Randomized control trials

Additional oligofructose/inulin does not increase faecal bifidobacteria in critically ill patients receiving enteral nutrition: A randomised controlled trial

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S U M M A R Y

Background & aims: Patients with diarrhoea during enteral nutrition (EN) have been shown to have low faecal bifidobacteria concentrations. Oligofructose/inulin selectively stimulate the growth of bifidobacteria in healthy humans. This study investigates the effect of additional oligofructose/inulin on the gastrointestinal microbiota, short-chain fatty acids (SCFA) and faecal output in patients receiving EN.

Methods: Adult patients in the intensive care unit (ICU) who were starting EN with a formula containing fibre were randomised to receive 7 g of additional oligofructose/inulin or an identically packaged placebo (maltodextrin). A fresh faecal sample was collected at baseline and following at least 7 days of supplementation. Faecal microbiota were analysed using fluorescent in-situ hybridisation and faecal output was monitored daily.

Results:Twenty-two patients (mean age 71 years) completed at least 7 days of intervention (mean 12 days). At the end of the intervention, there were no significant differences in the concentrations of bifidobacteria between the groups, after adjusting for baseline values (oligofructose/inulin 6.9 log10 cells/g dry faeces, P > 0.05), but there were significantly lower concentrations of Faecalibacterium prausnitzii (7.0 ± 1.0 vs. 8.4 ± 1.3 log10 cells/g, P = 0.01) and Bacteroides-Prevotella (9.1 ± 1.0 vs. 9.9 ± 0.9 log10 cells/g, P = 0.05) in patients receiving additional oligofructose/inulin. There were no differences in faecal concentrations of any SCFA, secretory IgA, daily faecal score or incidence of diarrhoea between the two groups.

Conclusions: Additional oligofructose/inulin did not increase faecal bifidobacteria in critically ill patients receiving EN, although it did result in lower concentrations of F. prausnitzii and Bacteroides-Prevotella.

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1. Introduction

Enteral nutrition is the most commonly utilised modality of nutritional support in the critically ill patient. However, diarrhoea can commonly occur in these patients, which can have a negative impact on outcome and is unpleasant for patients and their carers. Diarrhoea during EN can be caused by gastrointestinal colonisation with enteropathogenic bacteria such as Clostridium difficile, by antibiotic administration and by abnormal secretion of fluid into the colonic lumen that can be reversed by short-chain fatty acids (SCFA). There may be an interaction between each of these mechanisms and the colonic microbiota and the SCFA that they produce, implying that manipulation of the colonic microbial population may help to protect against diarrhoea during EN.

Prebiotics are dietary compounds that selectively stimulate the growth and/or activity of one or a limited number of microbial genera/species of the gastrointestinal microbiota and confer health benefits to the host. The most extensively investigated prebiotics are the inulin–type fructans oligofructose (degree of polymerisation 2–9) and inulin (degree of polymerisation 10–60). Oligofructose...
and inulin have properties that may help prevent diarrhoea during EN. Firstly, they stimulate the growth of bifidobacteria, low concentrations of which have been found in patients receiving long-term EN\(^\text{a}\) and short term EN,\(^\text{b}\) particularly in those who develop diarrhoea.\(^\text{c}\) Secondly, they might suppress \textit{C. difficile} colonisation,\(^\text{d}\) with a study in patients not receiving EN demonstrating that oligofructose increases bifidobacterial numbers and reduces \textit{C. difficile}-associated diarrhoea.\(^\text{e}\) Thirdly, oligofructose and inulin can stimulate immune function,\(^\text{f}\) with studies showing that supplementation of infant solutions increases secretory Immunoglobulin A (sIgA),\(^\text{g}\) an important component of mucosal immunity. Standard enteral solutions do not contain any prebiotics, however, prebiotics are increasingly being added to solutions, especially those also containing fibre, although the effect of these on the microbiota in patients is unclear.

