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Short communications

Microbial shifts of faecal microbiota using enteral nutrition *in vitro*

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ABSTRACT

Enteral nutrition (EN) formulas of polymeric type ordinarily have similar content of intact macronutrients but may vary in prebiotic saccharides and micronutrients. These components can play an important role in the intestinal microbiota modulation. The aim of this study was to investigate microbial changes of faecal samples after their *in vitro* anaerobic cultivation in four polymeric EN formulas using plate technique method, metabolite analysis, and microbiota profiling using 16S rRNA sequencing. Detected cultivable commensal groups (bifidobacteria, lactobacilli, *Escherichia coli*) in faecal samples of donors were able to grow in EN formulas. However, their counts varied depending on the individual donor and the type of EN formula. Similar trend was found in detected metabolites such as acetate, lactate, and butyrate. Also, taxonomic composition and diversity of original and cultivated faecal microbiota of one individual on different EN formula indicate a possible effect of the prebiotics and micronutrients to modulate gut microbiota.

1. Introduction

Nutrients in diet are the key factor of the microbiota configuration, through modulation of the abundance of specific species and their functions. Moreover, the effects of a diet on individuals in the population differ from person to person and may be influenced by a combination of host and microbiome features (David et al., 2014; Kolodziejczyk, Zheng, & Elinav, 2019; Shanahan, Van Sinderen, O'Toole, & Stanton, 2017; Yang et al., 2020). Enteral nutrition (EN) is a common artificial nutritional support for patients who are unable to achieve their nutritional requirements through oral diet. Exclusive EN represents the use of a complete liquid diet, with the exclusion of normal dietary components for a defined time, except water. In addition, exclusive EN providing a complete diet and simultaneously a therapeutic measure to induce remission of Crohn's disease (CD) in up to 80% of cases (Ashton et al., 2018; Forbes et al., 2017; MacLellan et al., 2017), especially in children and adolescents newly diagnosed with active CD (Cameron et al., 2013; Hradsky, Copova, Zarubova, Nevoral, & Bronsky, 2016). Therefore, exclusive EN is used as the first line therapy for CD patients (Ruemmele et al., 2014). There is significant evidence of this therapy efficacy in the microbiota changes such as specific species appearance, broad taxonomic shifts, and functional changes (Ashton et al., 2017; Ashton, Gavin, & Beattie, 2018; D'Argenio et al., 2013; Kaakoush, Day, Leach, Lemberg, & Mitchell, 2016; Quince et al., 2015). The effect on microbiota varies among patients, as well as the methods used to estimate bacterial distribution and diversity are heterogeneous (Gatti et al., 2017). It ought to be mentioned, that the gut microbiome composition of each individual is unique (Johnson, 2020) and the EN formulas used in the studies differ in type and composition (Cámara-Martos & Iturbide-Casas, 2019), especially in the presence of prebiotics, vitamins, and other nutrients and parameters, which can affect the colon microbiota (Klingbeil & de La Serre, 2018; Walker & Lawley, 2013; Yang et al., 2020). Above that, the time of exclusive EN therapy is usually at least 6 weeks and patients may change brands of formulas and at thus the composition of the EN during the therapy. The aim of this study was to investigate the microbial changes of faecal microbiota after their *in vitro* cultivation on EN formulas of polymeric type with variable prebiotic and nutrient composition.

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2. Materials and methods

2.1. Experimental design

Microbial composition of faecal samples (FS; n = 10) before and after their *in vitro* anaerobic cultivation in polymeric EN formulas (n = 4) were determined using plate technique method, metabolite analysis, and microbiota profiling of selected samples (n = 3, resp. 3 + 12) by 16S rRNA sequencing (Fig. 1**).**

2.2. Faecal inoculum

One g of fresh FS from human healthy donors (n = 10), aged from 5 to 18 years, with diversified adult-like gut microbiota, without recent intake of antibiotics for the previous 3 months, was collected into sterile anaerobically prepared tubes with 10 mL of dilution buffer consisting of tryptone (5 g L^{-1}), nutrient broth No. 2 (5 g L^{-1}), yeast extract (2.5 g L^{-1}; all Oxoid, UK), L-cysteine (0.5 g L^{-1}), and Tween® 80 (1 mL L^{-1}, both Sigma-Aldrich, USA). Tubes also contained glass pearls for homogenization and were prepared in an oxygen-free carbon dioxide environment according to Hungate (1969). The samples were tested within 3 h of defecation.

2.3. Enteral nutrition formulas as cultivation media

Four polymeric enteral nutrition (EN) formulas of different brands were used as media for FS cultivation: Fortini Multi Fibre (Nutricia, Zoetermeer, Netherlands) – labelled as EN_A, Fresubin Energy Fibre (Fresenius Kabi Deutschland GmbH, Germany) – EN_B, Renutryl Booster (Nestlé Clinical Nutrition, France) – EN_C, and Ensure Plus Advance (Abbott Laboratories, Czech Republic) – EN_D (Table 1**)**. The sterile tubes were filled with 10 mL of tested EN formulas and treated with CO_2 to establish an oxygen-free environment.

