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Oat bran, but not its isolated bioactive β-glucans or polyphenols, have a bifidogenic effect in an in vitro fermentation model of the gut microbiota

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1 Oat bran, but not its isolated bioactive β-glucans or polyphenols, have a bifidogenic

2 effect in an *in vitro* fermentation model of the gut microbiota[1-4]

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19

20   Abstract

21   Wholegrain oats are known to modulate the human gut microbiota and have prebiotic

22   properties (increase the growth of some health promoting bacterial genera within the colon).

23   Research to date mainly attributes these effects to the fibre content; however, oat is also a rich

24   dietary source of polyphenols, which may contribute to the positive modulation of gut

25   microbiota. *In vitro* anaerobic batch-culture experiments were performed over 24 h to evaluate

26   the impact of two different doses (1 and 3 % w/v) of oat bran, matched concentrations of β-

27   glucan extract or polyphenol mix, on the human faecal microbiota composition using 16S RNA

28   gene sequencing and short chain fatty acid analysis (SCFA). Supplementation with oats

29   increased the abundance of Proteobacteria ($p < 0.01$) at 10 h, Bacteroidetes ($p < 0.05$) at 24h

30   and concentrations of acetic and propionic acid increased at 10 and 24 h compared to negative

31   control. Fermentation of the 1 % w/v oat bran resulted in significant increase in SCFA

32   production at 24 h (86 ± 27 mM vs. 28 ± 5 mM; $p < 0.05$) and a bifidogenic effect, increasing

33   the relative abundance of *Bifidobacterium unassigned* at 10h and *Bifidobacterium adolescentis*

34   ($p < 0.05$) at 10 and 24 h compared to negative control. Considering the β-glucan treatment

35   induced an increase in the phylum Bacteroidetes at 24 h, it explains the Bacteriodetes effects

36   of oats as a food matrix. The polyphenol mix induced an increase in Enterobacteriacea family

37   at 24 h. In conclusion, in this study, we found that oats increased bifidobacteria, acetic acid and

38   propionic acid, and this is mediated by the synergy of all oat compounds within the complex

39   food matrix, rather than its main bioactive β-glucan or polyphenols. Thus oats as a whole food

40   led to the greatest impact on the microbiota.

43

44   1. Introduction

45   A large body of evidence from prospective and intervention studies suggests that a diet rich in

46   oats could significantly reduce the risk of bowel disease [1, 2], cardiovascular disease [3-5]

47   and lowers high blood cholesterol levels [4-7]. Recent reports suggest that oats may act as a

48   prebiotic, modulating the gut microbiota and impacting on metabolic disease risk [8, 9]. To

49   date, it is believed that the protective effect of whole grain oats is mainly due to the presence

50   of dietary fibre, in particular, soluble β-glucan [6, 10-12], and resistant starch [13]. β-glucan is

51   known to lower cholesterol and bile acid absorption through formation of viscous gels in the

52   upper gut [14] and/or directly binding of cholesterol or bile acids. Resistant starch leads to the

53   production of short chain fatty acids (SCFA) in the colon and may also increase the growth of

54   some health promoting bacterial genera within, such as *Bifidobacterium* and *Lactobacillus* [15-

55   18]. Species of these genera influence the cholesterol metabolism through increasing bile-salt

56   hydrolase enzyme activity and the deconjugation of bile acids [19].

57   Oats, however, are also rich dietary sources of polyphenols, including avenanthramides and

58   phenolic acids [20]. These are likely to contribute to the health effects of a diet rich in oats [21-

59   23] but have not yet been examined in detail. Phenolic acids are found in three different forms

60   within the oat food matrix: as soluble free acids, as soluble conjugates esterified to low

61   molecular weight components such as sugars, and as insoluble bound acids esterified to high

62   molecular weight components including lignin, cell wall polysaccharides and storage proteins

63   [20]. A large proportion of oat polyphenols are bound via an ester bond, and hence are poorly

64   absorbed in the upper intestine and reach the colon [24], where they may beneficially modulate

65   the microbiota [8]. There are no esterases in human tissues that break these ester links [25];

66   therefore, the main catalytic site is in the colon through cinnamoyl esterase activity of human

67   faecal microbiota, such as *Escherichia coli* (three isolates), *Bifidobacterium lactis* and

68   *Lactobacillus gasseri* (two strains) [26, 27].

69   Gut microbiota (GM) alterations by whole grain oats have been observed in a number of *in*

70   *vitro* fermentation models [9, 28-32] and animal studies [13, 33-35]. The fermentation of oats

71   has led to increased *Bifidobacterium, Lactobacillus* [8, 9, 30, 31, 33, 34] and *Bacteroides* [32]

72   populations and, at the same time decreased *Clostridium* [30, 33].

73   The soluble β-glucans have been one of the most commonly studied components of oats [28,

74   36, 37]. However, oat-derived isolated β-glucan only impacted on *Bacteroides* growth, not on

75   *Bifidobacterium* in 24h pH-controlled anaerobic batch culture fermenters [28]. Furthermore,

76   Crittenden *et al.* [38] reported that β-glucan was fermented by *Bacteroides* spp. but not by

77   *Lactobacillus* or *Bifidobacterium.* The viscosity and molecular weight of β-glucan may

78   strongly influence its ability to lead to SCFA production and act as a prebiotic [39].

79   Additionally, these studies have used the relatively high concentration of 1% v/w of β-glucan

80   dose [31, 40] which is much higher than the physiological β-glucan concentration delivered

81   from eating oats. To date, none of the oat or β-glucan *in-vitro* fermentation studies assessed the

82   polyphenol content of their treatment [9, 28, 32]. Thus, little information exists on the ability

83   of oat-derived avenanthramides, hydroxycinnamic and hydroxybenzoic acids to influence the

84   GM.

85   The main purpose of the present *in vitro* study was to examine if a physiologically relevant

86   dose of polyphenol mix (avenanthramide, hydroxycinnamic acids and benzoic acid

87   derivatives), β-glucan extract or digested oat bran as whole food matrix beneficially modulates

88   the GM. Secondly, the study aimed to assess the impact of different doses of digested oat bran

89   on the GM, notably using an oat bran that was well characterised in terms of physicochemical

90   properties.

91

92

93 2. Materials and Methods

94 2.1. Reagents

95 All chemicals and reagents were purchased from Sigma-Aldrich Chemical Co Ltd. (Pool,
96 Dorset, UK) or Fisher (Loughborough, Leics, UK) unless stated otherwise. Mixed-linkage β-
97 glucan kit was supplied by Megazyme Co (Wicklow, Ireland). The anaerobic jar (AnaerojarTM
98 2,5L) and gas-generating kit (AnaeroGen TM) were obtained from Oxoid Ltd (UK), the
99 dialysis tube from Spectrum (VWR International). Media and instruments were autoclaved at
100 121°C for 15 min. HPLC column and guard cartridges were obtained from Phenomenex
101 (Cheshire, UK). PowerSoil®DNA Isolation Kit was purchased from Mo Bio Laboratories, Inc
102 (USA), the primers for the 16S rRNA gene amplification from Integrated DNA Technologies,
103 BVBA (Belgium), AccuPrimeTM SuperMix II from Life Technologies (CA, USA), AMPure
104 XP beads from Beckman Coulter Genomic (CA, USA).

105 2.2. Oats and Controls

106 The oat bran was purchased from White's (Tandragee, Northern Ireland). The oat
107 macronutrient composition was analysed by Campden BRI laboratories (Total carbohydrate,
108 Available carbohydrate, Total dietary fibre- AOAC 991.43 method, Total fat – BS 4401, and
109 protein – AOAC 981.10 method), whereas the detailed polyphenol content was measured in
110 our laboratory at the University of Reading based on a previous method [41]. The beta-glucan
111 method used was employed specifically to quantify 1,3:1,4-β-D-glucan. Synergy1 the
112 oligofructose-enriched inulin was supplied by Beneo (Belgium) and 94%-β-glucan extract
113 from Megazyme Co (Wicklow, Ireland). Polyphenols were purchased from Sigma-Aldrich
114 Chemical Co Ltd. (Pool, Dorset, UK).

115   2.3. *In vitro* digestion of oat bran (from mouth to small intestine)

116   The method employed was adapted from Mills *et al.* [42]. Oat bran was digested *in vitro* in

117   three phases: the oral phase, the gastric phase and the small intestinal phase. 60 g of oat bran

118   was mixed with 150 ml of sterile and distilled water and homogenised, transferred into a 500

119   ml Duran bottle, and microwaved for 1 minute. In the oral phase, 20 mg of α-amylase was

120   dissolved in 6.25 ml $CaCl_2$ (1 mM, pH 7.0) and added to the solution, then incubated at 37°C

121   for 30 minutes on a shaker set at 120 *xg*. After incubation, the pH was adjusted to 2.0 with 6

122   M HCl solution and the gastric phase introduced by adding 2.7 g pepsin in 25 ml HCl (0.1 M)

123   and further incubated for 2 hours under the same conditions. In the small intestinal phase, 560

124   mg pancreatin and 3.5 g bile were mixed with 125 ml $NaHCO_3$ (0.5 M) and dispensed into the

125   mix. The pH was adjusted to 7.0 then incubated for 3 hours at 37 °C with shaking. Finally, the

126   sample solution was transferred to a seamless semi-permeable 100-500 Dalton molecular

127   weight cut-off regenerated cellulose dialysis tubing and dialysed against NaCl (0.01 M at 5°C)

128   to remove low molecular mass digestion products. After 15 hours, the dialysis fluid was

129   changed and the process continued for an additional 2 hours. The digested oat bran mix was

130   collected and transferred into several 250 ml clear plastic containers, frozen to -80° and freeze-

131   dried for 5 days to remove all fluid content.

