



In vitro fermentation of gum acacia - impact on the faecal microbiota

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Accepted Version

Alarifi, S., Bell, A. and Walton, G. (2018) In vitro fermentation of gum acacia - impact on the faecal microbiota. *International Journal of Food Sciences and Nutrition*, 69 (6). pp. 696-704. ISSN 1465-3478 doi: <https://doi.org/10.1080/09637486.2017.1404970> Available at <http://centaur.reading.ac.uk/75203/>

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To link to this article DOI: <http://dx.doi.org/10.1080/09637486.2017.1404970>

Publisher: Informa Healthcare

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1 **Title page**

2 ***In vitro* fermentation of gum acacia - impact on the faecal microbiota**

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23 ***In vitro* fermentation of gum acacia - impact on the faecal microbiota**

24 **Abstract**

25 Interest in the consumption of gum acacia (GA) has been associated with beneficial health
26 effects, which may be mediated in part by prebiotic activity. Two doses of GA and
27 fructooligosaccharide (FOS) (1% and 2%) were tested for their efficacy over 48 h in pH- and
28 temperature-controlled anaerobic batch cultures inoculated with human faeces. Samples were
29 taken after 0, 5, 10, 24, and 48 h of fermentation. The selective effects of GA (increases in
30 *Bifidobacterium* sp. and *Lactobacillus* sp.) were similar to those of the known prebiotic FOS.
31 The 1% dose of substrates showed more enhanced selectivity compared to the 2% dose. The
32 fermentation of GA also led to SCFA production, specifically increased acetate after 10, 24,
33 and 48 h of fermentation, propionate after 48 h, and butyrate after 24 and 48 h. Additionally,
34 FOS led to significant increases in the main SCFAs. These results suggest that GA displays
35 potential prebiotic properties.

36 **Key words**

37 Gum acacia (GA); Prebioitcs; Intestinal bacteria; *In vitro* fermentation.

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50 **Introduction**

51 The colon has the most abundant and diverse population of bacteria in the human body and is
52 inhabited by around 1000 different bacterial species, which can reach 10^{14} colony-forming
53 units CFU (Gibson and Roberfroid 1995). The human microbiome is a complex and dynamic
54 system that plays an important role in human health (Eckburg et al. 2005, Kaiko and
55 Stappenbeck 2014). By interacting with consumed material, colonic inhabitants ferment
56 undigested food and secrete end products such as gases and SCFA (Gibson and Roberfroid
57 1995). Moreover, the composition of the bacterial population may shift, resulting in increases
58 in bacteria associated with beneficial effects. Inulin and FOS are known prebiotics that are
59 commercially used worldwide (Mandalari et al. 2008, Steer et al. 2003). A prebiotic is
60 defined as “a selectively fermented ingredient that results in specific changes in the
61 composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon
62 host health” (Gibson et al. 2010).

63 GA is derived from acacia trees of the *Leguminosae* family. It is an arabinogalactan protein
64 complex with an approximate molecular weight of 350-850 K Da (Mahenran.T et al. 2008,,
65 Williams and Phillips 2000.). It is a polysaccharide consisting of branched chains of (1-3)
66 linked β -D galactopyranosyl units. Side chains, 2 to 5 units in length, are attached by (1-6)
67 units to the main chain. Both the main and side chains contain α -L-arabinofuranosyl, α -L-
68 rhamnopyranosyl, β -D-glucuronopyranosyl, and 4-O-methyl- β -D-glucuronopyranosyl units
69 (Bliss et al. 2013). GA is widely used in the pharmaceutical and food industries as an additive
70 , a stabilising, thickening, and an emulsifying agent (Dauqan and Abdullah 2013) (Verbeken
71 et al. 2003).

72 GA is not digestible in the small intestine and is fermented in the large intestine, and has been
73 observed to lead to increases in *Bifidobacterium* spp. (Calame et al. 2008) (Terpend et al.
74 2013) (Marzorati et al. 2015). *Bifidobacterium* spp. have been shown to inhibit the growth of

75 pathogenic bacteria, modulate the immune system, and produce SCFAs, which reduce the pH
76 in the colon, imparting antimicrobial activity against pathogens (Gibson and Roberfroid
77 1995). A variety of GA doses ranging from 5 to 40 g/d have been reported to be effective in
78 increasing *Bifidobacterium* spp. and *Lactobacillus* spp. populations (Calame, Weseler,
79 Viebke, Flynn and Siemensma 2008, Cherbut et al. 2003). These lines of evidence indicate
80 that GA has bifidogenic potential in healthy humans. However optimal effective doses have
81 not been defined. The results from most previous studies cannot be directly compared, as
82 different methodologies, population groups, and types of GA have been studied. In addition,
83 high daily doses of GA could result in the manifestation of adverse effects such as mild
84 diarrhoea and bloating (Babiker et al. 2012).

85

86 *In vitro* batch culture systems are used to simulate the main physiological and
87 microbiological processes in the distal colon and can be combined with metabolic and
88 molecular analyses. Therefore, the aim of the following study was to investigate the impact
89 of two GA doses on the faecal microbiota following 48 h fermentation in pH-controlled
90 batch culture systems, as compared to the known prebiotic FOS.

91

92 **Material and methods**

93 ***Substrates***

94 GA (KLTA-MF-Kerry Ingredients, U.K.) was in spray dried form as a water soluble, free-
95 flowing powder (food-grade). **Table (1)** shows the composition of GA used in this study,
96 dietary fibre were analysed by Campden BRI Laboratories (AOAC method 991.43). The
97 FOS used was Orafti® P95 (Beneo, Belgium) extracted from chicory root.

98 [Table 1 near here].

99

100 ***In vitro Upper Gut Digestion***

101 Upper gut digestion was performed according to the protocol of Mills *et al.*(2008) (Mills et
102 al. 2008). Briefly, 60 g of GA powder was added to 150 ml of distilled water and the solution
103 mixed with 20 mg α -amylase in 6.25 ml CaCl_2 (1 mM) and incubated on a shaker at 37 °C
104 for 30 minutes. This simulated the initial oral digestion. Subsequently, 2.7 g of pepsin in
105 25ml of HCl (0.1M) was used to facilitate gastric breakdown of the sample. The pH was then
106 reduced progressively to 2 by adding 6 M HCl, before incubating on a shaker at 37°C for 2
107 hours. A further 560mg of pancreatin and 3.5g of bile, in 125 ml of NaHCO_3 solution, was
108 added to simulate the effect of the small intestine on the gum sample. The pH was increased
109 to 7 by adding NaOH (6M) and the resulting suspension incubated on a shaker at 37°C for 3
110 hours. Samples were transferred to cellulose dialysis membrane (1 KDa molecular weight),
111 purchased from Cheshire biotech Cheshire, UK, and dialysed against 10Mm of NaCl solution
112 at 5°C to remove low molecular mass digestion products. After 15 hours, the dialysis fluid
113 was changed and dialysis continued for additional 2 h. The sample within the dialysis tubing
114 was freeze dried (5 days) prior to use in batch culture systems. The final sample was weighed
115 and GA loss in the upper gut was subsequently calculated.

