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RUNNING HEADER: HOST GENETIC COMPONENT OF THE MICROBIOME

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SHORT REPORT: Signs of host genetic regulation in the microbiome composition in

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cattle

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ABSTRACT

19 Previous studies have revealed certain genetic control by the host over the microbiome
20 composition, although in many species the host genetic link controlling microbial
21 composition is yet unknown. This potential association is important in livestock to study all
22 factors and interactions that rule the effect of the microbiome in complex traits. This report
23 aims to study whether the host genotype exerts any genetic control on the microbiome
24 composition of the rumen in cattle. Data on 16S and 18S rRNA gene-based analysis of the
25 rumen microbiome in 18 dairy cows from two different breeds (Holstein and Brown Swiss)
26 were used. The effect of the genetic background of the animal (through the breed and Single
27 Nucleotide Polymorphisms; SNP) on the relative abundance (RA) of archaea, bacteria and
28 ciliates (with average relative abundance per breed >0.1%) was analysed using Bayesian
29 statistics. In total, 13 genera were analysed for bacteria (5), archaea (1), and ciliates (7). All
30 these bacteria and archaea genera showed association to the host genetic background both for
31 breed and SNP markers, except RA for the genera *Butyrivibrio* and *Ruminococcus* that
32 showed association with the SNP markers but not with the breed composition. Relative
33 abundance of 57% (4/7) of ciliate analysed showed to be associated to the genetic
34 background of the host. This host genetic link was observed in some genus of *Trichostomatia*
35 family. For instance, the breed had a significant effect on *Isotricha*, *Ophryoscolex* and
36 *Polyplastron*, and the SNP markers on *Entodinium*, *Ophryoscolex* and *Polyplastron*. In total,
37 77% (10/13) of microbes analysed showed to be associated to the host genetic background
38 (either by breed or SNP genotypes). Further, the results showed a significant association
39 between DGAT1, ACSF3, AGPAT3 and STC2 genes with the relative abundance *Prevotella*
40 genus with a false discovery rate lower than 15%. The results in this study support the

41 hypothesis and provide some evidence that there exist a host genetic component in cattle that
42 can partially regulate the composition of the microbiome.

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45 **Keywords:** genomic, breed, SNP, Holstein, Brown Swiss, microbiome, NGS

46 **Abbreviations:** FDR: False Discovery Rate; NGS: Next Generation Sequencing; MAF:

47 Minor Allele Frequency; OTU: operational taxonomic units; PC: Principal components; RA:

48 Relative abundance; SNP: Single Nucleotide Polymorphisms.

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52 The research interest on the microbiome and its effects on the host, both in humans [1,2] and
53 livestock [3,4], is raising in the last years. The microbiome plays an important role in the
54 phenotypic expression of many phenotypes such as feed efficiency, disease status, or methane
55 emission. Traditionally, microbes have been studied in the lab, without considering their
56 effect on complex features and their interaction with the host. In the particular case of
57 livestock, the traits of interest are usually related to productive, health or environmental
58 factors. In the last decade, more attention has been focused on the interactions between
59 microbes and diet [5–8], methane emissions [9–13] and the microbiome compositions across
60 hosts, environment and age [4,7,14]. It has also been proposed as a predictor of complex
61 traits [13,15].

62 Therefore, there is an increasing interest on determining whether a host genetic control on the
63 microbiome composition exists. Recent studies show some evidences that support the
64 hypothesis that there is some sort of host control over the composition of the microbiome in
65 mammals. For instance, Weimer et al. (2010) reported that after a near-total exchange of
66 ruminal contents, the ruminal bacterial composition returned to a similar status to that prior
67 the exchange. More recently, [17] showed differences between sire progeny groups on the
68 archaea:bacteria ratio in Aberdeen Angus and Limousin cattle breeds, and [18] reported
69 heritabilities above 0.20 for the relative abundance of several microbes in a twin human
70 study.