2.4. In vitro cultivation of FS in EN formulas

FS (0.1 g per 10 mL of tested EN formula) was anaerobically inoculated into four prepared tubes with different EN formulas and incubated under anaerobic conditions (GENbag anaer, bioMérieux, France) at 37 °C for 24 h. FS inoculum and cultivated FS in EN formulas (marked as FS + EN_A, FS + EN_B, FS + EN_C, FS + EN_D) were further analysed.

2.5. Quantification of selected cultivable bacterial group

Microbiological analysis of FS before and after cultivation in the EN formulas was performed using the plate technique with media for total counts of anaerobic bacteria, bifidobacteria, lactobacilli, and *E. coli* according to Modrackova et al. (2019) with serial dilution of samples using above mentioned dilution buffer. Wilkins-Chalgren agar supplemented with soya peptone (WSP; 5 g L^{-1}, Oxoid), L-cysteine (0.5 g L^{-1}), and Tween® 80 (1 mL L^{-1}, both Sigma-Aldrich, USA) was used for the determination of total counts of anaerobic bacteria, WSP agar with mupirocin (100 mg L^{-1}, Oxoid) and acetic acid (1 mL L^{-1}, Sigma-Aldrich) for bifidobacteria, Rogosa Agar (Oxoid) with acetic acid (1.32 mL L^{-1}) for lactobacilli, and chromogenic T.B.X. medium (Oxoid) for *Escherichia coli*. Cultivation was performed under anaerobic conditions (GENbag anaer) for bifidobacteria and total anaerobes at 37 °C for 48 h, whereas microaerophilic conditions were used for lactobacilli at 37 °C for 48 h, and aerobic conditions for *Escherichia coli* at 37 °C for 24 h.

2.6. Short-chain fatty acid analysis by ion chromatography with suppressed conductivity detection

The main short chain fatty acids (SCFAs) acetate, propionate, butyrate, and two intermediate products, lactate and formate, were measured in fermentation supernatants of batch fermentation samples by capillary high-pressure ion-exchange chromatography with suppressed conductivity detection. Samples were centrifuged at 13 000 × g for 5 min. Supernatants were diluted (500×) and filtered through a 0.45 µm nylon membrane, and analysed using a Dionex ICS 4000 system equipped with IonPac AS11-HC 4 µm guard and analytical columns (Thermo Scientific, USA). Eluent composition was as follows: 0–10 min isocratic: 1 mM KOH; 10–20 min linear gradient: 1–60 mM KOH; and 20–25 min again isocratic: 60 mM KOH. The flow rate was set to 0.012 mL min^{-1}. An ACES 300 suppressor (Thermo Scientific, USA) was used to suppress eluent conductivity, while a carbonate Removal Device 200 (Thermo Scientific) was implemented to suppress carbon dioxide baseline shift. Chromatograms were processed with Chromeleon 7.20 (Thermo Fisher). Standards were prepared from 1 g L^{-1} stock solutions (Analytika, Czech Republic; Inorganic Ventures, USA). Deionised water (conductivity < 0.055 µS cm^{-1}) was used for eluent and standard preparation (0.1–40 mg L^{-1}).

2.7. Microbiota profiling with 16S rRNA sequencing

To investigate microbiota composition, three FS were randomly

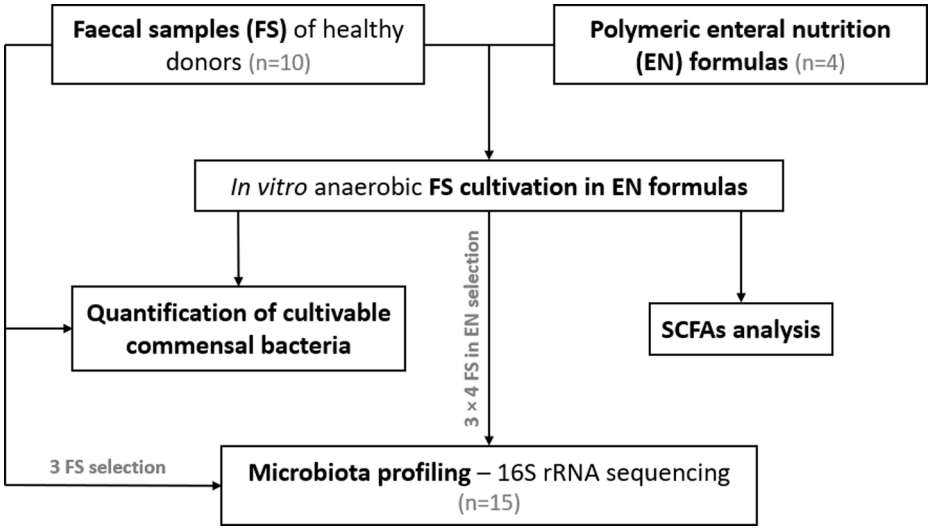

Fig. 1. Experimental design.

