), cellulose (11·3 %), gum arabic (15·0 %) and resistant starch (11·1 %). The choice for a standard or energy-enriched paediatric feed was dependent on the energy needs of the child. Children receiving a fibre-containing enteral feed before the study were allocated to a fibre-free feed during a run-in period of 1 month before the first phase of the study.

All children were then randomised (following a computer-generated randomisation list) to start the study intervention period on either one of the paediatric feeds supplemented with the Multi Fibre mixture (TMF) or one of the control feeds without fibre (TEN) for the first phase of 3 months. For the second phase, children were switched to the other study feeds. Between the two intervention phases, all children followed a washout period of 1 month on TEN.

Assessments were made at the beginning and end of each study phase (at the start of the study, and after 3, 4 and 7 months).

Dietary intake

As the children had a stable medical condition and a stable intake of EN before the study, energy intake from tube feeding was assumed to be constant during the entire study period. The daily energy intake from tube feeding during the study period was estimated by multiplying the reported volume consumed at baseline by the energy content of the study feed received

Table 1. Nutritional composition of the paediatric study formulas per 100 ml

Nutrients	Tentrini®	Tentrini® MF	Tentrini® energy	Tentrini® energy MF
Energy (kJ)	418	418	628	628
Energy (kcal)	100	100	150	150
Protein (g)	3·3	3·3	4·9	4·9
Carbohydrate (g)	12·3	12·3	18·5	18·5
Fat (g)	4·2	4·2	6·3	6·3
Fibre (MF6) (g)	–	1·1	–	1·1
Trace elements				
Fe (mg)	1·3	1·3	2·0	2·0
Zn (mg)	1·1	1·1	1·7	1·7
Se (μg)	4·9	4·9	7·4	7·4
Vitamins				
A (μg RE)	61	61	92	92
D (μg)	0·7	0·7	1·1	1·1
E (mg α-TE)	1·3	1·3	1·9	1·9
K (μg)	4·5	4·5	6·8	6·8

RE, retinol equivalent; α-TE, tocopherol equivalent.

during each phase. At each visit, a 48 h dietary recall (recording all foods and drinks consumed orally over the previous 2 d) was completed. From these data, the daily energy intake from the oral diet was estimated. Twenty of twenty-seven patients had no oral intake and for the seven patients who continued to eat, oral food intake remained limited. Overall, the EN amounted for 80–85 % of the total energy intake of the patients. Compliance was not directly monitored in the present study, but the study product was delivered on a monthly basis by the centre to the patient's house as a method of accountability.

Concomitant medication

Any changes in the medication prescribed or therapeutic procedures carried out on the child were recorded at each visit. Except for antibiotics that were adapted to the clinical situation of the patient, care was taken that the medical treatment (anticonvulsive drugs and laxatives) remained the same during the two phases of the study.

Anthropometry

Children were weighed and measured for height and mid-upper arm circumference at each visit (at the start of the study and after 3, 4 and 7 months of intervention). Skinfold thickness was also measured at these visits (three measures at four sites (biceps, triceps, sub-scapular and supra-iliac)) on the left side of the body using Holtain calipers[16]. Bioelectrical impedance absorptiometry (BIA) was carried out for each child at the same time points. Fat mass (FM) was quantified using skinfold thickness measurements and using either Brook's equation[17] for children aged 7–11 years or Durnin & Rahaman's equation[18] for children aged >12 years. Siri's equation[19] was then used to calculate the percentage of FM. Fat-free mass (FFM) was determined by BIA using Schaefer's equation[20]. The percentages of FM and FFM were obtained by averaging the results of both methods (skinfold and BIA).

Bowel movements and gastrointestinal symptoms

Parents were requested to record stool frequency and consistency (0: hard; 1: normal; 2: soft; and 3: liquid) during the 15 d preceding each visit to the clinic. During the same periods, GI symptoms (belching, flatulence, bloating, nausea, vomiting and abdominal pain) were also reported by the parents and rated for their severity (0: absent; 1: light; 2: moderate; or 3: severe) in order to assess tolerance.