71 It is of interest to provide more evidences on the host genetic control of the microbiome
72 composition because some selection intensity could be applied to select individuals with a
73 favourable microbiome for a given breeding goal, as the reduction of methane yield or the
74 improvement of the feed efficiency, for example.

75 This trial was carried out in accordance with Spanish Royal Decree 53/2013 for the
76 protection of animals used for experimental and other scientific purposes. In this study,

77 ruminal content was sampled from 18 dairy cows (10 Holstein and 8 Brown Swiss) from
78 Fraisoro Farm School (Zizurkil, Gipuzkoa, Spain). These cows were undergoing a nutrition
79 experiment. They were randomly assigned to one of two experimental concentrate
80 supplements. Concentrates were formulated to contain cold-pressed rapeseed cake or palm as
81 fat sources, and to provide equal amounts of crude protein, energy and fat. Both breeds were
82 fed both diets. The effect of the treatment was adjusted as a 2-levels factor in the statistical
83 analyses, but results are not reported here as this is not the objective of this study.

84 Rumen samples were taken 4 times over two consecutive days. Sampling began at 00:00 and
85 12:00 h on d 1, and 06:00 and 18:00 h on d 2; each sampling taking approximately 2 h.

86 Ruminal samples were collected from each dairy cow using a stomach tube connected to a
87 mechanical pumping unit. About 100 ml of each ruminal extraction were placed into a
88 container and were frozen immediately after the extraction and then stored at $-20\pm 5^{\circ}\text{C}$ until
89 analysis.

90 Then, samples were gradually thawed overnight at refrigeration ($5\pm 3^{\circ}\text{C}$) and squeezed
91 through four layers of sterile cheesecloth to separate solid (solids with a particle size smaller
92 than the diameter of the tube) from liquid digesta phases. This latter phase was subsequently
93 separated into planktonic organisms and bacteria associated with the liquid fraction. The solid
94 phase was separated in associated and adherent fractions. Fractionation procedures were
95 carried out following the methodology described in [19]. The four fractions were lyophilized
96 and composited to obtain a unique sample with the four fractions represented proportionally
97 (on dry matter basis).

98 After composition, DNA extraction was performed using the commercial Power Soil DNA
99 Isolation kit (Mo Bio Laboratories Inc, Carlsbad, CA, USA) following manufacturer's
100 instructions. The extracted DNA was subjected to paired-end Illumina sequencing of the V4
101 hypervariable region of the 16S rRNA [20] and of the V7 region of the 18S rRNA genes. The

102 libraries were generated by means of Nextera kit. The 250 bp paired-end sequencing
103 reactions were performed on a MiSeq platform (Illumina, San Diego, CA, USA).

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105 Sequence data were processed using the QIIME software package version 1.9.1 [21].
106 Sequences below 220 bp in length and Phred score below 20 were discarded. In total,
107 3,261,168 and 3,431,242 reads from the 16S and 18S rRNA regions respectively, were
108 analysed. Sequence data were grouped into operational taxonomic units (OTU) sharing 97%
109 sequence similarity, and assigned to phylogenetic groups by BLAST [22].

110 Bacterial and archaeal 16S rRNA genes were assigned using the GreenGenes database (May
111 2013 version) and ciliate protozoal 18S rRNA genes against SILVA database (March 2015
112 version). Data were summarised at the genus level. Relative abundance (RA) of genera in
113 each animal was calculated after excluding those genera that appeared in <0.1% proportion in
114 the previous step. Only genera showing average RA>0.1% in both breeds were kept for
115 subsequent analyses.

116 Genotypes from animals under study were also obtained with the Illumina 9K chip (Illumina,
117 Inc, San Diego, CA, USA). A total of 9,146 SNPs with minor allele frequency (MAF) >0.05
118 in the whole genotyped Spanish population were kept (data provided by the Spanish Holstein
119 association www.conafe.com from more than 3,000 individuals).

