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

Article

Accepted Version

1 **Title page**

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

3 Sehad Alarifi, Alan Bell, Gemma Walton

4 Corresponding author Gemma Walton. Email address g.e.walton@reading.ac.uk

5 Department of Food and Nutritional Sciences

6 PO Box 226

7 Whiteknights

8 Reading

9 RG6 6AP

10 Sehad Alarifi PhD student in University of Reading

11 Dr Alan Bell Lecturer in University of Reading

12 Dr Gemma Walton Lecturer in University of Reading

13

14

15

16

17

18

19

20

21

22

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.

38

39

40

41

42

43

44

45

46

47

48

49

Introduction

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

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

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

3

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 investigateof 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

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.

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).

5

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), K_2HPO_4 (0.04 g/l),

128   KH_2PO_4 (0.04 g/l), $NaHCO_3$ (2 g/l), $MgSO_4•7H_2O$ (0.01 g/l), $CaCl_2•6H_2O$ (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 diffrences 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 µL of cold 1×PBS, then 150 µL 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 µL of a 50 ng/µL stock of probe plus 50 µL 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 µL 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 Gallenkap 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 either 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 ^{o}C for the first 5 minutes,

197   temperature of the column was increased over 5 minutes to 240 ^{o}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 individial donors in three separate experiments. The log $_{10}$ numbers of

205   specific bacteria were expressed as mean values and standard diviation. 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± 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 ≤ 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, 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].

Discussion

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

Several studies have shown that GA can undergo a slow fermentation, specifically a more distal fermentation, whereas existing prebiotics typically undergo proximal fermentation (Macfarlane et al. 1992), as proteolytic fermentation develops in the distal colon; therefore, this substrate may be able to be saccharolytically fermented in this part of colon. In this present study GA fermentation shows selectivity in bifidobacteria and *Lactobacillus* spp at time 10 h and continues to 24 h which could indicate slower fermentaion and is inline with others work (Cherbut, Michel1, Raison1, Kravtchenko and Severine 2003). However, it is worth noting that the bifidogenic effect of the 1% was not maintained at 48 hours in current study.

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

12

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 (Ouwehand et al. 2009). The 2% dose did not lead to the same lactobacilli

292   impact. The use of a higher doasge 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   Michel1, Raison1, 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 has 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

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

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

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

The pH-controlled stirred batch culture systems enabled rapid analysis of the effects of GA on the faecal microbiota. In the absence of absorption, colonic secretions, and epithelial 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.

355

356

360 *Words count:*

361 7126

362

363

364

365

366

367

368

369

370

371

372

373

374

375

References

Adiotomre J, Eastwood MA, Edwards CA, Brydon WG. 1990. Dietary fiber-In vitro methods that anticipate nutrtion 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, Michel1 C, Raison1 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   glycated 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, Daguet 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.

588
589

590

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

592

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

594

595

596

597

598

599

600

601

602

603

604

605

606

607

608

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})						

610

611

612

613

	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 ± 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 ± SD.*, significant differences from the 0 h
value within the same treatment, p<0.05. small letters differences from the negative control. 1%
620 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%.
621

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

622

623