Table 1
The composition (per100 mL) of used enteral nutrition formulas for *in vitro* testing.

Enteral Nutrition		Fortini Multi Fibre	Fresubin Energy Fibre	Renutryl Booster	Ensure Plus Advance
Energy value (kJ/kcal)		**640/153**	**630/150**	**840/200**	**631/150**
Osmolarity (mOsm/L)		380	410	580	557
Nutrients	**Fat (g)**	6.8	5.8	7	4.8
	of which saturates (g)	0.7	0.4	0.9	0.42
	monounsaturates (g)		3.8	4	
	polyunsaturates (g)		1.6	1.3	
	Carbohydrate (g)	18.8	17.8	24	16.8
	of which sugars (g)	4.6	5.6	7	6.8
	lactose (g)	<0.025	< 0.26	< 0.05	
	Fibre* (g)	1.5	2	0	0
	Protein (g)	3.4	5.6	10	9.1
	Salt (g)	0.17	0.43		0.43
Minerals	Sodium (mg)	67	80	95	160
	Chloride (mg)	100	100	85	139
	Potassium (mg)	140	135	240	270
	Calcium (mg)	84	135	229	227
	Phosphorus (mg)	75	80	153	120
	Magnesium (mg)	17	21	50	25
	Iron (mg)	1.5	2	1.7	2.1
	Zinc (mg)	1.5	1.5	2.5	1.75
	Copper (mg)	0.135	300	250	0.45
	Iodine (μg)	15	30	25	22
	Selenium (μg)	4.5	10	13	8.5
	Manganese (mg)	0.23	0.4	0.16	0.45
	Chromium (μg)	0.23	10	21	10
	Molybdenum (μg)	6	15	7	15
	Fluorine (mg)	0.11	0.2	0.4	
Vitamins	Vitamin A (μg)	61	120	117	60
	Vitamin D (μg)	1.5	2	1.7	5.7
	Vitamin E (mg α-TE)	1.9	3	3.4	2.5
	Vitamin K (μg)	6	16.7	12	15
	Vitamin C (mg)	15	15	20	16
	Vitamin B1 (mg)	0.23	0.23	0.2	0.26
	Vitamin B2 (mg)	0.24	0.32	0.27	0.34
	Vitamin B6 (mg)	0.18	0.33	0.37	0.734
	Niacin (mg)	0.88	3	4.5	3
	Folic acid (μg)	23	50	67	35
	Vitamin B12 (μg)	0.26	0.6	0.8	0.65
	Pantothenic acid (mg)	0.5	1.2	0.83	1.1
	Biotin (μg)	6	7.5	10	6
Other nutrients	Carotenoids (mg)	0.15			
	L-carnitine (mg)	3			18
	Cholin (mg)	30	26.7		70
	Taurine (mg)	11			
	Betacarotene (μg)		300		60
	Linoleic acid (g)			1.1	
	α-linolenic acid (mg)			210	
	Fructooligosaccharides (g)				0.75
	Hydroxymethylbutyrate (g)				0.55

Footnotes: *Fibre content in Fortini Multi Fibre –soy polysaccharides, inulin, oligofructose, resistant starch, gum arabic, and cellulose; in Fresubin inulin – chicory

selected before and after cultivation in four different EN formulas. Total genomic DNA was extracted from 500 μl of 10 × diluted FS or FS in EN using the Fast DNA SPIN kit for soil (MP Biomedicals, Illkirch, France) according to the manufacturer's instructions. DNA concentration and quality were accessed by absorbance measurements at 260 nm on a NanoDrop® ND-1000 Spectrophotometer (Witec AG, Littau, Switzerland), and samples were stored at − 20 °C prior to the molecular analyses.

The bacterial V4–V5 16S rRNA region was amplified with the primers BactBF (GGATTAGATACCCTGGTAGT) and BactBR (CACGACACGAGCTGACG) according to Fliegerova et al. (2014). The obtained PCR products were purified using the QIAquick® PCR purification kit (Qiagen, Germany).

Purified amplicons were used for library preparation using the NEBNext® Fast DNA Library Prep Set for Ion Torrent (NEB, USA). The Ion Xpress Barcode Adapters were used to label each sample specifically. The sequencing template was prepared on the Ion OneTouch 2 system using the Ion PGM OT2 HiQ View Kit and sequenced on the PGM platform (both, Thermo Fisher Scientific) using the Ion PGM TM Hi-Q TM Sequencing Kit and the Ion 316 TM v2 chip, both according to manufacturer's protocols.

A quality control of the resulting sequences was performed using FastQC package v0.11.8. The resulting sequences were analysed using the Qiime2 (Bolyen et al., 2019) software package with the DADA2 v2019.10.0 (Callahan et al., 2016) pipeline for IonTorrent error prediction (see ***SuplementaryFile1***). The resulting set of ASVs was normalized by sub-sampling to even depth of the lowest sample. Relative bacterial abundance was subsequently plotted on the phylum and family level. Taxonomic groups that accounted for<0.1% of total sequences in each sample were pulled together into a low abundance category for increased legibility. Furthermore, the community diversity was expressed as Shannon's entropy. These procedures are detailed in the ***SupplementaryFile2***.

