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1 **Microbiota of healthy dogs demonstrate a significant decrease in richness and**
2 **changes in specific bacterial groups in response to supplementation with resistant**
3 **starch, but not psyllium or methylcellulose, in a randomized cross-over trial.**

4 Silke Salavati Schmitz^{1§}, Jorge Perez-Accino Salgado^{1*}, Laura Glendinning²,

5 ¹ Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, College of Medicine
6 and Veterinary Medicine, University of Edinburgh, Easter Bush Campus, Midlothian, EH25
7 9RG, UK

8 ² The Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG,
9 UK

10 * This author's current affiliation is: Hospital Canis, Carrer Can Pau Birol 38, 17006 Girona,
11 Spain

12 § corresponding author:

13 Prof. Silke Salavati Schmitz

14 University of Edinburgh, College of Medicine and Veterinary Medicine

15 Hospital for Small Animals, Royal (Dick) School of Veterinary Studies

16 Easter Bush Campus, Midlothian, EH25 9RG, UK

17 Telephone: +44(0)131-6507650

18 Fax: +44(0)131-6507672

19 Email: Silke.Salavati@ed.ac.uk

20

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23 **Abstract**

24 Even though dietary fibers are often used as prebiotic supplements in dogs, the effect of
25 individual types of fibers on canine microbiota composition is unknown. The objective of this
26 study was to assess changes in fecal microbiota richness, diversity and taxonomic
27 abundance with 3 different fiber supplements in dogs. These were psyllium husk, resistant
28 starch from banana flour and methylcellulose. They were administered to 17 healthy dogs in
29 a cross-over trial after transition to the same complete feed. Fecal scores and clinical activity
30 indices were recorded, and fecal samples collected before and at the end of
31 supplementation, as well as 2 weeks after each supplement (washout). Illumina NovaSeq
32 paired-end 16S rRNA gene sequencing was performed on all samples. After quality control
33 and chimera removal, alpha diversity indices were calculated with QIIME. Differences in
34 specific taxa between groups were identified using Metastats. Methylcellulose significantly
35 increased fecal scores but had no effect on microbiota. Psyllium resulted in minor changes
36 of specific taxa abundance, but with questionable biological significance. Resistant starch
37 reduced microbiota richness and resulted in the most abundant changes in taxa, mostly a
38 reduction in short-chain fatty acid producing genera of the Bacillota phylum, with an increase
39 in genera within the Bacteroidota, Pseudomonadota, Actinomycetota and Saccharibacteria.
40 In conclusion, while psyllium and methylcellulose led to few changes in the microbiota
41 composition, the taxonomic changes seen with resistant starch may indicate a less favorable
42 composition. Based on this, the type of resistant starch used here cannot be recommended
43 as a prebiotic in dogs.

44

45 **Data summary:**

46 The paired-read fastq data that support the findings of this study have been deposited in the
47 European Nucleotide Archive with the accession code PRJEB67805
48 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB67805>). For the purpose of open access, the
49 authors have applied a Creative Commons Attribution (CC BY) license to any Author
50 Accepted Manuscript version arising from this submission.

51

52 **Introduction**

53 A number of health benefits are associated with the consumption of dietary fiber (DF),
54 including compositional as well as functional changes of the intestinal microbiota (IM); for
55 example plant and carbohydrate rich diets result in increased IM richness and bacterial short
56 chain fatty acid (SCFA) production (1).

57 A low intake of DF does not only lead to reduced IM diversity, but also shifts the gut
58 microbial metabolism away from SCFAs towards less favorable bacterial metabolites, often
59 derived from amino acids (e.g. branched-chain fatty acids, ammonia, phenolic and indolic
60 compounds and hydrogen sulfide), which can be detrimental to host health (1). The cytotoxic
61 and pro-inflammatory nature of these metabolites contributes to the development of chronic
62 diseases, particularly an increased prevalence of inflammatory bowel disease (IBD) and
63 colorectal cancer (2).

64 A number of DFs are also considered prebiotics, defined as “a substrate that is selectively
65 utilized by host microorganisms conferring a health benefit” (3). DFs are additionally
66 classified by their physicochemical properties (water solubility and viscosity), as well as their
67 fermentability. In most cases, soluble fiber types are fermented for example into short chain
68 fatty acids (SCFAs) more quickly than insoluble types (1).