Faecal microbiology, pH and SCFA

In the week preceding each visit (at the start of the study and after 3, 4 and 7 months), parents were asked to collect a fresh stool sample immediately after defaecation. In the event that antibiotic treatment was used during the course of the study, the faecal sample was collected before the start of antibiotic therapy or otherwise delayed to 15 d following the end of the medication. Samples were immediately stored at −20°C until further processing. For each faecal sample, pH and DM were determined. Quantification of distinct groups of bacteria was performed using the fluorescent *in situ* hybridisation[21]. Specific bacteria were stained with Cy3-labelled 16S rRNA-targeted oligonucleotides (Bif164 for bifidobacteria[21], Lab158 for lactobacilli[22], Ec1531 for *Escherichia coli*[23] and Chis150 and Clit135 for subgroups of clostridia[24]), and the absolute numbers of bacteria per gram faeces were obtained by counterstaining with the DNA-binding dye 4′,6-diamidino-2-phenylindole[25]. The oligonucleotide probes used in the present study, their sequence and their targeted micro-organisms are given in Table 2. Hybridisation and washing steps were performed according to the conditions described in the cited references, except for the Ec1531 probe for which no formamide was used, and the samples were hybridised at 50°C instead of at 37°C.

Faecal samples were thawed, and the pH was measured directly at room temperature using a Handylab pH meter (Schott Glas, Mainz, Germany) equipped with an Inlab 423 pH electrode (Mettler-Toledo, Columbus, OH, USA).

Acetic, propionic, *n*-butyric, iso-butyric and *n*-valeric acids were quantitatively determined by a Varian 3800 GC (Varian, Inc., Walnut Creek, CA, USA) equipped with a flame ionisation detector as described previously[26].

Micronutrients

Blood samples were collected at the beginning and end of each phase (0, 3, 4 and 7 months), and were analysed in the same laboratory. Hb, mean corpuscular volume and ferritin were analysed using standard methods. Plasma vitamin E was determined by HPLC[27]. Vitamin C was determined with an automated enzymatic procedure,[28] and all the samples were analysed within 1 h. Se was analysed by inductively coupled plasma-MS. Zn was analysed by flame atomic absorption spectrometry. Glutathione peroxidase concentration

Table 2. List of the 16S rRNA-targeted oligonucleotides used in the present study

Probe name	Probe sequence (5′ to 3′ end)	Target bacteria	References
Bif164	CATCCGGCATTACCACCC	Bifidobacteria	21
Lab158	GGTATTAGCA(C/T)CTGTTTCCA	*Lactobacillus*/*Enterococcus*	22
Ec1531*	CACCGTAGTGCCTCGTCATCA	*Escherichia coli*	23
Clit135†	GTTATCCGTGTGTACAGG	*Clostridium lituseburense*	24
Chis150†	TTATGCGGTATTAATCT(C/T)CCTTT	*Clostridium histolyticum*	24

* The hybridisation and washing conditions for this probe were modified as follows: the hybridisation buffer contained 0·9 M-NaCl, 20 mM-Tris–HCl (pH 7·2), and 0·1 % (w/v) SDS and no formamide was used. The hybridisation and washing temperature was set to 50°C.
† The Clit135 and Chis150 probes were used in combination.

was analysed using a RANSEL kit (Randox Limited, Crumlin, County Antrim, UK) following the method of Paglia and Valentine[29], and superoxide dismutase was analysed using a RANSOD kit (Randox Limited) following the method described by Mac Cord & Fridovich[30], both of which were then adapted to a Hitachi 911 (Roche, Meylan, France) spectrophotometer.

Statistical analysis

Baseline characteristics of the children were analysed as frequency and descriptive data. Outcome variables were analysed on the basis of intention to treat. Normality was assumed for the Shapiro–Wilk test with $P>0{\cdot}05$. Differences in continuous variables were expressed as mean with standard error or mean and standard deviation when the data were normally distributed and compared between groups using the Student *t* test. For non-parametric data, values were expressed as median (range) and compared using the Mann–Whitney *U*-test. The statistical model took into account the treatment, period and carry-over effects as described by Altman[31]. All data analyses were done using Statistical Package for the Social Sciences 12.0.1 version for Windows (SPSS, Inc., Chicago, IL, USA). Statistical significance was set at $P<0{\cdot}05$.

Results

Twenty-seven children, of whom twenty-two had neurodisabilities, were recruited between September 2003 and June 2005. Twenty children completed the study (eighteen had neurodisabilities), with seven early terminations that included abdominal surgery because of a digestive fistula post gastrostomy, two consent withdrawals, three disturbed GI functions with diarrhoea and one loss to follow-up.