116 ***Faecal sample preparation***

117 Faecal samples were obtained from three healthy volunteers (31- 35 years of age), who had
118 not been consuming antibiotics for at least 6 months before the study and had no history of
119 gastrointestinal disease. Volunteers were not consumers of probiotic or prebiotic
120 supplements. Samples were prepared on the day of the experiment and within 1 hour of
121 production and were diluted to 1:10, w/v in anaerobic phosphate buffer (0.1 M; pH7.4).

122 Samples were homogenised in a stomacher for 2 min, the resulting slurry was inoculated into
123 batch culture fermenters.

124 ***Batch cultures***

125 Three independent batch culture experiments were carried out using faeces from a different
126 donor each time. Vessels were autoclaved and then aseptically filled with 135 ml of basal
127 medium (peptone water (2 g/l), yeast extract (2 g/l), NaCl (0.1 g/l), K₂HPO₄ (0.04 g/l),
128 KH₂PO₄ (0.04 g/l), NaHCO₃ (2 g/l), MgSO₄•7H₂O (0.01 g/l), CaCl₂•6H₂O (0.01 g/l), tween
129 80 (2 ml/l), hemin (50 mg/l), vitamin K1 (10 ml/l), L-cysteine (0.5 g/l), bile salts (0.5 g/l), 0.5
130 ml/l of 10% cysteine –HCl, resazurin (1 mg/l)). Vessels were left overnight with nitrogen
131 pumping (15mL/min) through the vessel to provide an anaerobic environment. Before
132 addition of faecal slurry, temperature of basal medium was set at 37 °C and pH was
133 maintained at 6.7-6.9 using a pH meters (Electrolab pH controller, Tewksbury, UK) by the
134 addition of 0.5 M HCl or 0.5M NaOH. The vessels were stirred using magnetic stirrers. 1.5g,
135 3g (1% w/v) of FOS and 0.6 g and 0.3 g (1:10 w/v) of pre-digested GA (taking to account the
136 loss of substrate in the upper gut from the predigestion) were added to the vessel 1% and
137 doubling the dose 2% just prior to the addition of 15 ml of faecal slurry (10%w/w). The
138 vessels were left for 48h, with samples taken at 0, 5, 10, 24 and 48h. Samples were
139 centrifuged in preparation for GC analysis, or prepared for microbial enumeration by FISH.

140 ***Florescence in situ hybridisation (FISH) analysis***

141 To asses differences in bacterial population, samples hybridised as described by Daims et al.,
142 1999 (Daims et al. 1999). A sample of 375-µl obtained from each vessel was fixed for four
143 hours 4°C in 1125 µ L (4% w/v) paraformaldehyde. Fixed samples were then centrifuged at
144 11,337g (Eppendorf centrifuge minispin, Eppendorf, UK) at room temperature for 5 minutes.
145 The supernatant removed and discarded. The pellet was resuspended in 1 ml of cold 1×PBS

146 by aspirating carefully using a pipette. This step was conducted twice. The washed cells were
147 suspended in 150 μ L of cold 1 \times PBS, then 150 μ L of ethanol (99%) was added and the
148 samples were stored at -20°C.

149 The oligonucleotide probes used were commercially synthesised and labeled with the
150 fluorescent dye Cy3 (Sigma Aldrich Co. Ltd. UK). These were: Bif164 for *Bifidobacterium*
151 *spp.* (BIF), Lab158 for *Lactobacillus/enterococcus* (LAB), Ato291 for *Atopobium cluster*
152 (*Atopobium, Coriobacterium, Collinsella spp.*) (ATO), Chis 150 for *Clostridium histolyticum*
153 group (CHIS) Erec 482 for *Eubacterium rectale – Clostridium coccoides* group (EREC),
154 Bac 303 for *Bacteroides–Prevotella* group (BAC). EUB 338 mixture consisting of EUB338,
155 EUB338II and EUB338III for total bacteria (Total) see **Table (2)**. For the hybridisation 20 μ
156 L of diluted sample was pipetted onto a teflon poly-L-lysine-coated six-well slide (Tekdon
157 Inc., Myakka City, FL). The samples were dried onto the slides at 46-50°C for 15 minutes
158 and after that dehydrated in an alcohol series 50, 80, and 96%. The ethanol was allowed to
159 evaporate from the slides before hybridisation buffer was added. A probe/hybridization buffer
160 mixture (5 μ L of a 50 ng/ μ L stock of probe plus 50 μ L of hybridization buffer). To
161 permeabilise the cells for use with probes Bif164 and Lab158, samples were treated with 20 μ
162 L of lysozyme at room temperature for 15 min before being washed briefly for 2–3 seconds
163 in water and then dehydrated in the ethanol series. Then slides were placed in hybridisation
164 oven for 4 hours (ISO 20 oven, Grant Boekel). For the washing step, slides were placed in 50
165 ml of washing buffer (0.9 M NaCl, 0.02 M Tris/HCl (pH 8.0), 0.005 M
166 ethylenediaminetetraacetic acid (EDTA) solution (pH 8.0, **Table (2)**), warmed at the
167 appropriate temperature for each probe and 20 μ L of 4,6-diamidino-2-phenylindole di
168 hydrochloride (DAPI) was added to the washing buffer for 15 min. They were then briefly
169 washed (2–3 s) in ice-cold water and dried under a stream of compressed air. Five microliters
170 of ProLong Gold antifade reagent (Invitrogen) was added to each well and a coverslip

171 applied. Slides were stored in the dark at 4 °C until cells were counted under a Nikon E400
172 Eclipse microscope. DAPI stained cells were examined under UV light, and a DM510 light
173 filter was used to count specific bacteria hybridised with the probes. For each slide, 15
174 random different fields of view were counted.

175 [Table 2 near here].