69 Chemically, DFs can be divided into 3 main classes: 1) non-starch polysaccharides (NSPs),
70 which includes non-fermentable fibers like psyllium seed husk [*Plantago ovata*] (PSY), 2)
71 resistant (non-digestible) oligosaccharides (for example fructo-oligosaccharides (4)); and 3)
72 resistant starch (RS), for example granular starches from green bananas (5).

73 There is evidence that supplementation with specific DF restores some of the health benefits
74 they can confer. Different types of RS (6), cellulose (7), and PSY have specific effects on the
75 IM. For example, RS has been shown to enrich *Bifidobacteria*, *Ruminococcus* and other
76 beneficial bacteria (1). PSY supplementation induces distinct IM community changes,
77 including higher relative abundance of *Bacteroides* and *Parabacteroides* (fiber-digesting
78 bacterial groups) up to 70% of the total IM bacteria, with a matching increase in SCFA
79 production; and reduction of *Enterobacteriaceae* and *Pseudomonadaceae* (8). Even the
80 nonfermentable fiber methylcellulose (MTC) has been shown to modulate IM composition
81 and diversity as well as fecal bile acid metabolism and prevent weight gain in mice fed a high
82 fat diet (9).

83 Not only do companion animals like dogs have a similar IM composition and richness to
84 people (10,11), as they have co-evolved to be able to digest similar food (12) and can hence
85 serve as appropriate model for IM related interventions; in “industrialized” populations, they
86 also suffer from similar emerging diseases, including chronic inflammatory gut conditions like
87 IBD (11,13). Different DFs (especially PSY) are commonly used in canine feed or given as
88 supplements to individual pet dogs for a variety of spontaneously occurring intestinal
89 conditions (e.g. “fiber-responsive” colitis (14), irritable bowel syndrome (15)), but very little is
90 known about the effect of specific DF on dogs’ IM composition or function. One study
91 showed that PSY results in a significant increase of the SCFAs propionate and n-butyrate in
92 fecal samples of dogs after 15 days of supplementation (16). In addition, PSY might protect
93 against colitis via activation of bile acid receptors in intestinal epithelial cells (17), which

94 could be particularly relevant, as severely altered fecal bile acid metabolism has recently
95 been identified as a hallmark of canine IBD (18).

96 The goal of this study was to investigate the effect of 3 commonly used DF types on the IM
97 composition and richness of healthy dogs to assess their potential health benefits and create
98 evidence-base for their use as prebiotics in both dogs and potentially people.

99 **Methods**

100 Animals, feeding interventions and clinical scores

101 Dogs recruited for the study were privately owned pets and deemed healthy based on the
102 absence of clinical signs and normal physical examination findings (table S1). They all had
103 to be regularly wormed and treated for ectoparasites, and not have travelled abroad. Dogs
104 on a prescription diet or any regular medication or supplements were excluded. Owners
105 were asked to collect a freshly voided fecal sample (day -14) before transitioning all dogs to
106 the same commercial complete dry dog food (Hill's Science Plan Advanced Fitness medium
107 adult tuna and rice) at 1.4-1.8 RER depending on lifestyle for 2 weeks, after which another
108 fecal sample was collected (day 0, baseline). After that, dogs were maintained on the same
109 diet and randomized to receive each of the 3 study fiber supplements for a duration of 2
110 weeks, followed by a 2-week washout period before the next supplement (figure 1).

111 Throughout the duration of the study, the dogs' diet was not permitted to be changed. Fresh
112 fecal samples were collected on the last day of each supplementation and washout period
113 (day 14, 28, 42, 56, 70, and 84). Owners were asked to keep a diary, that included daily
114 confirmation of supplement administration (which were labelled A, B and C in otherwise plain
115 dispensing bags), and scoring of each naturally voided fecal sample's consistency using a
116 pictorial template of a well-established tool (Purina Fecal Score; PFS), where score 1
117 represents very hard and dry feces to score 7, which represents watery feces with no
118 texture. In addition, owners were asked to fill in a validated questionnaire, the Canine IBD

119 Activity Index (CIBDAI (19)) at day -14 and 0, on the last day of each supplement (day 14,
120 42, and 70) and after a final washout of 2 weeks (end of study, day 84).