120 We used two strategies to analyse the host genetic effect on the microbiome composition.
121 Our response variable was the RA of the most common ruminal microbes previously found,
122 and the model adjusted by diet treatment (2 groups, with or without cold-pressed rapeseed
123 cake) and age (primiparous vs multiparous) groups and days in milk as a covariate. In the first
124 strategy, differences at the breed level (Holstein vs Brown Swiss) were estimated (Model 1).

125 Model 1)

126 $RA_{ijklm} = \mu + diet_j + age_group_k + lactation_group_l + breed_m + e_{ijklm}$

127 The second strategy included the first two principal components (PC) of a genomic
128 relationship matrix instead of the breed effect as

129 Model 2)

$$130 \quad RA_{ijklmn} = \mu + diet_j + age_group_k + lactation_group_l + PC_1_m + PC_2_n + e_{ijklmn}$$

131 This genomic relationship matrix was calculated as in [23], where the genome relationship
132 between individuals i and j can be calculated as

133

$$134 \quad G_{ij} = \frac{1}{L} \sum_{k=1}^L \frac{(g_{ik} - \hat{p}_k)(g_{jk} - \hat{p}_k)}{\hat{p}_k(1 - \hat{p}_k)}$$

135

136 where g_{ik} refers to the gene frequency value genotypes AA , Aa and aa , coded as 1, 0.5 and 0,
137 respectively, of individual i or j at locus k ($k = 1, 9146$). Gene frequency is half the number of
138 copies of the reference allele A . Then, \hat{p}_k was the estimated allele frequency in the whole
139 genotyped population as provided by CONAFE. The first PC of this matrix aims to detect
140 stratification at the breed level (Holstein vs Brown Swiss), whereas the subsequent PC are
141 expected to capture genomic differences between individuals.

142

143 Bayesian analyses were performed to estimate the breed and principal component effects [24]
144 using an in-home suite of programs written in R software [25]. Evidence of a host genetic
145 effect was considered when the 80% of the posterior distribution for the breed or the PC had
146 the same sign (either positive or negative). This is, 80% of the posterior probability for the
147 respective effect fell either above or below zero. Those microorganisms that showed evidence
148 of a host genetic control were selected to implement genome wide association analyses. Here,
149 the RA of those microorganisms was used as a dependent variable, and the SNP markers
150 were selected as explicative variables in a single marker linear regression model, including

151 breed and diet as environmental factors. The p-values were adjusted on false discovery rate
152 (FDR).

153 The gene content of the significant SNP was examined using the bovine genome annotation
154 in BioMart tool of Emsembl (ensembl.org/biomart) using Emsembl Genes 75 database. The
155 National Center for Biotechnology Information (NCBI) database and PubMed were
156 employed to investigate the potential biological relation of the genes that contained the SNP
157 and the microbes in order to propose candidate genes that underlie the detected associations.

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RESULTS AND DISCUSSION

160 The results from the 16S rRNA region showed a 98:2 for the bacteria:archaea ratio. The more
161 abundant bacterial phyla were *Bacteroidetes* (58%), *Firmicutes* (33%) and TM7 (*Candidatus*
162 *Saccharibacteria*) (4%). Methanobacteria was the most abundant clade among the archaeas.
163 Taxa composition was similar to those reported before in other ruminal microbiome
164 communities [7,13], being mainly microbes related to peptide and cellulose degradation or to
165 the synthesis of microbial protein and volatile fatty acids.

166

167 *Bacteria and archaea*

168 The RA of genera analysed are shown in Figure 1. *Prevotella* was the most abundant
169 bacteria-archaea genus in both breeds, followed by *Butyrivibrio* and *Succiniclasicum*. The
170 archaea *Methanobrevibacter* was more abundant than the rest of the archaea genera detected
171 in the samples.

172 Table 1 shows the results from the statistical analyses of the host genetic component on the
173 different RA. The analyses showed differences between breeds for 4 (*Methanobrevibacter*,
174 *Succiniclasicum*, *Prevotella* and *YRC22*) out of the 6 archaea and bacteria genera analysed

175 from 16S rRNA region. However, either the first or second genomic PC were significant for
176 all other genus analysed (Table 1).