176 *Preparation sample for short chain fatty acids*

177 Samples were extracted and derivatised as previously described (Richardson et al. 1989).
178 Samples were defrosted and 1ml of each sample or standard solution was transferred into a
179 labeled 100 mm × 16mm glass tube with the internal standard of 50µl of 2- ethyl butyric acid
180 (0.1M). 0.5 ml concentrated HCl and 2 ml of diethyl ether was added to each glass tube and
181 samples vortexed for 1 min. samples were centrifuged at 2000 g for 10 min (SANYO MSE
182 Mistral 3000i; Sanyo Gallenkamp PLC, Middlesex, UK). The diethyl ether (the upper layer)
183 was transferred in a new glass tube. A second extraction was conducted by adding 1 ml of
184 diethyl ether to the sample followed by vortex and centrifugation. 400 µl of pooled ether
185 extract and 50 µl N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) was
186 added in a GC screw-cap vial. Samples were heated at 80°C for 20 minutes and then left at
187 room temperature for 48 hours to allow lactic acid in the samples to completely derivatise.

188 A 5890 SERIES II Gas Chromatograph (Hewlett Packard, UK) using an Rtx-1 10m×0.18mm
189 column with a 0.20µm coating (Crossbond 100% dimethyl polysiloxane; Restek,
190 Buckinghamshire, UK) was used for analysis of SCFA. Temperatures of injector and detector
191 were 275°C, with the column programmed from 63°C for 3 minutes to 190°C at 10°C min⁻¹
192 and held at 190°C for 3 minutes. Helium was the carrier gas (flow rate 1.2 ml min⁻¹; head
193 pressure 90 MPa). A split ratio of 100:1 was used. The standard solution contained (mM):
194 sodium formate, 10; acetic acid, 30; propionic acid, 20; isobutyric acid, 5; n-butyric acid, 20;

195 iso-valeric acid, 5; n-valeric acid, 5; sodium lactate, 10; sodium succinate, 20. The sample
196 was injected onto the column, which was maintained at 140 °C for the first 5 minutes,
197 temperature of the column was increased over 5 minutes to 240 °C. To maintain appropriate
198 calibration after injection of every 20 samples an external standard solution, with known
199 concentrations of SCFAs was injected. Peaks and response factors within samples were
200 calibrated and calculated using ChemStation B.03.01 software (Agilent Technologies,
201 Cheshire, UK).

202 *Statistical analysis*

203 GA and FOS both doses were tested in batch cultures inoculated with faecal samples
204 collected from three individual donors in three separate experiments. The log₁₀ numbers of
205 specific bacteria were expressed as mean values and standard deviation. Statistical tests were
206 performed using SPSS, (SPSS Statistical Software, Inc., Chicago, IL, USA), version 18.0 A
207 repeated measures one-way analysis of variance ANOVA to test the effect of time with the
208 factor subjects, with five levels (0 h, 5 h, 10 h, 24 h and 48 h) and to assess the significant
209 differences between the two subjects in the same time points. Significant differences between
210 times point were represented by “*” p < 0.05, “**” p < 0.01 and “***” p < 0.001.

211 **Results**

212 *Effects of different doses of GA and FOS on human faecal bacteria*

213 To assess the impact of GA on the intestinal microbiota composition, pH-controlled,
214 anaerobic, faecal batch cultures were conducted using FOS as a positive prebiotic control.
215 Samples were taken after 0, 5, 10, 24, and 48 h of fermentation. Bacteria were enumerated by
216 FISH.

217 The fermentation of GA at the 1% dose led to increased numbers of *Bifidobacterium* spp.
218 after 5, 10, and 24 h of fermentation and of *Lactobacillus* spp. after 5 and 24 h compared with
219 the levels at 0 h, as shown in **Tables (3a, 3b)**. However, a small but statistically significant
220 drop in *Bifidobacterium* spp. compared with the negative control was seen after 24 h
221 (7.53 ± 0.10 , 7.22 ± 0.37 GA 1%) (7.46 ± 0.21 , 7.64 ± 0.29 control).

222 Fermentation of 2% GA a led to a significant increase in *Bifidobacterium* spp. after 5 and 10
223 h compared with the baseline levels (time 0 h). Total bacteria increased after the fermentation
224 of 2% GA for 48 h compared with the baseline levels.

225 The 1% dose of FOS led to a significant increase in *Bifidobacterium* spp. after 5 h compared
226 with baseline (7.39 ± 0.21 , 7.83 ± 0.06). *Lactobacillus* spp. increased following fermentation
227 of FOS at a dose of 1% at 5, 10, and 24 h. The 2% FOS dose significantly increased
228 *Bifidobacterium* spp. numbers after 24 h compared with the negative control (8.12 ± 0.16 ,
229 7.78 ± 0.17) and after 5 h compared with baseline levels ($p = 0.03$), whereas an increase in
230 *Lactobacillus* spp. was observed after 5 h (7.75 ± 0.14 , 7.29 ± 0.07) and 24 h (7.51 ± 0.10 ,
231 7.29 ± 0.04) ($p = 0.01$ and 0.02 , respectively) compared with the negative control.

232 The number of bacteria in the *C. histolyticum* group decreased after fermentation of 1% FOS
233 and 1% GA for 5 and 10 h, respectively, compared with the baseline levels. Additionally, GA
234 and FOS enhanced the growth of *Atopobium* spp. after 5 h of fermentation compared with the
235 baseline levels. The *C. coccoides-Eubacterium rectale* group did not change with any of the
236 tested substrates. Additionally, *Atopobium* also increased after 5 h of fermentation of 2%
237 FOS.

238 In the current study both substrates led to increases in *Bacteroides* spp.; these changes
239 occurred after 5 h of fermentation of GA and FOS at the 1% dose ($p = 0.01$ and $p = 0.02$,
240 respectively) and with the 2% dose of GA and FOS compared with the baseline levels ($p =$

241 0.02 and $p = 0.00$, respectively). Moreover, 1% FOS increased *Bacteroides* spp. after 24 h (p
242 $= 0.01$). On the other hand, the prebiotic FOS at the 1% dose enhanced the growth of total
243 bacteria, achieving statistical significance after 10 h and 24 h. Additionally, total bacterial
244 growth was enhanced with 2% FOS after 5 h compared with the negative control.

245 [Tables 3a & 3b near here].

