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Bilberry pomace in rabbit nutrition: effects on growth performance, apparent digestibility, caecal traits, bacterial community and antioxidant status

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Corresponding Author:	Francesco Gai, PhD ITALY
First Author:	Sihem Dabbou, PhD
Order of Authors:	Sihem Dabbou, PhD Ilario Ferrocino, PhD Attawit Kovitvadhi, PhD Samia Dabbou, PhD Stefania Bergagna, DVM Daniela Dezzutto, DMV Achille Schiavone, Prof. Luca Simone Cocolin, Prof. Francesco Gai, PhD Valentina Santoro Laura Gasco, Prof.
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Abstract:	<p>The aim of this study was to estimate the nutritive value and potential use of bilberry pomace (BP) for growing rabbits. One hundred and forty-four Grimaud rabbits (35 days old) were allotted four groups and fed with a diet containing increasing level of BP: BP0 (basal diet), BP 5, BP 10 and BP 15 containing 0, 50, 100 and 150 g/kg respectively. Growth trial lasted 48 days; apparent digestibility was evaluated, starting at 46 days of age, over four consecutive days. The nutritive value of BP was measured using the mean digestibility of the experimental diets. At 83 days of age, rabbits were slaughtered: blood, and liver and kidney samples were collected in order to determine the blood parameters and the antioxidant enzyme activities of the tissues. Moreover, caecal content was sampled and gut microbiota assessed by means of amplicon-based high-throughput 16S rRNA sequencing and PCR-Denaturing Gradient Gel Electrophoresis. The digestible protein was estimated to 104 g/kg of DM while digestible energy to 9.44 MJ/kg DM for incorporation rate up to 150 g/kg. During the finishing period, average daily feed intake and feed conversion ratio showed linear response to BP increase ($P=0.008$ and $P<0.001$, respectively). During all the period, both parameters decreased linearly and quadratically with increasing BP inclusion levels ($P<0.001$) up to 10% of BP.</p> <p>A significant effect of the antioxidant status was found in the kidneys and liver ($P<0.05$) where the glutathione peroxidase activity increased as the BP increased. As far as gut microbiota is concerned, BP increased the relative abundance of the Clostridium, Oscillospira, Ruminococcus and Ruminococcaceae species which were clearly associated with the BP inclusion level. In conclusion, BP showed a potential use as an</p>

Dear authors,

Please find strong suggestions to improve your manuscript, particularly about the nutritive value and the characterization of the BP.

Yours sincerely

=====

The calculation of the nutritive value of the BP should appear in the result section.

The calculation of the nutritive value has been added in the result section of the revised version of the manuscript.

Respect to "l 292" : "Extrapolating to 100%, the DP content was estimated to 110.81 g / kg DM"

First please remove the decimals.

We agree with the suggestion of the referee. The decimals in DP have been removed and we attributed two decimals in DE.

Secondly, 11.1% of DP in the BP means that proteins of the BP has a 78% digestibility coefficient ... this seems very high for such a fibrous product. Furthermore, the digestibility coefficients were similar among the four diets. This means, that more or less the BP protein digestibility is around 71% (the mean of the four diets). Please revise your calculations procedure, since the regression procedure is here not adapted to your design with low incorporation rate of BP. I suggest you calculate the mean digestibility of your 4 feeds, and to attribute to the BP since there is no effect of BP incorporation. This means that DP level of BP would be around 10.1%.

Similarly, for the DE content of the BP: it COULD NOT have such a high DE concentration, first according to its chemical composition (high in fibre). But, as for DP, the DE digestibility should be calculated without the regression procedure, but according to the mean of the four diets. However, here, it seems that already for your control diet (BP0) the level of digestible energy is very high : 10.93MJ/kg (as fed) that correspond to 2613 kcal/kg !! This is rather high for a feed containing 20% of ADF (please provide two decimals for energy concentration expressed in MJ/kg). I suspect either an analytical overestimation or analytical error (DM vs crude basis?). Please control carefully your original data. Anyway the DE content of the BP is apparently similar to that of BP0 diet (not 14.55 MJ/kg !!) , also since the feed conversion ratio are similar among groups or even a bit lower with BP.

For this discussion, make clear that the incorporation rate are low, and the DE and DP content are applicable only in the tested range of incorporation (<15%).

The authors checking the original data found a calculation error in the excel spreadsheet about the nutrient digestibilities. The new corrected data are now reported in table 2.

In order to uniform all the experimental data for all the parameters analyzed, authors take the opportunity of this second revision to revise the statistical analysis. Data about digestibility coefficients growth performances, caecal traits and blood parameters has been analyzed by polynomial contrasts and values are reported in the respective tables (2, 3 and 4).