177

178 *Ciliate*

179 Figure 2 shows the relative abundance of the analysed ciliate in both breeds. The genus
180 *Entodinium* was the most abundant among the ciliate protozoal, followed by *Isotricha*.
181 Phenotypically, *Ophryoscolex*, *Diplodinium* and *Polyplastron* were more abundant in
182 Holstein, whereas *Dasytricha* showed larger RA in Brown Swiss. The breed effects showed
183 differences in 3 (*Isotricha*, *Ophryoscolex* and *Polyplastron*) out of 7 ciliate genus analysed.
184 The genomic PCs were also statistically significant for these genera, except for *Isotricha*,
185 where the posterior distribution did not show a significant effect for the PCs (Table 2).

186

187 Despite the small sample size, RA for 77% (10/13) of the genera analysed were found to be
188 regulated by some host genetic factor (breed, SNP marker, or both), which suggest that the
189 microbiome composition is regulated by some genetic mechanisms in the host. The host
190 genetic background showed to have effect on a larger proportion of bacteria-archaea, in
191 comparison to ciliates. We did not find a host genetic effect on the relative abundance of
192 genera *Trichostomatia*, *Dasytricha*, and *Diplodinium*. These microbes might be more
193 influenced by diet than by the host genetic effect, and larger sample sizes might be necessary
194 to detect differences between breeds or host genetic effects.

195 [18] also showed host genetic effect on the RA of different genera and families of *Firmicutes*
196 and *Euryarchaeota* (e.g. *Turicibacter*, *Blautia Clostridiaceae*, *Ruminococcaceae* or
197 *Methanobrevibacter*) in humans. Their study also showed a host genetic effect on some
198 *Tenericutes*, *Proteobacteria* (Family *Oxalobacteraceae*) and *Actinobacteria* (Genera
199 *Bifidobacterium* and *Actinomyces*). Our study also shows a host genetic effect on some

200 genera of Firmicutes but also on some *Bacteroidetes* differently to [18] and ciliate which
201 were not analysed in the human study as they are not abundant in the human gut. Roehe et al.
202 [17] showed differences in the microbial community of progeny daughters from different
203 cattle breeds and sires, suggesting that even under the same diet and environmental
204 circumstances, individuals can differ in their microbial communities depending on their
205 progenitors.

206

207 Microbial networks for 16S and 18S-gene rRNA regions were constructed using the
208 algorithm described by [26] and their graphical representations are shown in Figures S1 and
209 S2. The microorganisms that showed to be related to the host genetics are relevant in the
210 composition of the ruminal environment and the degradation of feed. For instance, bacteria
211 from the *Prevotella*, the most abundant group, and *Paraprevotella* genera are involved in the
212 metabolism of proteins and peptides in the rumen. They break down protein and
213 carbohydrates in feed [27], synthesize *de novo* peptides and use products of cellulose
214 degradation from other cellulolytic bacteria [28,29]. Further, bacteria from the genus
215 *Ruminococcus* break down cellulose, hemicellulose and produce succinic acid as a major
216 fermentation product together with acetic and formic acids, hydrogen and CO₂. These
217 products are then used by other bacteria, some from the *Succinivibrio* genus, which
218 convert succinate to propionate as an energy-yielding mechanism. *Butyrivibrio* bacteria are
219 proteolytic bacteria and are involved in the degradation of hemicellulose walls, and lipid
220 hydrogenation. They produce mainly butyrate, that is metabolized through the rumen wall to
221 produce energy. Further, archaeas from the *Methanobrevibacter* genus use hydrogen and CO₂
222 products and by-products from other microorganism (e.g. *Ruminococcus*) to synthesise
223 methane. The archaea, mainly organisms related with genera *Methanobrevibacter* and
224 *Methanosphaera*, are highly associated with methane emission in ruminants [27].