121 DF supplements used were commercially available food-grade additives, namely PSY husk
122 (Colon Care Plus, Holland & Barrett, UK), a resistant starch (Green Banana Flour; Natural
123 Evolution, UK) and MTC (Methocel, SpecialIngredients, UK). Dosing was identical for all 3
124 DFs, twice daily and based on individual dog's body weight: dogs < 5 kg received 2 g, dogs
125 5-10 kg 4 g, dogs >10-30 kg 8 g, dogs >30-50 kg 12 g and dogs > 50 kg 16 g of each
126 supplement. This dosage was derived from empirically available doses for PSY (20).
127 Supplements were advised to be given with the normal food ration and using a specific
128 measuring spoon provided with specific dosing instructions for each supplement and dog to
129 allow administration of the correct amount.

130 Fecal DNA extraction, 16S rRNA gene amplification, and sequencing

131 Fecal samples were aliquoted into sterile 5 ml Bijoux tubes within 60 minutes of receipt at
132 the hospital and stored at -80° C until the time of analysis. DNA extraction was performed as
133 described previously (21) using the DNeasy PowerLyzer PowerSoil Kit (Qiagen).

134 Extracted genomic DNA was sent to a commercial service provider (Novogene, Cambridge,
135 UK; www.novogene.com) for 16S rRNA gene PCR amplification, DNA sample quality
136 control, amplicon library preparation and Illumina NovaSeq paired-end sequencing with 30x
137 coverage. Briefly, DNA concentration and purity was assessed on 1% agarose gels and
138 DNA diluted to 1 ng/ml. The V4 region of the 16S rRNA was amplified using the primers
139 GTGCCAGCMGCCGCGGTAA and GGACTACHVGGGTWTCTAAT with barcodes. All PCR
140 reactions were carried out with Phusion High-Fidelity PCR Master Mix (New England
141 Biolabs), resulting in an amplicon of ~300 bp in size. PCR products were mixed at equal
142 density ratios, purified with Qiagen Gel Extraction kit, and the library generated with
143 NEBNext® Ultra DNA Library Prep kit for Illumina (quantified via Qubit and q-PCR).

144 Sequence data processing, OTU clustering, taxonomic annotation and diversity analysis

145 Primer sequences were removed, then paired-end reads were merged using FLASH
146 (v.1.2.7) (22). Quality filtering was used to obtain high-quality clean reads using the
147 split_libraries_fastq.py command from Qiime (v.1.7.0) (23,24). Chimeras were detected
148 using UCHIME (25) with the SILVA reference database (v.138.1) (26), then removed. OTU
149 clustering at $\geq 97\%$ similarity was performed using UPARSE (v.7.0.1090). A representative
150 sequence for each OTU underwent taxonomic assignment, using the Qiime (v.1.7.0)
151 command assign_taxonomy.py (mothur method) with the SILVA Database (26).

152 Statistical analysis

153 OTU counts were normalized by subsampling to the sample with the least counts (39,442
154 reads). Relative abundance values are reported throughout as a value between 0 and 1,
155 where 0 indicated the complete absence of the taxa and 1 indicates that the taxa is 100%
156 abundant. Subsequent alpha and beta diversity analyses were all performed on these
157 normalized counts. Alpha diversity indices, including Observed-species (OTUs), Chao1 and
158 Shannon, were calculated with QIIME (v.1.7.0). Significant differences in specific taxa
159 between groups were identified using Metastats (27), with the Benjamini and Hochberg
160 False Discovery Rate (27) used for correcting for multiple tests (adjusted p-value = q-value).
161 NMDS graphs were constructed using values produced by metaMDS from the vegan
162 (v.2.6.4) package, using Bray-Curtis dissimilarity values. PERMANOVA analyses were
163 conducted using Bray-Curtis dissimilarity values, and the adonis2 command from the vegan
164 (v.2.6.4) package. The significance of differences in abundance between groups of specific
165 genera of interest was calculated using the Wilcoxon test.

166 Clinical data (CIBDAI and PFS) as well as alpha diversity indices were analyzed and
167 compared using GraphPad Prism 9.5.1 for Windows, GraphPad Software, San Diego,
168 California USA (www.graphpad.com). Data were tested for normality using Shapiro-Wilk
169 tests and compared using Kruskal-Wallis tests with Dunn's multiple comparison test as post
170 hoc analysis.

171

172 **Results**

173 Animal characteristics

174 A total of 24 dogs were initially recruited. Of those, 5 did not reach the end of their first
175 supplementation stage due to palatability issues with the supplement. Two further dogs were
176 removed from the study as they developed unrelated medical problems that prevented them
177 from completing the study. Data from the remaining 17 dogs was included in the analysis.
178 However, from 2 of those dogs, no fecal sample was collected at day 0 as they were
179 accidentally transitioned to the first supplement too early. Another 2 dogs completed the first
180 and second supplementation and washout phases but developed diarrhea with the third
181 supplement (MTC), so they were taken off this supplement and a final sample collected at
182 day 84. Hence, a complete dataset was available for 13/17 dogs.