Baseline characteristics of the children are presented in Table 3. All children were fed via gastrostomy. Interpretable 48 h recalls were collected for nine patients at visit 1, seven patients at visit 2, six patients at visit 3 and five patients at visit 4. Oral energy intake as assessed by these dietary recalls was, on average, 1912·1 kJ/d (457 kcal/d) at visit 1, 2267·7 kJ/d (542 kcal/d) at visit 2, 2606·6 kJ/d (623 kcal/d) at visit 3 and 3092 kJ/d (739 kcal/d) at visit 4. Seventeen subjects required the high-energy version of the study feed, eight subjects required the standard energy version and two subjects needed a combination of both densities. During the TMF phase, children consumed an estimated 8·4 (SD 2·2) g/d of total fibre from the enteral formula. During each phase of the study, children gained weight compared with baseline (Table 4). However, no significant changes in height or body composition were observed (Table 4). Neither stool frequency nor consistency differed significantly between the two formulas, but an increase in stool consistency was observed during the TEN period. Furthermore, no significant changes in the frequency or severity of GI symptoms (belching, nausea, vomiting, pain, flatulence and bloating) were recorded between the two diets. A tendency towards a carry-over effect for both the frequency ($P=0{\cdot}061$) and severity ($P=0{\cdot}056$) of nausea was observed. Twelve of the twenty-seven subjects were using polyethylene glycol laxatives (Forlax®) upon inclusion (ten out of twenty upon completion) and administration remained chronic and stable throughout the study. Overall drug use remained high in the present study population (Table 5).

A carry-over effect was observed for clostridia and vitamin C, respectively. Therefore, we excluded these parameters from further analysis.

A significant increase in the proportion of bifidobacteria (+16·6 %) was observed in the faeces of children in the fibre (TMF) period than in the faeces of those in the fibre-free (TEN) period (−11·3 %) (Table 6). No significant differences were observed in the faecal counts of lactobacilli, *E. coli* or specific subgroups of clostridia between the two diet phases (Table 6). Faecal pH was reduced (−0·3) when

Table 3. Baseline general characteristics of patients in each group

(Mean values and standard deviations for parametric variables or median (range) for non-parametric variables, frequency)

General characteristic of patients	Group A (*n* 13)*		Group B (*n* 14)*	
	Mean	SD	Mean	SD
Centre CHRU/Centre Vendin le Viel	7/6		10/4	
Sex (M/F)	11/2		9/5	
Age (years)	11·2	4·4	12·4	3·6
Weight (kg)	21·9	6·0	27·2	7·6
Height (cm)	122·2	16·0	132·2	16·4
Z-score (wt/ht)	−1·01	2·11	−0·95	2·07
Number of years on enteral nutrition	5·6	4·3	4·0	3·3
Usual energy intake from enteral nutrition (kcal/d)	600 (300–1125)		700 (500–1200)	
Severity of immobility				
Mobile	3		2	
On a chair	2		1	
Bed-bound	8		11	
Baseline feed				
Nutricia (Tentrini®)	7		8 (1)	
Other	6		6	

M, male; F, female.

* Group A: Tentrini®, then Tentrini® Multi Fibre; Group B: Tentrini® Multi Fibre, then Tentrini®.

Table 4. Changes in growth and body composition of children after 3 months
(Mean values with their standard errors)

	TEN					TMF							
	Baseline		Endpoint			Baseline		Endpoint			ΔTMF − ΔTEN		Treatment
	Mean	SEM	Mean	SEM	ΔTEN	Mean	SEM	Mean	SEM	ΔTMF	Mean	SEM	*P*
Weight (kg)	26·7	0·3	27·6	0·2	0·96*	26·7	0·3	27·5	0·2	0·75*	−0·22	0·45	0·638
Height (cm)	132·9	0·3	132·9	0·4	0·07	132·7	0·3	133·1	0·4	0·43	0·36	0·56	0·528
Z-score W/H	−0·65	0·18	−0·27	0·15	0·38	−0·63	0·18	−0·36	0·15	0·27	−0·11	0·31	0·721
FFM (kg)	22·0	0·2	22·1	0·3	0·11	22·2	0·2	22·5	0·3	0·32	0·21	0·66	0·754
FM (kg)	5·3	0·3	6·1	0·4	0·82	5·9	0·3	6·0	0·4	0·16	−0·66	0·71	0·379
FM skinfold (kg)†	4·5	0·2	4·3	0·2	−0·21	4·5	0·2	4·8	0·2	0·22	0·43	0·48	0·389