132   2.4. Extraction and analyses of polyphenols from undigested and digested oat bran

133   Polyphenols were extracted from undigested (raw) and digested (after *in vitro* digestion) oat

134   bran in two separate fractions (i.e. free and conjugated or bound) using the method of Schar *et*

135   *al.* [41]. The phenolic acids and avenanthramides in oat extracts were identified and quantified

136   using a high-performance liquid chromatography (HPLC) Agilent 1100 series (Agilent

137   Technologies Ltd) equipped with a quaternary pump, autosampler, column thermostat, sample

138   thermostat and photo diode array detector. Compound separation was achieved by a Kinetex

139   biphenyl column (100 A 250 x 4.6 mm length, 5 uM particle size; Phenomenex) and using a

140   gradient elution. Mobile phase A consisted of 0.1 % (v/v) formic acid in HPLC water (A),

141   while mobile phase B was 0.1 % (v/v) formic acid in methanol. The following optimised

142   gradient protocol was run: 0 min, 95% A, 5 % B; 20 min, 75 % A, 25 % B; 25 min, 74 % A,

143   26 % B; 30 min, 65 % A, 35 % B; 40 min, 64 % A, 36 % B; 53 min, 30 % A, 70 % B; 56 min,

144   5 % A, 95 % B; 61 min, 5 % A, 95 % B; 62 min, 95 % A, 5 % B; 65 min, 95 % A, 5 % B. The

145   flow rate of the mobile phase was 1.0 ml/min and the sample injection volume was 20 µl [43].

146   The absorbance was recorded at 254, 280 and 320 nm and quantification was based on 12 point

147   linear calibration curves (mean $R^2 > 0.99$) and as a ratio to the internal standard (i.e. 3,5-

148   dichloro-4-hydroxybenzoic acid) to account for losses during extraction ($R^2 \geq 0.99$).

149

150   2.4. Determination of the β-glucan content

151   The β-glucan content was analysed with the 1,3:1,4 mixed-linkage β-glucan kit. The assay uses

152   lichenase and β-glucosidase to metabolize β-glucan to β-gluco-oligosaccharides and

153   subsequently glucose. Glucose then reacts with GOPOD reagent and its absorbance was

154   measured at 510 nm by UltroSpec 1100 photo spectrometer (Scinteck Instruments LLC, USA).

155   The final β-glucan content was calculated by the Megazyme Mega-CalcTM tool [44].

156   The molecular weight of the β-glucan was determined by size-exclusion HPLC. The

157   chromatography system consisted of three serially connected columns (Shodex SB-G, Shodex

158   SB-806M, Shodex SB-804 HQ) and an UV-MALLS-Viscometer-dRI detector. The column

159   temperature was set at 40°C, the mobile phase was MiliQ water (Millipore, Bedford, MA)

160   containing 0.02 % sodium azide, and the flow rate set at 0.5 ml/min. Samples were prepared at

161   a concentration of 10 mg/ml, heated at 60°C for 3 hours under constant shaking, syringe filtered

162   (0.45 µm PVD; Whatman, NY) and diluted to a concentration of 1 mg/ml before injection.

163   2.5. pH controlled faecal batch culture fermentation

164   Substrate ability to modulate gut microbiota was determined using anaerobic, stirred, pH and

165   temperature controlled faecal batch cultures. Batch culture fermentation vessels (300 ml

166   volume: one vessel per treatment) were sterilised and filled with 135 ml of sterile basal medium

167   (g/l: 2 g peptone, 2 g yeast extract, 0.1 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g

168   MgSO4·7H_2O, 0.01 g CaCl2·6H_2O, 2 g $NaHCO_3$, 2 ml Tween 80, 0.05 g Hemin (dissolved in

169   a few drops of 1 M NaOH), 10 μl vitamin K, 0.5 g L-cysteine HCl, 0.5 g bile salts and 4ml of

170   resazurin solution (0.02 %)). Before addition to the vessel, the medium was adjusted to pH 7.0

171   and autoclaved. The sterile medium was gassed overnight with O_2-free N_2 (15 ml / min) to

172   establish anaerobic conditions. To mimic the proximal region of the human large intestine, pH

173   was held in the range of 6.7 - 6.9 by automatic addition of 0.5 M NaOH or 0.5 M HCl and

174   controlled via pH meter controllers (Electrolab, UK) and the temperature was kept at 37 °C.

175   Faecal samples were collected from three healthy female donors, who were between 25 and 40

176   years old, with no history of bowel disorders, who had not received antibiotic treatment for at

177   least 6 months before the study and had not consumed pre- or probiotic supplements one month

178   before the study. All donors were informed of the study aims and procedures, and provided

179   their verbal consent for stool samples to be used for the experiments in compliance with the

180   ethics procedures required at the University of Reading. Samples were collected in anaerobic

181   jars and used within one hour of collection. Samples were diluted 1:10 (w/v) with anaerobic

182   phosphate buffer (0.1 M, pH 7.4) and homogenised in a stomacher for 2 mins; the batch culture

183   systems were inoculated with 15 ml faecal slurry from an individual sample. The vessels were

184   treated with the following substrate: 1 % w/v, 1.5 g digested oat bran (OAT1.5); 3 % w/v, 4.5

185   g digested oat bran (digestibility of oats see McCane et al.[45]) (OAT4.5); 0.12 % w/v, 180 mg

186   94 % β-glucan extract (BG); 0.01 % w/v, 1.7 mg polyphenol mix (same profile as 4.5 g digested

187   oats) (POLY); 1 % w/v, 1.5 g Synergy1 (positive control, PC); an additional vessel was

188   prepared under the same conditions but without any substrate, negative control (NC) (Table 2).

189   The Synergy 1 is an inulin enriched with oligofructose, has been proved their prebiotic effects

190   [46]. The 3 % w/v oat was chosen as the highest dose since it would be the concentration

191   reached by an average person consuming 60 g oat bran (i. e. assuming 30 g oat bran reaches

192   the colon and colonic total volume is 1L) [45]. Based on this calculation, β-glucan extract and

193   polyphenol mix treatments were matched to the dose present in 3 % w/v oat bran. Treatment

194   with OAT1.5 was added to the experiment to monitor the impact of the oat dose on the prebiotic

195   effect. Five millilitre samples were collected at 0, 5, 10, 24h, of which 1 ml aliquots were

196   centrifuged at 13,000 *xg* for 10 min. Supernatants and pellets were stored separately at -20°C

197   until analysis.

198

199   2.6. High throughput sequencing of the gut microbiota

200   The GM compositions and dynamics were determined using tag-encoded 16S rRNA gene

201   MiSeq-based (Illumina, CA, USA) high throughput sequencing. DNA was extracted from 2 ml

202   of faecal samples from fermentation vessel using power soil DNA isolation kit. The bead-

203   beating was performed in 3 cycles of 15 second each at 6.5 pulse / s. (The FastPrep-24™ 5G

204   Instrument, MP Biomedicals). DNA concentrations and purity were determined using

205   Nanodrop 1000 (ThermoScientific, USA). The V3 region of the 16S rRNA gene was amplified

206   using primers compatible with the Nextera Index Kit NXt_338_F: 5'-

207   TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACWCCTACGGGWGGCAGCAG

208   -3'   and   NXt_518_R: 5'-

209   GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG -3'¨

210   [47]. PCR reactions and library preparation were conducted as described in [48]. Briefly, PCR

211   reactions containing 12 µl AccuPrime SuperMix II, 0.5 µl of each primer (10 µM), 5 µl of

212   genomic DNA (∼10 ng / µl), and nuclease-free water to a total volume of 20 µl were run on a

213   SureCycler 8800 (Agilent, CA, USA). Applied cycling conditions were: denaturation at 95 °C

214   for 2 min; 35 cycles of 95 °C for 15 s, 55 °C for 15 s and 68 °C for 40 s; followed by final

215   elongation at 68°C for 5 min. To incorporate primers with adapters and indices, PCR reactions

216   contained 12 µl Phusion High-Fidelity PCR Master Mix, 2 µl P5 and P7 primer, 2 µl PCR

217   product and nuclease-free water for a total volume of 25 µl.

218   Cycling conditions applied were: 98°C for 1 min; 12 cycles of 98°C for 10 s, 55°C for 20 s and

219   72°C for 20 s; elongation at 72°C for 5 min. The amplified fragments with adapters and tags

220   were purified using AMPure XP beads. Prior to library pooling, clean constructs were

221   quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and mixed in

222   approximately equal concentrations to ensure even representation of reads per sample. 180 bp

223   pair-ended MiSeq (Illumina) sequencing was then performed according to the instructions of

224   the manufacturer. The raw dataset containing pair-ended reads with corresponding quality

225   scores was trimmed using CLC Genomic Workbench (CLC bio, Arhus, Denmark). Trimming

226   settings were set to a low-quality limit of 0.01, with no ambiguous nucleotides allowed, and

227   trimming off the primer sequences. Merging overlapped reads was performed using the "Merge

228   overlapping pairs" tool using default settings. The Quantitative Insight Into Microbial Ecology

229   (QIIME) tool (version. 1.8.0; Open source software) was used for further analysis [49].