Studies in healthy humans have shown that mixtures of oligofructose and inulin increase the numbers of bifidobacteria when used as supplements\(^\text{h}\) or when added to enteral solutions,\(^\text{i}\) however the evidence for their effect in modifying the microbiota in patients receiving EN is equivocal. Two randomised trials in patients receiving long-term EN in the community showed either no impact\(^\text{j}\) or an increase\(^\text{k}\) in bifidobacterial numbers with solutions supplemented with fibre and prebiotics. Meanwhile, in a non-randomised trial in hospitalised patients receiving EN no significant differences in concentrations of faecal bifidobacteria were found between patients receiving a standard formula and one supplemented with fibre, inulin and oligofructose.\(^\text{l}\)

One possible reason for the absence of an effect of oligofructose/inulin on faecal bifidobacteria in these studies is the inadequate quantities in such solutions. Dose-response studies in healthy subjects indicate that supplements of 10 g/d of oligofructose/inulin are effective at increasing bifidobacterial numbers,\(^\text{m}\) and this is in addition to background dietary intakes of approximately 5 g/d from normal diets.\(^\text{n}\) The studies showing that prebiotic supplemented solutions did not impact on bifidobacteria used doses of oligofructose and inulin between 4.8–7.6 g/d\(^\text{o}\) and 2.5–9.0 g/d,\(^\text{p}\) depending upon the volume of formula delivered. In contrast, in the study that demonstrated an increase in bifidobacterial numbers, the dose of oligofructose averaged 11.1 g/d.\(^\text{q}\)

The aim of the present study was to investigate the effect of providing additional inulin/oligofructose, at doses known to increase bifidobacterial numbers in healthy human subjects, on the colonic microbiota, faecal SCFA and immune status of patients receiving EN.

### 2. Methods

This was a multi-centre, randomised, double-blind controlled trial in adult patients who were starting exclusive EN with mixed fibre formula. This study was approved by an NHS Research Ethics Committee (COREC 07/H0702/41) and was registered with the International Standard Randomised Controlled Trial Number (ISRCTN06446184).

#### 2.1. Subjects

Consecutive patients were recruited from the intensive care units (ICU) of Guy’s and St Thomas’ NHS Foundation Trust and King’s College Hospital NHS Foundation Trust, London. Adult patients on the ICU starting exclusive nasogastric EN with a mixed-fibre formula expected to last for more than three days were screened for inclusion. Exclusion criteria were patients undergoing gastrointestinal (GI) surgery\(^\text{r}\) or GI radiation therapy\(^\text{s}\) or chemotherapy or had GI diseases (e.g. inflammatory bowel diseases)\(^\text{t}\) or diarrhoea\(^\text{u}\) or constipation as these factors are known to affect the GI microbiota. Patients were also excluded if they had received lactulose, because it is a prebiotic.\(^\text{v}\) Patients who discontinued EN, were transferred to another hospital or received palliative treatment prior to providing a baseline or follow-up sample were also excluded. Informed, written consent was obtained from all patients or their personal legal representative.

The sample size calculation was based on detecting a change in faecal bifidobacterial numbers between baseline and post-intervention that differed between groups by 1 log\(_{10}\) cells/g, in line with previous observations in healthy subjects.\(^\text{w}\) In order to achieve 90% power with \(P < 0.05\), 20 subjects were required in each group.

#### 2.2. Enteral solutions

The choice of the type and volume of formula was decided by the clinical team based on their assessment of the patient’s requirements. Patients received one of two fibre/prebiotic-enriched solutions (Nutrison Multifibre, Nutrison Protein Plus Multifibre, Nutricia UK). Both contained the same six sources of non-digestible carbohydrate (soy polysaccharides, resistant starch, arabic gum, cellulose, inulin and oligofructose), of which 10% was oligofructose and 20% was inulin. Both solutions contained 7.6 g/100 mL soluble fibre and 0.8 g/100 mL insoluble fibre. Of the 22 patients who completed the trial, four were temporarily switched to a different enteral formula during the trial based upon the decision of the clinical team. Three were temporarily switched to a standard formula (Nutrison Standard; Nutrison Low Sodium; Nutrison Concentrated, Nutricia UK) and one to a renal formula that contained only oligofructose (Nepro, Abbott UK). However, this temporary switch to standard formula only occurred for 2–5 days before patients were transferred back to the fibre/prebiotic-enriched solutions.