TEN, tentrini; TMF, tentrini MF; MF, multifibre; FFM, fat-free mass; FM, fat mass.
*Mean values were significantly different ($P<0·05$).
† Determined using skinfold thickness measurements at four sites – triceps, biceps, supra-iliac and subscapular.

subjects were fed TMF ($P<0·001$; Table 6). However, this was not accompanied by any significant changes in SCFA levels, possibly due to the very low number of stool samples available for this analysis (Table 6).

The majority of blood parameters did not change across time or treatment (Table 7). Glutathione peroxidase ($P=0·058$) and superoxide dismutase ($P<0·05$) levels showed trends of increasing after 3 months on TMF, though no significant differences across diets were observed. However, there was a significantly increased Fe status in patients on TEN, as evidenced by their raised ferritin levels ($P<0·01$), which was not observed in the children during the TMF period. However, ferritin levels remained within safe and normal ranges (20–300 ng/ml) during both intervention phases.

Discussion

To the best of our knowledge, the present study is the first to evaluate the effect of fibre-supplemented formula on gut microbiota in enterally fed children. There are only a limited number of studies available assessing the benefits of fibre supplementation in paediatric nutritional support, and most of them are reported only in abstract form[12–15]. The present study clearly shows a strong effect of multifibre on bifidobacteria, with increased counts (+16·6 %) in children when fed TMF than when fed TEN.

The multifibre mixture (MF6™) used in the present study contains soya polysaccharides, fructo-oligosaccharides, cellulose, inulin, gum arabic and resistant starch, and has been designed to allow fibres to be fermented along the large intestine[32–35] and to yield SCFA by-products that fuel colonocytes and exert a trophic effect on enteric bacteria[36]. However, while stool pH significantly decreased during the multifibre period, no changes in faecal SCFA levels were observed in the present study. A previous study using the same fibre mixture, but at a higher level (1·5 g/100 ml feed), has shown an increase in butyrate and acetate after feeding adult patients the multifibre-supplemented feed for 14 d[36]. However, this did not translate into any changes in the counts of dominant gut bacteria, as observed in the present study. Therefore, this led us to believe that the relationship between the stimulation of SCFA production and proliferation of gut microbiota is not a simple linear one[37]. Another reason for the lack of effect on stool SCFA levels could be the very small sample size (n 5) available for SCFA analysis. Therefore, we would suggest a sample consisting of minimum ten patients as described in the study done by Schneider *et al.*[36]. Furthermore, stool SCFA levels do not necessarily reflect the grade of SCFA production in the gut[37]. The fibre intake of the children may have been too low (8·4 (SD 2·2) g/d) from the fibre-supplemented formula) and/or the period of fibre supplementation may have been too short for an effect on stool SCFA levels. Finally, significant levels of lactate are only found in baby faeces, therefore it was not included in the analysis plan. Unfortunately, we did not have suitable samples left to measure lactate also.

In the present study, we targeted representative bacterial groups as biomarkers to study the dynamics of the faecal microbiota of the children, with bifidobacteria and lactobacilli being potentially health-promoting bacteria, and *E. coli* and a specific group of clostridia also containing potentially pathogenic strains. Members of the *Clostridium histolyticum*/*Clostridium lituseburense* group comprise pathogenic *Clostridium difficile* and *Clostridium perfringens* species[24] that are generally known to be harmful toxin-producing species causing diarrhoea and food poisoning. Our findings of an increase in the proportion of stool bifidobacteria and

Table 5. Percentage of children on medication throughout the study

	Users (%)
Total drugs*	95·6
Antibiotics	29·6
Benzodiazepine**	51·9
Anti-convulsants†	44·4
Laxatives	44·4
Corticosteroids	14·8
Neuroleptics	48·1

Mean values were significantly different (higher) in centre V *v.* centre L: *$P<0·001$, **$P<0·05$.
† Trend $P=0·057$.