230   Purging the dataset from chimeric reads was performed using USEARCH, while the usearch61

231   method was used for Operational Taxonomic Units (OTUs) selection [50]. The Greengenes

232   (version 12.10) 16S rRNA gene database and EzTaxon were used as reference [51, 52].

233   High throughput sequencing yielded 12465219 sequences free from chimeric reads, providing

234   an average ± SD of 178202 ± 80036 sequences per sample (Range 292-447040), five baseline

235   samples and one 10h sample were excluded due to low reads (292-509).

236   2.7. Short-chain fatty acid analysis

237   The defrosted supernatant samples were filtered (0.22 μm, Millipore) and spiked with 50 μl

238   internal standard (2-ethyl butyric acid, 100 mM). SCFAs were measured in an ion exclusion

239   HPLC system (Agilent 1100 Series) equipped with DAD detector (G-1315B), autosampler

240   (G1316A) and Aminex HPX-8711 column (300 x 7.8mm) heated to 84°C. Samples (20 μl)

241   were injected in duplicate, and UV absorption was measured at 214 nm. The mobile phase was

242   2.5 mM aqueous sulphuric acid run at a flow rate of 0.6 ml/min. Peaks were integrated using

243   Agilent ChemStation software (Agilent Technologies, Oxford, UK) and single point internal

244   standard method. Peak identity and quantification were determined using a mixture of

245   standards of acetic, butyric, iso-butyric, propionic, lactic, formic, valeric and isovaleric acids.

246   Quantification was based on the 10-point calibration curve of analytical standards ($R^2 \geq 0.99$).

247   2.8. Statistics

248   For calculation of alpha and beta diversity measurement of the sequencing data, the d- and e-

249   values were set to 9800 reads per sample (85 % of the sequence number of the most indigent

250   sample). Alpha diversity measures expressed with an observed species with Chao1, the

251   sequence similarity 97 % OTUs value were computed for rarefied OTU tables using the alpha

252   rarefaction workflow. Differences in alpha diversity were determined using a t-test-based

253   approach employing the non-parametric (Monte Carlo) method (999 permutations)

254   implemented in the compare alpha diversity workflow with QIIME.

255   The relative distribution of the genera registered was calculated and summarised at the genus

256   level OTU tables, followed by Principal Coordinates Analysis (PCoA) plots generated with the

257   Jackknifed beta diversity workflow based on 10 distance matrices calculated using 10

258   subsampled OTU tables with QIIME. The *p*-value and the conservative FDR-corrected *p*-value

259   for multiple comparisons are reported. 3D plots were constructed from the three primary PCs

260   from the PCoA of the MiSeq analysis to visualise group differences in the composition of the

261   GM.

262   For the rest of the data analysis, GraphPad Prism statistics software package version 7 was

263   used. One-way ANOVA was used to determine differences between fermentation treatments

264   (OAT1.5, OAT4.5, POLY, BG) at the same time point (0, 5, 10 or 24 h), followed by the least

265   significant difference (Bonferroni) post hoc test. A repeated measures ANOVA was used to

266   explore the differences within the same treatment (OAT1.5, OAT4.5, POLY, BG) with all the

267   time points (0, 5, 10 and 24 h) with Bonferroni as the post hoc test. In addition to these analyses,

268   the p values were corrected using false discovery rate (FDR), $p \leq 0.05$ was considered

269   statistically significant.

270   3. Results

271   3.1. The composition of undigested and digested oat bran

272   The macronutrient, fibre and phenolic content of undigested and digested oat bran are reported

273   in Table 1. The *in vitro* oral, gastric and small intestine digestion reduced the carbohydrate

274   content by 53 %, available carbohydrate by 62 %, while the dietary fibre and β-glucan content

275   remained stable, losing only 2 % and 12 %, respectively. Free and conjugated, bound

276   polyphenol compounds decreased by 48 % and 26 %, respectively.

277   3.2. Polyphenols and β-glucan content in oat bran and extract

278   The OAT4.5 contained 1.73 mg polyphenols (total amount of free + conjugated and bound),

279   the POLY intervention were matched to OAT4.5 (Table 2). OAT1.5 and OAT4.5 delivered

280   56.67 mg and 170 mg β-glucan, respectively; the BG vessel to match OAT4.5 180 mg of 94%

281   β-glucan extract was added (Table 2). The molecular weight of the β-glucan in the digested oat

282   bran and the extract were similar (mean $\pm$ SD 335.5 $\pm$1.0 % kDa and 387.3$\pm$1.0 % kDa; $p >$

283   0.05).

284   3.2. Changes in alpha and beta diversity

285   Independent of the donor, alpha diversity decreased with oats treatments over the 24h

286   fermentation period (Chao1 2618$\pm$15 at 0h vs. 1577$\pm$22 at 24h for OAT1.5) but was constant

287   over time in the negative control (Chao1 2639$\pm$87 at 0h vs. 2488$\pm$77 at 24h) and POLY (Chao1

288   2655$\pm$138 at 0h vs. 2241$\pm$320 at 24h).

289   PCoA analyses based on unweighted UniFrac distance matrices of all samples showed a clear

290   donor effect (Figure 1.A). However, the donor effect was no longer significant when using

291   weighted UniFrac distance matrix analysis (ANOSIM test, Donor 1. Vs. Donor 2 p =1, Donor

292   1 vs Donor 3 p =1, Donor 2. Vs. Donor 3 p =1, Figure 1.B).

293   Across all samples, seven bacterial phyla were classified and one phylum designated as

294   unassigned. Phyla composition was similar for all donors ($p < 0.06$), although at baseline the

295   third donor had lower Tenericutes ($p < 0.01$), while the second donor had higher Actinobacteria

296   ($p < 0.05$). At baseline, the bacterial communities, were dominated by Firmicutes (57-67 %)

297   and Bacteroidetes (32-41 %), while the remaining five phyla including Actinobacteria (0.8-1.4

298   %), Verrucomicrobia, Cyanobacteria, Tenericutes and Proteobacteria (0.4-0.9 %) constituted

299   < 1.5 % of the community (Figure 2.).

300   3.3. Compositional Shifts

301   Supplementation with OAT4.5 led to a lower abundance of Firmicutes ($p < 0.02$) at 5 to 24 h

302   and to an increased abundance of Proteobacteria ($p < 0.01$) and Bacteroidetes at 24 h compared

303   to NC. A similar trend was observed for OAT1.5 (p values)(Figure 2).

304 The OAT1.5 treatment had a significant effect on the relative abundance of Actinobacteria at

305 10 h. The relative abundance of Actinobacteria differed between OAT1.5 and OAT4.5 at

306 baseline (1.1 % and 0.8 %, respectively). Following 10 h fermentation, Actinobacteria were

307 almost four times higher in OAT1.5 compared to OAT4.5 (4.9 % and 1.3 %, respectively),

308 however, differences did not persist over 24 h (4.2 % vs. 3.4 %, respectively). The treatment

309 of BG and POLY did not promote the growth of Actinobacteria, even though their dose was

310 matched to the OAT4.5 treatment. The PC, Synergy1 resulted in the strongest bifidogenic

311 effect and the relative abundance of Actinobacteria (baseline 1.1 % vs. 5 h at 31.1 %, 10 h at

312 11.5 %, $p < 0.05$) (Figure 2.).

313 Focusing on changes over 24 h, the abundance of Bacteriodetes increased with BG (49.3 % vs.

314 NC for 21 %), whereas Proteobacteria significantly decreased in comparison to the NC (14 %

315 vs. 33.5 %). POLY treatment, the abundance of Proteobacteria increased over the course of

316 fermentation (45 % vs. NC for 33.5 %) and decreased for Bacteriodetes ($p < 0.04$) (18.6 % vs.

317 46.8 % for OAT4.5). The oats and BG treatments promoted the growth of Bacteriodetes (Figure

318 2.).

319 At the lower taxonomic level and across all samples, 59 genera and 69 species were identified

320 when setting the cut-off at a relative abundance above 0.1 % of the community (Table 3). 5 h,

321 the OAT4.5 treatment led to a significantly lower abundance of *Ruminococcus* (0.1 %), and

322 *Coprococcus* (0.1 %) genus and, at the same time, increased the proportion of unassigned

323 *Enterobacteriaceae* (61.2 %) compared to NC (25.8 %) (Table 3). Most notably, OAT1.5

324 increased the abundance of *Bifidobacterium unassigned* (1.5 % vs. 0.2 for NC, $p < 0.05$) at 10

325 h and *Bifidobacterium adolescentis* at 10 h (1.9 % vs. 0.3 % for NC, $p < 0.04$) and at 24 h (2

326 % vs. 0.4 for NC, $p < 0.02$) (Table 3), whereas OAT4.5 did not significantly impact the relative

327 abundance of these species due to the high standard deviation among donors. Using EzTaxon

328 database, we were able to further identify the species designation of several OTUs at 24 h with

329 BG and POLY treatments. At 24 h, the BG treatment had higher abundances in *Bacteroides*

330 *ovatus* (13.5 %)*, B. uniform* (12.9 %) and *B. xylaniosolvens* (3.7 %) species, while POLY led

331 to the most abundant levels of *Enterobacteriaceae* species, *Hafnia alvei* (21.7 %). However,

332 these changes mediated by BG and POLY treatments were not significantly different compared

333 to NC.