2.8. Statistics

Counts of bacterial colonies (log CFU g^{-1}) within the groups FS, FS + EN_A, FS + EN_B, FS + EN_C, and FS + EN_D are shown as boxplots

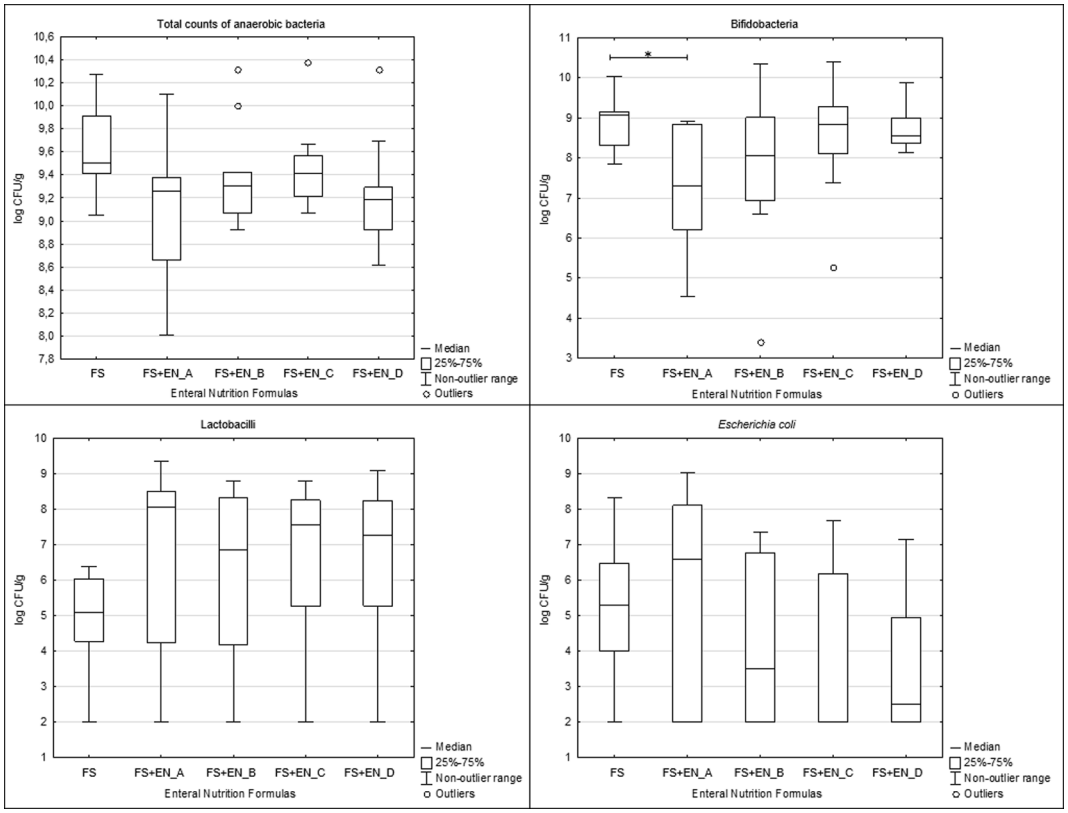

Fig. 2. Enumeration of bacterial groups after FS incubation in four EN formulas. Counts of cultivable commensal groups of bacteria are shown as averages in log CFU g^{-1}. Four different media were used for quantification of total counts of anaerobic bacteria, bifidobacteria, lactobacilli, and *Escherichia coli* within the incubated faecal sample (FS) obtained from ten individual donors (n = 10) in four different polymeric enteral nutrition (EN) formulas; EN_A (Fortini Multi Fibre), EN_B (Fresubin Energy Fibre), EN_C (Renutryl Booster), and EN_D (Ensure Plus Advance). Scheffe's test of one-way ANOVA and Kruskal-Wallis test were used for assessment of statistically significant differences ($P < 0.05$), shown as s horizontal bar, between FS and EN formulas using STATISTICA software (StatSoft, Czech Republic).

(Fig. 2). SCFA levels (mM) of lactate, acetate, propionate, formate, and butyrate are expressed as averages with standard deviations (Fig. 3). The normality of data was evaluated by Shapiro-Wilk W test ($P < 0.05$). Depending on the fulfilment of the testing conditions, Scheffe's method for multiple hypothesis testing adjustment of P-values of One-Way ANOVA or Kruskal-Wallis test were used ($P < 0.05$). All statistical analyses were processed by STATISTICA software (StatSoft, Czech Republic).