225 *Methanobrevibacter* has been associated to methane emissions in many previous studies, e.g.
226 [13,30,31].

227 *Entodinium* ciliate are able to engulf small plant particles and degrade cellulose [27,32]. They
228 are considered as cellulolytic microorganisms. *Isotricha* and *Dasytricha* use soluble sugar,
229 and many carbohydrates enzymatic activities have been detected. *Polypastron* ciliates can
230 actively ingest large cellulosic fibres of the rumen fluid [27,33]. The products of rumen
231 ciliates are more or less similar and include acetate, butyrate, and lactate. They also produce
232 CO₂ and hydrogen during the synthesis that can be converted to methane by methanogenic
233 archaea and protozoa. Ciliates interact with other rumen microorganism as they can ingest
234 bacteria as protein source. A host genetic effect on the RA of these microorganisms explain
235 the heritability found in related traits such as feed efficiency or methane yield [34–36].

236

237 Genome-wide association analyses was performed for the RA the four microorganisms that
238 showed significant effect on both breed and PC1 effect (*Methanobrevibacter*,
239 *Succiniclasticum*, *Prevotella*, and *Polyplastron* genera). The generalized linear model
240 implemented included the breed, diet and the bovine SNP marker effects, and p-values were
241 adjusted on false discovery rate (FDR). As expected, the small sample size caused that most
242 of the markers with significant P-values (<0.01) presented a large FDR. We chose the
243 threshold of FDR<0.15 (equivalent P-value of 1.81×10^{-4}) to report significant SNP markers.
244 After this adjustment, significant bovine SNP markers were found for *Prevotella* genus RA
245 (Table 3). Most of these markers were within known genes with functions related mainly to
246 metabolic pathways and signalling on the central neural system. The role of the microbiome
247 in the metabolic status and the development of several central system disorders have been
248 well establish in humans [37,38], and our results suggest that there are also associations
249 between genes involved in metabolic and neural processes and the rumen microbiota

250 compositions. It must be highlighted that we found association between the DGAT1 gene and
251 the RA of *P. Prevotella*. The DGAT1 gene is a major gene with a large effect on the fat
252 composition in milk [39–41]. The association found in this study shows that the effect of the
253 DGAT1 on the milk fat composition may be partially regulated by some effect on the
254 microbiome composition, where individuals carrying the A (A/G) allele of the ARS-BFGL-
255 NGS-4939 SNP tend to host a larger proportion of *Prevotella* microorganisms which are also
256 involved in the protein and peptide degradation in the rumen, in the production of saturated
257 fatty acids as well as in saccharolytic pathways. Other genes with significant association to
258 the *Prevotella* RA were the ACSF3, AGPAT3 and STC2, all of them previously associated to
259 fatty acids or cell metabolism.

260

261 The results in this study provide some evidence that support the hypothesis of a host genetic
262 component that can partially regulate the composition of the microbiome, and indirectly some
263 metabolic pathways. In this sense, it seems that there is a genetic component in the regulation
264 of some groups of H₂- producing microorganisms included in the *Firmicutes* phylum and
265 ciliate protozoa and H₂-utilizers bacteria associated to *Bacteroidetes*. This is relevant because
266 diets and management practices can be specifically designed to compensate those genotypes
267 that are more susceptible to harbour less efficient microorganisms from a nutritional and
268 energetic point of view. Results from this study must be considered carefully due to the
269 reduced sample size. Future studies should allow to better estimate heritability of the
270 microbiome composition in cattle, as well as covariance components with other traits of
271 interest (e.g. feed efficiency, productivity, or methane emissions). Still, if these results were
272 confirmed, breeding strategies could be developed to select future livestock generations prone
273 to harbor a favourable microbiome composition that improves feed digestion and utilization,
274 while precluding presence of harmful microbes or composition thereof.

