Title

Colonic fermentation of water soluble fiber fraction extracted from Sugarcane (*Sacchurum officinarum* L.) bagasse in murine models.

Authors and Affiliations

Samanthi Wathsala PELPOLAGE[a,b], Yuka GOTO[a], Ryuji NAGATA[a,b], Naoki FUKUMA[a,c], Toma FURUTA[d], Masami MIZU[d], Kyu-Ho HAN[a,c], Michihiro FUKUSHIMA[a,b*]

[a] Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, West 2-11, Inada, Obihiro, Hokkaido, Japan, 080-8555.

[b] United Graduate School of Agricultural Sciences- Iwate University, 3-18-8 Ueda, Morioka, Japan, 020-8550.

[c] Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, West 2-11, Inada, Obihiro, Hokkaido, Japan, 080-8555.

[d] Mitsui Sugar Corporation Limited, 30-1, Nihonbashi, Chuo-ku, Tokyo, Japan, 103-0015.

***Corresponding author**

Michihiro Fukushima - fukushim@obihiro.ac.jp

Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, West 2-11, Inada, Obihiro, Hokkaido, Japan, 080-8555.

Telephone - +81-155-49-5557

Authors and email addresses

Samanthi Wathsala Pelpolage – samanthipelpola@gmail.com

Yuka Goto- yukag10@gmail.com

Ryuji Nagata- boc.10rj24@gmail.com

Naoki Fukuma- n.fukumax@obihiro.ac.jp

Toma Furuta- Toma.Furuta@mitsui-sugar.co.jp

Masami Mizu- Masami.Mizu@mitsui-sugar.co.jp

Kyuho Han – kyuho@obihiro.ac.jp

Michihiro Fukushima* - fukushim@obihiro.ac.jp

41 **Abstract**

42 Biochemical effects of the water soluble fiber fraction of sugarcane bagasse (BSF) fermented in the
43 colon was examined to evaluate its potential health promoting effects. A feeding experiment involving
44 Fischer 344 rats, was conducted with 3 experimental diets containing, cellulose (CON), a commercial
45 xylo-oligosaccharide (XYO) and BSF (BGS). Cumulative feed intake was significantly lower in XYO
46 group while cecal weight was significantly higher. Acetic and propionic acid contents in the cecal
47 content were significantly higher in the BGS and XYO, respectively. Total short chain fatty acid
48 content was significantly higher in BGS and XYO resulting significantly lower cecal pH. Beneficial
49 bacteria such as *Bifidobacterium*, *Blautia*, *Akkermansia* and *Roseburia* abundance was significantly
50 higher in the XYO and BGS groups. Further, mucin and immunoglobulin-A contents were
51 significantly higher in BGS group compared to CON group. Thus, BSF exhibited its ability to enhance
52 the intestinal and systemic health upon fermentation in the colon.

53

54 **Key words:** sugarcane bagasse, xylo-oligosaccharides, short chain fatty acids, *in vivo* colonic
55 fermentation, gut microbiota, mucin

56

57 **Chemical compounds studied:** Acetic acid (PubChem CID: 176); Butyric acid (PubChem CID:
58 264); Propionic acid (PubChem CID: 1032); Ammonia- nitrogen (PubChem CID: 6857397);
59 Cellulose (PubChem CID: 16211032); Xylobiose (PubChem CID: 439538); Xylotriose (PubChem
60 CID: 10201852)

1. Introduction

Xylo-oligosaccharides (XOS) are xylose sugar oligomers with a degree of polymerization (DP) varying from 2 to 10 resulted from xylan (DP up to 200) hydrolysis via chemical, enzymatic, combined methods or auto-hydrolysis under high temperature and pressure (Samanta et al., 2015). XOS are considered to be recalcitrant to small intestinal digestion due to the absence of enzymes that cleave β-(1,4)-xylosidic bonds within the human carbohydrate hydrolytic enzyme repertoire, thus reach the colon intact and provide substrates for bacterial fermentation, promoting colonic and systemic health, similar to inulin and fructo-oligosaccharides (Brienzo, Carvalho, de Figueiredo, & Neto, 2016) .

Daily consumption of XOS has been found to regulate insulin secretion from the pancreas, increase mineral absorption from the large intestine, increase fecal moisture content, regulate bowel function and maintain stool frequency in the normal range, effectively reduce constipation in pregnant women, reduce fecal pH and improve abdominal conditions without complications such as diarrhea and flatulence as in the case of inulin (Aachary & Prapulla, 2011; Samanta et al., 2015). Moreover, substitution of dietary starch and sucrose by XOS has been found to improve hepatic lipid profile and alleviate diabetic, cancerous and stress symptoms (Gobinath, Madhu, Prashant, Srinivasan, & Prapulla, 2010; Samanta et al., 2015). The beneficial physiological effects of XOS intake are linked to the prebiotic properties of XOS, such as increased cecal weight (CW), improved *Bifidobacterium* and *Lactobacillus* abundance, altered short chain fatty acid (SCFA) composition, immune stimulation, decreased colonic pH and suppressed production of nitrogenous end products and pro-carcinogenic

enzymes (Aachary & Prapulla, 2011; Brienzo et al., 2016; Samanta et al., 2015).

Bagasse is the main fiber residue of the industrial juice extraction process from sugarcane (*Saccharum officinarum* L.), mainly comprising of cellulose (40-45%) and hemicellulose (20-25%) that are embedded in a lignin (18-24%) matrix (Gupta & Bhatnagar, 2015). Sugarcane bagasse is identified as a rich source of xylan [L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan], the dominant hemicellulose type in sugarcane bagasse, which is a promising resource for commercial XOS production (Brienzo et al., 2016). It has been reported that XOS obtained via xylan hydrolysis from alkali pre-treated sugarcane bagasse, are prebiotic (Brienzo et al., 2016; Samanta et al., 2015). Further, many beneficial biological effects related to gastrointestinal health, such as anti-inflammatory, anti-tumor, anti-cancer, anti-microbial effects have been reported for acidic XOS and derivatives containing uronic acid substitutions as in sugarcane bagasse (Aachary & Prapulla, 2011).

Despite the reported beneficial biological and physiological effects, incorporation of XOS in human diet is limited, mostly due to the less production and availability (Brienzo et al., 2016). As previously mentioned sugarcane bagasse is a promising resource for XOS production, which might yield XOS of versatile characteristics and benefits, depending on the production methods and processing conditions (Brienzo et al., 2016). In agreement with its higher XOS content, we hypothesized that the water soluble fiber fraction (BSF) obtained from hydrothermal decomposition of sugarcane bagasse could be a promising substrate for gut microbial fermentation, which might improve colonic health by selective stimulation of beneficial bacteria and beneficial metabolite production. Thus, in this study we aimed to characterize the biochemical effects of colonic fermentation of BSF in murine models.

2. Materials and Methods

2.1. Preparation of experimental diets

BSF obtained by hydrothermal decomposition (200°C, 1.8 MPa) followed by membrane ultrafiltration (2500 g/mol) was provided by Mitsui Sugar Co. Ltd., (Tokyo, Japan) and commercial XOS (DP: 2-3; 95% purity) preparation was purchased from B Food Science Co. Ltd., (Aichi, Japan). All three experimental diets, cellulose (CON), commercial XOS diet (XYO) and BSF diet (BGS) were prepared according to the AIN-93G diet guidelines (Oriental Yeast Co., Ltd., Tokyo, Japan). Diet composition is provided in the Supplementary Table I. All chemicals used were of analytical grade.

2.2. Animal experimental design, care for laboratory animals and post-mortem excision of organs

Eighteen Fischer 344 male rats (7 weeks old; average body weight 125-155 g) were purchased from Charles River Laboratories Japan Inc., (Yokohama, Japan). The rats were acclimatized for one week prior to the experiment on a commercial diet (Standard powder diet for mouse, rat, hamster, Oriental Yeast Co.,) and were grouped into 3 similar body weight groups (≃ 170 g) at the end of the acclimatizing period. Followed by grouping, rats were fed with experimental diets with free access to *ad libitum* water. Each rat was housed individually, a feeder (≃ 25g) and a drinker (≃ 150 ml) were allocated to each animal, which were replenished every morning at 8 o' clock. The cages were maintained at 23±1˚C temperature and 60±5% relative humidity under a 12 h light/dark cycle. Body weight and feed intake were measured once a week and daily, respectively. After the experimental

period of 4 weeks, the final body weight (FBW) was measured and the animals were sacrificed (Sodium pentobarbital, 40 mg/kg body weight, Abbott Laboratories, Chicago, Illinois, United States). Following the sacrifice cecum was excised. CW, cecal content weight (CCW) and cecal tissue weight (CTW) were measured and a part of the cecal content (≃1g) was diluted (×5) in sterilized distilled water for microbial analysis (section 2.3.1), pH measurement (Han et al., 2016) and other analyses (sections 2.4, 2.5, 2.6 and 2.7), while the rest was stored at -30 ˚C (used in the section 2.3.2). The animal experiment was conducted according to the guidelines of "*Guide for the Care and Use of Laboratory Animals*" and all the procedures were approved by the Animal Care and Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine (License no: 29-94).

2.3. Rat cecal bacterial population analyses

2.3.1. Viable plate count method for anaerobes and coliform

One milliliter from each diluted cecal content sample was cultured to enumerate specific bacterial counts by viable plate count method with selective media according to the method previously reported by Han et al. (2016). After the specific incubation periods, colonies were visually counted and were expressed in decadic logarithm of colony forming units per milliliter ($\log_{10}$ CFU/ mL) of the working volume.

2.3.2. Cecal bacterial DNA extraction, next-generation sequencing (NGS) and the analysis of 16S

ribosomal RNA (16S rRNA) gene sequences

Bacterial DNA was extracted from the cecal content samples (non-diluted) employing the modified phenol-free repeated beads beating plus column (RBB+C) method described by Yu & Morrison (2004). Following the extraction, the genomic DNA was purified via sequential digestions with RNase and proteinase K using QIAamp columns from QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, California, United States). The concentration of extracted community DNA was measured by Nano Drop 2000c spectrophotometer (Thermo Fisher Scientific, Tokyo, Japan) and the concentration was adjusted to 5 ng/µL with Tris-EDTA buffer.