We agree with the reviewer about our calculation procedure. We calculated the nutritive value by the mean digestibility of four feeds. The digestible protein was estimated to 104 g/kg of DM while digestible energy to 9.44 MJ/kg DM for incorporation rate up to 150 g/kg.

As it was suggested in the previous revision by the reviewer 3, we calculated the nutritive value by the regression method with the new data of protein and energy digestibilities:

Using the digestibility coefficient for GE and CP obtained for the four feeds, the linear regression method was used in order to apply the equation to predict the digestible protein (DP (g / kg) = 122.074 – 17.346 BP (%); R² = 0.036; P=0.257) and energy (DE (MJ / kg DM) = 10.936+2.614 BP (%); R² = 0.073; P=0.106) of BP. Accordingly and using the calculation procedure proposed by Villamide et al. (2001), the DP of the BP decreased with the inclusion rate of BP. Extrapolating to 100%, the DP content was estimated to 104.7 g / kg DM. Our estimation was associated to a high standard error for the predicted value (15%). The DE was estimated to 13.55 MJ / kg DM with standard error of 1.58 MJ / kg DM.

The two method of estimation, gave similar DP values but different DE values for BP. An overestimation of the nutritive value with a high standard error of the predicted value was found in our study. Since the risk of error is increased for low incorporation levels and due also of higher impact of the basal mixture.

Remove lines 300 to 304, in the light of previous comment

Lines from 300 to 304 were deleted as requested.

L320 : you declare that 10% is optimal... but, we need to see if statistically the FC of the BP10 differ clearly from the others or not.

Thus, in table 3, specify which group differ when P<0.05 ; with superscript letters : a, b...

The superscript letters were added as requested in the revised version of the manuscript.

Conclusions: should be largely rewritten.

L407-408 you CANNOT state "with a possible positive impact on the rabbit health status", since you did not measure the health effect. Please remove. You should state, that growth performances and health should be studied with a much larger number of rabbits with "commercial" housing (collective cages).

The conclusion was improved as requested.

The reference INRA 2004 should be move at the right place in the list

Corrected as requested.

In the M&M section, please specify that you analyse ADL with the modified Van Soest procedure (and also in table1, as a footnote)

Corrected as requested.

It is important to deeply characterised your product "BP". You assume that BP is rich in "bioactive components" such Phenolic compounds etc...

According to the suggestion with the collaboration of another research team supplementary analysis of the phenolic compounds of BP in terms of phenolic acids, flavanols, flavonols and anthocyanins were performed by HPLC-DAD-ESI-HRMS as described in supplementary material S1, the quantity of the phenolic compound values are added in the supplementary Table S1 and are commented in the Nutritive value of BP and digestibility trial paragraph of the discussion section.

Could you provide analysis of these components (or other , such aminoacids, etc...)

Similarly, you should provide further fibre criteria: crude fibre, TDF (IDF and SF water soluble fibre, NDSF?)..or value from literature?

For the determination of both requested components (aminoacids and TDF) specific laboratory equipments (aminoacid analyser and GDE Enzymatic Digester + Filtration Unit) are necessary and unfortunately are not available in our labs. Moreover we have also asked for a price quotation to perform outside these analyses in a private lab but the expensive costs requested us could not be covered by the funds of this project.

1 **Bilberry pomace in rabbit nutrition: effects on growth performance, apparent**
2 **digestibility, caecal traits, bacterial community and antioxidant status**

3

4 S. Dabbou^{1,5}, I. Ferrocino¹, A. Kovitvadhi², S. Dabbou³, S. Bergagna⁴, D. Dezzuto⁴,
5 A. Schiavone^{5,6}, L. Cocolin¹, F. Gai^{6*}, **V. Santoro⁷**, L. Gasco^{1,6}

6

7 *¹Department of Agricultural, Forest and Food Sciences, University of Turin, Largo P.*
8 *Braccini 2, 10095 Grugliasco, Italy*

9 *²Department of Physiology, Kasetsart University, 50 Ngam Wong Wan Road,*
10 *Chatuchak, 10900 Bangkok, Thailand*

11 *³Dentistry Faculty, University of Monastir, Avicenne Street, 5019 Monastir, Tunisia*

12 *⁴Veterinary Medical Research Institute for Piemonte, Liguria and Valle d'Aosta, Via*
13 *Bologna 148, 10154, Turin, Italy*

14 *⁵Department of Veterinary Sciences, University of Turin, Largo P. Braccini 2, 10095*
15 *Grugliasco, Italy*