334

335 3.4. Production of SCFA

336 OAT4.5 led to a high production of total SCFA compared to NC at 10 h (98.2 ± 22 mM vs 12.8

337 ± 4 mM respectively) and 24 h (151.5 ± 43 mM vs 28.1 ± 5 mM). The concentration of butyric

338 acid was significantly increased after 24 h fermentation of OAT1.5 ($p < 0.05$), and PC at 10 h

339 ($p < 0.05$) and 24 h ($p < 0.01$) compared to NC (Figure 3). Acetic acid was the most abundant

340 SCFA. Both oat treatments resulted in a significant increase of acetic acid at 10 h ($p < 0.01$),

341 and 24 h ($p < 0.01$) compared with baseline and NC (Figure 3.). At 24 h, OAT4.5, OAT1.5 and

342 PC significantly increased the concentration of propionic acid compared to NC (48 ± 24 mM,

343 16.7 ± 3 mM, 21 ± 9 mM and 4.5 ± 0.3 mM, respectively $p < 0.01$, 0.05 and 0.05, respectively).

344 Lactic and formic acids increased upon fermentation of OAT4.5 after 10 h compared to

345 baseline, but did not reach significance due to the large variation among donors. BG led to a

346 small production of acetic and butyric acids (not significant) whereas POLY did not induce

347 SCFA production. Overall, there was a dose-response effect between the two oats doses in total

348 SCFA at 10 h (98.2 ± 22 mM vs 58 ± 19 mM respectively, $p < 0.05$) and 24 h (151.5 ± 43 mM

349 vs 86.1 ± 27 mM, $p < 0.05$).

350

351   4. Discussion

352   This study aimed to identify the impact of different doses of oats and its isolated bioactive

353   compounds (i.e. β-glucan or polyphenols) on the faecal gut microbiota using *in vitro* systems.

354   Digested oat bran decreased alpha diversity and had a bifidogenic effect but isolated β-glucan

355   or polyphenol mix given at a matched dose did not induce a similar effect. (Figure 2.). This is

356   likely to be because these compounds alone did not provide enough energy for bacterial growth.

357   We have demonstrated that OAT1.5 significantly increased the proliferation of

358   *Bifidobacterium adolescentis* (Table 3). An increase in *Bifidobacterium* (genus level) was

359   reported by Connolly *et al.* [9] and Kedia *et al.* [30] after fermentation of 1 % and 5 % oat

360   grains in similar *in vitro* studies. *B. adolescentis* has a number of benefits including prevention

361   of the development of diabetes by stimulating insulin secretion. Furthermore *B. adolescentis*

362   has the ability to synthesise and secrete the neuroactive substance gamma-aminobutyric acid

363   (GABA). GABA facilitates communication between bacteria and the human nervous system,

364   enabling release of other neurotransmitters from specific epithelial intestinal cells [53, 54]. Our

365   main findings suggest that oat bran could have beneficial effects on the host through increasing

366   the relative abundance of *B. adolescentis.*

367   In accordance with the literature [55, 56], the compositional analysis of digested oat bran

368   showed limited degradation of β-glucan in the stomach and the small intestine (Table 1). BG

369   (180 mg – 387 kDa) did not introduce changes to Actinobacteria phyla (Figure 2.), which is

370   supported by previous studies on β-glucan [28, 38]. However, BG tended to promote the growth

371   of beneficial *Bacteroides uniformis, Bacteroides ovatus* and *Bacteroides xylaniosolvens*

372   compared with NC (Table 3), which is in line with reports of Wang *et al.* [57]. Additionally,

373   Hughes *et al.* [28] used a similar anaerobic batch culture system for fermentation of 0.5 g β-

374   glucan and also showed an increase in a member of Bacteroidetes, specifically in the

375 *Bacteroides-Prevotella* group following 24 h fermentation. However, the dose of β-glucan used

376 by Hughes *et al.,* was almost three times higher in concentration compared with what was

377 found in 60 g oat bran (containing 0.17 g β-glucan), which would equate to consumption of

378 150 g oat bran.

379 While human digestive enzymes cannot degrade plant cell wall polysaccharides, gut

380 xylanolytic bacteria (e.g. *B. xylaniosolvens, B. uniformis* and *B. ovatus)* can*,* while producing

381 SCFA with potential health-beneficial effects [58-60]. Certain strains of *B. uniformis*

382 downregulate gene and protein expression of pro-inflammatory cytokines, notably iNOS and

383 PPAR-γ, IFN-γ, resulting in reduced inflammatory status [61], suggesting that oat β-glucan

384 could have beneficial effects on human health. More powered studies should be applied to

385 confirm this effect.

386 *In vitro* upper gut digestion (mouth to small intestine) reduced polyphenols in the free -

387 conjugated, bound fractions by 48 % and 26 %, respectively (Table 1). This is in agreement

388 with findings by Dall`Asta *et al.* [62] on phenolic bioaccessibility in durum wheat aleurone

389 fractions where caffeic and sinapic acids appeared as the most bioaccessible (83.3 % and 79.5

390 %) while total ferulic acid and *p*-coumaric acid were less bioaccessible (29.5 % and 40.7%)

391 with *in vitro* digestion. Moreover, several phenolic metabolites, including vanillic acid, 4 - and

392 3 - hydroxyhippuric acids, sulfate-conjugates of benzoic and ferulic acids are derived from the

393 hepatic and microbial metabolism of oat brans [41]. Schar *et al.* [41] found relevant

394 concentrations in urinary excretion of 30 different phenolics, and amounted to a total excretion

395 of 33.7 ± 7.3 μmol, suggesting that a high proportion of oat phenolics are bioavailable with

396 absorption occurring both in the small intestine and then in the large intestine within eight hours

397 of consumption.

398   In the current study, POLY did not change the abundance of the Actinobacteria phyla, which

399   is similar to the research conducted by Gwiazdowska *et al.* [63], which showed that 20 μg/ml

400   polyphenols had an effect on *Bifidobacterium* after 1 h incubation, but no effect at 24 h. The

401   effects of polyphenols on microbial composition, may also be related to the fact that there is

402   no carbohydrate energy available for this fermentation. Therefore, at least part of the change

403   may also be due to utilisation of protein as an energy source. However, in the current study a

404   POLY- induced increase in Enterobacteriaceae phyla and *Hafnia alvei* species was detected

405   [64]. Several studies connected this group with polyphenol-degrading metabolism [65, 66].

406   While Wen Gu *et al.* [67] and Hunter *et al.* [68] observed that Enterobacter strains could

407   transform ferulic acid to vanillin via the non-oxidative decarboxylation, Kuntz *et al.* [69]

408   believed that the *H. alvei* significantly attenuated the expression of adhesion molecules and

409   cytokine secretion (IL-8 and IL-6), resulting in reduced inflammation [69]. The higher relative

410   abundance of Enterobacteriaceae phyla and *H. alvei* species in POLY and digested oat bran

411   treatments might be associated with the metabolic activity of these microbial groups towards

412   plant-derived polyphenols and saccharides [70].

413   Previous oat fermentation studies have not shown significant increases in the growth of

414   Enterobacteriaceae phyla and *H. alvei* species [30], most likely because the fluorescence *in situ*

415   hybridisation (FISH) method used did not target Enterobacteriaceae or allow analysis at the

416   species level. We have detected an increased presence of Proteobacteria phyla (Figure 2) in all

417   3 vessels including NC, which might be due to the artificial conditions of batch culture systems

418   [71, 72]. Facultative anaerobic microbes are indeed less abundant in the human colon [73] but

419   this batch culture model may not be able to achieve a strict anaerobic environment, causing an

420   increase in Proteobacteria phyla [72].

421 Despite *in vitro* enzymatic digestion (mimicking the digestion in the upper intestine prior to

422 colon fermentation), the available carbohydrate content of the oat bran remained high (62 %)

423 (Table 1). Wholegrain oats are a source of starch (60 % of the total dry matter of the oat grain),

424 consisting of 7 % rapidly digestible starch, 22 % slowly digestible and 25 % resistant starch

425 [74] all of which contribute to the available carbohydrates. The findings of Englyst *et al.*

426 suggest that human digestive enzymes do not break down dietary resistant starch [75].

427 Strikingly, 30 % resistant starch type 4 also led to species level alterations in a colon and

428 increases in *B. adolescentis.* [76]. A previous study [40] reported that 1 % (w/v) arabinoxylan,

429 a dietary fibre found in wholegrain including oats, has a bifidogenic effect using a similar 24 h

430 batch culture fermentation *in vitro* model, yet oats generally have about 3.8 − 13.2 % of

431 arabinoxylan [20]. This, in combination with our findings, suggests that interactions among a

432 wide range of dietary polysaccharides may explain the bifidogenic effect of oats [77].

433 The current results indicate that digested oat bran treatments increase SCFA production dose-

434 dependently, with a higher dose inducing higher acetate and propionate production but lower

435 butyrate (Figure 3.). Lactic acid disappeared at 24 h, possibly because it can be further

436 metabolised by bacteria to acetic and propionic acids [78]. Acetate and lactate are widely

437 produced by different bacteria; however, bifidobacteria have a distinct pathway, transforming

438 glucose to acetate and lactate [79]. SCFA can create acidic conditions in the human colon

439 inhibiting the growth of pathogens and reducing harmful enzyme activities in the human

440 intestine, while they also act as an energy source for gut epithelial cells [80, 81]. Furthermore,

441 propionate derived from carbohydrate fermentation is a substrate for gluconeogenesis in

442 humans, and also inhibits the utilisation of acetate for cholesterol synthesis in the colon and

443 liver [82, 83]. Therefore oat bran may have a beneficial impact on human health through SCFA

444 production.