246 *Impact of GA and FOS on SCFA production*

247 **Table (4)** shows that within 1% GA, the concentration of acetate significantly increased after
248 24 h of fermentation ($p \leq 0.05$) and exhibited an increasing trend after 5, 10, and 48 h ($p =$
249 0.06, 0.06, and 0.08, respectively). Acetate levels were elevated at all time points after FOS
250 fermentation compared with the levels at 0 h ($p = 0.00, 0.00, 0.04, \text{ and } 0.00$, respectively),
251 and 1% FOS led to an increase after 5 h compared with the negative control ($p = 0.01$).
252 Butyrate production was significantly enhanced following the fermentation of FOS between 0
253 and 24 h ($p = 0.02$) and following GA 1% fermentation after 24 and 48 h ($p = 0.03, 0.02$);
254 this was also the case at 24 h when compared with the negative control ($p = 0.04$). Compared
255 with the levels at 0 h, propionate production increased following the fermentation of GA1%
256 for 48 h ($p = 0.03$) and the fermentation of 1% FOS for 24 h ($p = 0.01$).

257 Additionally, with 2% FOS, acetate increased after 24 and 48 h ($p = 0.00$ and 0.01,
258 respectively), and with 2% GA, acetate increased after 48 h compared with the baseline levels
259 ($p = 0.02$). Butyrate production increased following the fermentation of 2% GA for 10 and 48
260 h compared with the levels at 0 h ($p = 0.01$ and 0.03, respectively). In addition, propionate
261 increased after 10 h of fermentation of 2% FOS compared with the negative control ($p =$
262 0.04) and the baseline values ($p = 0.01$).

263 [Table 4 near here].

264 **Discussion**

265 Prior studies have noted the importance of the effect of GA on improving human health
266 (Terpend, Possemiers, Daguet and Marzorati 2013). GA is not digestible in the upper
267 gastrointestinal tract, therefore it can reach the large intestine where it is fermented by
268 intestinal bacteria (Adiotomre et al. 1990),(Annison et al. 1995), (Bourquin et al. 1996).
269 Therefore this study aimed to determine the effects two doses of GA on human intestinal
270 bacteria and to assess prebiotic potential as compared to prebiotic FOS. As such pH-
271 controlled batch culture fermentation systems were used to evaluate the selectivity of GA
272 when fermented with healthy human gut microbiota compared with FOS at two different
273 doses.

274 Several studies have shown that GA can undergo a slow fermentation, specifically a more
275 distal fermentation, whereas existing prebiotics typically undergo proximal fermentation
276 (Macfarlane et al. 1992), as proteolytic fermentation develops in the distal colon; therefore,
277 this substrate may be able to be saccharolytically fermented in this part of colon. In this
278 present study GA fermentation shows selectivity in bifidobacteria and *Lactobacillus* spp at
279 time 10 h and continues to 24 h which could indicate slower fermentaion and is inline with
280 others work (Cherbut, Michell, Raison, Kravtchenko and Severine 2003). However, it is
281 worth noting that the bifidogenic effect of the 1% was not maintained at 48 hours in current
282 study.

283 In the present study, GA significantly enhanced the growth of *Bifidobacterium* spp..
284 *Bifidobacterium* is considered an important group related to human health, having a
285 favourable impact in the large intestine (Gibson and Wang 1994),(Russell et al. 2011). These
286 results are consistent with those of Calame *et al* (2008) in which the consumption of 10 g of
287 GA daily by healthy adults had a beneficial effect on the gut microbial composition, and
288 increases in bifidobacteria (Calame, Weseler, Viebke, Flynn and Siemensma 2008).

289 Furthermore, the 1% dose also led to increases in lactobacilli. Lactobacilli has long been
290 considered a positive microbial group; as such, stimulation of this genera offers potential
291 benefits to the host (Ouweland et al. 2009). The 2% dose did not lead to the same lactobacilli
292 impact. The use of a higher dosage could have impacted on selectivity through a cross
293 feeding network (Guiot 1982).

294 Furthermore, after 10 h, the numbers of bacteria in the *C. histolyticum* group decreased
295 following the fermentation of 1% GA, which also agreed with results of *in vivo* studies
296 (Wyatt et al. 1986), (Calame, Weseler, Viebke, Flynn and Siemensma 2008), (Cherbut,
297 Michell, Raison, Kravtchenko and Severine 2003). This group of bacteria has sometimes
298 been associated with inflammation and large bowel disease (Hughes 2008), (Gibson and
299 Roberfroid 2008). It is thought that increased numbers of beneficial bacteria could lower the
300 pH within the colon, therefore making the environment unfavourable for pathogenic groups;
301 the results of the present study indicate that the fermentation of GA selectively increased the
302 number of beneficial bacteria and reduced the number of harmful bacteria. This result further
303 suggests the lower dose to offer improved selective potential.

304 In the current study GA fermentation resulted in a similar bacterial profile to FOS. Several
305 *in vivo* and *in vitro* studies have confirmed that FOS can regulate the gut through the selective
306 stimulation of the gut microbiota (Hidalgo et al. 2012), (Palframan et al. 2002), (Cueva et al.
307 2013), (Tuohy et al. 2001), therefore, GA could have potentially prebiotic properties. GA can
308 be incorporated into baked goods, therefore could provide an alternative prebiotic source for
309 inclusion in the diet.

310 Moreover, an increase in *Bacteroides* spp. was observed with in GA and FOS, this group is
311 associated with a range of colonic activities (Pool-Zobel et al. 2002), (Nakamura et al. 2002).
312 *Bacteroides* spp. constitute a large proportion of the microbial population in the healthy adult
313 gastrointestinal tract (Walton 2006). In previous *in vivo* and *in vitro* studies on GA

314 fermentation, increased propionate levels were associated with *Bacteroides* spp. and
315 *Prevotella* spp. which has relevance to the improving in lipid metabolism (Frost et al.
316 2014),(Tulung et al. 1987).

317 The results show that both doses of GA were selectively fermented. The higher dose was
318 arguably less selective as the impact on lactobacilli and *C. histolyticum* groups were no
319 longer apparent. In fact Calame et al (2008) noted that an increase in the concentration of
320 substrates results in less selectivity (Calame, Weseler, Viebke, Flynn and Siemensma 2008).
321 This might be explained by competition for substrate, at higher dose other bacterial strains
322 have easier access to the substrate and subsequently, become less selectively than the lower
323 dose.

324 GA fermentation induced modulation of the colonic microbiota, with increased levels of
325 acetate, propionate, and butyrate. Acetate is produced mainly through the fructose-6-
326 phosphate phosphoketolase pathway by bifidobacteria, and the increased production of this
327 acid could be related to increased numbers of this group (Miller and Wolin 1996). Acetate
328 plays an important role in controlling inflammation and resisting invasion by pathogens
329 (Rigottier-Gois et al. 2003). Propionate may be produced by Cluster IX Clostridia groups;
330 indeed an increase in this bacterial groups was observed during GA fermentation Hosseini *et*
331 *al* (2011). Propionate may have a direct role in central appetite regulation; increasing satiety
332 and reducing food intake by the host (Russell et al. 2013), (Brown et al. 2003), (Chambers et
333 al. 2015), (Cherbut et al. 1998). Whilst butyrate is often used as an energy source by
334 epithelial cells; as such . these SCFA increases could offer benefits to the host.