V3 and V4 variable regions of 16S rRNA gene were amplified using the following bacterial overhang adapters and universal primers in the first stage polymerase chain reaction (PCR); forward primer (*5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′*) and the reverse primer (*5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATTACHVGGGTATCTAATCC-3′*). In the second stage PCR, Illumina sequencing adapters and dual index barcodes were added to the amplicons using Nextera® XT Index Kit (Illumina Inc., San Diego, California, United States). After quantifying the concentration of the PCR products from the second stage PCR using Quantus™ fluorometer (QuantiFluor® dsDNA System, Promega, Madison, Wisconsin, United States), the successful PCR products were pooled in one tube with equal volumes and were subjected to paired-end sequencing by Illumina MiSeq System (Illumina Inc.,). The preparation of 16S rRNA gene amplicon was done according to the method described in the 16S Metagenomic Sequencing Library Preparation Guide (Part # 15044223 Rev. B).

The analysis of retrieved raw 16S rRNA gene sequences was conducted according to the method reported by Warren et al. (2018). The generated biome table was normalised using an equal subsampling size of 13674 sequences. Calculation of distances between bacterial communities in different samples by the weighted UniFrac distance metric and preparation of Principle Coordinate Analysis (PCoA) plot were conducted in QIIME (Lozupone & Knight, 2005). Calypso version 8.72 (Zakrzewski et al., 2017) was used to generate hierarchical clustering plots at phylum, genus and species levels and Least Discriminant Analysis effect size (LEfSe) plots.

2.4. Rat cecal SCFA analysis by high performance liquid chromatography (HPLC)

SCFA content in the cecal digesta of rats were analyzed by HPLC (Shimadzu LC-10AD, Kyoto, Japan). Samples for HPLC were prepared according to the method described in Han et al. (2016) and the analytical specifications were as follows; column, RSpak KC-811 (8.0 mm x 300 mm, Shodex, Tokyo, Japan); eluent and flow rate, 2 mM $HClO_4$ at 1 mL/min; column temperature, 47 °C; reaction reagent and flow rate, ST3-R (×10 diluted, Cat. No. F56120000, Shodex) at 0.5 mL/min; UV detector wavelength, 450 nm. Quantification of SCFA concentration was performed using the external standard quantitation method (images of chromatograms obtained for CON, XYO and BGS groups are presented in the supplementary figure I). Injection volume was 20 µL. The formula for calculation was as follows;

$$\text{SCFA concentration} = \frac{\text{Sample peak area}}{\text{Standard peak area}} * \text{Injected standard amount}$$

181 2.5. Determination of cecal ammonia-nitrogen content

182 Ammonia-nitrogen content in the diluted samples of cecal content was analyzed using a commercially
183 available kit (Wako Pure Chemical Industry Ltd., Tokyo, Japan) according to manufacturer's
184 instructions.

185

186 2.6. Analysis of physical barrier function of gut by mucin content in rat cecal digesta

187 Mucin in the rat cecal digesta was fractionated according to the method by Bovee-Oudenhoven et al.
188 (1997) and was analyzed by the fluorometric assay procedure described by Crowther and Wetmore
189 (1987).

190

191 2.7. Analysis of immunological barrier function in rat cecal digesta by Immunoglobulin A (IgA)
192 content

193 IgA content was analyzed by ELISA quantitative kit for Rat IgA provided by Bethyl Laboratories Inc.
194 (Texas, United States) as per the manufacturer's instructions.

195

196 2.8. Statistical analysis

197 All data except the microbial community data were analyzed for their significance (p<0.05) by
198 analysis of variance (ANOVA) using SPSS statistical software version 17.0 (IBM Co., Armonk, New
199 York, United States). When significant differences among the test groups were revealed, mean scores
200 were compared by Tukey's test (p<0.05). Statistical significance of Shannon's diversity (H) index

was determined by Dunn's Multiple comparisons test (post hoc Kruskal-Wallis H test) in Prism version 7.0a (GraphPad Software, La Jolla, California, United States). Relative abundance and statistical significance of phyla, genera and species among the 3 diet groups were compared using Kruskal-Wallis H test in Calypso (version 8.72). A *p* value less than 0.05 was considered as statistically significant.

3. Results & Discussion

3.1. Effects of XYO, BGS and CON diets on cumulative feed intake (FI), body weight and cecal parameters

As presented in Table 1, FI at the end of the experimental period was significantly different ($p<0.05$) between CON and XYO, where CON and XYO reported the highest and lowest intakes, respectively. FBW and body weight gain (BWG) were not significantly different among the diet groups, a trend which has been similarly reported in previous studies also (Hsu, Liao, Chung, Hsieh, & Chan, 2004).

CW, CTW and CCW followed a similar trend among the three diet groups (Table 1). Both CW and CTW were significantly higher ($p<0.05$) in the XYO group, which can be attributed to the prominent microbial fermentation that took place in the ceca of XYO fed animals (Montagne, Pluske, & Hampson, 2003). Cecal pH, which was significantly lower ($p<0.05$) in XYO and BGS groups compared to the CON group, also provided evidence for colonic fermentation, despite the similar CW,

CTW and CCW of BGS group to that of the CON group (Aachary & Prapulla, 2011; Fukuda et al., 2011; Hsu et al., 2004). Increased total weight of cecum has been previously observed upon XOS intake, followed by the increased abundance of beneficial gut microbiota (discussed in sections 3.3-3.6) and acidification of colonic environment, similar to the observations in the present study (Samanta et al., 2015). The higher CW and CTW can be attributed to the trophic effect of SCFA production in the cecum, especially butyric acid and subsequently acetic acid, where cecal wall cell density would be increased by the enhanced normalized colonic epithelial cell proliferation, which would subsequently decrease mucosal atrophy (Hsu et al., 2004; Montagne et al., 2003).

BGS diet was formulated with 5% w/w BSF, which contained 51% oligosaccharides (dry weight basis) in the carbohydrate fraction. The hydrothemal treatment employed in this study for treating the ligno-cellulosic material in sugarcane bagasse, is known to yield mainly soluble oligosaccharides, which might explain the higher oligosaccharide fraction in BSF (Aachary & Prapulla, 2011). XYO diet also contained a 5% w/w commercial XOS preparation (95% purity) which was composed of xylobiose and xylotriose. XOS are linked together by β-(1,4)-xylosidic linkages that cannot be hydrolyzed in the small intestine, which get transferred to the colon (Aachary & Prapulla, 2011; Brienzo et al., 2016). Thus, both XYO and BGS diets might have provided favorable substrates for cecal microbial fermentation which was manifested by the above observations for cecal parameters (Hsu et al., 2004).

3.2. BGS and XYO diets favored SCFA production in the rat cecum

240 Among the major individual SCFAs, acetic acid was produced in the highest proportion in all diet
241 groups (Table 1). It was produced significantly (p<0.05) abundantly in BGS fed animals followed by
242 XYO, which was similar to a previous study, where acetic acid content was markedly higher even
243 than that of inulin and β-glucan, upon continuous intake of XOS (Aachary & Prapulla, 2011; Carlson,
244 Erickson, Hess, Gould, & Slavin, 2017). Branched XOS sources, with different types of branch chains
245 such as arabinofuranosyl residues or 4-*O*-methyl derivatives of α-D-glucopyranosyl uronic acid
246 groups as in the case of sugarcane bagasse xylan, possess different biological properties compared to
247 their linear counterparts, which might explain the different fermentation capacities of the two XOS
248 sources in this study (Aachary & Prapulla, 2011). Acetic acid is found to be metabolized by muscle
249 tissue, kidney, heart and brain upon absorption into the blood stream (Madhukumar & Muralikrishna,
250 2012).
251 Propionic acid content in the cecal digesta of XYO fed rats was significantly higher (p<0.05)
252 compared to CON fed rats, while it was similar to that of the BGS fed rats (Table 1). Significantly
253 lower FI in the XYO group can be attributed to the higher propionic acid content, while the propionic
254 acid contents and FI in BGS and CON groups also exhibited a similar trend. Propionic acid is known
255 for its ability to improve satiety and reduce feed intake (Carlson et al., 2017; Ottman, Geerlings,
256 Aalvink, de Vos, & Belzer, 2017). Propionic acid production by XOS fermentation has been reported
257 to be significantly lower compared to the other prebiotic substrates such as inulin and β-glucan,
258 similar to the present study, where propionic acid content was 6 and 12 folds lesser compared to the
259 specific acetic acid contents in the XYO and BGS diets, respectively (Carlson et al., 2017). Further,

it has been previously reported that the main products of linear and branched XOS fermentation to be acetic acid and lactic acid (Aachary & Prapulla, 2011; Abbeele et al., 2011).

Butyric acid content was similar between XYO and BGS groups, both of which were significantly higher (p<0.05) than that of the CON group (Table 1). Butyric acid contents in the cecal digesta of rats fed XYO and BGS were almost 3 folds higher than that of the CON fed animals, which reflected the presence of butyrogenic substrates in the XYO and BGS diets (Giuberti, Gallo, Moschini, & Masoero, 2013; O'Callaghan & van Sinderen, 2016). Butyric acid is known for its versatile beneficial effects such as anti-cancer and anti-inflammatory activities by influencing healthy colonocyte proliferation and enhancing gut barrier function by decreasing bacterial translocation (Thursby & Juge, 2017).