445   In previous studies [84, 85] differences were observed between the activity of pure and of food

446   matrix derived β-glucan on small intestine digestion and lipolysis.Purified β-glucan was

447   observed to have less gel forming capacity and a lesser effect on lipolysis and cholesterol

448   metabolism, than the matrix derived β-glucan. In addition, dietary fibre induced SCFA

449   production has been shown to interact with phenolics [86], where butyric acid increased the

450   ferulic acid absorption in the colon, potentially translating to higher concentrations of ferulic

451   acid in systemic circulation *in vivo*. These findings indicate positive interactions of fibres and

452   polyphenols within the food matrix on cholesterol metabolism and the bioavailability of

453   phenolics, and also highlights the importance of considering the structure and physicochemical

454   properties of foods, and not just the nutrient content.

455   In conclusion, our study has shown that oat bran as a complex food matrix beneficially

456   increases the number of *B. adolescentis* and the amount of SCFA production. In contrast, a

457   matched dose of the isolated bioactive compounds, β-glucan and other polyphenols did not

458   show any effect on the abundance of Actinobacteria. However, by regulating the Bacteroides

459   and Enterobacteriacea families, they may also contribute to further health benefits.

464   CONTRIBUTIONS

465   The authors' contributions were as follows: AK, MYS and GW designed the study; OK

466   analysed the β-glucan molecular weight, AK and PH conducted the *in vitro* fermentation

467   experiments; MW and AK conducted the 16S rRNA gene sequencing, AK and MW conducted

468   the statistical analyses. AK, MW, MYS, GW, GK, GS, SA and JS interpreted the data and AK,

469 MW, MYS, GM and JS drafted the manuscript. AK had primary responsibility for the final

470 content. All authors agreed on the final version of the manuscript.

471 The authors declare no conflict of interest

472 **References**

473 1. Haboubi, N.Y., S. Taylor, and S. Jones, *Coeliac disease and oats: a systematic review.*
474 Postgrad Med J, 2006. 82(972): p. 672-8.

475 2. Thies, F., et al., *Oats and bowel disease: a systematic literature review.* Br J Nutr, 2014.
476 112 Suppl 2: p. S31-43.

477 3. Ye, E.Q., et al., *Greater whole-grain intake is associated with lower risk of type 2*
478 *diabetes, cardiovascular disease, and weight gain.* J Nutr, 2012. 142(7): p. 1304-13.

479 4. Thies, F., et al., *Oats and CVD risk markers: a systematic literature review.* Br J Nutr,
480 2014. 112 Suppl 2: p. S19-30.

481 5. Ruxton, C.H.S. and E. Derbyshire, *A systematic review of the association between*
482 *cardiovascular risk factors and regular consumption of oats.* British Food Journal,
483 2008. 110(11): p. 1119-1132.

484 6. Whitehead, A., et al., *Cholesterol-lowering effects of oat beta-glucan: a meta-analysis*
485 *of randomized controlled trials.* Am J Clin Nutr, 2014. 100(6): p. 1413-21.

486 7. Bao, L., et al., *Effect of oat intake on glycaemic control and insulin sensitivity: a meta-*
487 *analysis of randomised controlled trials.* Br J Nutr, 2014. 112(3): p. 457-66.

488 8. Connolly, M.L., et al., *Hypocholesterolemic and Prebiotic Effects of a Whole-Grain*
489 *Oat-Based Granola Breakfast Cereal in a Cardio-Metabolic "At Risk" Population.*
490 Frontiers in Microbiology, 2016. 7: p. 1675.

491 9. Connolly, M.L., J.A. Lovegrove, and K.M. Tuohy, *In vitro fermentation characteristics*
492 *of whole grain wheat flakes and the effect of toasting on prebiotic potential.* J Med
493 Food, 2012. 15(1): p. 33-43.

494 10. Tosh, S.M., *Review of human studies investigating the post-prandial blood-glucose*
495 *lowering ability of oat and barley food products.* Eur J Clin Nutr, 2013. 67(4): p. 310-
496 7.

497 11. Tosh, S.M. and Y. Chu, *Systematic review of the effect of processing of whole-grain*
498 *oat cereals on glycaemic response.* Br J Nutr, 2015. 114(8): p. 1256-62.

499 12. Tappy, L., E. Gugolz, and P. Wursch, *Effects of breakfast cereals containing various*
500 *amounts of beta-glucan fibers on plasma glucose and insulin responses in NIDDM*
501 *subjects.* Diabetes Care, 1996. 19(8): p. 831-4.

502 13. Drzikova, B., G. Dongowski, and E. Gebhardt, *Dietary fibre-rich oat-based products*
503 *affect serum lipids, microbiota, formation of short-chain fatty acids and steroids in rats.*
504 Br J Nutr, 2005. 94(6): p. 1012-25.

505 14. Zhang, Y., et al., *The effect of oat beta-glucan on in vitro glucose diffusion and glucose*
506   *transport in rat small intestine.* J Sci Food Agric, 2016. 96(2): p. 484-91.

507 15. Ovando-Martínez, M., et al., *Effect of hydrothermal treatment on physicochemical and*
508   *digestibility properties of oat starch.* Food Research International, 2013. 52(1): p. 17-
509   25.

510 16. Rasane, P., et al., *Nutritional advantages of oats and opportunities for its processing as*
511   *value added foods - a review.* J Food Sci Technol, 2015. 52(2): p. 662-75.

512 17. Gangopadhyay, N., et al., *A Review of Extraction and Analysis of Bioactives in Oat and*
513   *Barley and Scope for Use of Novel Food Processing Technologies.* Molecules, 2015.
514   20(6): p. 10884-909.

515 18. Gibson, G.R., et al., *Dietary modulation of the human colonic microbiota: updating the*
516   *concept of prebiotics.* Nutr Res Rev, 2004. 17(2): p. 259-75.

517 19. Oner, O., B. Aslim, and S.B. Aydas, *Mechanisms of cholesterol-lowering effects of*
518   *lactobacilli and bifidobacteria strains as potential probiotics with their bsh gene*
519   *analysis.* J Mol Microbiol Biotechnol, 2014. 24(1): p. 12-8.

520 20. Shewry, P.R., et al., *Phytochemical and fiber components in oat varieties in the*
521   *HEALTHGRAIN Diversity Screen.* J Agric Food Chem, 2008. 56(21): p. 9777-84.

522 21. Chen, C.Y., et al., *Avenanthramides and phenolic acids from oats are bioavailable and*
523   *act synergistically with vitamin C to enhance hamster and human LDL resistance to*
524   *oxidation.* J Nutr, 2004. 134(6): p. 1459-66.

525 22. Liu, S., et al., *Antioxidant Effects of Oats Avenanthramides on Human Serum.*
526   Agricultural Sciences in China, 2011. 10(8): p. 1301-1305.

527 23. Kern, S.M., et al., *Absorption of hydroxycinnamates in humans after high-bran cereal*
528   *consumption.* J Agric Food Chem, 2003. 51(20): p. 6050-5.

529 24. Del Rio, D., et al., *Dietary (poly)phenolics in human health: structures, bioavailability,*
530   *and evidence of protective effects against chronic diseases.* Antioxid Redox Signal,
531   2013. 18(14): p. 1818-92.

532 25. Andreasen, M.F., et al., *Esterase activity able to hydrolyze dietary antioxidant*
533   *hydroxycinnamates is distributed along the intestine of mammals.* J Agric Food Chem,
534   2001. 49(11): p. 5679-84.

535 26. Couteau, D., et al., *Isolation and characterization of human colonic bacteria able to*
536   *hydrolyse chlorogenic acid.* J Appl Microbiol, 2001. 90(6): p. 873-81.

537 27. Raimondi, S., et al., *Role of bifidobacteria in the hydrolysis of chlorogenic acid.*
538   MicrobiologyOpen, 2015. 4(1): p. 41-52.

539 28. Hughes, S.A., et al., *In vitro fermentation of oat and barley derived beta-glucans by*
540   *human faecal microbiota.* FEMS Microbiol Ecol, 2008. 64(3): p. 482-93.

541 29. Connolly, M.L., J.A. Lovegrove, and K.M. Tuohy, *In vitro evaluation of the microbiota*
542   *modulation abilities of different sized whole oat grain flakes.* Anaerobe, 2010. 16(5): p.
543   483-8.

544 30. Kedia, G., et al., *In vitro fermentation of oat bran obtained by debranning with a mixed*
545   *culture of human fecal bacteria.* Curr Microbiol, 2009. 58(4): p. 338-42.

546 31. Connolly, M.L., K.M. Tuohy, and J.A. Lovegrove, *Wholegrain oat-based cereals have*
547   *prebiotic potential and low glycaemic index.* Br J Nutr, 2012. 108(12): p. 2198-206.

548 32. Chappell, A.J., et al., *The effect of in vitro fermentation of oats (Avena sativa) and*
549   *barley (Hordeum vulgare) on the faecal gut microbiota.* Proceedings of the Nutrition
550   Society, 2015. 74(OCE1): p. null-null.

551 33. Zhou, A.L., et al., *Whole grain oats improve insulin sensitivity and plasma cholesterol*
552   *profile and modify gut microbiota composition in C57BL/6J mice.* J Nutr, 2015. 145(2):
553   p. 222-30.

554 34. Berger, K., et al., *Cereal byproducts have prebiotic potential in mice fed a high-fat diet.*
555   J Agric Food Chem, 2014. 62(32): p. 8169-78.

556 35. Immerstrand, T., et al., *Effects of oat bran, processed to different molecular weights of*
557   *beta-glucan, on plasma lipids and caecal formation of SCFA in mice.* Br J Nutr, 2010.
558   104(3): p. 364-73.