335 The pH-controlled stirred batch culture systems enabled rapid analysis of the effects of
336 GA on the faecal microbiota. In the absence of absorption, colonic secretions, and epithelial
337 interactions, the system has limitations. However, processes such as SCFA production can

338 still be monitored away from the impact of additionally dietary factors. Thus, batch culture
339 systems provide an alternative way of assessing how bacteria ferment a substrate and the end
340 products they produce (Ohashi et al. 2012).

341 The comparisons of the substrates in the pH-controlled batch cultures indicated that GA
342 has selective abilities that are at least similar to those of the known prebiotic FOS, as
343 indicated by the bacteriology results and increased concentrations of acetate, butyrate, and
344 propionate. These results could be relevant to improving host health by increasing the levels
345 of the bifidobacteria group, especially in individuals with lower numbers such as elderly
346 population. Tuohy *et al* (2001) reported that prebiotics can alter the gut microbiota in those
347 with initially low numbers of bifidobacteria (Tuohy, Kolida, Lustenberger and Gibson 2001).
348 This may be particularly relevant in elderly people. Elderly are experiencing negative
349 changes in their gut microbiota.

350 **Conclusion**

351 The aim of the current study was to use *in vitro* batch cultures to assess the effects of GA on
352 the microbiota compared to FOS. Here, we showed that GA modulated the gut microbiota
353 similarly to FOS, furthermore, the 1% dose showed additional selective potential. As such
354 GA holds the potential to be used as a novel prebiotic source.

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357 ***Acknowledgements:***

358 We thank Saudi Arabian Ministry of Education, Kingdom of Saudi Arabia for their
359 sponsorship of this study.

360 *Words count:*

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References

Adiotomre J, Eastwood MA, Edwards CA, Brydon WG. 1990. Dietary fiber-In vitro methods that anticipate nutrition and metabolic- activity in humans Am J Clin Nutr. Jul;52:128-134.

Annison G, Trimble RP, Topping DL. 1995. Feeding Australian Acacia gums and gum arabic leads to non-starch polysaccharide accumulation in the cecum of rats. J Nutr. Feb;125:283-292. Epub 1995/02/01.

Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. 2012. Effects of gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. Nutrition journal. 2012;11:111.

Bliss DZ, Weimer PJ, Jung H-JG, Savik K. 2013. In Vitro Degradation and Fermentation of Three Dietary Fiber Sources by Human Colonic Bacteria. J Agric Food Chem.

Bourquin LD, Titgemeyer EC, Fahey Jr GC. 1996. Fermentation of various dietary fiber sources by human fecal bacteria. Nutrition Research. 7//;16:1119-1131.

395

396 Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI,
397 Wigglesworth MJ, Kinghorn I, Fraser NJ, et al. 2003. The Orphan G protein-coupled
398 receptors GPR41 and GPR43 are activated by propionate and other short chain
399 carboxylic acids. *The Journal of biological chemistry*. Mar 28;278:11312-11319.
400 Epub 2002/12/24.

401

402 Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD. 2008. Gum arabic establishes
403 prebiotic functionality in healthy human volunteers in a dose-dependent manner. *Br J*
404 *Nutr*. Dec;100:1269-1275. Epub 2008/05/10.

405

406 Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK,
407 MacDougall K, Preston T, Tedford C, Finlayson GS, et al. 2015. Effects of targeted
408 delivery of propionate to the human colon on appetite regulation, body weight
409 maintenance and adiposity in overweight adults. *Gut*. Nov;64:1744-1754.

410

411 Cherbut C, Ferrier L, Roze C, Anini Y, Blottiere H, Lecannu G, Galmiche JP. 1998. Short-
412 chain fatty acids modify colonic motility through nerves and polypeptide YY release
413 in the rat. *Am J Physiol*. Dec;275:G1415-1422. Epub 1998/12/09.

414

415 Cherbut C, Michel C, Raison V, Kravtchenko T, Severine M. 2003. Acacia Gum is a
416 Bifidogenic Dietary Fibre with High Digestive Tolerance in Healthy Humans.
417 *Microbial Ecology in Health and Disease* 2003; 15: 43/50.

418

419 Cueva C, Sanchez-Patan F, Monagas M, Walton GE, Gibson GR, Martin-Alvarez PJ,
420 Bartolome B, Victoria Moreno-Arribas M. 2013. In vitro fermentation of grape seed
421 flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and
422 phenolic metabolites. *Fems Microbiology Ecology*. Mar;83:792-805.

423

424 Daims H, Brühl A, Amann R, Schleifer K-H, Wagner M. 1999. The Domain-specific Probe
425 EUB338 is Insufficient for the Detection of all Bacteria: Development and Evaluation
426 of a more Comprehensive Probe Set. *Syst Appl Microbiol*. 9//;22:434-444.

427

428 Dauqan E, Abdullah A. 2013. Utilization of gum arabic for industries and human health.
429 *American Journal of Applied Sciences*, 2013 Received 2012-11-07, Revised 2013-
430 06-04; Accepted 2013-09-03;10 (10): :1270-1279.

431

432 Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson
433 KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. *Science*.
434 Jun;308:1635-1638.

435

436 Franks AH, Harmsen HJM, Raangs GC, Jansen GJ, Schut F, Welling GW. 1998. Variations
437 of bacterial populations in human feces measured by fluorescent in situ hybridization

438 with group-specific 16S rRNA-Targeted oligonucleotide probes. *Appl Environ*
439 *Microbiol. Sep*;64:3336-3345.
440

441 Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J,
442 Ghourab S, Hankir M, Zhang S, et al. 2014. The short-chain fatty acid acetate reduces
443 appetite via a central homeostatic mechanism. *Nature communications*.5:3611. Epub
444 2014/05/02.
445

446 Gibson GR, Roberfroid MB. 1995. Dietary Modulation of the Human Colonie
447 Microbiota:Introducing the Concept of Prebiotics. *J Nutr*.125:1401-1412.
448

449 Gibson GR, Roberfroid MB. 2008. Handbook of prebiotics. Times Cited: 0.
450

451 Gibson GR, Scott KP, Rastall RA, Tuohy KM, Hotchkiss A, Dubert-Ferrandon A, Gareau M,
452 Murphy EF, Saulnier D, Loh G, et al. 2010. Dietary prebiotics: current status and new
453 definition. *Food Science and Technology Bulletin: Functional Foods*. 2010;7:1-19.
454
455