Total SCFA content followed the same trend as the acetic acid content, where significantly higher (p<0.05) content of acetic acid might have contributed to the significant increment in the total SCFA content in BGS fed rats, in comparison to the other two diet groups (Table 1). Both XYO and BGS fed rat cecal digesta had significantly higher (p<0.05) total SCFA content which was similar to a previous study where the highest total SCFA production was obtained from XOS fermentation, among a variable array of oligosaccharides (Aachary & Prapulla, 2011). The higher SCFA content in BGS and XYO groups can be attributed to the recalcitrant nature of XOS to the vertebrate digestive enzymes, which facilitates their passage into colon without any compromise in the structural integrity and the subsequent fermentation processes by the resident gut microbiota, which yield SCFAs as the

279 end products Samanta et al. (2015). Significantly lower cecal pH in XYO and BGS groups also can
280 be ascribed to the significantly higher total SCFA contents compared to the CON group, a beneficial
281 health effect of XOS intake (Fukuda et al., 2011; Vazquez-Gutierrez, de Wouters, Werder, Chassard,
282 & Lacroix, 2016). And due to the established correlations between colonic pH and abundance of
283 pathogenic and beneficial bacteria, reduction in cecal pH can be used as a marker of prebiotic effects
284 of XOS fermentation in XYO and BGS groups (Madhukumar & Muralikrishna, 2012). Thus, as
285 hypothesized beneficial metabolites were produced significantly abundantly in BGS fed rats, as well
286 as in XYO fed rats due to XOS fermentation.
287
288 3.3. XYO and BGS groups were different in cecal microbial diversity and possessed different
289 characteristic microbial genera
290 Mammalian colon is considered to be almost devoid of oxygen, and thus the microbial community
291 reside in the colon is considered to be predominantly anaerobic and/or a smaller percentage of
292 facultative anaerobes (Thursby & Juge, 2017). Similarly, both XYO and BGS favored the growth and
293 proliferation of anaerobes significantly (p<0.05) in the cecum compared to the CON group (Fig. 1-a).
294 The higher abundance of anaerobes in the cecal digesta of XYO and BGS might have implicated their
295 ability to feed and maintain a healthy gut microbial community (Vieira, Teixeira, & Martins, 2013).
296 Interestingly, in the present study, coliform count was significantly higher (p<0.05) in the XYO group,
297 irrespective of its significantly lower cecal pH compared to the CON group (Fig. 1-b). In contrast,
298 coliform count in the BGS group was significantly lower (p<0.05) in agreement with the substantially

299 higher total SCFA content and significantly lower cecal pH. It has been reported that the enzymes
300 expressed by *Escherichia coli* are capable of degrading XOS derived from xylan, and thus the higher
301 coliform abundance in the XYO group might implicate the availability of degradable XOS by
302 coliforms (Aachary & Prapulla, 2011). Moreover, a previous study by Vazquez-Gutierrez et al. (2016)
303 also had reported that *E. coli* was not affected by the incubation at low pH and high organic acid
304 concentrations produced by bifidobacteria strains in *in vitro* co-cultures.

305 Metagenomic analysis of bacterial community DNA in the cecal digesta showed distinct differences
306 in the microbial diversity among the test groups. As presented in Fig. 2-a, according to the values
307 obtained for *H* index, CON group possessed a significantly higher diversity, which was followed by
308 the BGS group and lastly the XYO group. Evenness (H_E) also followed the same trend, where a higher
309 evenness of species diversity was observed in both CON and BGS groups compared to the XYO
310 group (data not shown). Thus, α-diversity indices of microbial community suggested that the
311 microbial communities observed in XYO and BGS groups were diverse in terms of the relative
312 abundance and the homogeneity of the observed species. PCoA plot for the β-diversity of the
313 microbial community data also revealed the presence of significantly distinct microbial community
314 structures among the diet groups, while the individual animals within each group exhibited similar
315 gut microbial community structures as reflected by the clear clustering based on the type of the test
316 diet (Fig. 2-b). Differences in the microbial diversity between XYO and BGS groups might reflect
317 the differences in the type and availability of the fermentable substrates and the selective fermentation

318 characteristics of the gut microbiota (Vieira et al., 2013). Linear and low molecular weight (282-414
319 g/mol) XOS in the XYO diet and high molecular weight (≥2500 g/mol) branched XOS in the BGS
320 diet might have selectively influenced the members of colonic microbiota in different degrees
321 (Aachary & Prapulla, 2011). It is a well-established fact that the DP or molecular weight and the chain
322 structure (linear or branched) affect the fermentability of XOS, as they determine the selectivity of
323 microbial degraders (Aachary & Prapulla, 2011).

324 As shown in Fig. 3-a, among the three diet groups, Firmicutes accounted for the most abundant
325 microbial phylum (Chen, Liu, Ling, Tong, & Xiang, 2012). Abundance of Firmicutes in BGS and
326 XYO groups was significantly lower (p<0.01) compared to the CON (Fig. 3-b), which might have
327 assigned Firmicutes as the signature phylum of the CON group (Fig. 3-c). Among the other major
328 phyla, Actinobacteria, Proteobacteria and Verrucomicrobia accounted for the signature phyla of the
329 XYO group. Albeit, BGS group did not inherit any signature phylum, significantly higher abundance
330 of Actinobacteria (p<0.01), Proteobacteria (p<0.01) and Verrucomicrobia (p<0.05) compared to the
331 CON group, was observed (Fig. 3-b). Phylum Bacteroidetes was not significantly different among the
332 three diet groups.

333 Analysis of bacterial community data revealed, 36 core bacterial species in all groups, 2 common
334 species in XYO and BGS, 5 unique species in CON and 1 in BGS (data not shown). In CON group,
335 *Ruminococcus flavefaciens*, *Coprococcus eutactus* and *Clostridium perfringens*, in XYO
336 *Bifidobacterium pseudolongum* and *Akkermansia muciniphila* and in BGS *Roseburia faecis* and

Blautia producta were among the characteristic species (Supplementary Figure II). As shown in Fig. 4, *Bifidobacterium adolescentis* ($p<0.01$), *B. pseudolongum* ($p<0.01$), unclassified *Bifidobacterium* ($p<0.01$), unclassified *Blautia* ($p<0.01$) were significantly higher in abundance in the XYO group compared to BGS, while the abundance of *B. producta* ($p<0.01$) and *R. faecis* ($p<0.01$) was significantly higher in the BGS group compared to XYO, apart from several other unclassified species presented in the supplementary figure III.

3.4. Bifidobacteria abundance manifested the linear and non-substituted XOS fermentation in the rat cecum

Among the diverse gut microbial members, bifidobacterial strains are proposed to inherit a strong affinity to ferment oligosaccharides and known to be the most efficient xylose based oligo and polysaccharide fermenters (Carlson et al., 2017; Madhukumar & Muralikrishna, 2012). The abundance of bifidobacteria in the gastrointestinal tract is known to positively correlated with decreased blood lipopolysaccharides and inflammatory reagents and negatively correlated with obesity and weight gain suggesting its importance as a marker of healthy gut (Carlson et al., 2017). XOS and its derivatives have been confirmed in various *in vivo* and *in vitro* studies, for their bifidogenic nature, similar to the observations in the XYO group in this study (Gobinath et al., 2010; O'Callaghan & van Sinderen, 2016). In the current study, two bifidobacterial strains were characterized along with an unclassified *Bifidobacterium* fraction (Fig. 4-a, b, c), namely, *B. pseudolongum* and *B. adolescentis*.

357 *B. pseudolongum*, one of the dominant species found in animals, is considered to be able to metabolize
358 the xylan backbone of arabinoxylooligosaccharides (AXOS) up until xylotetraose moiety and its
359 ability to utilize substitutions on xylan backbone is considered to be limited (O'Callaghan & van
360 Sinderen, 2016). On the other hand, some strains of *B. adolescentis* abundantly found in the human
361 gut, are considered to be unable to utilize either substitutions or xylan backbone of AXOS, but able
362 to utilize xylose and arabinose monomers, while certain other strains are known to possess similar
363 capabilities as *B. pseudolongum* (O'Callaghan & van Sinderen, 2016). Thus, xylobiose and xylotriose
364 being the solo fermentable substrates in the XYO diet, *B. adolescentis* might not have been presented
365 with a niche advantage which was justified by its lower abundance. In the BGS group also, the key
366 bifidobacterial strain was *B. pseudolongum*, but the abundance was significantly very low compared
367 to the XYO group (Fig. 4-a), which might have been due to its limited capacity to utilize substitutions
368 on xylan backbone as previously mentioned (O'Callaghan & van Sinderen, 2016). Further, all
369 currently characterized bifidobacterial arabinoxylan (AX) degrading enzymes are predicted to be
370 intracellular, which suggested the fact that these bifidobacterial strains rely on the other members of
371 the gut microbiota for fermentative substrates, who are encoded for extracellular hydrolytic enzymes
372 (O'Callaghan & van Sinderen, 2016). This could be another reason behind the lower abundance of
373 bifidobacteria in BGS fed animals over XYO fed animals. Above mentioned limitations in
374 bifidobacteria might have resulted the higher selectivity between linear and branched structures as
375 fermentative substrates in this study, where they were found to prefer low substituted linear structures
376 (Aachary & Prapulla, 2011).

377

378 3.5. Specialist mucin degrader abundance and mucin contents were improved in XYO and BGS
379 groups

380 *A. muciniphila* is a specialist mucin degrader in the gut and considered as a marker of the salubrious
381 status of host health, as it is found to negatively correlate with inflammatory bowel disease,
382 appendicitis, obesity, autism, atopy and diabetes (Belzer & De Vos, 2012; Ottman et al., 2017). It
383 constitutes 1-4% of total gut microbial population in a healthy subject (Ottman et al., 2017), which
384 was similar to the findings of the present study and its abundance was significantly (p<0.05) higher
385 in BGS and XYO diet groups compared to CON (Fig. 4-d). Several previous studies also have
386 reported that the abundance of *A. miciniphila* was stimulated by feeding AXs to rats (Abbeele et al.,
387 2011; Belzer & De Vos, 2012; Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016).