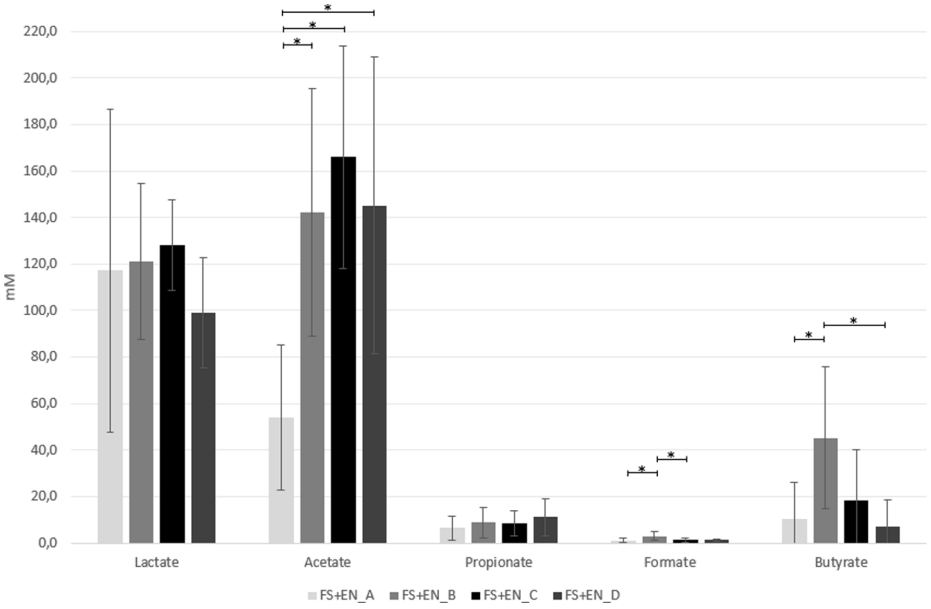

Fig. 3. Short chain fatty acid analysis. Formation of lactate, acetate, propionate, formate, and butyrate was measured in fermentation supernatants of batch fermentation samples within the incubated faecal sample (FS) obtained from individual donors (n = 10) in four different polymeric enteral nutrition (EN) formulas; EN_A (Fortini Multi Fibre), EN_B (Fresubin Energy Fibre), EN_C (Renutryl Booster), and EN_D (Ensure Plus Advance). The measurement was performed after 24 h of *in vitro* cultivation by ion-exchange chromatography with suppressed conductivity. Scheffe's test of one-way ANOVA and Kruskal-Wallis test were used for assessment of statistically significant differences ($P < 0.05$), shown as a horizontal bar, between the concentrations of fermentation metabolites of FS in EN formulas. The statistics was performed using STATISTICA software (StatSoft, Czech Republic) and Microsoft Office Professional Plus 2016.

3. Results

3.1. Detected counts of cultivable bacterial commensal groups

The counts of cultivable commensal groups of bacteria naturally present in FS (n = 10), which were inoculated in four different EN formulas, were quantified by desk-plate method using selective media and factors for their cultivation. The resulting bacterial counts display of a wide spread that was caused by inter-individual differences among the FS, and due the detection limit 10^2 (especially for *E. coli*). Total counts of anaerobic bacteria reached more than 10^9 CFU g^{-1} and were similar among each variant of EN formula as growth media, and as well in comparison to the FS itself (Fig. 2). The same trend was also detected in the other two monitored groups. Lactobacilli exhibited average numbers of (6.21 ± 2.43)–(6.71 ± 2.59) log CFU g^{-1} that is almost 1.5 order of magnitudes higher than in FS (5 log CFU g^{-1}), and *E. coli* of (3.50 ± 1.86)–(6.21 ± 2.74) log CFU g^{-1}. Bifidobacteria were detected in significantly decreased numbers 7.14 ± 1.56 log CFU g^{-1} in Fortini Multi Fibre in comparison with FS 8.86 ± 0.68 log CFU g^{-1}. Other EN formulas do not appear to have enhanced their growth and enabled bifidobacteria to grow in range 10^7–10^9 CFU g^{-1}.

3.2. Metabolic profile of fermentation supernatants

The production of main detected metabolites, such as acetate, propionate, formate, and butyrate, differed among used EN formula as the fermentation substrates (Fig. 3). In general, acetate and lactate reached the highest levels, as main metabolites of bifidobacteria, as well as lactobacilli. The acetate levels after FS incubation in Fortini Multi Fibre were significantly decreased (53.94 ± 31.33 mM) in comparison with other EN formulas where the concentrations were almost triplicated (142.32 ± 53.31 mM in Fresubin Energy Fibre, 165.98 ± 47.90 mM in Renutryl Booster, and 145.22 ± 63.82 mM in Ensure Plus Advance). The low amount of acetate correlates with lower abundance of bifidobacteria in FS incubated in Fortini Multi Fibre. In addition, fermentation supernatant of FS in Fortini Multi Fibre showed the inverse acetate: lactate ratio in favour of lactate compared to other EN formulas. Formate levels were significantly lower in FS in Fortini Multi Fibre (1.14 ± 0.85 mM) and Renutryl Booster (1.41 ± 0.94 mM) compared to FS in Fresubin Energy Fibre (3.05 ± 1.82 mM). Similarly, the butyrate levels were significantly lower in FS in Fortini Multi Fibre (10.45 ± 15.70 mM) and Ensure Plus Advance (7.01 ± 11.49 mM) compared to the FS in Fresubin Energy Fibre (45.26 ± 30.57 mM). In contrast to that, the propionate levels were similar among all tested EN.