The volume of enteral formula prescribed for each patient was calculated by the attending dietitian based on the patient’s estimated energy requirement, calculated using Schofield equations. The prescription of enteral formula was sufficient to achieve reference nutrient intakes for all vitamins and minerals.\(^\text{x}\) Delivery of the enteral formula was recorded daily by the researcher based on either paper or electronic versions of enteral feeding input and output charts.

#### 2.3. Study design

In addition to EN, patients were randomly assigned to receive either an additional 7 g/d of oligofructose/inulin (Synergy-1, Beneo-Orafti, Tienen, Belgium) or 7 g/d of an identically packaged non-prebiotic carbohydrate (maltodextrin). This dose was chosen because the formula already provided 4.5 g/L of prebiotics (equivalent to 6.75 g/d in a patient receiving 1.5 L of formula), therefore an additional 7 g/d of oligofructose/inulin would increase the dose into the range previously shown to increase bifidobacteria.\(^\text{y}\)\(^\text{,z}\) The supplements were supplied by Beneo-Orafti, Belgium and were prepared in identical sachets with a blinding code and the contents were similar in terms of volume, colour and texture. The randomisation sequence was generated using a randomisation website (http://www.randomization.com) by the principal investigator. All researchers were blinded to whether patients were receiving the intervention or placebo. A copy of the randomisation list was kept by the intensive care research nurse to be opened should an adverse event occur. Patients were randomised as soon as they had been recruited and consent had been obtained.

Supplementation was begun immediately after the baseline faecal sample had been collected. The sachet contents were dissolved in 50 ml of sterile water and administered via the
nasogastric feeding tube daily by the nurse in charge, assisted by the principal investigator. Supplements were administered daily for up to 14 days.

The following data were recorded: date of birth; diagnosis; estimated weight and height; drug and antibiotic prescription at day 0, during the study and on collection of the post-intervention stool sample (drug, dose, route, duration); date EN started; volume of formula prescribed daily; volume of formula delivered daily; confirmation of administration of sachets (placebo or oligofructose/inulin), stool output monitoring daily (King’s stool chart).

2.4. Sample collection

A baseline faecal sample was collected as soon as possible after recruitment. The post-intervention sample was collected following 7–14 days of receiving the placebo/ intervention, depending upon when a sample was passed. Since the samples need to be analysed fresh after an hour of defecation, the principal investigator will be collecting the samples at these time periods (post-intervention at least 7 days or at least 12 days) since some patients had frequent bowel opened and some did not. Faecal samples were subsequently analysed for microbiota using fluorescent in situ hybridisation (FISH), SCFA using gas liquid chromatography, secretory IgA and C. difficile enterotoxins A/B using enzyme linked immunoabsorbent assay (ELISA) and water content using lyophilisation.

2.5. Analytical methods

2.5.1. Fluorescent in situ hybridisation

Samples were prepared as described previously. The probes used detected bifidobacteria (Bifidobacterium bifidum 5′-GCC GCA TTA CCA CCC-3′), lactobacilli and enterococci (Lab158 5′-GGTT TAC CAC TGT TCGA AAC-3′), lactobacilli (Bac303 5′-ATG TGG GGC ACC TT-3′), Faecalibacterium prausnitzii (FPr459T 5′-CTG CAAC TAC TCA AGA AAA AC-3′) and Clostridia cocoides-Eubacterium rectale (Ere482 5′-GCT TCT TAG TCA RGT ACC G-3′). Total cells were quantified using the nucleic acid stain 4,6-diamidino-2-phenylindole (DAPI). The hybridised microbiota were quantified manually in duplicate using an Axioplan 2 microscope (Carl Zeiss, Oberkochen, Germany) equipped with an HBO-100 fluorescent lamp (Ossram, Siemens AG, Munich, Germany). All samples were counted independently by two microscopists who were both blinded to the timing and group allocation of the samples and to each other’s results.