388 *A. muciniphila* uses mucin as the only carbon and nitrogen substrate for its metabolism, gaining a
389 competitive advantage in the gut in niche utilization, even at times of adversity, such as fasting,
390 malnutrition or total parenteral nutrition (Belzer & De Vos, 2012). *A. muciniphila* is known to produce
391 oligosaccharides and SCFAs, specifically, acetate and propionate via mucin degradation, within the
392 mucus layer which is easily available for the host cells (Belzer & De Vos, 2012; Tailford, Crost,
393 Kavanaugh, & Juge, 2015). Thus, significantly very high acetic acid contents in the BGS and XYO
394 groups and propionic acid content in the XYO group might suggest the fact that *A. muciniphila* might
395 have contributed significantly to the acetic and propionic acid contents by mucin degradation (Belzer
396 & De Vos, 2012). Further, SCFAs and oligosaccharides produced by *A. muciniphila*, are known to

397 stimulate other microbial members who are closely associated with the mucus layer, such as
398 *Roseburia* spp. (Abbeele et al., 2011; Belzer & De Vos, 2012).
399 Mucus is a protective bilayer which shields the intestinal epithelia from physical, chemical and
400 biological hazards along the gastrointestinal tract (Montagne et al., 2003; Tailford et al., 2015). Mucin
401 is a polymeric glycoprotein secreted from the goblet cells in the intestinal epithelium and the major
402 structural component of mucus (Tailford et al., 2015). Mucin content in the cecal digesta of the BGS
403 group was significantly higher (p<0.05) than the CON group and similar to that of the XYO group
404 (Fig. 5-a). Abundance of *A. muciniphila* could have been a major reason for the higher mucin contents
405 in XYO and BGS groups, as it was suggested to influence the secretion of mucin from goblet cells,
406 as a part of the normal turn-over process (Tailford et al., 2015). It might have facilitated the
407 maintenance of a healthy mucus layer which was frequently refreshed (Belzer & De Vos, 2012).
408 Mucus lining is in a dynamic equilibrium between the erosion (physical, chemical and enzymatic) on
409 the luminal side and the synthesis and secretion by the goblet cells in the epithelium (Montagne et al.,
410 2003). The relationship between the dietary fiber ingestion and the increment of mucin in the intestine
411 is considered as a result of the erosion driven recovery process of mucus, caused by the physical
412 abrasion, either by insoluble dietary fiber or swollen soluble fiber (Montagne et al., 2003). Thus, the
413 highest mucin content with a similar abundance of *A. muciniphila* in the BGS group compared to the
414 XYO group, can be attributed to the differences in the physicochemical properties of the two XOS
415 sources in XYO and BGS diets (Montagne et al., 2003). For example, AXOS in BGS inherit a higher
416 inherent viscosity due to their higher molecular weight (≥2500 g/mol) and are able to improve digesta

viscosity due to both the higher molecular weight and the branched nature, which in turn causing a considerable mechanical irritation compared to small XOS (molecular weight 280-415 g/mol) in XYO (Charmet, Guillon, & Ame, 2007; Montagne et al., 2003).

3.6. BGS diet favored the abundance of the Clostridium cluster *XIVa* members in rat cecum

Another significantly abundant bacterial group in the cecal digesta of XYO and BGS fed animals was genus *Blautia* (Fig. 4-e, f). Genus *Blautia* belongs to the family *Lachnospiraceae,* one of the major bacterial groups found in the human gut microbiota, who are capable of degrading complex polysaccharides into SCFAs (Eren et al., 2015). Furthermore, these bacteria are placed within the *Clostridium coccoides* group, which is also referred to as Clostridium cluster *XIVa*, or otherwise known as a butyrate producing cluster (Bajaj et al., 2012). Yet it has been reported that *Blautia* does not produce butyrate, which was further backed by the similar butyric acid contents in XYO and BGS groups despite the higher abundance of *B. producta* in the BGS group (Eren et al., 2015). Genus *Blautia* is found to be an autochthonous genera associated with mucosa and considered to be a marker of healthy gut (Bajaj et al., 2012). In a study involving diabetic and normal Japanese subjects, genus *Blautia* was found to be negatively correlated with the prevalence of type 2 diabetes, hemoglobin A_{1C} and fasting plasma glucose levels (Inoue et al., 2017). And also *Blautia* abundance was found to be significantly lower in cancerous tissue compared to the healthy lumen, in patients with colorectal cancers (Chen et al., 2012). It has been reported that the major end products of carbohydrate metabolism by genus *Blautia* are acetate, lactate and succinate apart from hydrogen and ethanol and

known to be an acetogenic bacteria (Liu, Finegold, Song, & Lawson, 2008). Further, as implicated by the LEfSe plot (Supplementary Figure II), *B. producta*, one of the principle feature microorganisms in the BGS group, could most likely be the reason behind the observed abundantly higher acetic acid content in the BGS group (Chen et al., 2012).

Roseburia spp. another member of the Clostridium cluster *XIVa*, is a prominent butyrate producer in the colon (Louis & Flint, 2009). Members of genus *Roseburia* are famously known to degrade non-digestible carbohydrates such as resistant starch and oligosaccharides such as inulin type fructans, xylans, AXs and fucose (Louis & Flint, 2009). *R. faecis* found in higher abundance in the BGS group, is known to breakdown complex polysaccharides and predominantly produce butyrate. Significantly higher ($p<0.05$) abundance of *R. faecis* (Fig. 4-g) in the cecal digesta of BGS fed rats suggested the fact that BGS might have contained favorable butyrogenic substrates in comparison to the CON and XOS (Abbeele et al., 2011; Rivière et al., 2016; Hatziioanou, Mayer, Duncan, Flint, & Narbad, 2013). A previous study has reported that the members of genus *Roseburia* are considerably specialized in carbohydrate utilization abilities, where *R. faecis* was identified as a type 1 arabinogalactan utilizer and *R. intestinalis* as a specialized plant cell wall material degrader (Sheridan et al., 2016). The present study sheds light to the fact that *R. faecis* also might possess ability to utilize plant cell wall materials such as AXs, as implicated by its significantly very high abundance in the BGS group. Reasons for the similar butyric acid contents in the XYO and BGS groups, despite the significantly higher abundance of *R. faecis* in the BGS group were not clear. Yet, it could be due to the rapid uptake by the host cells, driven by the increased the bioavailability of butyric acid in the mucosal layers, as the

457 members of genus *Roseburia* are known to colonize the mucus layer and govern mucosal butyric acid
458 production (Abbeele et al., 2011; Rivière et al., 2016).
459
460 3.7. Ammonia-nitrogen in XYO and BGS fed rats was derived from different origins
461 Ammonia-nitrogen content was similar in both CON and BGS groups while it was significantly higher
462 (p<0.05) in the XYO group compared to the BGS group (Fig. 5-b). Ammonia in the large intestine
463 has two origins, either from hydrolysis of urea by ureases (gut microbial ureases and mucosal ureases)
464 or deamination of undigested protein and other nitrogenous substances by the gut microbiota (Vince,
465 Dawson, Park, & O'Grady, 1973). Among the members of gut microbiota, members of *Bacteroides*,
466 *Bifidobacterium*, *Clostridia*, *Proteus* and *Klebsiella* are well known to possess urease expression
467 while one of the dominant gram negative aerobic bacilli, *E. coli* is known to produce ammonia by
468 deamination of substances other than urea and considered as one of the most active ammonia
469 producers in the gut (Vince et al., 1973). It has been previously suggested that the depressive effect
470 on ammonia production by pH reduction in the colon, upon introduction of a fermentable dietary fiber
471 depends on the existing dominant origin of ammonia, either urea hydrolysis or deamination of
472 porteinous substances, where the greatest effect was found to be on the former (Vince et al., 1973).
473 Thus, it was suggested that, in XYO diet fed rats, the main source of ammonia could have been the
474 deamination of porteinous substances, as implicated by its higher ammonia-nitrogen content and
475 coliform abundance, despite the significantly lower cecal pH and higher *Bifidobacterium* abundance.

476 Generally, XOS consumption has been reported to reduce the concentration of putrefactive substances
477 as a repercussion of the bifidogenic activity associated with the reduction of putrefactive enzymes,
478 which was in contrast to the observations in the XYO group, where the ammonia-nitrogen content
479 and the abundance of bifidobateria were both significantly higher (Samanta et al., 2015). Vazquez-
480 Gutierrez et al. (2016) also had reported that *E. coli* was not affected by the incubation at low pH and
481 high organic acid concentrations produced by bifidobacteria strains in *in vitro* co-cultures, which
482 might explain the observations in the XYO group. Moreover, it has been previously reported that the
483 ammonia production in the colon was suppressed by XOS fermentation by inhibiting the enteric
484 colonization of ammonia producing anaerobes such as *Bacteroides*, whose abundance also was not
485 significantly different among the three diet groups (Supplementary Figure III-b) in the present study
486 (Aachary & Prapulla, 2011). Significantly lower ammonia-nitrogen content and lower coliform
487 abundance in BGS compared to XYO, suggested that the main origin of ammonia in the BGS group
488 could have been urea hydrolysis (Vince et al., 1973). Above fact was further backed by the lower
489 abundance of *C. perfringens* in the BGS group (Fig. 4-h), a prominent ammonia producer via urea
490 hydrolysis in gut, whose growth might have been restricted by the lower cecal pH as a result of AXOS
491 fermentation (Montagne et al., 2003; Vince et al., 1973).