456 Gibson GR, Wang X. 1994. Regulatory effects of bifidobacteria on the growth of other
457 colonic bacteria. *J Appl Bacteriol. Oct*;77:412-420. Epub 1994/10/01.
458

459 Guiot HF. 1982. Role of competition for substrate in bacterial antagonism in the gut. *Infect*
460 *Immun. Dec*;38:887-892. Epub 1982/12/01.
461

462 Harmsen HJM, Elfferich P, Schut F, Welling GW. 1999. A 16S rRNA-targeted probe for
463 detection of lactobacilli and enterococci in faecal samples by fluorescent in situ
464 hybridization. *Microb Ecol Health Dis. March*;11:3-12.
465

466 Harmsen HJM, Wildeboer-Veloo ACM, Grijpstra J, Knol J, Degener JE, Welling GW. 2000.
467 Development of 16S rRNA-based probes for the Coriobacterium group and the
468 Atopobium cluster and their application for enumeration of Coriobacteriaceae in
469 human feces from volunteers of different age groups. *Appl Environ Microbiol.*
470 *Oct*;66:4523-4527.
471

472 Hidalgo M, Oruna-Concha MJ, Kolida S, Walton GE, Kallithraka S, Spencer JPE, Gibson
473 GR, de Pascual-Teresa S. 2012. Metabolism of Anthocyanins by Human Gut
474 Microflora and Their Influence on Gut Bacterial Growth. *J Agric Food Chem.*
475 2012/04/18;60:3882-3890.
476

477 Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. 2011. Propionate as a health-
478 promoting microbial metabolite in the human gut. *Nutr Rev. May*;69:245-258. Epub
479 2011/04/28.
480

- 481 Hughes S. 2008. The effect of cereal cell wall fractions on fermentation by the human gut
482 microbiota, with a focus upon health promoting effects The University of Reading.
483
- 484 Kaiko GE, Stappenbeck TS. 2014. Host-microbe interactions shaping the gastrointestinal
485 environment. *Trends in Immunology*. Nov;35:538-548.
486
- 487 Langendijk PS, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MHF, Welling
488 GW. 1995. Quantitative fluorescence in-situ hybridization of bifidobacterium spp
489 with genus-specific 16s ribosomal-rna-targeted probes and its application in fecal
490 samples. *Appl Environ Microbiol*. Aug;61:3069-3075.
491
- 492 Macfarlane GT, Gibson GR, and , Cummings JH. 1992. Comparison of fermentation
493 reactions in different regions of the human colon. *J Appl Bacteriol*.72:57-64.
494
- 495 Mahenran.T, Williams.P.A, Philips.G.O, AL-Assaf.S. 2008,. New Insights into the Structural
496 Characteristics of the Arabinogalactan-Protein (AGP) Fraction of Gum Arabic. *J*
497 *Agric Food Chem* 56,:9269–9276.
498
- 499 Mandalari G, Nueno-Palop C, Bisignano G, Wickham MSJ, Narbad A. 2008. Potential
500 prebiotic properties of almond (*Amygdalus communis* L.) seeds. *Appl Environ*
501 *Microbiol*. Jul;74:4264-4270.
502
- 503 Manz W, Amann R, Ludwig W, Vancanneyt M, Schleifer KH. (1996). Application of a suite
504 of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the
505 phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology*
506 ,.142:1097-1106.
507
- 508 Marzorati M, Qin B, Hildebrand F, Klosterbuer A, Roughead Z, Roessle C, Rochat F, Raes J,
509 Possemiers S. 2015. Addition of acacia gum to a FOS/inulin blend improves its
510 fermentation profile in the Simulator of the Human Intestinal Microbial Ecosystem
511 (SHIME®). *J Funct Foods*. 6//;16:211-222.
512
- 513 Miller TL, Wolin MJ. 1996. Pathways of acetate, propionate, and butyrate formation by the
514 human fecal microbial flora. *Appl Environ Microbiol*. May;62:1589-1592.
515
- 516 Mills DJS, Tuohy KM, Booth J, Buck M, Crabbe MJC, Gibson GR, Ames JM. 2008. Dietary
517 glycosylated protein modulates the colonic microbiota towards a more detrimental
518 composition in ulcerative colitis patients and non-ulcerative colitis subjects.
519
- 520 Nakamura J, Kubota Y, Miyaoka M, Saitoh T, Mizuno F, Benno Y. 2002. Comparison of
521 four microbial enzymes in *Clostridia* and *Bacteroides* isolated from human feces.
522 *Microbiology and immunology*.46:487-490. Epub 2002/09/12.
523

- 524 Ohashi Y, Harada K, Tokunaga M, Ishihara N, Okubo T, Ogasawara Y, Juneja LR, Fujisawa
525 T. 2012. Faecal fermentation of partially hydrolyzed guar gum. *J Funct Foods*.
526 Jan;4:398-402.
527
- 528 Ouwehand AC, Tiihonen K, Saarinen M, Putaala H, Rautonen N. 2009. Influence of a
529 combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly:
530 intestinal and immune parameters. *Br J Nutr*. Feb;101:367-375. Epub 2008/07/19.
531
- 532 Palframan RJ, Gibson GR, Rastall RA. 2002. Effect of pH and Dose on the Growth of Gut
533 Bacteria on Prebiotic Carbohydrates in vitro. *anaerob*. 10//;8:287-292.
534
- 535 Pool-Zobel B, van Loo J, Rowland I, Roberfroid MB. 2002. Experimental evidences on the
536 potential of prebiotic fructans to reduce the risk of colon cancer. *Br J Nutr*. May;87
537 Suppl 2:S273-281. Epub 2002/06/29.
538
- 539 Richardson AJ, Calder AG, Stewart CS, Smith A. 1989. Simultaneous determination of
540 volatile and non-volatile acidic fermentation products of anaerobes by capillary gas
541 chromatography. *Letters in Applied Microbiology*.9:5-8.
542
- 543 Rigottier-Gois L, Rochet V, Garrec N, Suau A, Dore J. 2003. Enumeration of *Bacteroides*
544 species in human faeces by fluorescent in situ hybridisation combined with flow
545 cytometry using 16S rRNA probes. *Syst Appl Microbiol*. Mar;26:110-118. Epub
546 2003/05/16.
547
- 548 Russell DA, Ross RP, Fitzgerald GF, Stanton C. 2011. Metabolic activities and probiotic
549 potential of bifidobacteria. *Int J Food Microbiol*. Sep 1;149:88-105. Epub 2011/07/19.
550
551
- 552 Russell WR, Hoyles L, Flint HJ, Dumas ME. 2013. Colonic bacterial metabolites and human
553 health. *Current opinion in microbiology*. Jun;16:246-254. Epub 2013/07/25.
554
- 555 Steer TE, Johnson IT, Gee JM, Gibson GR. 2003. Metabolism of the soyabean isoflavone
556 glycoside genistin in vitro by human gut bacteria and the effect of prebiotics. *B J*
557 *Nutr*. Sep;90:635-642.
558
- 559 Terpend K, Possemiers S, Daguët D, Marzorati M. 2013. Arabinogalactan and fructo-
560 oligosaccharides have a different fermentation profile in the Simulator of the Human
561 Intestinal Microbial Ecosystem (SHIME®). *Environmental Microbiology*
562 *Reports*.n/a-n/a.
563
- 564 Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J,
565 Grosse J, Reimann F, Gribble FM. 2012. Short-chain fatty acids stimulate glucagon-