2.5.2. Gas liquid chromatography

Samples for analysis of SCFA were stored at −80 °C prior to analysis. The SCFA were extracted from defrosted fecal samples using an extraction buffer (1.0% H3PO4; 0.1% HgCl2) containing an internal standard (2,2-dimethylbutyric acid) and analysed for SCFA as described previously.

2.5.3. Lyophilisation

Faecal water content was determined by measuring the weight loss of a sample following complete lyophilisation (LSL Secffroid, Aclens, Switzerland) for a minimum of four days until constant weight.

2.5.4. Faecal secretory IgA assay

Faecal samples were stored at −80 °C prior to analysis for sIgA, which was measured using the sIgA ELISA kit (Immundiagnostik AG, Bensheim, Germany). The analysis was done according to the manufacturers’ instructions.

2.5.5. C. difficile enterotoxin assays

Fresh stool samples were analysed for C. difficile-toxins A and B by the routine clinical microbiology service using the Premier Toxins A and B ELISA kit according to manufacturer’s instructions (Meridian Bioscience Inc, Cincinnati OH, US).

2.5.6. Faecal output

Faecal output was monitored daily by nursing staff using the King’s Stool Chart and a daily stool score and the prevalence of diarrhoea were calculated. Briefly, nurses visualised each stool passed during the 7–14 day intervention period and recorded stool frequency, consistency and quantity. The King’s Stool Chart applies a score to each stool which is summed into a daily faecal score, with a score of ≥15 on any one day classified as diarrhoea.

2.6. Statistical analysis

The data were analysed using SPSS software for Windows (Version 17.0, Chicago, IL, US). The normality of the distributions was tested using the Kolmogorov–Smirnov test and where necessary variables were log transformed to ensure good fit to the normal distribution. Post-intervention values for faecal microbiota, SCFA and sIgA concentrations were compared between groups using a univariate analysis of covariance (ANCOVA) with baseline values as a covariate. Other comparisons between the two groups were made using Student’s t-test for continuous variables and the chi-squared test or Fisher’s exact test for categorical variables.

Differences were considered significant when P < 0.05.

3. Results

Out of 381 patients screened (Fig. 1), 47 were recruited and randomised. Twelve patients discontinued the study before passing a baseline stool sample, for the reasons shown in Fig. 1, so that 35 patients (21 males, 14 females) started the intervention. Of these patients, 22 completed the study for >7 days and were able to provide a post-intervention stool sample. Of the remaining 13 patients (6 from the placebo group, 7 from the oligofructose/inulin group) discontinued the study because they ceased exclusive EN (n = 11) or were transferred to another hospital (n = 2) prior to 7 days of supplementation and therefore did not provide a post-intervention stool sample. Of the 22 patients who completed the study, 10 were in the placebo group and 12 were in the oligofructose/inulin group. There were no significant differences in age, serum albumin concentrations, body mass index or nutrient intake between the two groups at baseline, nor in the number of days from starting EN until the first faecal sample was collected (Table 1).

Data were analysed on an intention-to-treat basis for the 22 patients who completed at least 7 days of EN (mean 13, SD 4 days). There were no differences in oligofructose/inulin intakes from the EN formula between the placebo group and the oligofructose/inulin group during the intervention (7.8 vs. 8.0 g/d, P = 0.78). However, when comparing the total intake of oligofructose/inulin from both the EN formula and the additional sachet, patients in the placebo group received 7.8 g/d (SD 2.4) compared with 14.8 g/d (SD 1.6) in the oligofructose/inulin group (P < 0.001).