559 36. Cloetens, L., et al., *Role of dietary beta-glucans in the prevention of the metabolic*
560   *syndrome.* Nutr Rev, 2012. 70(8): p. 444-58.

561 37. Ibrugger, S., et al., *Extracted oat and barley beta-glucans do not affect cholesterol*
562   *metabolism in young healthy adults.* J Nutr, 2013. 143(10): p. 1579-85.

563 38. Crittenden, R., et al., *In vitro fermentation of cereal dietary fibre carbohydrates by*
564   *probiotic and intestinal bacteria.* Journal of the Science of Food and Agriculture, 2002.
565   82(8): p. 781-789.

566 39. Kim, H.J. and P.J. White, *Optimizing the molecular weight of oat beta-glucan for in*
567   *vitro bile acid binding and fermentation.* J Agric Food Chem, 2011. 59(18): p. 10322-
568   8.

569 40. Hughes, S.A., et al., *In vitro fermentation by human fecal microflora of wheat*
570   *arabinoxylans.* J Agric Food Chem, 2007. 55(11): p. 4589-95.

571 41. Schar, M.Y., et al., *Excretion of Avenanthramides, Phenolic Acids and their Major*
572   *Metabolites Following Intake of Oat Bran.* Mol Nutr Food Res, 2017.

573 42. Mills, D.J., et al., *Dietary glycated protein modulates the colonic microbiota towards*
574   *a more detrimental composition in ulcerative colitis patients and non-ulcerative colitis*
575   *subjects.* J Appl Microbiol, 2008. 105(3): p. 706-14.

576 43. Shewry, P.R., *HEALTHGRAIN Methods: Analysis of Bioactive Components in Small*
577   *Grain Cereals.* Vol. 290pp. 2010.

578 44. Zygmunt, L.C. and S.D. Paisley, *Enzymatic method for determination of (1-->3)(1--*
579   *>4)-beta-D-glucans in grains and cereals: collaborative study.* J AOAC Int, 1993.
580   76(5): p. 1069-82.

581 45. McCance, R.A. and E.M. Glaser, *The Energy Value of Oatmeal and the Digestibility*
582   *and Absorption of its Proteins, Fats and Calcium.* British Journal of Nutrition, 1948.
583   2(03): p. 221-228.

584 46. Stiverson, J., et al., *Prebiotic Oligosaccharides: Comparative Evaluation Using In*
585   *Vitro Cultures of Infants' Fecal Microbiomes.* Appl Environ Microbiol, 2014. 80(23):
586   p. 7388-97.

587 47. Ovreås, L., et al., *Distribution of bacterioplankton in meromictic Lake Saelenvannet,*
588   *as determined by denaturing gradient gel electrophoresis of PCR-amplified gene*
589   *fragments coding for 16S rRNA.* Applied and Environmental Microbiology, 1997.
590   63(9): p. 3367-3373.

591 48. Kristensen, K.H., et al., *Gut Microbiota in Children Hospitalized with Oedematous and*
592 *Non-Oedematous Severe Acute Malnutrition in Uganda.* PLoS Negl Trop Dis, 2016.
593 10(1): p. e0004369.

594 49. Caporaso, J.G., et al., *QIIME allows analysis of high-throughput community*
595 *sequencing data.* Nat Methods, 2010. 7(5): p. 335-6.

596 50. Edgar, R.C., et al., *UCHIME improves sensitivity and speed of chimera detection.*
597 Bioinformatics, 2011. 27(16): p. 2194-200.

598 51. McDonald, D., et al., *An improved Greengenes taxonomy with explicit ranks for*
599 *ecological and evolutionary analyses of bacteria and archaea.* Isme j, 2012. 6(3): p.
600 610-8.

601 52. Kim, O.S., et al., *Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence*
602 *database with phylotypes that represent uncultured species.* Int J Syst Evol Microbiol,
603 2012. 62(Pt 3): p. 716-21.

604 53. Dyachkova, M.S., et al., *Draft Genome Sequences of Bifidobacterium angulatum*
605 *GT102 and Bifidobacterium adolescentis 150: Focusing on the Genes Potentially*
606 *Involved in the Gut-Brain Axis.* Genome Announc, 2015. 3(4).

607 54. Averina, O.V. and V.N. Danilenko, *Human intestinal microbiota: Role in development*
608 *and functioning of the nervous system.* Microbiology, 2017. 86(1): p. 1-18.

609 55. McCleary, B.V., et al., *Determination of insoluble, soluble, and total dietary fiber*
610 *(CODEX definition) by enzymatic-gravimetric method and liquid chromatography:*
611 *collaborative study.* J AOAC Int, 2012. 95(3): p. 824-44.

612 56. Knudsen, K.E.B., B.B. Jensen, and I. Hansen, *Digestion of polysaccharides and other*
613 *major components in the small and large intestine of pigs fed on diets consisting of oat*
614 *fractions rich in β-D-glucan.* British Journal of Nutrition, 2007. 70(2): p. 537-556.

615 57. Wang, Y., et al., *High Molecular Weight Barley β-Glucan Alters Gut Microbiota*
616 *Toward Reduced Cardiovascular Disease Risk.* Frontiers in Microbiology, 2016. 7: p.
617 129.

618 58. Ulsemer, P., et al., *Preliminary safety evaluation of a new Bacteroides xylanisolvens*
619 *isolate.* Appl Environ Microbiol, 2012. 78(2): p. 528-35.

620 59. Hosseini, E., et al., *Propionate as a health-promoting microbial metabolite in the*
621 *human gut.* Nutrition Reviews, 2011. 69(5): p. 245-258.

622 60. Beckmann, L., O. Simon, and W. Vahjen, *Isolation and identification of mixed linked*
623 *beta -glucan degrading bacteria in the intestine of broiler chickens and partial*
624 *characterization of respective 1,3-1,4-beta -glucanase activities.* J Basic Microbiol,
625 2006. 46(3): p. 175-85.

626 61. Fernández-Murga, M.L. and Y. Sanz, *Safety Assessment of Bacteroides uniformis*
627 *CECT 7771 Isolated from Stools of Healthy Breast-Fed Infants.* PLoS ONE, 2016.
628 11(1): p. e0145503.

629 62. Dall'Asta, M., et al., *In Vitro Bioaccessibility of Phenolic Acids from a Commercial*
630 *Aleurone-Enriched Bread Compared to a Whole Grain Bread.* Nutrients, 2016. 8(1): p.
631 42.

632 63. Gwiazdowska, D., et al., *The impact of polyphenols on Bifidobacterium growth.* Acta
633 Biochim Pol, 2015. 62(4): p. 895-901.

634 64. Lindsay, R.F. and F.G. Priest, *Decarboxylation of Substituted Cinnamic Acids by*
635 *Enterobacteria: the Influence on Beer Flavour.* Journal of Applied Bacteriology, 1975.
636 39(2): p. 181-187.

637 65. Grbic-Galic, D., *O-Demethylation, dehydroxylation, ring-reduction and cleavage of*
638 *aromatic substrates by Enterobacteriaceae under anaerobic conditions.* Journal of
639 Applied Bacteriology, 1986. 61(6): p. 491-497.

640 66. Huang, J., et al., *Different Flavonoids Can Shape Unique Gut Microbiota Profile In*
641 *Vitro.* J Food Sci, 2016. 81(9): p. H2273-9.

642 67. Gu, W., et al., *Structural Basis of Enzymatic Activity for the Ferulic Acid*
643 *Decarboxylase (FADase) from Enterobacter sp. Px6-4.* PLoS ONE, 2011. 6(1): p.
644 e16262.

645 68. Hunter, W.J., D.K. Manter, and D. van der Lelie, *Biotransformation of ferulic acid to*
646 *4-vinylguaiacol by Enterobacter soli and E. aerogenes.* Curr Microbiol, 2012. 65(6): p.
647 752-7.

648 69. Kuntz, S., et al., *Inhibition of Low-Grade Inflammation by Anthocyanins after*
649 *Microbial Fermentation in Vitro.* Nutrients, 2016. 8(7): p. 411.

650 70. Wust, P.K., M.A. Horn, and H.L. Drake, *Clostridiaceae and Enterobacteriaceae as*
651 *active fermenters in earthworm gut content.* Isme j, 2011. 5(1): p. 92-106.

652 71. Saulnier, D.M., G.R. Gibson, and S. Kolida, *In vitro effects of selected synbiotics on*
653 *the human faecal microbiota composition.* FEMS Microbiol Ecol, 2008. 66(3): p. 516-
654 27.

655 72. Kemperman, R.A., et al., *Impact of polyphenols from black tea and red wine/grape*
656 *juice on a gut model microbiome.* Food Research International, 2013. 53(2): p. 659-
657 669.

658 73. Eckburg, P.B., et al., *Diversity of the human intestinal microbial flora.* Science, 2005.
659 308.

660 74. Menon, R., et al., *Oats-From Farm to Fork.* Adv Food Nutr Res, 2016. 77: p. 1-55.

661 75. Englyst, H.N. and J.H. Cummings, *Digestion of the polysaccharides of some cereal*
662 *foods in the human small intestine.* Am J Clin Nutr, 1985. 42(5): p. 778-87.

663 76. Martínez, I., et al., *Resistant Starches Types 2 and 4 Have Differential Effects on the*
664 *Composition of the Fecal Microbiota in Human Subjects.* PLoS ONE, 2010. 5(11): p.
665 e15046.