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DECLARATIONS

278 **Ethics approval and consent to participate**

279 It was not considered to ask for an authorization because the procedures used in animals were
280 those used under a common clinical veterinary procedure, therefore not subject to regulation
281 by the Spanish and European Legislation related with the protection of animals used for
282 scientific purposes. Nevertheless, the animals were manipulated according to the Spanish
283 Policy for Animal Protection RD 53/2013, which meets the European Union Directive 86/609
284 about the protection of animals used in experimentation.

285

286 **Consent for publication**

287 Not applicable

288

289 **Availability of data and material**

290 The datasets during and/or analysed during the current study available from the
291 corresponding author on reasonable request, and the authors plan to upload them to a data
292 repository soon.

293

294 **Competing interests**

295 The authors declare that they have no competing interests.

296

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300 **Authors' contributions**

301 OGR performed the analyses of sequence data, managed the genotyping of the animals,
302 implemented the statistical analyses, discuss the results and wrote the first draft of the
303 manuscript. AGR, IZU and RAT made the experimental design and executed the
304 experiments, collected and analyzed the samples, discussed the results and helped to write the
305 manuscript. AHU performed the preparatory actions for the sequencing analysis and helped
306 writing the manuscript. All authors read and approved the final manuscript.

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476 **Table 1.** Effect¹ of the breed (Holstein vs Brown Swiss) and the two first principal component
 477 of a genomic relationship matrix based on genotypes on the relative abundance of different
 478 bacteria and archaea genera. Only genera that are present with average relative abundance
 479 larger than 0.1% in both breeds are shown.

Domain	Phylum	(Family) Genus	Breed	PC1	PC2
Archaea	<i>Euryarchaeota</i>	<i>Methanobacteriaceae</i> <i>Methanobrevibacter</i>	*	*	*
Bacteria	<i>Firmicutes</i>	<i>Lachnospiraceae</i> <i>Butyrivibrio</i>	N.S.	N.S.	*
Bacteria	<i>Firmicutes</i>	<i>Veillonellaceae</i> <i>Succiniclasticum</i>	*	*	N.S.
Bacteria	<i>Firmicutes</i>	<i>Ruminococcaceae</i> <i>Ruminococcus</i>	N.S.	N.S.	*
Bacteria	<i>Bacteroidetes</i>	<i>Prevotellaceae</i> <i>Prevotella</i>	*	*	*
Bacteria	<i>Bacteroidetes</i>	<i>Paraprevotellaceae</i> <i>YRC22</i>	*	N.S.	*

480 ¹ * states that >80% of the posterior distribution of the effect was either larger or lower than
 481 zero, suggesting a significant effect of the breed or of the principal component on the relative
 482 abundance. N.S. states otherwise.

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485 **Table 2.** *Effect¹ of the breed (Holstein vs Brown Swiss) and the two first principal component*
 486 *of a genomic relationship matrix based on genotypes on the relative abundance of different*
 487 *ciliate genera. Only genera that are present with average relative abundance larger than*
 488 *0.1% in both breeds are shown.*

Domain	Order	(Family) Genus	Breed	PC1	PC2
Eukaryota	<i>Ciliophora</i>	<i>Litostomatea Trichostomatia</i>	N.S.	N.S.	N.S.
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Dasytricha</i>	N.S.	N.S.	N.S.
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Entodinium</i>	N.S.	N.S.	*
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Isotricha</i>	*	N.S.	N.S.
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Diplodinium</i>	N.S.	N.S.	N.S.
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Ophryoscolex</i>	*	N.S.	*
Eukaryota	<i>Ciliophora</i>	<i>Trichostomatia Polyplastron</i>	*	*	*

489 ¹ * states that >80% of the posterior distribution of the effect was either larger or lower than
 490 zero, suggesting a significant effect of the breed or of the principal component on the relative
 491 abundance. N.S. states otherwise.

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501 **Table 3.** Genes contained within significant bovine SNP markers for the relative abundance
 502 of *P. Prevotella*, and their position.