16 *⁶Institute of Science of Food Production, National Research Council, Largo P.*
17 *Braccini 2, 10095 Grugliasco, Italy*

18 *⁷Department of Molecular Biotechnology and Health Sciences, University of Turin,*
19 *Via P. Giuria 5, 10125, Turin, Italy*

20

21

22 *Corresponding author: Francesco Gai. E-mail: francesco.gai@ispa.cnr.it

23

24 Running head: Bilberry pomace in rabbit diets

25

26 **Abstract**

27 The aim of this study was to estimate the nutritive value and potential use of bilberry
28 pomace (BP) for growing rabbits. One hundred and forty-four Grimaud rabbits (35
29 days old) were allotted four groups and fed with a diet containing increasing level of
30 BP: BP0 (basal diet), BP 5, BP 10 and BP 15 containing 0, 50, 100 and 150 g/kg
31 respectively. Growth trial lasted 48 days; apparent digestibility was evaluated,
32 starting at 46 days of age, over four consecutive days. The nutritive value of BP was
33 measured using **the mean digestibility of the experimental diets**. At 83 days of age,
34 rabbits were slaughtered: blood, and liver and kidney samples were collected in order
35 to determine the blood parameters and the antioxidant enzyme activities of the
36 tissues. Moreover, caecal content was sampled and gut microbiota assessed by
37 means of amplicon-based high-throughput 16S rRNA sequencing and PCR-
38 Denaturing Gradient Gel Electrophoresis. **The digestible protein was estimated to**
39 **104 g/kg of DM while digestible energy to 9.44 MJ/kg DM for incorporation rate up to**
40 **150 g/kg**. During the finishing period, average daily feed intake and feed conversion
41 ratio showed linear response to BP increase ($P=0.008$ and $P<0.001$, respectively).
42 During all the period, both parameters decreased linearly and quadratically with
43 increasing BP inclusion levels ($P<0.001$) up to 10% of BP.

44 A significant effect of the antioxidant status was found in the kidneys **and liver**
45 ($P<0.05$) where the glutathione peroxidase activity increased as the BP increased.

46 As far as gut microbiota is concerned, BP increased the relative abundance of the
47 *Clostridium*, *Oscillospira*, *Ruminococcus* and *Ruminococcaceae* species which were
48 clearly associated with the BP inclusion level. In conclusion, BP showed a potential
49 use as an alternative protein and fibre sources for growing rabbits.

50

51 **Keywords:** Rabbit, *Vaccinium myrtillus*, by-product, digestibility, microbiota

52

53 **Implication**

54 Bilberry pomace (BP) contains fibers and different beneficial antioxidant compounds.
55 The current paper, which had the purpose of studying the BP nutritive value and its
56 potential use in rabbits, has focused on its effect on several parameters. Results
57 showed that BP can be used as an alternative protein and fiber sources for growing
58 rabbits.

59

60 **Introduction**

61 The high cost and limitation of feedstuff resources are critical issues for the livestock
62 section, especially in the rabbit production field. However, several agricultural by-
63 products could be used as alternative raw materials in rabbit nutrition as they have
64 been found to be highly nutritious and low cost feeding sources (Dabbou *et al.*, 2014;
65 Dabbou *et al.*, 2017a).

66 Fruit pomace is a by-product of industrial fruit processing, and it is composed
67 of the cell wall compounds, stems and seeds of the fruit. Vegetable by-products and
68 pomace can be incorporated into animal diets, without adverse results on growth
69 performances and digestibility (Dabbou *et al.*, 2014), and can help to reduce the feed
70 costs. Moreover, due to the presence of bioactive components they can prevent the
71 harmful effects of oxidation (Saura-Calixto, 2011) and improve the function of the
72 intestinal ecosystem (Silva *et al.*, 2013). Nevertheless, Gidenne *et al.* (2010)
73 demonstrated that the use of fibrous sources rich in insoluble fibre fractions, even if
74 of low nutritional value, affects the retention rate of the digesta, microbial activity,
75 fibre fermentability and caecal turnover. Gut microbiota play important roles in

76 mammal's health and, in rabbits, the control of the microbiota could therefore
77 improve digestive efficiency or immune status (Zeng *et al.*, 2015). Improved digestive
78 efficiency through optimization of the composition of the microbiota has a direct
79 impact on feed costs. Moreover, the control of the microbiota could limit digestive
80 problems around weaning, considering its barrier effect and partly through its role as
81 immune stimulator (Combes *et al.*, 2013).