666 77. Grootaert, C., et al., *Microbial metabolism and prebiotic potency of arabinoxylan*
667 *oligosaccharides in the human intestine.* Trends in Food Science & Technology, 2007.
668 18(2): p. 64-71.

669 78. Macfarlane, S. and G.T. Macfarlane, *Regulation of short-chain fatty acid production.*
670 Proc Nutr Soc, 2003. 62(1): p. 67-72.

671 79. Pokusaeva, K., G.F. Fitzgerald, and D. van Sinderen, *Carbohydrate metabolism in*
672 *Bifidobacteria.* Genes & Nutrition, 2011. 6(3): p. 285-306.

673 80. Fooks, L.J. and G.R. Gibson, *Probiotics as modulators of the gut flora.* Br J Nutr, 2002.
674 88 Suppl 1: p. S39-49.

675 81. Russell, W.R., et al., *Colonic bacterial metabolites and human health.* Curr Opin
676 Microbiol, 2013. 16(3): p. 246-54.

677 82. Wolever, T.M., P. Spadafora, and H. Eshuis, *Interaction between colonic acetate and*
678 *propionate in humans.* Am J Clin Nutr, 1991. 53(3): p. 681-7.

679 83. Alvaro, A., et al., *Gene expression analysis of a human enterocyte cell line reveals*
680 *downregulation of cholesterol biosynthesis in response to short-chain fatty acids.*
681 IUBMB Life, 2008. 60(11): p. 757-64.

682 84. Cheickna, D. and Z. Hui, *Oat Beta-Glucan: Its Role in Health Promotion and*
683 *Prevention of Diseases.* Comprehensive Reviews in Food Science and Food Safety,
684 2012. 11(4): p. 355-365.

685 85. Grundy, M.M.L., et al., *The impact of oat structure and β-glucan on in vitro lipid*
686 *digestion.* Journal of Functional Foods, 2017. 38: p. 378-388.

687 86. Ziegler, K., et al., *Butyric acid increases transepithelial transport of ferulic acid*
688 *through upregulation of the monocarboxylate transporters SLC16A1 (MCT1) and*
689 *SLC16A3 (MCT4).* Archives of Biochemistry and Biophysics, 2016. 599: p. 3-12.

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Table 1. Macronutrient, fibre and phenolic content of oat bran before and after *in vitro* digestion

and bioaccessibility (%) through digestion

Components	Before digestion	After digestion	Bioaccessibility %
Amount (g)	60	40	33
Total Carbohydrate (g)	37.8	17.6	53
Available Carbohydrate (g)	32.6	12.5	62
Total dietary fibre(g)	5.2	5.1	2
β-glucan (g)	1.7	1.5	12
Total protein (g)	7	5	29
Fat (g)	3	1.1	63
Free+conjugated polyphenols	6.2	3.2	48
Hydroxybenzoic acids (mg)	1.2	1.1	8
Hydrocinnamic acids (mg)	4.6	2.1	54
Avenanthramides (mg)	0.4	0.1	75
Bound polyphenols	16.4	12.2	26
Hydroxybenzoic acids (mg)	1	0.4	60
Hydrocinnamic acids (mg)	15.4	11.8	23
Avenanthramides (mg)	ND	ND	-
Total polyphenols (mg)	22.6	15.4	32

Bioaccessibility - evaluated following in vitro upper gut digestion procedures, we have used the term bioavailability to indicate the percentage of compound remaining, thus available to the large gut microbiota. This term was first defined by Dall'Asta et al. in Nutrients 2016, 8(1), 42.

Table 2. *In vitro* fermentation treatments

Treatment	β-glucan (mg)	Polyphenol (mg)
OAT1.5	56.67	0.58
OAT4.5	170	1.73
BG (94% of extract)	180	0.1
POLY	ND	1.73
PC	ND	ND
NC	ND	ND

Digested oat bran 1.5g (OAT1.5) and 4.5g (OAT4.5), β-glucan extract (BG), polyphenol mix (POLY), Synergy 1 positive control (PC) and negative control (NC), not detected (ND)

Table 3. Significant changes (FDR p<0.05) in relative abundance (%) of bacterial taxa at 5h, 10h and 24h of *in vitro* batch culture fermentation inoculated with faeces and administered with digested oat bran 1.5g (OAT1.5) and 4.5g (OAT4.5), β -glucan extract (BG), polyphenol mix (POLY), Synergy 1 (PC) and negative control (NC) as substrates. This experiment was conducted three times, with a different faecal donor used for each run.

Phylum	Family	Genus	Species	Time	Treatment[1]						
					OAT 1.5	OAT 4.5	BG	POLY	PC	NC	*p value*
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	*adolescentis*	5h	2.73±1.9	0.82±0.7	0.31±0.1	0.38±0.1	18.48±3.5*	0.39±0.2	0.001
Firmicutes	Ruminococcaceae	*Ruminococcus*	*unassigned*	5h	0.2±0*	0.1±0.1	0.52±0.1	0.67±0.1	0.32±0.1	0.74±0.1	0.03
Firmicutes	Lachnospiraceae	*Coprococcus*	*unassigned*	5h	0.2±0.1*	0.12±0*	0.48±0.2	0.44±0	0.76±0.3	0.54±0	0.02
Proteobacteria	Enterobacteriaceae	*unassigned*	*unassigned*	5h	55.46±8.4*	61.2±9*	23±6	31.9±8.6	11.33*±3.7	25.9±11	0.05
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	*unassigned*	10h	1.17±10.1*	1.1±0.9	0.19±0.1	0.17±0.1	4.12±2.1*	0.27±0.1	0.03
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	*adolescentis*	10h	1.93±0.5*	0.66±0.5	0.19±0.1	0.27±0.1	6.7±2.6*	0.26±0	0.02
Firmicutes	Ruminococcaceae	*unassigned*	*unassigned*	10h	4.26±5	0.05±0*	8.96±2.8	8.68±1.7	9.76±10	8.85±0.8	0.03
Firmicutes	Lachnospiraceae	*unassigned*	*unassigned*	10h	5.14±4	0.08±0*	10.1±2.4	8.14±5	5.11±1.2	10.1±0.2	0.03
Firmicutes	Lachnospiraceae	*Blautia*	*unassigned*	10h	0.35±0.1*	0.02±0*	1.39±0.3	1.21±0.4	1.15±0.8	1.14±0.4	0.03
Proteobacteria	Enterobacteriaceae	*unassigned*	*unassigned*	10h	50.2±12.2	67.2±14*	12.75±6*	36.1±9.2	6.47±3.7*	29.2±1.4	0.02
Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	*adolescentis*	24h	2.03±0.1*	1.91±1.8	0.31±0.1	0.31±0.2	6.87±3.7	0.41±0	0.05

[1] *The difference in relative abundance of taxa between treatments within the same time points was assessed by ANOVA The p-value after correction for multiple tests (69taxa) with the FDR method.*

* indicate significant differences (p<0.05) to NC at the same time point. Values are mean ± SE

Figure Legend

Figure 1. Principal coordinates analysis (PCoA) plots of 16S rRNA gene profiles based on (A) unweighted and (B) weighted phylogenetic Unifrac distance matrices calculated from 10 rarefied OTU tables (9800 reads per samples) unweighted (A) showing clear clustering according to donors (ANOSIM test, $p = 0.01$). The degree of variation between 10 jackknifed replicates of PCoA is displayed with confidence ellipsoids around each sample. Weighted (B) quantitative information used to generate the bacterial relative abundance of donors showed no clear clustering (ANOSIM test, $p > 0.05$) for a whole dataset (24h *in vitro* batch culture fermentation inoculated with 3 healthy donors faeces and administered with digested oat bran 1.5g (OAT1.5) and 4.5g (OAT4.5), β-glucan extract (BG), polyphenol mix (POLY), Synergy 1 (PC) and negative control (NC) as the substrates). Each colour represents a different donor

Figure 2. Changes in bacterial phyla (relative abundances (%)) 0, 5, 10 and 24h *in vitro* batch culture fermentation. This experiment was conducted three times, with a different faecal donor used for each run. The media was supplemented with digested oat bran 1.5g (OAT1.5) and 4.5g (OAT4.5), β-glucan extract (BG), polyphenol mix (POLY), Synergy 1 (PC) and negative control (NC). Samples were analysed at 0, 5, 10, 24 h. Values are mean (%).

Figure 3. Change in concentrations of acetic acid (A), propionic acid (B), butyric acid (C) from negative control (mM) throughout 24h in *vitro* batch culture fermentation. This experiment was conducted three times, with a different faecal donor used for each run. The media supplemented with digested oat bran 1.5g (OAT1.5) and 4.5g (OAT4.5), β-glucan extract (BG), polyphenol mix (POLY), Synergy 1 (PC) as the substrates. Samples were analysed at 0, 5, 10, 24 h

Figure 1.