3.3. Bacterial composition of the original and cultivated faecal microbiota

Microbiota profile of the analysed FS (n = 3) and FS incubated in four different EN formulas Fortini Multi Fibre, Fresubin Energy Fibre, Renutryl Booster, and Ensure Plus Advance was determined by sequencing the V4–V5 regions of the 16S rRNA gene amplicons. The bacterial diversity in each sample was expressed as the Shannon's entropy. The cultivation samples displayed a considerable spread of values, however the median diversity remained similar to the FS sample (Fig. 4).

The relative abundance of the microbiota of the collective FS was similar in phyla Actinobacteria and Firmicutes (both ≈ 40.53%), followed by Proteobacteria (5.98%), and Bacteroidetes (2.30%). The taxonomic families *Bifidobacteriaceae* (33.73%), *Enterobacteriaceae* (5.52%), and *Lactobacillaceae* (0.16%) were further analysed for comparison with the cultivation data and the SCFAs profiles (Fig. 5). Fresubin Energy Fibre displayed the highest increase of *Bifidobacteriaceae* from 34 to 53%, followed by the Renutryl Booster (42%). In contrast to that, bifidobacteria were decreased in Fortini Multi Fibre to 10%. The *Lactobacillaceae* was nearly undetectable in the FS samples and Fresubin Energy Fibre and Ensure Plus Advance, whereas they sharply increased in Fortini Multi Fibre and Renutryl Booster to 34 and 33%, respectively. The relative abundance of *Enterobacteriaceae* was not strongly affected by any of the EN formulas as fermentation substrates. Further microbial shifts were detected. *Streptococcaceae* extensively multiplied in Fortini

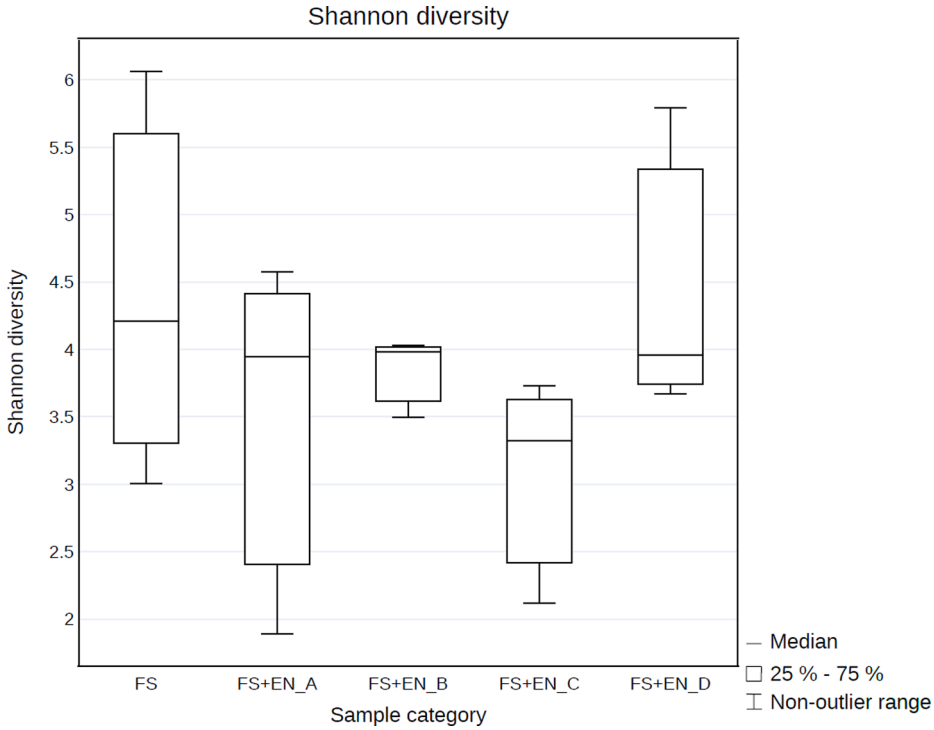

Fig. 4. Shannon's entropy. The bacterial diversity in each sample, where the faecal sample (FS) obtained from three individual donors (n = 3) was incubated in four different polymeric enteral nutrition (EN) formulas; EN_A (Fortini Multi Fibre), EN_B (Fresubin Energy Fibre), EN_C (Renutryl Booster), and EN_D (Ensure Plus Advance); was expressed as the Shannon's entropy.