183 For the 17 dogs, the order of DF supplements given can be found in table S2 and the
184 available samples in table S3.

185 Methylcellulose, but not psyllium husk or resistant starch, causes an increase in fecal 186 scores, which returned to normal at the end of washout periods:

187 Fecal scores at the start of the study were a median of 2 (range 1-4) and did not significantly
188 change with the dietary transition (median of 2, range 1-3) ($p > 0.99$). There was also no
189 difference between baseline samples and supplements with the exception of MTC, resulting
190 in a median PFS of 5 (range 3-7) (figure 2A); and no significant change in fecal scores
191 between the different washout periods ($p = 0.7$; figure 2B).

192 CIBDAI values were low at the start (day -14) with a median of 2 (range 1-4), and remained
193 low throughout the supplementation periods, with no significant differences between DF
194 supplements (see figure 3).

195 Dietary change did not significantly influence the intestinal microbiota:

196 Prior to quality filtering, samples contained $99,501 \pm 17,945$ (mean \pm SD) OTUs. After quality
197 filtering and clustering of OTUs, 1964 OTUs were identified, and samples contained OTU
198 counts of $82,104 \pm 14,848$ (mean \pm SD). All samples were then sub-sampled to 39,442 OTU
199 counts prior to further analysis (table S4, S5). Rarefaction curves for samples plateaued,
200 indicating that the sequencing depth was adequate. Based on these plots, observed OTU
201 numbers reduced upon dietary change from baseline, but not significantly so (figure S1).
202 Similarly, diversity indices remained unchanged (figure 4). Based on this, data from day 0
203 were considered the “baseline” for all subsequent analyses.

204 Resistant starch but not psyllium husk or methylcellulose reduce microbiota richness, but
205 changes recovered during the washout period

206 Of the different supplements, only RS resulted in significant IM changes, as evidenced by a
207 significant reduction in observed OTUs and Chao1, but unaltered Shannon diversity (figure
208 5).

209 When assessing relative abundances, there was no meaningful difference on the phylum
210 level (figure S2). The average composition of the most abundant families and genera were
211 also similar across groups (figure S3).

212 Baseline and DF treated samples did not cluster significantly separately by their overall
213 community composition (PERMANOVA: $P > 0.05$, figure 6). Using Metastats to identify taxa
214 that differed significantly between groups, 12 genera were found to be significantly differently
215 abundant between baseline samples and PSY treated samples ($q < 0.05$, Sup_D-vs-
216 P_metastats_genera.xls), with 8 that were more abundant in baseline samples
217 (*Deinococcus*, *Hydrogenophilus*, *Anaerotruncus*, *IS-44*, *Kocuria*, *Exiguobacterium*,
218 *Psychroglaciecola* and *A2*) and 4 that were more abundant in treated samples
219 (*Psychrobacter*, *Anaerovibrio*, *Pseudoalteromonas*, *Lysinibacillus*). However, all of these
220 genera were low in abundance, with the most abundant per group being *Psychrobacter*, at

221 0.00024 ± 0.00023 (mean ± SE). No genera were found to be significantly different between
222 baseline and MTC treated samples (Sup_D-vs-M_metastats_genera.xls).

223 Thirty-seven genera were found to differ between baseline samples and samples after
224 supplementation with RS (Sup_D-vs-R_metastats_genera.xls), with 21 being more abundant
225 in baseline samples and 16 being more abundant in treated samples (table 1 and figures 7
226 and 8).

227 The relative abundance of specific bacterial groups of interest (e.g. part of the “dysbiosis
228 index” [(28)] or associated with gut health in dogs in the literature) was also assessed. Using
229 Wilcoxon rank test, there was a significant decrease in *Faecalibacterium* and
230 *Peptoclostridium* with RS supplementation (figure 9), but this significance was not upheld
231 after false discovery rate control. There was no difference in the abundance of any of those
232 selected bacteria with the other DF supplements compared to baseline.