566 like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes.
567 Feb;61:364-371. Epub 2011/12/23.
568

569 Tulung B, Remesy C, Demigne C. 1987. Specific effect of guar gum or gum arabic on
570 adaptation of cecal digestion to high fiber diets in the rat. J Nutr. Sep;117:1556-1561.
571 Epub 1987/09/01.
572

573 Tuohy KM, Kolida S, Lustenberger AM, Gibson GR. 2001. The prebiotic effects of biscuits
574 containing partially hydrolysed guar gum and fructo-oligosaccharides - a human
575 volunteer study. B J Nutr. Sep;86:341-348.
576

577 Verbeken D, Dierckx S, Dewettinck K. 2003. Exudate gums: occurrence, production, and
578 applications. Appl Microbiol Biotechnol. Nov;63:10-21. Epub 2003/06/13.
579

580 Walton GE. 2006. The bacterial metabolism of dietary prebiotics and the potential for
581 protection against colorectal cancer - University of Reading, .
582

583 Williams P, Phillips G. 2000. . Handbook of Hydrocolloids: CRC Press, Boca Raton, FL, pp.
584
585

586 Wyatt GM, Bayliss CE, Holcroft JD. 1986. A change in human fecal flora in response to
587 inclusion of gum arabic in the diet B J Nutr. Mar;55:261-266.

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591 **Table 1:** Composition and nutritional profile of GA used in the present study

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Analysis	Results
Energy (kcal)	1205kJ/100g
Protein	2.1g/100g
Total carbohydrate (by difference)	82.6g/100g
Carbohydrate (avail)	56.5g/100g
Total Suger*	0.6g/100g
Fibre	26.1g/100g
Fat	0.1g/100g
Sodium	11.0mg/100g
Moisture	11.8g/100g
Ash	3.49g/100g

593 * Total sugars are the sum of glucose, sucrose and fructose expressed as monosaccharides

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609 **Table 2 :** Hybridisation and washing conditions for oligonucleotide probes

Probe name	Sequence (5' to 3')	Hybridisation pre-treatment	Formamide (%) in hybridisation buffer	Hybridisation temperature (°C)	Washing temperature (°C)	Reference
Ato 291	GGTCGGTCTCTCAACCC	Lysozyme	0	50	50	(Harmsen et al. 2000)
Lab 158	GGTATTAGCAYCTGTTTCCA	Lysozyme	0	50	50	(Harmsen et al. 1999)
Bif 164	CATCCGGCATTACCACCC	Lysozyme	0	50	50	(Langendijk et al. 1995)
Erec 482	GCTTCTTAGTCARGTACCG	None	0	50	50	(Franks et al. 1998)
Chis 150	TTATGCGGTATTAATCTYCCTTT	None	0	50	50	(Franks, Harmsen, Raangs, Jansen, Schut and Welling 1998)

Bac 303	CCAATGTGGGGGACCTT	None	0	46	48	(Manz et al. (1996))
EUB338*	GCTGCCTCCCGTAGGAGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
EUB338II*	GCAGCCACCCGTAGGTGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
EUB338III*	GCTGCCACCCGTAGGTGT	None	35	46	48	(Daims, Brühl, Amann, Schleifer and Wagner 1999)
* These probes are used together in equimolar concentrations (all at 50 ng μl^{-1})						

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	Bif164		Lab158		Ato291		Bac303		Erec482		Chis150		EubI-II-III	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Control 0h	7.46	0.21	7.24	0.09	7.40	0.18	7.85	0.24	7.96	0.20	6.91	0.24	8.26	0.35
Control 5h	7.76	0.20	7.29	0.07	7.60	0.15	8.04	0.20	7.36	0.07	6.42	0.38	8.45	0.08
Control 10h	7.75	0.24	7.47	0.07	7.56	0.02	8.25	0.28	7.51	0.07	6.99	0.16	8.44	0.04
Control 24h	7.78	0.16	7.29	0.04	7.54	0.14	8.12	0.21	7.54	0.08	5.73	0.47	8.53	0.36
Control 48h	7.64	0.29	7.14	0.50	7.50	0.61	7.76	0.17	7.51	0.06	5.68	0.73	8.42	0.18
FOS 0h	7.39	0.21	7.31	0.08	7.52	0.09	7.80	0.11	7.72	0.2	6.62	0.53	8.21	0.14
FOS 5h	7.83 *	0.06	7.78 **	0.04	7.83 *	0.10	8.16 *	0.09	7.41	0.16	5.71 *	0.55	8.78	0.11
FOS 10h	8.00	0.07	7.53 **	0.09	7.77	0.30	8.30	0.10	7.83	0.29	6.23	1.09	8.72 *	0.08
FOS 24h	8.17	0.23	7.75 *	0.14	7.79	0.31	8.24 *	0.01	7.50	0.12	6.27	0.88	8.83 *	0.14
FOS 48h	7.82	0.15	7.44	0.15	7.58	0.18	7.93	0.18	7.28	0.19	5.65	0.69	8.57	0.17
GUM 0h	7.53	0.10	7.00	0.16	7.43	0.05	7.70	0.22	7.81	0.37	6.08	0.50	8.29	0.22
GUM 5h	7.92 *	0.11	7.32 *	0.05	7.80 **	0.06	7.97 *	0.19	7.42	0.11	6.64	0.42	8.54	0.18
GUM 10h	7.90 *	0.14	7.46	0.11	7.60	0.26	8.01	0.35	7.44	0.166	5.87 *	0.82	8.79	0.71
GUM 24h	7.93 *	0.06	7.59 **	0.22	7.66	0.22	8.08	0.52	7.65	0.17	5.87	0.82	8.53	0.25
GUM 48h	7.22 a	0.37	7.39	0.11	7.53	0.40	8.20	0.24	7.39	0.49	6.28	0.86	8.49	0.56

Table 3-b: changes in the bacterial composition figures are presenting the mean bacterial populations in pH-controlled batch cultures at 0, 5, 10, 24, and 48h. Values are mean \pm SD. *, significant differences from the 0 h value within the same treatment, $p < 0.05$. small letters differences from the negative control. 1% faecal batch culture inoculated with vessel 1 negative control, vessel 2 FOS 1%, vessel 3 FOS 2%, vessel 4 GA 1%, vessel 5 GA 2%.