3.1. Faecal microbiota

There were no significant differences in any of the microbiota measured at baseline between patients randomised to the placebo group and those randomised to the oligofructose/inulin group (P > 0.05, t-test). At follow-up, there were significantly lower concentrations of F. prausnitzii (P = 0.01) and Bacteroides-Prevotella (P = 0.05) in the oligofructose/inulin group after adjusting for...
baseline values (ANCOVA), but no significant differences in the concentrations of any of the other bacterial species tested (Table 2).

3.2. Short-chain fatty acids

There were no significant differences in SCFA concentrations at baseline or follow-up between the two groups (Table 3). In addition, there were no statistically significant differences in faecal pH between the groups either at baseline or at follow-up (Table 3).

3.3. Secretory IgA and C. difficile toxin

There were no statistically significant differences in sIgA concentrations between the groups either at baseline or at follow-up (Table 3). None of the patients recruited into the study had a positive C. difficile enterotoxin test at any time point.

3.4. Faecal output

There were no statistically significant differences in faecal frequency or daily faecal score between the two groups (Table 4). On days when the faecal score was greater than 15, patients were considered to be experiencing diarrhea.25 There was no significant difference between the two groups in the mean number of days of diarrhea or in the number of patients experiencing diarrhea on either one or two or more consecutive days (Table 4).

4. Discussion

Oligofructose and inulin are prebiotics that increase faecal bifidobacteria when added to enteral formula and consumed by healthy human subjects12,13 whereas studies in patients show discordant findings with either no effect5,6 or only a modest effect14.
Many commonly used antibiotics suppress anaerobic bacteria such as bifidobacteria, lactobacilli, clostridia and bacteroides. An in vitro study has shown that the antibiotic clindamycin prevents oligofructose and inulin from stimulating the growth of bifidobacteria, and indeed the addition of these actually resulted in a greater reduction in bifidobacteria than when clindamycin alone was added. Inulin has been shown to increase F. prausnitzii in healthy subjects consuming a normal diet. Hence, finding that F. prausnitzii decreased in response to prebiotic supplementation was unexpected. F. prausnitzii has been shown to decrease in healthy subjects on exclusive EN for two weeks, irrespective of whether the formula contained fibre and prebiotics. F. prausnitzii produces butyrate and it has been shown to affect Gl immune function through blocking NF-kappa b activation and IL-8 production. However, the clinical relevance of the decrease in F. prausnitzii numbers in the present study is unknown. Additional prebiotics in this study had shown to change the composition of the microbiota but it is unclear such changes is beneficial or not to these patients since the impact towards diarrhoea is not shown.

There were no significant differences between the groups in faecal SCFA following the intervention, despite the decrease in F. prausnitzii, a major producer of butyrate. It is possible that there was a compensatory increase in the numbers of other microbiota that produce butyrate, such as Roseburia, which were not measured here. It should be emphasised that faecal SCFA reflects both colonic SCFA production and absorption rates.

Faecal sIgA plays a role in preventing the adherence of pathogenic bacteria and viruses to the mucosal surface by agglutination. The effect of prebiotics on faecal sIgA has not previously been investigated in patients receiving EN, although increased faecal sIgA concentrations have been shown in infants receiving infant solutions containing mixed prebiotics. No difference between groups in faecal sIgA was found in the present study. Faecal sIgA may not be the most accurate measurement for total IgA but this biomarker was investigated in other studies. Although patients receiving EN are at greater risk of C. difficile infection, no patient was tested positive for C. difficile enterotoxin. The absence of C. difficile colonisation in this study removes one confounding factor that may influence the faecal microbiota.