492

493 3.8. BGS and XYO fed rats showcased an improved immunological barrier function

494 Another important component of the gut barrier function that defines the structure of gut microbiota,

is the host secretory immune system which limits the opportunistic invasion by the pathogenic members of the gut microbiota, in which IgA is a key player (Thursby & Juge, 2017). IgA content was significantly higher (p<0.05) in the XYO group followed by BGS, both of which had significantly higher (p<0.05) IgA contents than the CON group (Fig. 5-c). IgA is the most abundant antibody isotype in the mucosal secretions, specialized in mucosal protection (immunological factor) which involves in limiting the translocation and exposure of inherently and/or opportunistically pathogenic bacteria to the intestinal epithelium (Gutzeit, C., Magri, G. and Cerutii, 2014; Thursby & Juge, 2017). It has been reported that the prebiotics maintain immune homeostasis and improve gut barrier function by influencing the production and secretion of immunoglobulins such as IgA, justifying the observations of this study (O'Flaherty, Saulnier, Pot, & Versalovic, 2010; Thursby & Juge, 2017; Vieira et al., 2013). Protective functions of IgA encompass the maintenance of non-invasive commensals and neutralization of invasive pathogens, which was similarly observed in BGS group for the abundance of *Bifidobacterium*, coliform and *C. perfringens* compared to CON (Gutzeit, C., Magri, G. and Cerutii, 2014). But, despite the higher abundance of bifidobacteria in the XYO group, coliform abundance was in discrepancy with the immune function of IgA. Further, the mechanisms of the immune homeostasis of prebiotics are attributed to the immunomodulatory effects of the effector molecules produced by the beneficial bacteria, such as SCFAs, as observed in both XYO and BGS groups (O'Flaherty et al., 2010). Gut microbial members are well-known for their various immunomodulatory mechanisms, such as competition for niches, induction of antimicrobial peptide secretion from intestinal epithelial cells, regulation of differentiation and proliferation of epithelial

515 cells, modulation of mucus production and induction of IgA production, which can be related to the
516 observed microbial species in XYO and BGS groups such as, *Bifidobacterium*, *Blautia*, *R. faecis* and
517 *A. muciniphila* (O'Flaherty et al., 2010; Vieira et al., 2013).
518
519 **4. Conclusions**
520 The present study revealed potentials of BSF as a promising prebiotic candidate. Significantly higher
521 total SCFA, acetic acid contents and significantly lower ammonia-nitrogen content, similar cecal pH
522 and butyric acid content compared to the XYO group suggested its superiority over xylobiose and
523 xylotriose, well-characterized prebiotic substrates. Moreover, significantly higher mucin and IgA
524 contents in the BGS group reflected its protective gut physical and immunological barrier functions
525 compared to XYO and CON, respectively. Bacterial structure was clearly different between the XYO
526 and BGS groups as reflected by the differences in microbial relative abundances, where the BGS
527 group exhibited a higher diversity and richness compared to XYO group. BSF fermentation in the
528 BGS group clearly influenced different groups of microbiota, for example *B. producta*, *R. faecis*, in
529 contrast to the *Bifidobacterium* species in XYO group. Thus, the upregulated metabolic pathways
530 could have been different between the two substrates. Future studies are recommended to characterize
531 the underlying metabolic pathways responsible for the unique differences observed between the two
532 XOS substrates used in this study.
533
534 **5. Acknowledgements**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

6. Appendix

Supplementary material

7. Conflict of interest

Authors declare no conflict of interest.

8. References

Aachary, A. A., & Prapulla, S. G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. *Comprehensive Reviews in Food Science and Food Safety*, *10*, 2–16. https://doi.org/10.1111/j.1541-4337.2010.00135.x

Abbeele, P. Van Den, Gérard, P., Rabot, S., Bruneau, A., Aidy, S. El, Derrien, M., & Possemiers, S. (2011). Arabinoxylans and inulin differentially modulate the mucosal and luminal gut microbiota and mucin-degradation in humanized rats. *Environmental Microbiology*, *13*, 2667–2680. https://doi.org/10.1111/j.1462-2920.2011.02533.x

Bajaj, J. S., Hylemon, P. B., Ridlon, J. M., Heuman, D. M., Daita, K., White, M. B., & Gillevet, P. M. (2012). Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic

555 encephalopathy and is linked to cognition and inflammation. *AJP: Gastrointestinal and Liver*
556 *Physiology*, *303*, G675–G685. https://doi.org/10.1152/ajpgi.00152.2012
557 Belzer, C., & De Vos, W. M. (2012). Microbes insidefrom diversity to function: The case of
558 Akkermansia. *ISME Journal*, *6*, 1449–1458. https://doi.org/10.1038/ismej.2012.6
559 Bovee-Oudenhoven, I. M. J., Termont, D. S. M. L., Heidt, P. J., & Van Der Meer, R. (1997). Increasing
560 the intestinal resistance of rats to the invasive pathogen Salmonella enteritidis: Additive effects
561 of dietary lactulose and calcium. *Gut*, *40*, 497–504. https://doi.org/10.1136/gut.40.4.497
562 Brienzo, M., Carvalho, A. F. A., de Figueiredo, F. C., & Neto, P. de O. (2016). Sugarcane bagasse
563 hemicellulose properties , extraction technologies. In G. L Riley (Ed.), *Food Waste: Practices,*
564 *Management and Challenges* (pp. 155-164). New York: Nova Science Publishers Inc.
565 Carlson, J. L., Erickson, J. M., Hess, J. M., Gould, T. J., & Slavin, J. L. (2017). Prebiotic dietary fiber
566 and gut health: Comparing the in vitro fermentations of beta-glucan, inulin and
567 xylooligosaccharide. *Nutrients*, *9*, 1361-1378. https://doi.org/10.3390/nu9121361
568 Charmet, G., Guillon, F., & Ame, U. M. R. (2007). Wheat arabinoxylans : Exploiting variation in
569 amount and composition to develop enhanced varieties, *46*, 261–281.
570 https://doi.org/10.1016/j.jcs.2007.06.014
571 Chen, W., Liu, F., Ling, Z., Tong, X., & Xiang, C. (2012). Human intestinal lumen and mucosa-
572 associated microbiota in patients with colorectal cancer. *PLoS ONE*, *7*, e39743.
573 https://doi.org/10.1371/journal.pone.0039743
574 Crowther, Roger S. and Wetmore, R. F. (1987). Fluorometric assay of O-linked glycoproteins by

reaction with 2-cyanoacetamide. *Analytical Biochemistry*, *163*, 170–174. https://doi.org/10.1016/0003-2697(87)90108-4

Eren, A. M., Sogin, M. L., Morrison, H. G., Vineis, J. H., Fisher, J. C., Newton, R. J., & McLellan, S. L. (2015). A single genus in the gut microbiome reflects host preference and specificity. *ISME Journal*, *9*, 90–100. https://doi.org/10.1038/ismej.2014.97

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., & Tobe, T. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, *469*, 543–547. https://doi.org/10.1038/nature09646

Giuberti, G., Gallo, A., Moschini, M., & Masoero, F. (2013). In vitro production of short-chain fatty acids from resistant starch by pig faecal inoculum. *Animal*, 7, 1446–1453. https://doi.org/10.1017/S1751731113001092

Gobinath, D., Madhu, A. N., Prashant, G., Srinivasan, K., & Prapulla, S. G. (2010). Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, *104*, 40–47. https://doi.org/10.1017/S0007114510000243

Gupta, G., & Bhatnagar, R. (2015). A Review on Composition and Properties of Bagasse Fibers. *International Journal of Scientific & Engineering Research*, *6*, 143–148.

Gutzeit, C., Magri, G. and Cerutii, A. (2014). Intestinal IgA production and its role in host-microbe interaction. *Immunological Reviews*, *260*, 1–23. https://doi.org/10.1088/1367-2630/15/1/015008.Fluid

Han, Kyu-Ho, Lee, Chang-Hyun, Kinoshita, Mikio, Oh, Chan-Ho, Shimada, Ken-ichiro and

Fukushima, M. (2016). Spent turmeric reduces fat mass in rats fed a high-fat diet. *Food and Function*, *7*, 1814–1824. https://doi.org/10.1039/C5FO00764J

Hatziioanou, D., Mayer, M. J., Duncan, S. H., Flint, H. J., & Narbad, A. (2013). A representative of the dominant human colonic Firmicutes, Roseburia faecis M72/1, forms a novel bacteriocin-like substance. *Anaerobe*, *23*, 5–8. https://doi.org/10.1016/j.anaerobe.2013.07.006

Hsu, C.-K., Liao, J.-W., Chung, Y.-C., Hsieh, C.-P., & Chan, Y.-C. (2004). Nutrition and Cancer Xylooligosaccharides and Fructooligosaccharides Affect the Intestinal Microbiota and Precancerous Colonic Lesion Development in Rats 1. *J. Nutr*, *134*, 1523–1528. https://doi.org/10.1093/jn/134.6.1523

Inoue, R., Kitano, R. O., Tsukahara, T., Tanaka, M., Masuda, S., & Inoue, T. (2017). functional profiles of gut microbiota from 16S rRNA metagenomic data provides a more robust evaluation of gut dysbiosis occurring in Japanese type 2 diabetic patients. *Journal of Clinical Biochemistry and Nutrition*, *61*, 217–221. https://doi.org/10.3164/jcbn.17

Liu, C., Finegold, S. M., Song, Y., & Lawson, P. A. (2008). Reclassification of Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoides gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov., Blautia producta comb. nov., Blautia schinkii comb. nov. and description of Blautia wexlerae . *International Journal of Systematic and Evolutionary Microbiology*, *58*, 1896–1902. https://doi.org/10.1099/ijs.0.65208-0

615 Louis, P., & Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing
616 bacteria from the human large intestine. *FEMS Microbiology Letters*, *294*, 1–8.
617 https://doi.org/10.1111/j.1574-6968.2009.01514.x
618 Lozupone, C., & Knight, R. (2005). UniFrac : a new phylogenetic method for comparing microbial
619 communities. *Applied and Environmental Microbiology*, *71*, 8228–8235.
620 https://doi.org/10.1128/AEM.71.12.8228
621 Madhukumar, M. S., & Muralikrishna, G. (2012). Fermentation of xylo-oligosaccharides obtained
622 from wheat bran and Bengal gram husk by lactic acid bacteria and bifidobacteria. *Journal of*
623 *Food Science and Technology*, *49*, 745–752. https://doi.org/10.1007/s13197-010-0226-7
624 Montagne, L., Pluske, J. R., & Hampson, D. J. (2003). A review of interactions between dietary fibre
625 and the intestinal mucosa, and their consequences on digestive health in young non-ruminant
626 animals. *Animal Feed Science and Technology*, *108*, 95–117. https://doi.org/10.1016/S0377-
627 8401(03)00163-9
628 O'Callaghan, A., & van Sinderen, D. (2016). Bifidobacteria and their role as members of the human
629 gut microbiota. *Frontiers in Microbiology*, *7*, Article 925.
630 https://doi.org/10.3389/fmicb.2016.00925
631 O'Flaherty, S., Saulnier, D. M., Pot, B., & Versalovic, J. (2010). How can probiotics and prebiotics
632 impact mucosal immunity? *Gut Microbes*, *1*, 293–300.
633 https://doi.org/10.4161/gmic.1.5.XXXXX
634 Ottman, N., Geerlings, S. Y., Aalvink, S., de Vos, W. M., & Belzer, C. (2017). Action and function of