233

234 **Discussion**

235 This is the first study to assess and compare detailed fecal microbiota changes associated
236 with supplementation of 3 types of commonly used DFs as specific supplements in dogs.
237 While other studies have determined microbiota changes with high-fiber extruded diets (for
238 example in comparison to hydrolyzed or high protein diets (29), for weight loss (30) or to
239 modulate intestinal postbiotics (31)) or functional properties of certain types of fiber naturally
240 occurring in raw ingredients (for example grains or cereals (32), miscanthus (33) or – recently
241 – red ginseng (34)) or fiber blends (31), there is no study that compares single DFs from
242 different broad fiber “categories” in the same dogs. This seems particularly surprising for
243 psyllium husk, as this is one of the most commonly recommended DF for the use in intestinal
244 disorders in dogs (35,36). For this reason, PSY was chosen as one of the supplements in the
245 present study. Representatives of the other DF categories were chosen based on their easy
246 availability (e.g. household ingredients), with green banana flour as a source of resistant

247 starch (RS) and pure methylcellulose (MTC; used as a gelling agent in baking, but also
248 occasionally as a laxative (37)) as a non-digestible fiber. The latter effect of MTC was
249 confirmed in this study, as it was the only one of the 3 supplements that caused a softer
250 fecal consistency (as evidenced by significant increases in fecal scores). This did, however,
251 not extend to any other unwanted adverse effects, as – based on the static CIBDAI indices –
252 activity levels, appetite and other clinical parameters remained unchanged.

253 Overall, the effects of all 3 DFs on the microbiota richness and composition as assessed by
254 16S sequencing in these healthy dogs was mild, with most numerous changes found with
255 RS supplementation, while there were no significant microbiota alterations with MTC. For
256 PSY, while significant, differences found were in low abundance genera, hence these may
257 not be biologically relevant. The most abundant change for PSY was for genus
258 *Psychrobacter*, which belongs to the family Moraxellaceae, of the order Pseudomonadales
259 within the class of Gammaproteobacteria. *Psychrobacter* is a widespread and evolutionarily
260 successful group of bacteria and is likely to have a role as a commensal, degrading various
261 dissolved organic carbon compounds other than sugars (38). Some species of the genus
262 have been isolated as cause of human infections (38), and as it is a Gram-negative
263 bacterium carrying hypoacylated lipopolysaccharides, it induces a TLR4-mediated
264 inflammatory response (39). While this study does not dispute any clinical benefit seen with
265 PSY supplementation in dogs with GI conditions, it does not support that any benefits are
266 derived from significant microbiota changes. However, it is possible that changes would be
267 more evident when giving PSY to dogs with specific GI conditions instead of healthy dogs. In
268 addition, we did not assess microbiota function directly (e.g. measuring SCFA production).
269 Prediction of metagenomic functions based on 16S data, for example using enrichment
270 analysis (40) or bioinformatic tools like PICRUSt2 (41), is unlikely to be meaningful, as
271 canine-specific databases are currently not of the desired quality, and tools are biased
272 towards human microbiota taxa. It is possible that PSY administration was not of sufficient

273 duration or dose to detect related microbiota changes or that changes are related to taxa or
274 species of very low abundance, which is difficult to capture with 16S sequencing alone.

275 Supplementation with RS led to the greatest number of taxa differing in comparison to
276 baseline samples. This may indicate that RS supplementation has a greater effect on fecal
277 microbiota composition. This is also supported by the significant differences that were seen
278 in richness (but not diversity indices) for this DF. Interestingly, in another study that used RS
279 as a supplement in healthy dogs, no changes of α - or β -diversity were observed (42).

280 Interpretation of the type of changes in microbiota abundance with RS supplementation and
281 their meaning is challenging, as the majority of genera are not well described with regards to
282 their microbiological niche, main physiological function or relevance in disease in dogs. The
283 overall observation when assessing the phylogeny is that Firmicutes (renamed Bacillota)
284 were generally reduced in their abundance (all of the ones belonging to the class of
285 Clostridia, and some of the class of Bacilli; compare figure 7), while Proteobacteria (now
286 Pseudomonata) and Bacteroidetes (now Bacteroidota) were increased (compare figure 8).