As the findings of the present study are in marked contrast with published findings of the effect of prebiotics in healthy humans, we interrogated our methodology and results for explanations. These findings could be due to chance differences in baseline characteristics, despite random allocation. These critically ill patients were admitted for heterogeneous clinical reasons and it was impractical to match the two groups based on disease status. However, there were no differences in age and serum albumin concentrations between the groups. Patients who completed the study (at least seven days of EN) were inevitably those who stayed longest in the ICU and were mostly older adults with more complex critical illness.

The enteral solutions were changed temporarily (less than five days) in four patients (two placebo, two oligofructose/inulin) on the day on which faeces were collected, but these differences were not significant.

Table 1

| Duration of intervention with placebo or oligofructose/inulin (days), mean (SD) | 14.6 (4.4) | 11.5 (3.0) | 0.10 |

**Table 1**

| Baseline nutrient intake, mean (SD) | 240 (2.4) | 263 (4.8) | 0.20 |

**Table 2**

| Duration of intervention with placebo or oligofructose/inulin (days), mean (SD) | 14.6 (4.4) | 11.5 (3.0) | 0.10 |

**Table 3**

EN: enteral nutrition.

a Differences between the two groups were tested using Student's t-test.

b Categorical data were analysed using the chi-squared test.
prior to starting the intervention. It was originally planned to perform an intention-to-treat analysis on all the randomised patients. However, due to the cessation of EN and the inability to maintain the intervention for up to 14 days, but very few patients continued exclusive EN for this long. Furthermore, detailed data regarding the duration of antibiotics prior to the intervention was not available. In addition, other parameters such as faecal cytotoxicity or fat and protein content were not measured in this study.

4.1. Limitations

The major limitation of this study is that the calculated sample size was not achieved (40 patients), despite recruitment continuing for 18 months. The reasons were manifold, including the many necessary exclusion criteria, the withdrawal of many patients during the study due to the cessation of EN and the inability to perform an intention-to-treat analysis on all the randomised patients (n = 47) since the primary outcome (microbiota) required both a baseline and follow-up stool sample during the period of exclusive EN. Despite not achieving the target recruitment number, significant differences were found in the concentrations of Bacteroides and F. prausnitzii, arguing against a type 2 error explaining the failure to find a significant difference in the concentration of bifidobacteria. Moreover, the 95% confidence interval for the difference in adjusted mean values for bifidobacteria was -0.32 to 2.01, indicating that any increase in faecal bifidobacteria in the underlying population was unlikely to be more than 0.3 log10 cells/dry faeces.

A further weakness was the limited duration of the intervention (mean 12 days) and the fact that the patients had been receiving EN for an average of 7.6 days before the intervention started. The latter was the result of having to wait for passage of a baseline stool prior to starting the intervention. It was originally planned to maintain the intervention for up to 14 days, but very few patients continued exclusive EN for this long. Furthermore, detailed data regarding the duration of antibiotics prior to the intervention was not available. In addition, other parameters such as faecal cytotoxicity or fat and protein content were not measured in this study.

4.2. Conclusion

In conclusion, the administration of an additional 7 g/d of oligofructose/inulin for at least 7 days to critically ill patients receiving EN with mixed fibre solutions resulted in decreased faecal concentrations of F. prausnitzii and Bacteroides-Prevotella but no change in bifidobacteria. These results contrast sharply with the effects of oligofructose and inulin in healthy subjects, suggesting that some aspect of critical illness and/or antibiotic usage profoundly alters the prebiotic potential of these unavailable carbohydrates. Based on the findings of the current study, there is no reason to advise that critically ill patients receiving EN are given additional oligofructose/inulin, beyond that which is already provided in the enteral formula. Further studies are required to investigate the optimal lower range of oligofructose/inulin to be provided in fibre-containing enteral solutions in this population and whether these findings are specific to critical care or whether similar findings occur in other hospitalised patients receiving EN.