635 Akkermansia muciniphila in microbiome ecology, health and disease. *Best Practice and*
636 *Research: Clinical Gastroenterology*, *31*, 637–642. https://doi.org/10.1016/j.bpg.2017.10.001
637 Rivière, A., Selak, M., Lantin, D., Leroy, F., & De Vuyst, L. (2016). Bifidobacteria and butyrate-
638 producing colon bacteria: Importance and strategies for their stimulation in the human gut.
639 *Frontiers in Microbiology*, *7*, Article 979. https://doi.org/10.3389/fmicb.2016.00979
640 Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., & Sridhar, M. (2015).
641 Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications.
642 *Bioactive Carbohydrates and Dietary Fibre*, *5*, 62–71.
643 https://doi.org/10.1016/j.bcdf.2014.12.003
644 Sheridan, P. O., Martin, J. C., Lawley, T. D., Browne, H. P., Harris, H. M. B., Bernalier-donadille, A.,
645 & D, H. J. F. (2016). Polysaccharide utilization loci and nutritional specialization in a dominant
646 group of butyrate-producing human colonic Firmicutes. *Microbial Genomics*, *2*, e000043.
647 https://doi.org/10.1099/mgen.0.000043
648 Tailford, L. E., Crost, E. H., Kavanaugh, D., & Juge, N. (2015). Mucin glycan foraging in the human
649 gut microbiome. *Frontiers in Genetics*, *5*, Article 81. https://doi.org/10.3389/fgene.2015.00081
650 Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, *474*,
651 1823–1836. https://doi.org/10.1042/BCJ20160510
652 Vazquez-Gutierrez, P., de Wouters, T., Werder, J., Chassard, C., & Lacroix, C. (2016). High iron-
653 sequestrating bifidobacteria inhibit enteropathogen growth and adhesion to intestinal epithelial
654 cells in vitro. *Frontiers in Microbiology*, *7*, 1–12. https://doi.org/10.3389/fmicb.2016.01480

Vieira, A. T., Teixeira, M. M., & Martins, F. S. (2013). The role of probiotics and prebiotics in inducing gut immunity. *Frontiers in Immunology*, *4*, 1–12. https://doi.org/10.3389/fimmu.2013.00445

Vince, A., Dawson, A. M., Park, N., & O'Grady, F. (1973). Ammonia production by intestinal bacteria. *Gut*, *14*, 171–177. https://doi.org/10.1136/gut.14.3.171

Warren, F. J., Fukuma, N. M., Mikkelsen, D., Flanagan, B. M., Williams, B. A., Lisle, A. T., & Gidley, M. J. (2018). Food Starch Structure Impacts Gut Microbiome Composition. *Molecular Biology and Physiology*, *3*, 1–13. https://doi.org/10.1128/mSphere.00086-18.

Yu, Z., & Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Bio Techniques*, *36*, 3–6.

Zakrzewski, M., Proietti, C., Ellis, J. J., Hasan, S., Brion, M. J., Berger, B., & Krause, L. (2017). Calypso: A user-friendly web-server for mining and visualizing microbiome-environment interactions. *Bioinformatics*, *33*, 782–783. https://doi.org/10.1093/bioinformatics/btw725

9. Figure captions

Figure 1. (a) Anaerobic and (b) Coliform counts in the rat cecal digesta.

Values presented are mean ± SE. a, b stand for significant differences at (p<0.05) as determined by Tukey's test. Abbreviations: CON: control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS: sugarcane bagasse soluble fiber (BSF) diet.

675 **Figure 2.** (a) Boxplot of Shannon's diversity (*H*) index and (b) PCoA plot for the β-diversity of the microbial
676 community data.
677 The red line within each box depict the average value of *H* index in (a). (**p<0.001). Abbreviations: CON:
678 control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS: sugarcane bagasse soluble fiber (BSF)
679 diet.
680
681 **Figure 3.** (a) Unclustered bar chart for microbial composition in each diet group at phylum level (b) Rank test
682 bar chart for relative abundance of microbial taxa at phylum level (c) Linear discriminant analysis (LDA) effect
683 size (LEfSe) plot at phylum level. Statistical significance was determined by Calypso (version 8.72) (*p<0.05;
684 **p<0.01). Abbreviations: CON: control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS:
685 sugarcane bagasse soluble fiber (BSF) diet.
686
687 **Figure 4.** Rank test bar charts for the relative abundance of selected microbial species in the rat cecal digesta.
688 Relative abundances of (a) *B. pseudolongum* (b) *B. adolescentis* (c) unclassified *Bifidobacterium* (d) *A.*
689 *muciniphila* (e) *B. producta* (f) unclassified *Blautia* (g) *R. faecis* and (h) *C. perfringens* in the three diet groups
690 are presented here. Statistical significance was determined by Calypso (version 8.72) (*p<0.05; **p<0.01).
691 Abbreviations: CON: control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS: sugarcane
692 bagasse soluble fiber (BSF) diet.
693
694 **Figure 5.** (a) Mucin content, (b) Ammonia-nitrogen content and (c) IgA content in the rat cecal digesta.

695 Values presented are mean ± SE. a, b and c stand for significant differences at (p<0.05) as determined by
696 Tukey's test. Abbreviations: CON: control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS:
697 sugarcane bagasse soluble fiber (BSF) diet.
698
699
700 **Supplementary Figure I.** HPLC chromatograms of the SCFA profiles of cecal digesta.
701 (a) Standard curve (b) control diet (CON) (c) commercial xylo-oligosaccharide (XOS) diet (XYO) and (d)
702 sugarcane bagasse soluble fiber (BSF) diet (BGS)
703 Abbreviations: AU; area under the curve, P1, P2 and P3; peaks 1, 2 and 3.
704 P1; acetic acid, P2; propionic acid, P3; butyric acid
705
706 **Supplementary Figure II.** Linear discriminant analysis (LDA) effect size (LEfSe) plot at species level.
707 Abbreviations: CON: control diet; XYO: commercial xylo-oligosaccharide (XOS) diet; BGS: sugarcane
708 bagasse soluble fiber (BSF) diet.
709
710 **Supplementary Figure III.** Rank test bar charts for the relative abundance of selected microbial genera in the
711 rat cecal digesta.
712 Relative abundance of (a) *Clostridium* (b) *Bacteroides* (c) unclassified *Holdemania* (d) unclassified *Sutterella*
713 (e) unclassified *Erysipelotrichaceae* (f) unclassified *Coriobacteriaceae* (g) unclassified *Adlercreutzia* and (h)
714 unclassified *Enterobacteriaceae* in the three diet groups are presented here. Statistical significance was

715 determined by Calypso (version 8.72) (*p<0.05; **p<0.01). Abbreviations: CON: control diet; XYO:

716 commercial xylo-oligosaccharide (XOS) diet; BGS: sugarcane bagasse soluble fiber (BSF) diet.

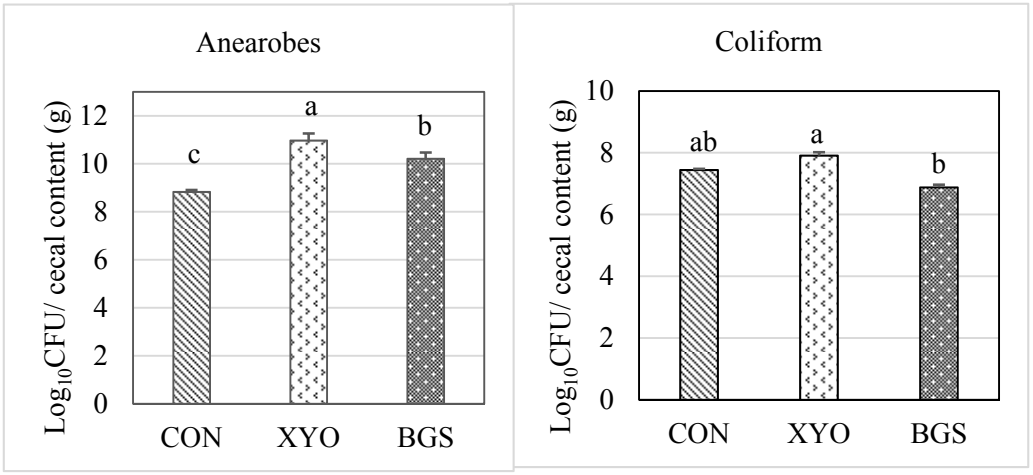
Table 1

Cumulative feed intake, body weight gain, final body weight, cecal parameters and short chain fatty acid (SCFA) contents of the rats fed CON, XYO and BGS diets for 28 days.

Parameter	CON			XYO			BGS		
FI (g/28 days)	379.00 ± 7.61	a		351.00 ± 4.61	b		363.00 ± 5.16	ab	
BWG (g/28 days)	68.20 ± 3.77	ns		60.30 ± 2.31	ns		61.00 ± 2.93	ns	
FBW (g)	237.81 ± 4.16	ns		230.14 ± 2.99	ns		231.11 ± 2.73	ns	
CW (g)	3.40 ± 0.38	b		4.53 ± 0.23	a		4.23 ± 0.23	ab	
CCW (g)	2.81 ± 0.30	ns		3.68 ± 0.22	ns		3.49 ± 0.21	ns	
CTW (g)	0.59 ± 0.09	b		0.85 ± 0.03	a		0.74 ± 0.05	ab	
pH	7.69 ± 0.06	a		6.66 ± 0.08	b		6.58 ± 0.07	b	
Acetic acid (µmol/CC)	228.60 ± 21.15	c		387.20 ± 36.00	b		625.40 ± 61.77	a	
Propionic acid (µmol/CC)	30.40 ± 2.48	b		56.90 ± 8.10	a		47.10 ± 5.47	ab	
Butyric acid (µmol/CC)	10.90 ± 1.92	b		30.40 ± 2.98	a		33.40 ± 4.01	a	
Total SCFA (µmol/CC)	269.82 ± 24.80	c		474.40 ± 42.71	b		705.91 ± 69.10	a	

Abbrev: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF diet); FI, cumulative feed intake; BWG, body weight gain; FBW, final body weight; CW, cecal weight; CCW, cecal content weight; CTW, cecal tissue weight; CC (within parenthesis), cecal content; ns, not significant

Values presented are mean±SE (n=6); values followed by different lowercase letters are significantly ($p<0.05$) different.