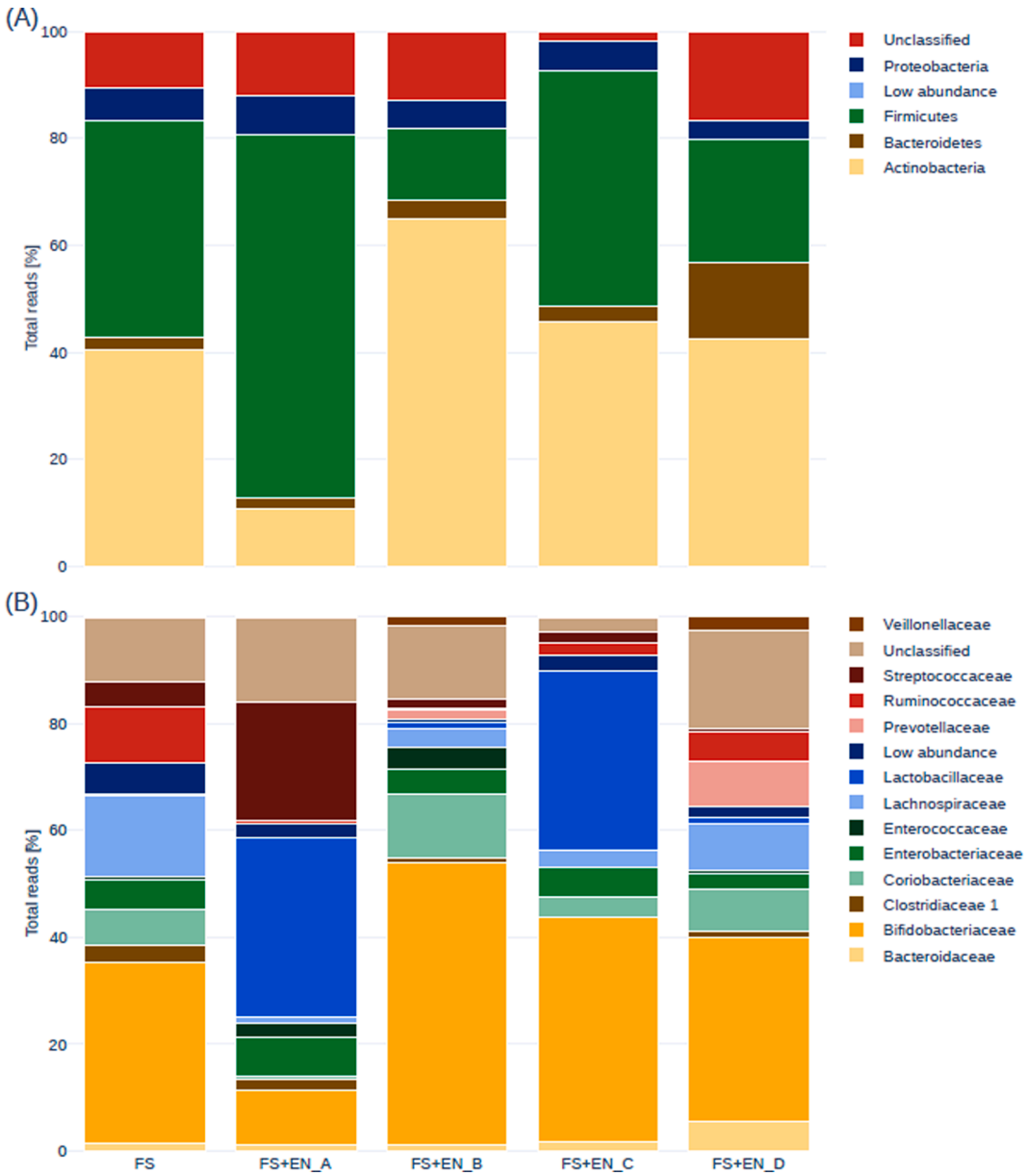

Fig. 5. Microbiota profiling with 16S rRNA sequencing. Microbial phyla (A) and families (B) in the faecal samples (FS) and FS incubated in four different EN formulas; EN_A (Fortini Multi Fibre), EN_B (Fresubin Energy Fibre), EN_C (Renutryl Booster), and EN_D (Ensure Plus Advance). Values presented are averages of the biological replicates (n = 3).

Multi Fibre up to 22% of reads in comparison with 5% in FS and<2% in other EN formulas. Likewise, *Coriobacteriaceae* thrived in Fresubin Energy Fibre (12%) and *Prevotellaceae* in the Ensure Plus Advance (8%). A notable decrease of *Lachnospiraceae*, when compared to the FS (15%), was detected in Ensure Plus Advance with 9%, Fresubin Energy Fibre and Renutryl Booster with 3%, and in Fortini Multi Fibre with tenths of percent reads. Microbial shift of gut representatives was significantly influenced by the brand of the used EN formulas as fermentation substrate and their diverse content.