	Bif164		Lab158		Ato291		Bac303		Erec482		Chis150		EubI-II-III	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Control 0h	7.46	0.21	7.24	0.09	7.40	0.18	7.85	0.24	7.96	0.20	6.91	0.24	8.26	0.35
Control 5h	7.76	0.20	7.29	0.07	7.60	0.15	8.04	0.20	7.36	0.07	6.42	0.38	8.45	0.08
Control 10h	7.75	0.24	7.47	0.07	7.56	0.02	8.25	0.28	7.51	0.07	6.99	0.16	8.44	0.04
Control 24h	7.78	0.16	7.29	0.04	7.54	0.14	8.12	0.21	7.54	0.08	5.73	0.47	8.53	0.36
Control 48h	7.64	0.29	7.14	0.50	7.50	0.61	7.76	0.17	7.51	0.06	5.68	0.73	8.42	0.18
FOS 0h	7.4	0.1	7.24	0.24	7.57	0.10	7.89	0.17	7.84	0.27	6.44	0.51	8.35	0.29
FOS 5h	7.93 *	0.15	7.75 a	0.14	7.98 **	0.12	8.38 *	0.02	7.74	0.3	6.45	0.40	8.89 a	0.083
FOS 10h	8.02	0.11	7.58	0.14	7.89	0.18	7.8	0.32	7.88	0.19	6.25	0.94	8.76	0.26
FOS 24h	8.12 a	0.17	7.51 a	0.1	7.81	0.19	8.07	0.3	7.82	0.19	5.88	0.85	8.56	0.08
FOS 48h	7.88	0.11	7.43	0.15	7.67	0.10	7.95	0.34	7.62	0.18	5.87	0.15	8.69	0.29
GUM 0h	7.33	0.05	7.38	0.14	7.58	0.18	7.73	0.22	7.77	0.2	6.39	0.58	8.11	0.17
GUM 5h	7.78 **	0.06	7.32	0.13	7.67	0.29	8.01 **	0.23	7.6	0.16	6.31	0.67	8.44	0.16
GUM 10h	7.98 *	0.13	7.6	0.31	7.70	0.25	8.21	0.54	7.58	0.2	6.09	0.75	8.74	0.47
GUM 24h	7.93	0.28	7.67	0.27	7.66	0.26	8.17	0.3	7.68	0.29	6.57	0.73	8.72	0.18
GUM 48h	7.69	0.149	7.34	0.12	7.78	0.27	8.2	0.25	7.46	0.3	6.04	0.91	8.66 *	0.17

618 **Table 4:** changes in the SCFA concentration are presenting in table 3 in pH-controlled batch
619 cultures at 0, 5, 10, 24, and 48h. Values are mean \pm SD. *, significant differences from the 0 h
620 value within the same treatment, $p < 0.05$. small letters differences from the negative control. 1%
621 faecal batch culture inoculated with vessel 1 negative control, vessel 2 FOS 1%, vessel 3 FOS
2%, vessel 4 GA 1%, vessel 5 GA 2%.

	ACETATE	PROPIONATE	BUTYRATE
Control			
0 h	1.68 \pm 1.16	0.19 \pm 0.12	0.15 \pm 0.09
5 h	4.49 \pm 0.99 a	0.82 \pm 0.86	2.15 \pm 3.19
10 h	6.47 \pm 1.34	2.51 \pm 1.19 a	1.47 \pm 0.35
24 h	8.88 \pm 1.71	4.50 \pm 2.29	1.73 \pm 0.85 a
48 h	10.57 \pm 3.57	5.40 \pm 3.11	3.10 \pm 2.41
FOS 1%			
0 h	0.98 \pm 0.04	0.10 \pm 0.03	0.08 \pm 0.02
5 h	8.84 \pm 3.53 ** a	1.86 \pm 0.31	0.66 \pm 0.24
10 h	13.12 \pm 2.02 **	8.45 \pm 6.98	4.42 \pm 2.02
24 h	11.89 \pm 4.22 *	8.13 \pm 2.10 *	8.26 \pm 2.98 *
48 h	10.00 \pm 1.11 **	4.32 \pm 3.42	1.21 \pm 1.58
FOS 2%			
0 h	1.35 \pm 0.26	0.19 \pm 0.10	0.13 \pm 0.06
5 h	14.28 \pm 4.93	4.24 \pm 3.54	2.32 \pm 2.10
10 h	15.08 \pm 2.93	5.80 \pm 1.04 * a	5.93 \pm 4.34
24 h	13.00 \pm 7.81 **	5.44 \pm 2.56	6.21 \pm 4.05
48 h	8.80 \pm 0.32 *	2.31 \pm 1.85	3.66 \pm 1.52
GUM 1%			
0 h	2.01 \pm 1.73	0.18 \pm 0.10	0.16 \pm 0.12
5 h	5.36 \pm 0.37	4.79 \pm 4.76	4.99 \pm 7.61
10 h	12.72 \pm 4.79	6.77 \pm 4.78	6.96 \pm 7.79
24 h	10.78 \pm 8.27	3.28 \pm 2.61	4.59 \pm 1.49 * a
48 h	11.64 \pm 4.10 *	4.79 \pm 1.46 *	4.01 \pm 0.88 *
GUM 2%			
0 h	2.37 \pm 2.29	0.19 \pm 0.12	0.16 \pm 0.12
5 h	5.24 \pm 0.56	5.13 \pm 5.93	0.73 \pm 0.42
10 h	16.67 \pm 7.59	12.03 \pm 8.32	2.40 \pm 0.51
24 h	11.41 \pm 9.15	3.28 \pm 2.51	3.09 \pm 2.49 *
48 h	13.63 \pm 3.17 *	6.15 \pm 2.24	6.24 \pm 2.22 *

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