287 Of the 21 taxa with lower abundance after RS supplementation, 8 have been associated with
288 gut homeostasis, repair or SCFA production (*Sellimonas* (43), *Negativibacillus* (44,45),
289 *Eubacterium* (46), *UCG-005* (47), *Intestinimonas* (48), *Rikenella* (49), *Anaerotruncus* (50,51),
290 *UCG-009* (48)). Some of these findings are in line with Beloshapka et al. (2021), who also
291 found *Anaerotruncus* to decrease with increased RS consumption. Furthermore, 7 of the 21
292 taxa are considered normal commensals of the oral microbiome (*Mogibacterium* (52)), GI
293 tract or environmental (*Cellulomonas & Porphyromonas* (51), *Paludicola* (48)
294 *Phenylobacterium* (53), *Pseudoxanthomonas* (54)), family XIII UCG-001 (55), and 4 have
295 been found to show differential abundance in human diseases or disease models compared
296 to healthy controls (*Desulfovibrio* (56), *Candidatus stoquefichus* (45), and *Clostridium*
297 *innocuum* (57) are associated with colitis, while *Peptostreptococcus* was shown to increase

298 in diabetic patients upon weight loss (58)). For 2 groups (A2 and *Kapabacteriales*) no
299 information could be found.

300 In contrast to the above, of the 16 taxa with increased abundance after RS treatment, 6
301 belonged to different classes of the phylum Pseudomonadota (previously Proteobacteria,
302 see figure 8), 5 to the phylum Actinomycetia (*Blastococcus*, *Microbacterium*,
303 *Saccharopolyspora*, *Brooklawnia* and *Propriociclava*), 3 to the phylum Bacillota/ Firmicutes,
304 1 to the phylum Bacteroidota (compare figure 7), and 1 to the phylum Saccharibacteria
305 (TM7X or *Nanosynbacter* (59)). Of these, the vast majority has been identified as being part
306 of the environment, e.g. soil dwelling, aquatic or part of plant root microbiota (60–62). Only 2
307 (*Vagococcus* and *Peptoniphilus* (63)) have been found as part of the human gut and
308 reproductive tract microbiota, some with possible pathogenic potential. While it is possible
309 that these samples had a contamination from the environment, it seems less likely that this
310 would only affect a specific subgroup of samples. Overall, the significance of these changes
311 remains unclear.

312 None of the DFs given showed any significant effect on bacterial groups comprising the
313 diagnostically used “dysbiosis index” (28), ie. there were no specific increases in “gut health”
314 markers like *Faecalibacterium* or *Clostridium sensu stricto1* (which contains *Clostridium*
315 *hiranonis*). Contrary to this, a significant increase in *Faecalibacterium* and *Roseburia* (both
316 Firmicutes) was seen with increased RS consumption in another study (42). These
317 differences might be due to different types and sources of RS. For example, Beloshapka et
318 al. (2021) used High-amylose maize cornstarch as source for RS.

319 Lastly, general limitations of next generation sequencing (NGS) workflows need to be
320 acknowledged. While we have strived to follow best practice in our methods, bias can be
321 introduced at all methodological stages during 16S rRNA analysis (64), and this can lead to
322 issues with reproducibility (65). This should be taken into account when interpreting results.

323

324 **Conclusion**

325 Overall, while MTC induced no discernable microbiota changes (but resulted in mild
326 diarrhea), microbiota changes seen with PSY supplementation were mild and of
327 questionable biological relevance. In contrast, microbiota changes induced by RS
328 supplementation did not seem favorable, albeit being based on limited available information
329 for the observed bacterial groups. Consequently, this particular RS would not be considered
330 a desirable prebiotic and its use cannot be recommended in dogs.

331

332 **Additional information:**

333 **Conflict of interest:** None of the authors declare a conflict of interest with regards to the
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340 **Ethical approval:** This study was approved by the institutional Veterinary and Human Ethics
341 Review Committees of the University of Edinburgh (VERC# 130.17; HERC# 222.18).

342 **Author’s contributions:** SS contributed conceptualization, funding acquisition, formal
343 analysis of parts of the data, project administration, supervision and writing of both the
344 original draft and editing. JPAS contributed to conceptualization, investigation, methodology,
345 project administration, parts of the formal analysis and review/ editing of the manuscript. LG
346 provided data curation, formal analysis, supervision of parts of the methodology, validation
347 and visualization as well as writing (reviewing/ editing) of the manuscript.

348 **Sequencing data:** The paired-read fastq data that support the findings of this study have
349 been deposited in the European Nucleotide Archive with the accession code PRJEB67805.
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551

552 **Tables**

553 **Table 1:** Metastats for baseline samples vs samples after resistant starch (RS)

554 supplementation, showing differentially abundant bacterial taxa only. Relative abundance is
555 reported as a value between 0 and 1, where 0 indicated the complete absence of the taxa
556 and 1 indicates 100% abundance. SE = standard error.