### Table 2

Faecal microbiota concentrations at baseline and during enteral nutrition with additional oligofructose/inulin (n = 12) or placebo (n = 10).

| Baseline | Post-intervention | Post-intervention Adjusted mean
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>Placebo</td>
<td>10.3 (0.3)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>10.3 (0.3)</td>
</tr>
<tr>
<td>Bacteroides-Prevotella</td>
<td>Placebo</td>
<td>9.0 (1.6)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>9.5 (1.2)</td>
</tr>
<tr>
<td>C. coccoides-E. rectale</td>
<td>Placebo</td>
<td>8.9 (1.1)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>8.5 (1.2)</td>
</tr>
<tr>
<td>F. prausnitzii</td>
<td>Placebo</td>
<td>8.0 (1.7)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>8.2 (1.6)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Placebo</td>
<td>8.3 (1.1)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>7.4 (1.5)</td>
</tr>
<tr>
<td>Lactobacillus-enterococci</td>
<td>Placebo</td>
<td>7.4 (1.3)</td>
</tr>
<tr>
<td>(log10 cells/g dry faeces)</td>
<td>Oligofructose/inulin</td>
<td>7.9 (1.7)</td>
</tr>
</tbody>
</table>

* Data were analysed by univariate analysis of variance, with post-intervention value as the outcome variable and baseline value as covariate to adjust for the baseline value (ANCOVA).

### Table 3

Faecal short-chain fatty acid concentrations, faecal pH and secretory IgA at baseline and post-intervention during enteral nutrition with additional oligofructose/inulin (n = 12) or placebo (n = 10).

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Post-intervention</th>
<th>Post-intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Adjusted mean a</td>
</tr>
<tr>
<td>Total short-chain fatty acids</td>
<td>Placebo</td>
<td>294 (220)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>226 (205)</td>
</tr>
<tr>
<td>Acetate</td>
<td>Placebo</td>
<td>148 (114)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>113 (97.5)</td>
</tr>
<tr>
<td>Propionate</td>
<td>Placebo</td>
<td>74.8 (71.3)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>81.0 (94.7)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>Placebo</td>
<td>47.2 (43.0)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>18.2 (11.8)</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>Placebo</td>
<td>7.9 (6.2)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>4.1 (3.1)</td>
</tr>
<tr>
<td>Valerate</td>
<td>Placebo</td>
<td>6.6 (3.2)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>3.9 (3.5)</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>Placebo</td>
<td>9.6 (6.4)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>5.7 (4.0)</td>
</tr>
<tr>
<td>Faecal pH</td>
<td>Placebo</td>
<td>6.6 (0.8)</td>
</tr>
<tr>
<td>μmol/g dry faeces</td>
<td>Oligofructose/inulin</td>
<td>6.6 (0.6)</td>
</tr>
<tr>
<td>Secretory IgA</td>
<td>Placebo</td>
<td>2.97 (3.15)</td>
</tr>
<tr>
<td>mg/ml wet faeces</td>
<td>Oligofructose/inulin</td>
<td>1.84 (1.36)</td>
</tr>
</tbody>
</table>

* Data were analysed by univariate analysis of variance, with post-intervention value as the outcome variable and baseline value as covariate to adjust for the baseline value (ANCOVA).

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The manuscript including authorship list. 

Statistical analysis of the study; Kevin Whelan (KW): contributed to the design, statistically analysed and interpreted the data, drafted the manuscript, conducted the study (including recruitment, lab analysis), and was the primary author.

Statement of authorship

Hazen Abdul Majid (HAM): contributed to the design of the study, conducted the study (including recruitment, lab analysis), statistically analysed and interpreted the data, drafted the manuscript; Jayne Cole (JC): contributed to microbiota analysis; Peter Emery (PE): contributed to the design, data analysis and interpretation of the study; Kevin Whelan (KW): contributed to the design, data analysis and interpretation of the study. All authors contributed to the final manuscript, read and approved the final version of the manuscript including authorship list.

Conflicts of interest

The authors do not have any additional conflict of interest to declare.

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