(a)

Fig. 1. (a) Anaerobic and (b) Coliform counts in the rat cecal digesta. Values presented are mean Å} SE. a, b stand for significant differences at ($p < 0.05$) as determined by Tukey's test. Abbreviations: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF) diet.

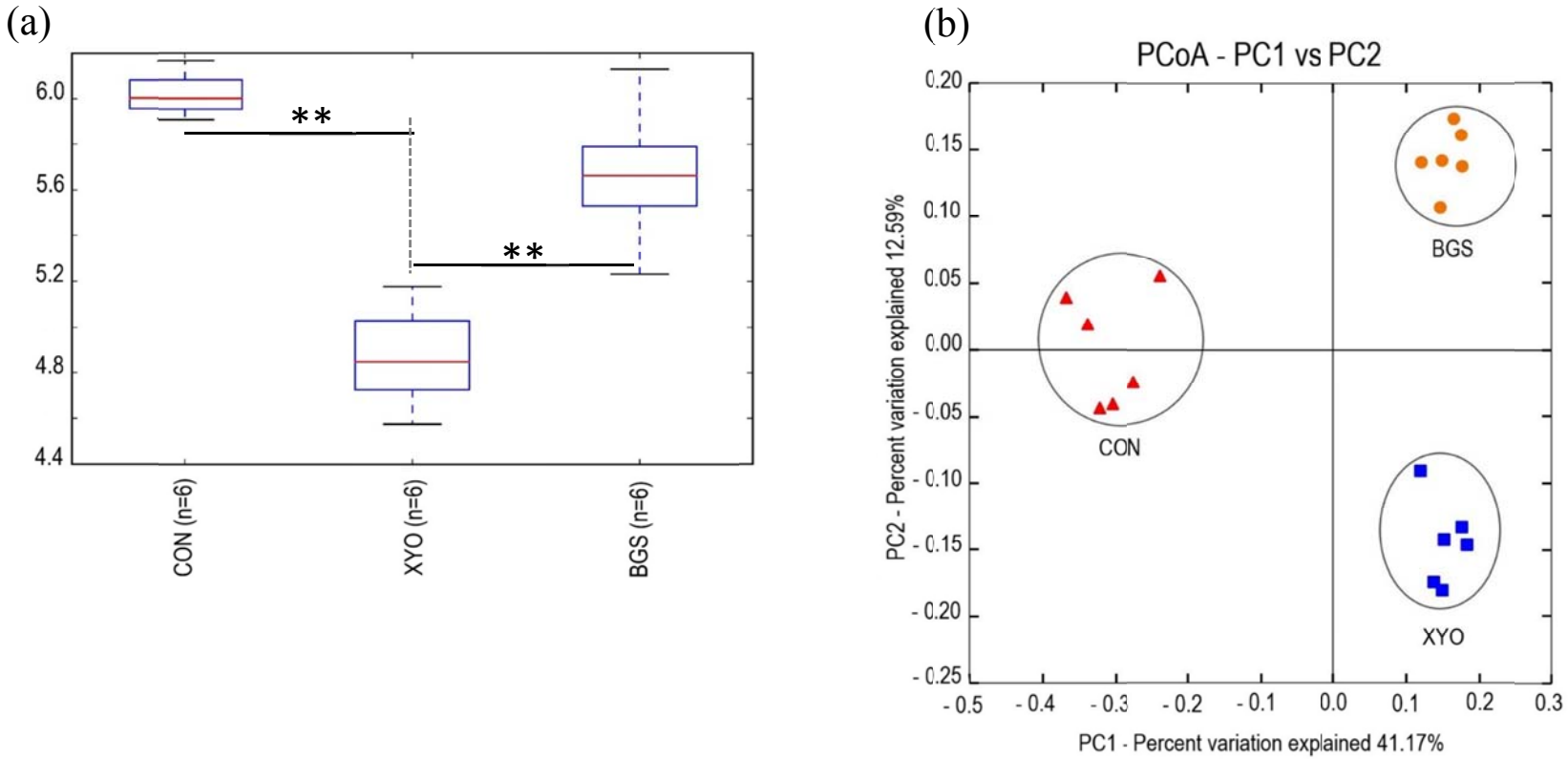


Fig. 2. (a) Boxplot of Shannon's diversity (H) index and (b) PCoA plot for the β-diversity of the microbial community data. The red line in each box depict the average value of H index in (a). Statistical significance was determined by Dunn's multiple comparisons test (post hoc Kruskal-Wallis H test) in Prism (version 7.0a) (**$p < 0.01$). β-diversity was determined by the weighted UniFrac distance metric in QIIME. Abbreviations: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF) diet.

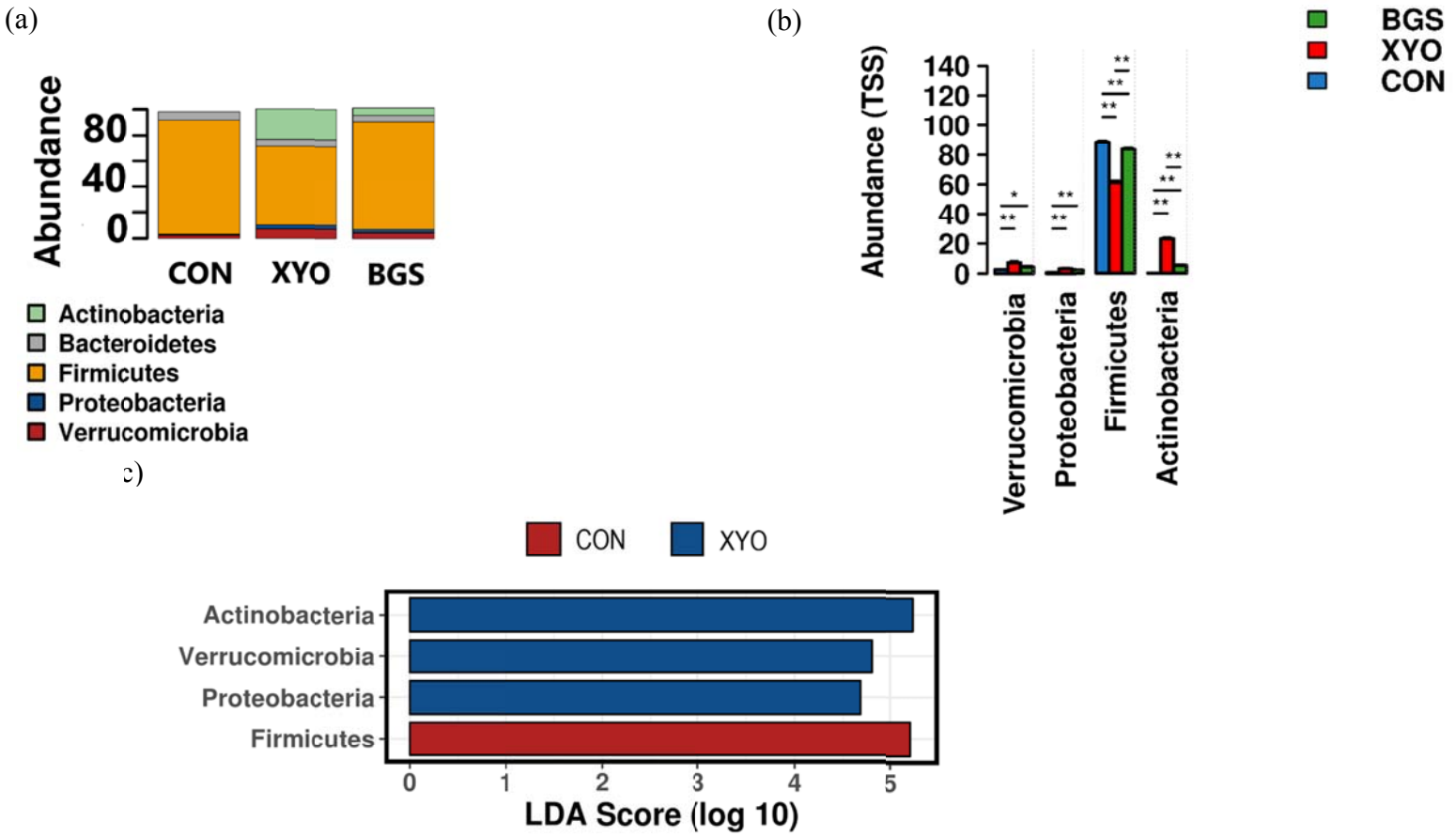
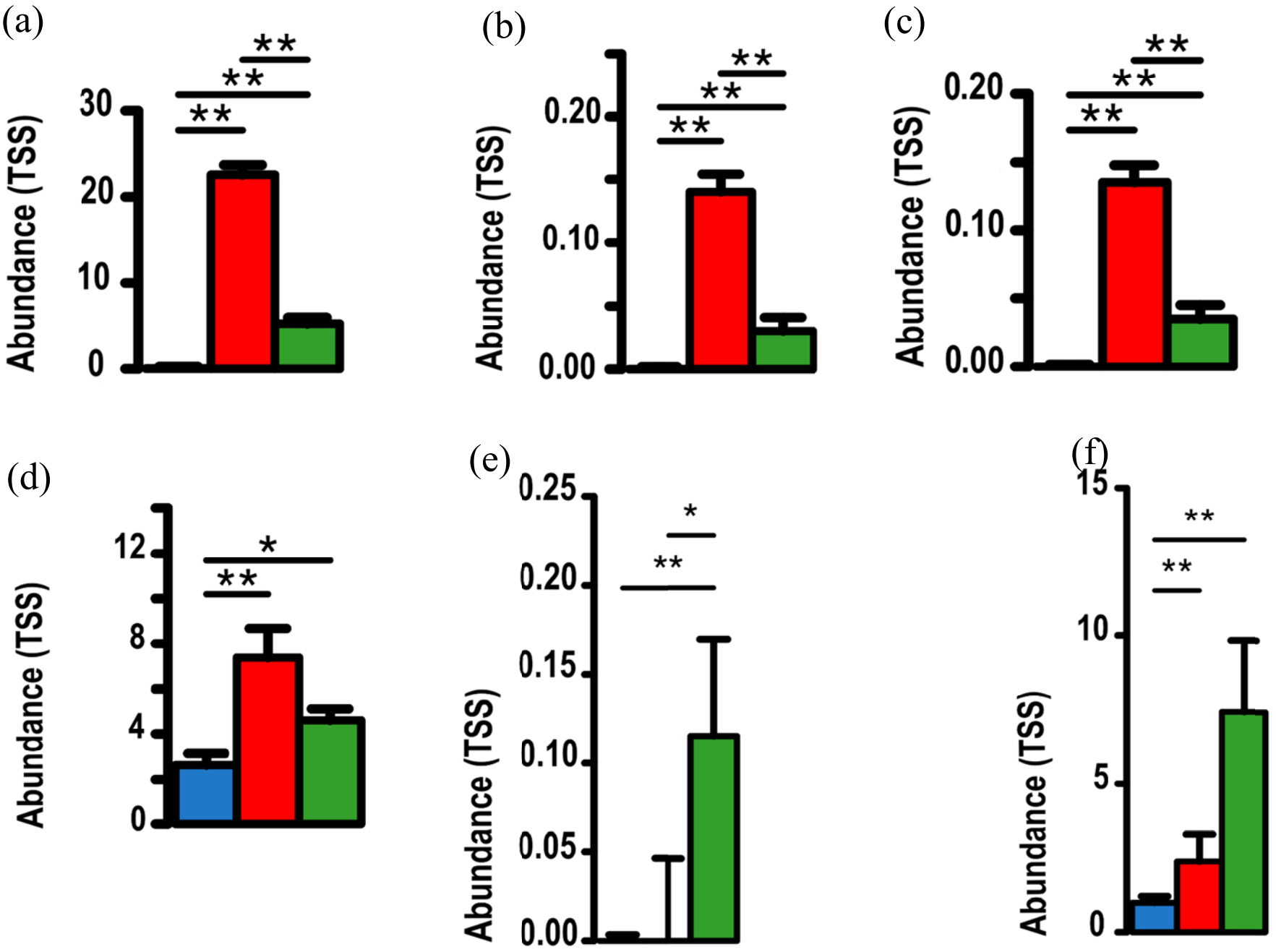