A
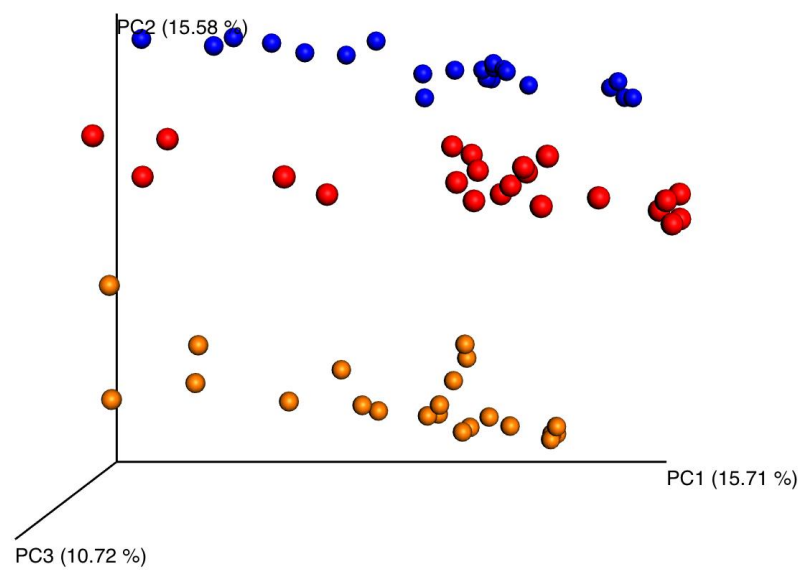
B
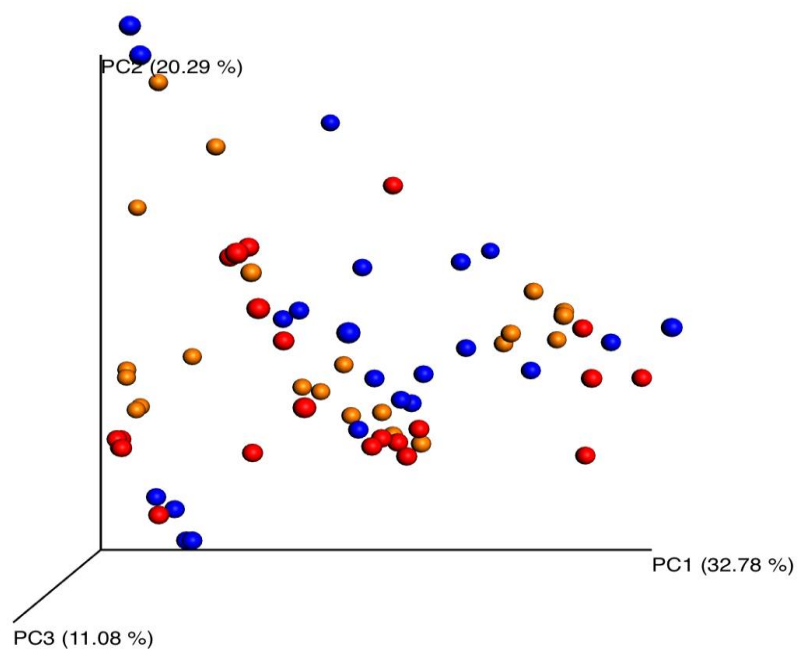
■ Donor 1   ■ Donor 2   ■ Donor 3

Figure 2.
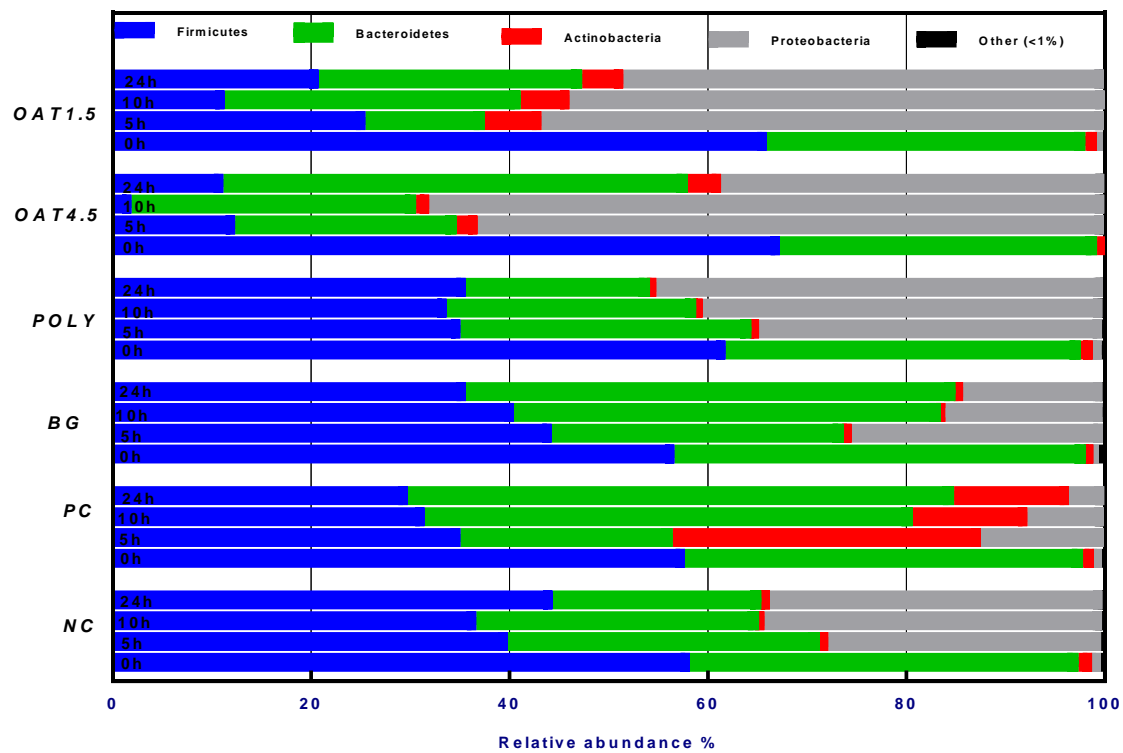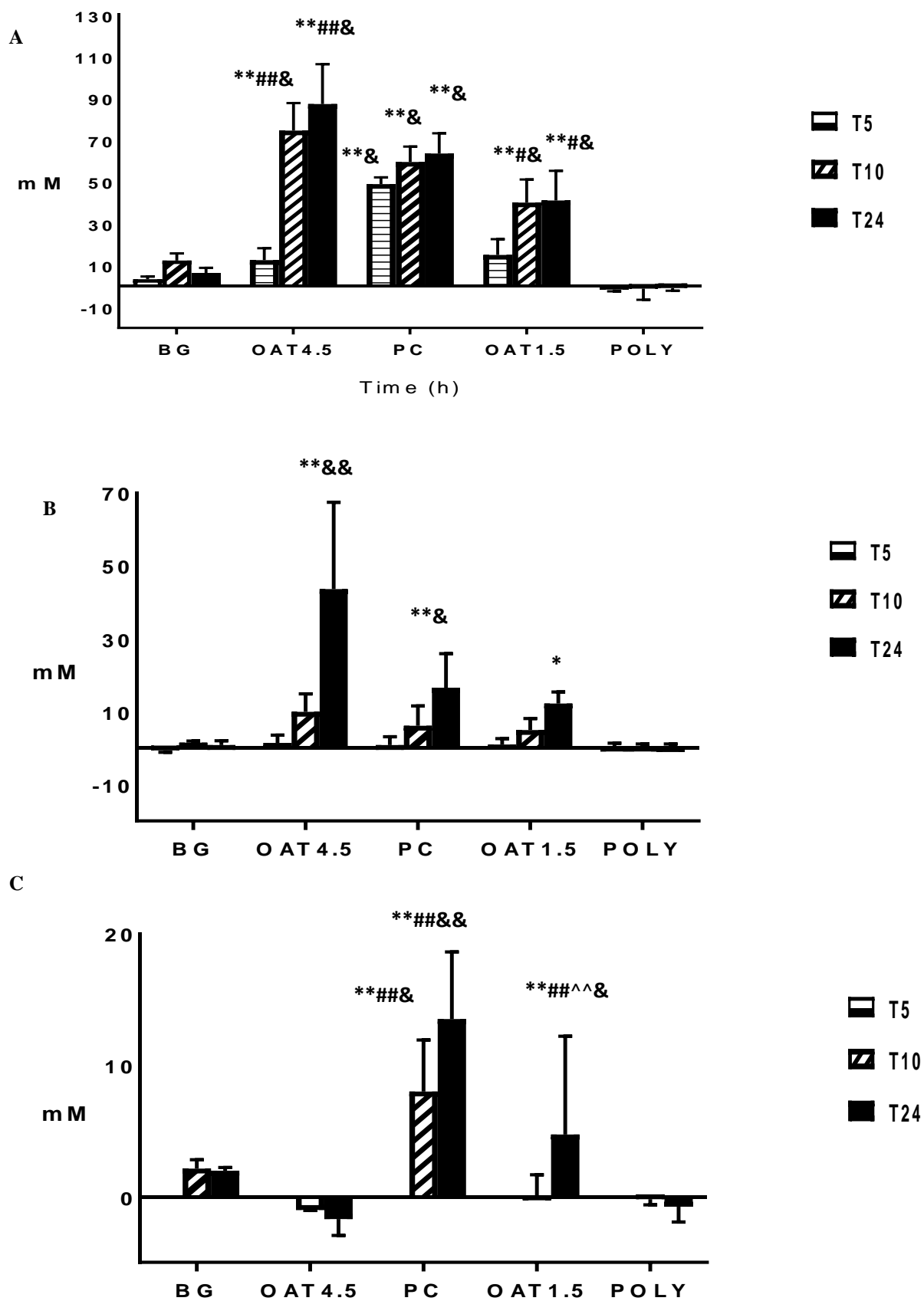
Figure 3.

Values are reported after subtracted of negative control value

Significant from initial value *p < 0.05, **p< 0.01; Significant from 5h value #p < 0.05, ##p< 0.01,

Significant from 10h value ^p < 0.05, ^^p< 0.01; Significant from negative control value at the same time

point &p < 0.05, &&p< 0.01