Table 6. Changes in faecal microbiota and SCFA of children after 3 months

(Mean values with their standard errors)

	n	TEN					TMF					ΔTMF − ΔTEN		Treatment
		Baseline		Endpoint			Baseline		Endpoint					
		Mean	SEM	Mean	SEM	ΔTEN	Mean	SEM	Mean	SEM	ΔTMF	Mean	SEM	*P*
pH†	12	7·55	0·05	7·59‡	0·07	0·04	7·66	0·05	7·32‡	0·07	−0·336***	−0·375	0·105	0·003
Total bacteria (× 10^{10} cells/g)	10	1·93	0·27	1·51‡	0·16	−0·42	1·92	0·27	2·16‡	0·16	0·23	0·66	0·43	0·154
Bifidobacteria (%)	10	15·0	5·27	3·7‡	4·47	−11·3	3·8	5·27	20·5‡	4·47	16·6	28·0	13·7	0·033§
Lactobacilli (%)	10	22·9	3·7	25·2	3·4	2·3	24·5	3·7	16·0	3·4	−8·6	−10·9	10·0	0·150§
Clostridia (%)‖	10	4·7	2·6	5·4	2·4	0·7	6·2	2·6	3·0	2·4	−3·3	−3·9	4·7	0·414
E. coli (%)	10	0·00	0·00	0·08	0·94	0·1	0·00	0·00	2·01	0·94	2·01	1·9	1·4	0·192
Acetic acid (mm/kg faeces)	5	35·38	5·32	35·46	7·09	0·08	25·29	5·32	35·54	5·32	10·25	10·17	13·41	0·473
Propionic acid (mm/kg faeces)	5	11·69	0·91	13·79	3·01	2·10	9·06	0·91	12·21	3·01	3·15	1·05	4·50	0·822
Isobutyric acid (mm/kg faeces)	3	3·10	0·36	2·52	0·52	−0·58	2·05	0·36	2·78	0·52	0·73	1·31	0·96	0·231
Butyric acid (mm/kg faeces)	5	9·04	1·85	6·50	1·47	−2·54	4·87	1·85	6·75	1·47	1·88	4·42	3·44	0·240
Isovaleric acid (mm/kg faeces)	3	4·47	0·55	3·25	0·67	−1·22	2·88	0·55	3·54	0·67	0·67	1·88	1·30	0·207
Valeric acid (mm/kg faeces)	2	2·67	0·25	2·89	0·59	0·22	2·38	0·25	2·69	0·59	0·31	0·09	1·01	0·931

TEN, tentrini; TMF, tentrini MF; MF, multifibre.
*** Mean values were significantly different ($P < 0·001$).
† Significant period effect ($P < 0·05$).
‡ Significant difference between both endpoints.
§ One-tailed test.
‖ Significant carry-over effect.

Table 7. Changes in blood parameters of children after 3 months

(Mean values with their standard errors)

	TEN					TMF					ΔTMF − ΔTEN		Treatment
	Baseline		Endpoint			Baseline		Endpoint					
	Mean	SEM	Mean	SEM	ΔTEN	Mean	SEM	Mean	SEM	ΔTMF	Mean	SEM	*P*
Hb (g/dl)	13·5	0·1	13·5	0·2	−0·05	13·2	0·1	13·1	0·2	−0·08	−0·04	0·32	0·907
MCV (10^{-15} l)	88·1	0·3	87·9	0·4	−0·15	88·2	0·3	88·3	0·4	0·09	−0·24	0·60	0·697
Ferritin (ng/ml)†	32·0	3·0	40·9	3·2	8·84*	38·0	3·0	34·9	3·2	−3·03	−11·88	3·86	0·008*
Zn (μg/100 ml)	68·2	2·1	69·2	2·0	1·05	66·9	2·1	65·3	2·0	−1·62	−2·68	4·30	0·545
Se (μg/l)	81·5	4·8	83·4	2·2	1·89	75·3	4·8	77·8	2·2	2·51	0·619	8·27	0·942
Vitamin C (μmol/l)‡	56·8	4·0	54·0	4·0	−2·83	69·1	4·0	60·9	4·0	−8·23	−5·40	6·09	0·391
Vitamin E (μmol/l)	19·7	0·9	20·6	0·6	0·87	19·5	0·9	19·6	0·6	0·12	−0·75	1·60	0·644
GPx (U/g Hb)	73·5	5·8	68·7§	7·8	−4·78	84·0	5·8	103·0§	7·8	18·97	23·76	13·48	0·100
SOD (U/g Hb)	1073·6	49·5	1192·7	73·9	119·1	1070·1	49·5	1313·1	73·9	243·0	123·9	164·9	0·465