Fig. 3. (a) Unclustered bar chart for microbial composition in each diet group at phylum level (b) Rank test bar chart for relative abundance of microbial taxa at phylum level (c) Linear discriminant analysis (LDA) effect size (LEfSe) plot at phylum level. Statistical significance was determined by Kruskal-Wallis H test in Calypso (version 8.72) (*$p < 0.05$; **$p < 0.01$). Abbreviations: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF) diet.


(a)
Abundance (TSS)
30
20
10
0
**
**
**
(b)
Abundance (TSS)
0.20
0.10
0.00
**
**
**
(c)
Abundance (TSS)
0.20
0.10
0.00
**
**
**
(d)
Abundance (TSS)
12
8
4
0
**
*
(e)
Abundance (TSS)
0.25
0.20
0.15
0.10
0.05
0.00
**
*
(f)
Abundance (TSS)
15
10
5
0
**
**

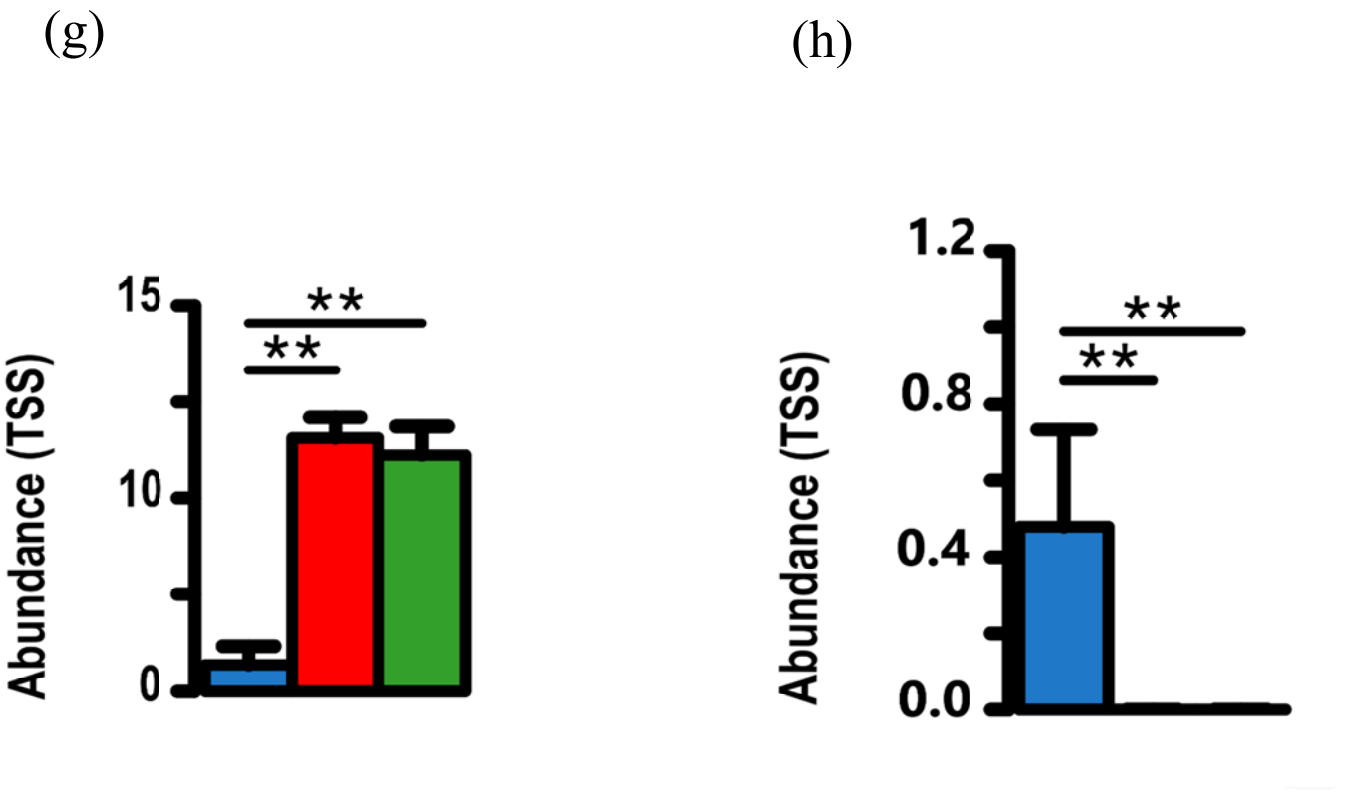


Fig. 4. Rank test bar charts for the relative abundance of selected microbial species in the rat cecal digesta. Relative abundances of (a) B. pseudolongum (b) B. adolescentis (c) unclassified Bifidobacterium (d) A. muciniphila (e) B. producta (f) unclassified Blautia (g) R. faecis and (h) C. perfringens in the three diet groups are presented here. Statistical significance was determined by Kruskal-Wallis H test in Calypso (version 8.72) (*$p < 0.05$; **$p < 0.01$). Abbreviations: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF) diet.

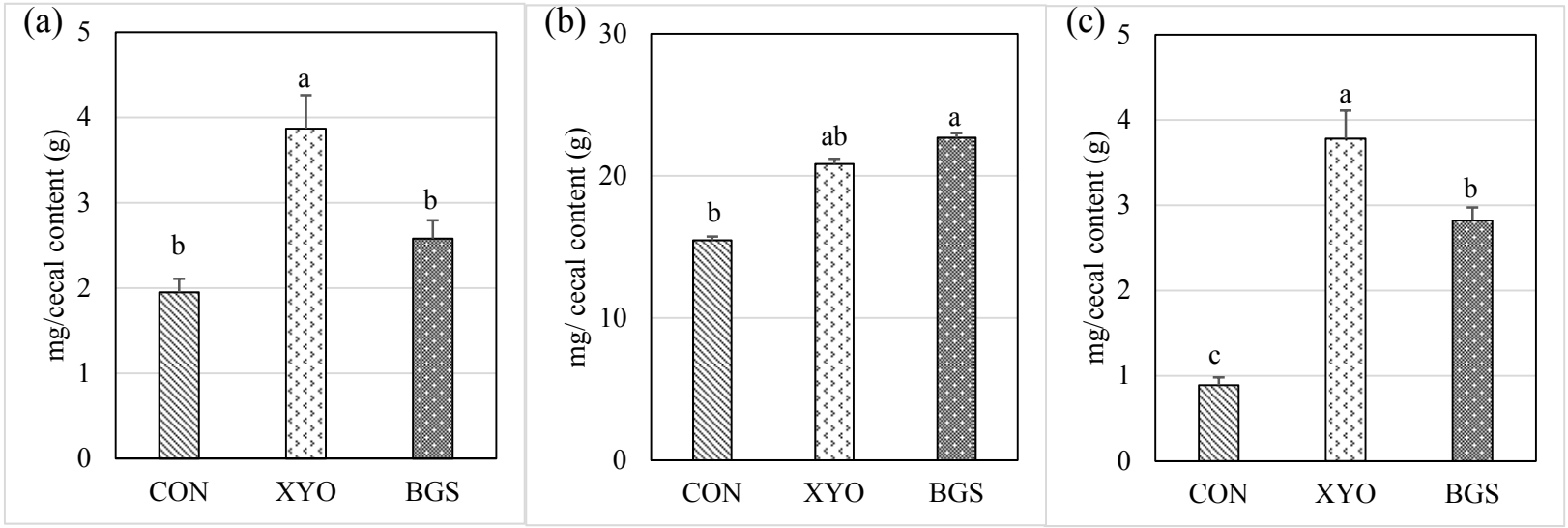


Fig. 5. (a) Mucin content, (b) Ammonia-nitrogen content and (c) IgA content in the rat cecal digesta. Values presented are mean Å} SE. a–c stand for significant differences at ($p < 0.05$) as determined by Tukey's test. Abbreviations: CON, control diet; XYO, commercial xylo-oligosaccharide (XOS) diet; BGS, sugarcane bagasse soluble fiber (BSF) diet.