Taxa	Mean baseline	SE baseline	Mean RS	SE RS	q value
<i>Sellimonas</i>	0.001674	0.000203	0.000978	0.00010 7	0.042502
<i>Negativibacillus</i>	0.001187	0.000185	0.000513	0.00011 5	0.042502
<i>Eubacterium_brachy_group</i>	0.000823	0.000175	0.000228	4.18E-05	0.042502
<i>UCG-005</i>	0.000749	9.37E-05	0.00027	4.31E-05	0.039722
<i>Desulfovibrio</i>	0.000194	8.41E-05	1.64E-05	1.02E-05	0.042502
<i>Intestinimonas</i>	0.000174	2.19E-05	8.39E-05	1.76E-05	0.042502
<i>A2</i>	0.000145	0.000145	0	0	0.024101
<i>Candidatus_Stoquefichus</i>	0.000129	2.40E-05	2.74E-05	1.32E-05	0.024101
<i>Rikenellaceae_RC9_gut_grou</i> <i>p</i>	0.000106	6.51E-05	0	0	0.042502
<i>Mogibacterium</i>	9.20E-05	3.75E-05	0	0	0.039722
<i>Family_XIII_UCG-001</i>	5.96E-05	1.72E-05	7.30E-06	5.64E-06	0.042502
<i>Paludicola</i>	3.07E-05	9.72E-06	0	0	0.042502
<i>Porphyromonas</i>	2.04E-05	1.39E-05	0	0	0.024101
<i>Anaerotruncus</i>	2.04E-05	2.04E-05	1.82E-06	1.82E-06	0.042502
<i>Cellulomonas</i>	1.70E-05	1.16E-05	0	0	0.039722
<i>Kapabacteriales</i>	1.53E-05	1.05E-05	0	0	0.042502
<i>Clostridium_innocuum_group</i>	1.53E-05	1.02E-05	0	0	0.042502
<i>UCG-009</i>	1.53E-05	1.36E-05	0	0	0.042502
<i>Peptostreptococcus</i>	1.53E-05	6.96E-06	0	0	0.042502
<i>Phenylobacterium</i>	1.53E-05	1.05E-05	0	0	0.042502
<i>Pseudoxanthomonas</i>	1.53E-05	1.53E-05	0	0	0.042502
<i>Blastococcus</i>	0	0	1.82E-05	1.12E-05	0.024101

<i>Microbacterium</i>	0	0	1.46E-05	7.91E-06	0.042502
<i>Brooklawnia</i>	0	0	1.64E-05	1.64E-05	0.032337
<i>Propioniciclava</i>	0	0	2.19E-05	2.00E-05	0.024101
<i>Saccharopolyspora</i>	0	0	2.92E-05	2.73E-05	0.024101
<i>Segetibacter</i>	0	0	0.00017	0.00015 3	0.024101
<i>Lysinibacillus</i>	0	0	3.65E-05	2.24E-05	0.024101
<i>Vagococcus</i>	0	0	4.20E-05	3.12E-05	0.024101
<i>Peptoniphilus</i>	0	0	1.82E-05	1.64E-05	0.024101
<i>TM7x</i>	0	0	0.000462	0.00037 7	0.024101
<i>Pleomorphomonas</i>	0	0	2.92E-05	2.25E-05	0.024101
<i>Ochrobactrum</i>	0	0	2.74E-05	2.74E-05	0.024101
<i>Novosphingobium</i>	0	0	1.46E-05	1.13E-05	0.042502
<i>Pelomonas</i>	0	0	6.39E-05	5.44E-05	0.024101
<i>Schlegelella</i>	0	0	4.38E-05	3.67E-05	0.024101
<i>Alkanindiges</i>	0	0	8.21E-05	8.21E-05	0.024101

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559

560 **Figure captions**

561 **Figure 1.** Illustration of the design and analysis of crossover fiber supplementation trial. Six
562 separate supplementation sequences were designed (A-F). Different coloured supplement
563 boxes represent the three different dietary fibers used. Filled stars indicate time points for
564 fecal sampling and owner questionnaires; open stars indicate time points for fecal sampling
565 only.

566 **Figure 2.** Fecal scores throughout the different supplementation phases (A) and at the end
567 of each washout (WA) phase (B) for 17 dogs participating in the study. The higher the score
568 the softer the stools. d -14 was before their change to a standardized diet at day 0, P =
569 psyllium, M = methylcellulose, R = resistant starch, WA = washout phase.