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SNP name	SNP position	Gene	Related function	P-value
ARS-BFGL-NGS-13121	1:146833973	AGPAT3	Metabolic pathways and glycerolipid metabolism	5.02x10 ⁻⁵
ARS-BFGL-NGS-106490	3:13635591	Unknown	-	1.60 x10 ⁻⁴
ARS-BFGL-NGS-28573	3:24081964	Unknown	-	2.05 x10 ⁻⁵
BTB-01512420	8:72495155	ADAMDEC1	Dendritic cell maturation and functions	1.56 x10 ⁻⁴
ARS-BFGL-NGS-32158	12:90983897	RASA3	Ras signalling pathway; control of intracellular signaling networks	1.81 x10 ⁻⁴
ARS-BFGL-NGS-4939	14:1801116	DGAT1	Conversion of diacylglycerol and fatty acyl CoA to triacylglycerol; metabolic status	1.81 x10 ⁻⁴
ARS-BFGL-NGS-31386	18:14208633	ACSF3	Fatty acid, triacylglycerol, and ketone body metabolism	1.81 x10 ⁻⁴
ARS-BFGL-NGS-112014	18:34794005	CES3	Fatty acyl and cholesterol ester metabolism	1.72 x10 ⁻⁴
ARS-BFGL-NGS-31292	20:4907906	STC2	Autocrine or paracrine functions and cell metabolism	1.81 x10 ⁻⁴
ARS-BFGL-NGS-31656	26:51426365	Unknown	-	1.81 x10 ⁻⁴
BTA-122892-no-rs	X:81638519	SLC16A2	Transporter of thyroid hormone and development of the central nervous system	1.81 x10 ⁻⁴

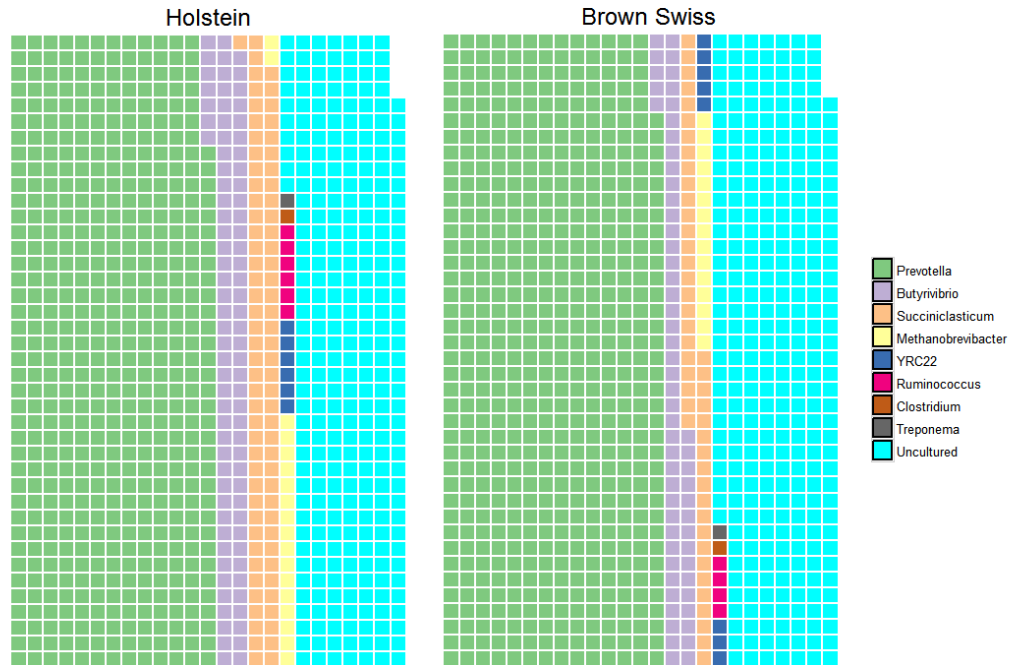
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507 **Figure 1.** Relative abundance of Bacteria and Euryarchaea with average relative abundance
508 larger than 0.1% in both breeds.