TEN, tentrini; TMF, Tentrini MF; MF, multifibre; MCV, mean corpuscular volume; GPx, glutathione peroxidase; SOD, superoxide dismutase.
* Mean values were significantly different ($P < 0·05$).
† Significant period effect $P < 0·05$.
‡ Significant carry-over effect.
§ Significant difference between both treatment endpoints.

a reduction in faecal pH during the TMF (fibre) period are assumed to be of benefit to the enterally fed children. A bifidobacteria-dominant microbiota may prevent pathogen invasion[38] and help activate the immune system[39], as well as synthesise vitamins and digestive enzymes[33], thus stimulating the development of a healthier gut. Furthermore, a lowered gut pH favours the selective proliferation of lactic acid bacteria over pathogens[40]. Such benefits are of direct interest in the paediatric population who are often fed enterally from a very early age, and this is continued for a prolonged period of time, sometimes even for a lifetime[1]. Despite heavy medication in this study population, TMF was effective at promoting the proliferation of bifidobacteria to yield a healthier gut microbiota. Clinical benefits related to these findings remain to be investigated in this population.

Use of a large amount of a single (insoluble or soluble) fibre source in EN has been reported to cause digestive side effects, including bloating, diarrhoea, flatulence, vomiting and abdominal pain[41,42]. In the present study, the use of the MF6™ mixture was well tolerated by children, and did not cause any GI symptoms. This finding is fully in line with results from previous studies in children and adults using formulas supplemented with the same multifibre mixture, where the fibre-containing feeds were reported to be well tolerated[13–15,36,43].

One of the expected benefits of using fibre was a reduction in constipation. Previous clinical studies using the multi-fibre blend (MF6™) have reported improvements in stool consistency in both paediatric and adult enterally fed patients[13,14,43,44]. In a study in paediatric burn patients, a tube feed enriched with the same fibre mixture was well tolerated and it reduced the use of laxatives compared with a fibre-free tube feed[14]. Furthermore, a 2-week, cross-over study in sixteen children, ten of whom had cerebral palsy, in which a multifibre-supplemented paediatric tube feed was compared with a fibre-free feed, showed a significant reduction in the number of days of constipation during the fibre period[13]. Vandewoude *et al.*[44] reported a reduction in the incidence of hard faeces and a change towards more soft pasty faeces following multifibre supplementation in enterally fed elderly patients. In addition, the use of an adult tube feed enriched with the multifibre blend (MF6) normalised whole gut transit time and re-established colonic activity after nasogastric bolus in adult patients[43]. A recent randomised, double-blind study in adults receiving fructo-oligosaccharides and fibre-supplemented enteral formula showed a positive correlation between gastrointestinal quality of life index score and the number of faecal bifidobacteria, suggesting that a change in intestinal microbiota could induce an increased quality of life in these patients[45].

However, in the present study, all patients who were constipated and received laxatives at study entry remained constipated during follow-up, independent of the type of formula they received. This could be partly due to inadequate total fibre intakes. Indeed, children were consuming less than 50 % of their recommended intake of fibre, even when combining fibre intake from the enteral feed (8·3 g) and from their oral diet (2·4 g). A recent study showed an improvement in constipation in children aged 8–14 years when their dietary fibre intakes were at least 14·5 g/d[46]. Furthermore, it was recently reported that giving children a high amount of a dietary fibre mixture (20 g/d in children of 15–20 kg and 30 g/d in those >20 kg) was effective in the treatment of childhood constipation[46].

In addition to this, the subgroup of neurologically impaired children often had a relatively low intake of enteral formula due to fluid restrictions and/or low energy requirements. Furthermore, these children often suffer from constipation[10], and therefore, may have higher requirements of fibre than healthy children[46].

Finally, the observation that stool consistency increased during the TEN period, but remained constant during the TMF period, could suggest that the multifibre mixture (MF6™) used in the present study may have a stabilising effect on stool consistency, but this should be investigated further in a larger trial with higher doses of fibre.