570 **Figure 3.** Canine chronic enteropathy clinical activity index (CIBDAI) for the 17 dogs
571 included in the feeding of different DF supplements. d -14 = before diet change, d 0 = after
572 diet change, P = Psyllium husk, R = resistant starch, M = methylcellulose.

573 **Figure 4.** Observed OTUs, Chao1 and Shannon diversity index comparison between the
574 baseline diet at day -14 and when all dogs were switched to a standardized diet at day 0.
575 Wilcoxon Rank comparison revealed p-values of 0.45 for OTUs, 0.35 for Chao1 and 0.15 for
576 Shannon.

577 **Figure 5.** Observed OTU numbers (A), Chao1 (B) and Shannon index (C) across all
578 supplementations and washouts (WO).

579 **Figure 6:** NMDS clustering samples using Bray-Curtis dissimilarity values (stress=0.14).
580 Samples originated from baseline (d 0) or from dogs that had received a DF supplement in
581 their diet (M = methylcellulose, P = psyllium husk, R = resistant starch). Groups did not
582 cluster significantly by composition (PERMANOVA: P =0.38).

583 **Figure 7:** Difference in abundance of bacterial groups belonging to the phylum of Firmicutes/
584 Bacillota from samples after supplementation with resistant starch. The upper panel

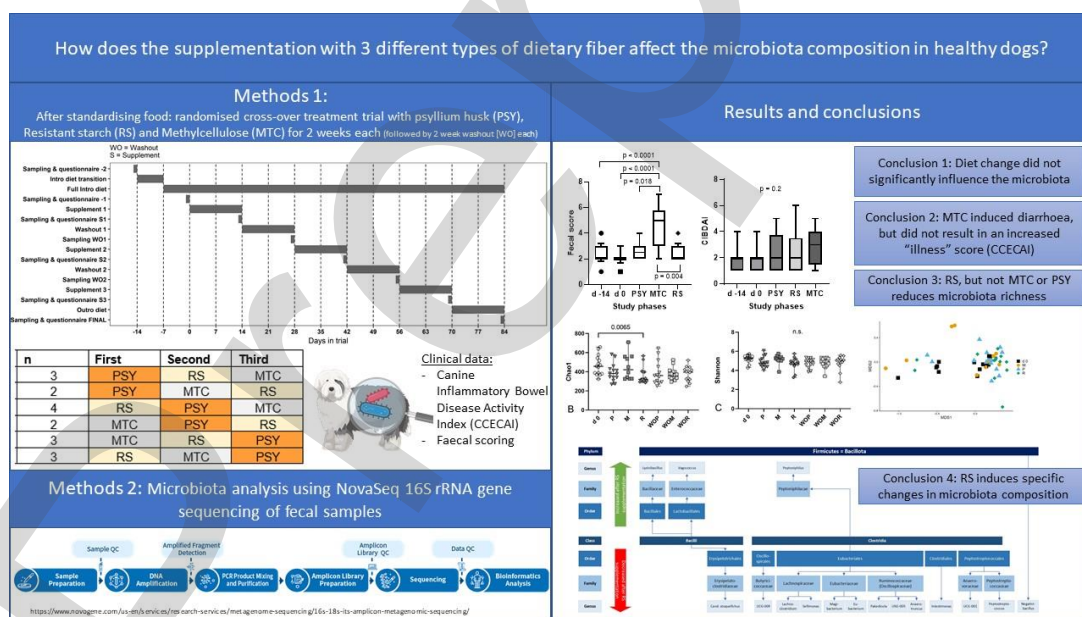
585 (indicated by green arrow) shows groups increased after supplementation; the lower panel
 586 (indicated by red arrow) shows groups decreased.

587 **Figure 8:** Difference in abundance of bacterial groups belonging to the phyla Bacteroidetes/
 588 Bacteroidota (left groups in shades of brown) and Pseudomonata/ Proteobacteria (right
 589 groups in shades of purple) from samples after supplementation with resistant starch. The
 590 upper panel (indicated by green arrow) shows groups increased after supplementation; the
 591 lower panel (indicated by red arrow) shows groups decreased.

592 **Figure 9.** Relative abundance of selected bacterial groups at baseline (day 0) and after
 593 supplementation with different DF (M = methylcellulose, P = psyllium husk, R = resistant
 594 starch).

595

596 **Graphical abstract:**



597

598 Graphical abstract legend: Microbiota of healthy dogs demonstrate significant changes in
 599 specific bacterial groups in response to supplementation with resistant starch (but not
 600 psyllium or methylcellulose) in this randomized cross-over trial.