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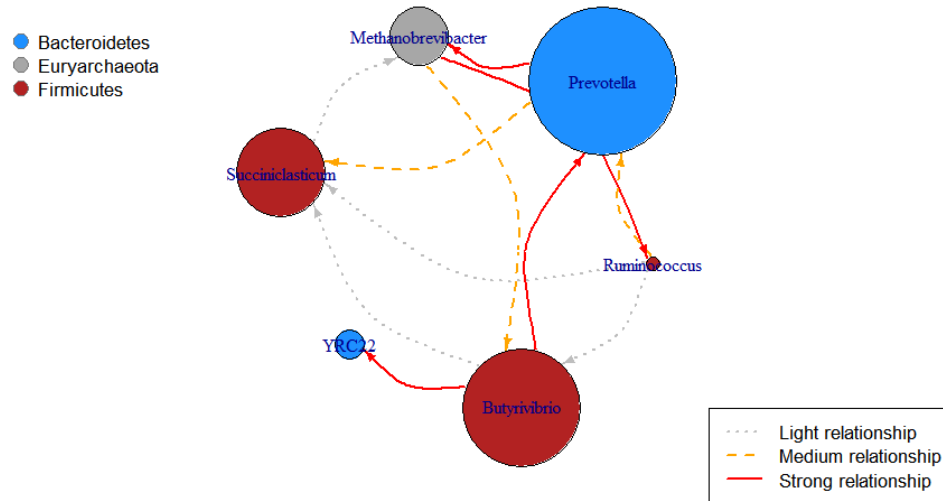
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513 **Figure 2.** Relative abundance of genera of ciliate with average relative abundance larger
514 than 0.1% in both breeds.



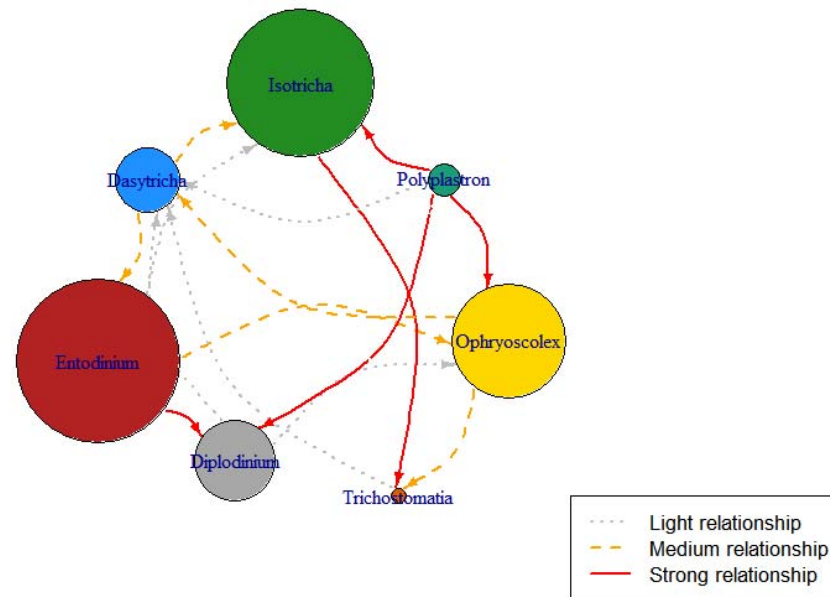
517 **Figure S1.** Microbial network based on 16S rRNA-gene based region for microorganism
518 with relative abundance larger than 0.1% in both breeds. The size of the nodes represents the
519 relative abundance of the genera.



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522 **Figure S2.** Microbial network based on 18S rRNA-gene based region for ciliates with
523 relative abundance larger than 0.1% in both breeds. The size of the nodes represents the
524 relative abundance of the genera.



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