During both formula phases of the study, the children gained weight. This is of major relevance in this patient group, as malnutrition in these children can result in serious sequelae affecting organ development and maturation, immune response, GI function, muscle strength, motor function and behaviour[47,48]. Other studies have reported similar effects on weight gain of gastrostomy tube feeding in children with neurological disabilities[48–50]. Improvements in linear growth have also been reported, but much less commonly[51]. Remarkably, in a recent study done by Sullivan *et al.*[52], a relatively low energy expenditure and high body-fat content were reported in gastrostomy-fed children with severe cerebral palsy, highlighting the potential risk of overfeeding in this patient population. In the present study, no significant changes in height, FM or FFM were observed.

A few questions can be raised regarding the use of BIA in this population with muscle spasticity and abnormal skin texture. Several studies have pointed out the lack of validity of BIA in children with cerebral palsy, indicating a good correlation with FFM, but no reliability for FM determination[53]. Attempts should be made to improve the techniques for measuring body composition in this group, as FFM appears to have an impact on motor function and therefore on the quality of life of children with cerebral palsy[54,55].

Very few data are available on micronutrient status in children receiving fibre-supplemented feeds. Daly *et al.*[56] found no differences in plasma Zn, Cu and Se status between children receiving a multifibre-supplemented sip feed for 12 weeks and those receiving a fibre-free feed during this period. Tolia *et al.*[12] observed no negative effect on micronutrient status with intakes of 8 to 10 soya fibre\d.

In the present study, most of the biological parameters remained unchanged. Nevertheless, plasma ferritin levels showed a significant diet effect. After the 3-month intervention period on fibre-free enteral feeding, plasma ferritin levels increased, whereas no change in the levels was observed during the period on the fibre-supplemented feeds. As plasma ferritin levels remained within normal physiological ranges during both phases of the study, we cannot conclude that a formula enriched with fibre has any impact on the Fe bioavailability of patients.

The data on plasma vitamin C status of the children were inconsistent due to oxidative loss of the vitamin during sample transport. Lastly, the observed increase in glutathione peroxidase and superoxide dismutase levels after 3 months on the fibre-enriched feed could indicate an improved antioxidant

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capacity, possibly relating to the potential effects of fibre on the gut microbiota. Large, long-term studies with higher levels of fibre are required to assess the effect of fibre-enriched formulas on micronutrient status of enterally fed children.

One of the major limitations of the present study was the small sample size. In addition, the pathology of the children included was very heterogeneous. This made the analysis and interpretation of the present study results quite difficult. Another limitation is the under-representation of the female sex in this study population. Although we are unaware of sex differences in the effects of fibre supplementation of enteral feeds on gut microbiota and bowel actions, this may merit further investigation.

Compliance was not assessed in the present study, but as the children had been already fed via gastrostomy before the study, there is no reason to doubt their compliance. Furthermore, weight gain was achieved during the study period, which is an indication of adherence to the feed prescription. Lastly, a carry-over effect was observed for several variables (clostridia, vitamin C and a trend for nausea and stool frequency). This might indicate the need for a longer washout period in subsequent cross-over nutritional intervention studies in enterally fed children.

Conclusion

In conclusion, the present study demonstrates that the use of a multifibre-supplemented paediatric tube feed in long-term enterally fed children is beneficial, as it is well tolerated, promotes the growth of bifidobacteria and reduces gut pH, suggesting an improved gut health.

Further studies are needed to establish the fibre requirements of long-term enterally fed children with neurological disabilities as well as to assess the effect of long-term fibre supplementation on micronutrient status.

Acknowledgements

We thank the children and their parents who took part in the present study. We are grateful to G. Descamp and B. Seignez for their invaluable help in performing the logistic of shipment of nutritional products in the present study. Data quality assurance and collection, logistics of blood samples and technical assistance were assumed by C. Marichez from the Clinical Investigation Center of Lille (CIC-9301-CH&U-Inserm). Stool checking and storage were assumed by the BRC (Biological Ressource Centre) of CIC-9301-CH&U-Inserm. The sponsor of the present study was Nutricia Advanced Medical Nutrition, Danone Research, Centre for Specialised Nutrition (The Netherlands). F. G. is a member of the Nutricia International Paediatric Advisory Board. K. B. A., A. G., J. S. and J. K. are employees of Danone Research. D. G., F. G., L. B. and A. G. contributed to the design of the study. L. B., S. N. and B. B. were responsible for data collection and analysis. J. K. performed stool analysis. D. G., F. G. and A. G. prepared the final manuscript.

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