4. Discussion

Modulation of the human microbiota is an evolving strategy to improve human health. The ability to shift the composition and metabolic outcomes of the gut microbial population is achieved via dietary or non-dietary interventions (David et al., 2014; Gibson et al., 2017; Walker & Lawley, 2013). Commercial enteral formulas usually differ in composition of prebiotic substrates and another specific nutrients, which can modulate gut microbiota profile (Klingbeil & de La Serre, 2018; Walker & Lawley, 2013; Yang et al., 2020). In this study, the prebiotic fibre content of the tested EN formulas varied. Fortini Multi Fibre contained soy polysaccharides, inulin, oligofructose, resistant starch, gum arabic, and cellulose, Fresubin Energy Fibre chicory inulin, and Ensure Plus Advance fructooligosaccharides. Renutryl Booster did not contain any prebiotic fibre, but linoleic and α-linolenic acids unlike the others. Knowledge of the content of these substances should be considered when choosing exclusive EN as the therapy. The effects of EN formulas on specific bacterial species occurrence and metabolite formation are variable and inconsistent between studies (D'Argenio et al., 2013; Guinet-Charpentier, Lepage, Morali, Chamaillard, & Peyrin-Biroulet, 2017; Tjellström et al., 2012), and EN components can play an important role in the intestinal microbiota modulation. Moreover, the presence of soluble dietary fibre can have effect on stool form and short-chain fatty acid production (Mizuno, Bamba, Abe, & Sasaki, 2020).

Dietary changes lead to significant shifts in the human gut microbiota, which can occur in a rapid and reproducible manner (David et al., 2014; Lang et al., 2018; Seo, Lee, Kim, & Park, 2020). The changes are highly variable among individuals, without strong population level trends (Lang et al., 2018). Heterogeneous and highly personalized microbial shifts have also been detected in response to carbohydrates, including dietary fibre with resistant starches and prebiotic carbohydrates (Lockyer & Nugent, 2017; Walker et al., 2011). Fortifying enteral formulas with prebiotics have been proposed as a method to increase beneficial species such as bifidobacteria to assist in colonisation resistance and to increase SCFAs production (Whelan, 2007; Whelan, Gibson, Judd, & Taylor, 2001). However, some results are not so convincing to support bifidobacterial counts (Majid, Emery, & Whelan, 2011; Schneider et al., 2006). According to Modrackova et al. (2019) EN formulas were found to be suitable growth media for commensal groups such as bifidobacteria, lactobacilli, and *E. coli* tested in single culture assay *in vitro*. On the other hand, the counts of cultivable commensal bacteria in the FS of CD children were not significantly affected by 6 weeks exclusive EN therapy *in vivo*. It is known that added prebiotic carbohydrates, which are also part of the EN formulas can also support the growth of other groups with a pathogenic potential (e.g. clostridia and Gram-negative bacteria) present in the intestinal microbiota (Bunesova et al., 2012; Rada et al., 2008). Differences in the prebiotic substrates available in the individual commercial EN formulas (Table 1) may be responsible for the different microbial and metabolic profiles obtained after culturing an identical FS on the tested polymeric EN formulas in this study. Bifidobacteria and lactobacilli detected in FS of donors were able to grow in the tested EN formulas *in vitro* with respect to the other present members of microbiota. In addition to that, microbiota profiling using 16S rRNA sequencing points to the growth also of other taxa than *Bifidobacteriaceae* and *Lactobacillaceae*, such as *Streptococcaceae, Coriobacteriaceae, Prevotellaceae,* and *Lachnospiraceae.*

Batch fermentation represents an opportunity to test the effect of prebiotics and other nutrients on microbial populations from single cell culture to complex faecal microbiota (Bunesova et al., 2012; Modrackova et al., 2019). Whereas, *in vitro* continuous intestinal fermentation technology with immobilized faecal microbiota, mimicking planktonic as well as sessile growth, can be used to produce controlled and stable "artificial" large intestinal microbiota with high cell densities and quantities, moreover is a potential alternative to faecal microbiota transplantation (Bircher, Schwab, Geirnaert, & Lacroix, 2018; Lacroix, de Wouters, & Chassard, 2015; Payne, Zihler, Chassard, & Lacroix, 2012). Both can be used for further research of EN components to modulate homeostatic and dysbiotic microbiota of CD patients.

5. Conclusion

Our results indicate that even slight composition differences of EN formulas can shift microbial profile. The efficacy of prebiotics and other nutrients in modulation of the gut microbiota in health and disease needs further investigation, and an individualized approach is merited, given the great (inter)individual variation in microbiota configurations.

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Ethics approval and consent to participate

The study was approved by the Ethics Committee of the authors' institution (the Ethics Committee Reference number is 1491/16).

CRediT authorship contribution statement

Nikol Modrackova: Software, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Ivana Copova:** Conceptualization, Methodology, Resources, Writing - review & editing. **Adam Stovicek:** Software, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization. **Marie Makovska:** Investigation. **Dagmar Schierova:** Investigation, Writing - review & editing. **Jakub Mrazek:** Investigation, Funding acquisition. **Monika Sabolova:** Investigation, Writing - review & editing. **Eva Vlkova:** Funding acquisition. **Ondrej Hradsky:** Conceptualization, Writing - review & editing. **Jiri Bronsky:** Conceptualization. **Jiri Nevoral:** Conceptualization, Writing - review & editing. **Vera Neuzil-Bunesova:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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