82 In this regard, bilberry (*Vaccinium myrtillus* L.) has been reported to be a
83 nutrient source that is high in bioactive components, including dietary fibre and
84 polyphenols. Bilberry pomace (BP) contains different beneficial phytochemicals,
85 including phenols, anthocyanins and flavonoids (Dabbou *et al.*, 2017b). BP has a
86 high antioxidant activity with about 65% of inhibition of 1,1-diphenyl-2-picrylhydrazyl
87 (DPPH) radical scavenging activity (Dabbou *et al.*, 2017b). In addition, BP has health
88 benefits by reducing plasma cholesterol and abdominal fat in rat (Khanal *et al.*,
89 2012). Moreover, from a microbiological point of view, Puupponen-Pimiä *et al.* (2005)
90 shown that berries and their phenolics selectively inhibit the growth of human
91 pathogenic bacteria through different possible mechanism of action.

92 The goal of this study was to determine the nutritive value of BP and to
93 evaluate its potential use in growing rabbits by assessing: growth performance, blood
94 parameters, bacterial community and organ oxidative status.

95

96 **Materials and Methods**

97 *Animals, diets and experimental design*

98 The trial was carried out at the experimental rabbitry of the Department of
99 Agricultural, Forest and Food Sciences (DISAFA) of the University of Turin (Italy).

100 The experimental protocol was designed according to the guidelines of the current

101 European and Italian laws on the care and use of experimental animals (European
102 Directive 86 609/EEC, put into law in Italy with D.L. 116/92) and approved by the
103 Ethical Committee of the University of Turin. At 35 days of age, 144 Grimaud rabbits
104 were individually caged (41 cm x 30 cm x 28 cm height) and randomly allotted to four
105 groups. Rabbits were housed at a temperature of $22\pm 2^{\circ}\text{C}$ (photoperiod 16L: 8D) and
106 had free access to clean drinking water. Diets were prepared using organic BP (Arc
107 en ciel Soc. Agric. Coop., Cafasse, Italy) dried in an oven at 60°C to a constant
108 weight and then ground finely. Rabbits were fed *ad libitum* with a basal diet (BP0),
109 which was tested against three assay diets, prepared by substituting 50, 100 and 150
110 g/kg of the BP0 diet with BP (BP5, BP10 and BP15, respectively Table 1), as
111 reported in Dabbou *et al.* (2017b).

112

113 *Digestibility trial*

114 A digestibility trial was conducted using 10 rabbits per dietary treatment and started
115 at 46 day of age according to the procedure described by the European Group on
116 Rabbit Nutrition (EGRAN, 2001). Faeces were collected individually and daily for four
117 consecutive days (09.00 a.m.). Each faecal sample was weighed and placed in a
118 two-layer plastic bag and immediately frozen at -20°C for chemical composition.

119 The nutrient digestibility coefficient was calculated using the total collection of faeces
120 for each rabbit and for each nutrient following the equation reported in Rotolo *et al.*
121 (2014).

122

123 *Growth performance*

124 During the trial, live weight was recorded at 35, 49 and 83 days of age, whereas feed
125 intake was recorded individually on a fortnightly basis.

126 Mortality was recorded daily. The average daily feed intake (ADFI), average daily
127 weight gain (ADG) and feed conversion ratio (FCR) were calculated. The trial lasted
128 48 days.

129

130 *Chemical proximate analysis*

131 The BP, diet and faeces samples were analysed for DM (2000 #930.15), ash (2000
132 #923.03), CP (2000 #984.13), EE (2003 #2003.05) and ADF (2000 #973.18)
133 contents according to the AOAC procedures (AOAC 2000 and 2003). NDF was
134 determined according to Van Soest *et al.* (1991), and ADL with the modified Van
135 Soest procedure adapted to tannins content with sodium bisulphate. Gross energy
136 (GE) was measured using an adiabatic bomb calorimeter (IKA C7000, Staufen,
137 Germany). Moreover the main BP polyphenols were identified and quantified as
138 described in Supplementary Material S1 and values are reported in Table S1.
139 Analyses were carried out on three replicates of each feed and two replicates of each
140 faeces sample.

141

142 *Caecal sampling*

143 At 83 days of age, 10 rabbits per group were slaughtered without fasting. The
144 caecum of each rabbit was separated from the digestive tract and weighed. The pH
145 value of the fresh caecal content was immediately measured (Crison Micro pH 2001
146 pH meter, Crison Instruments, Barcelona, Spain). The caecal content was then
147 removed, put into sterilized polyethylene bags (using a sterilized spatula), and kept at
148 -20°C to evaluate the bacterial community. The remaining empty caecum was
149 washed with distilled water, dried with blotting paper and weighed. The weights of the
150 full, empty and caecal contents were expressed in % of body weight (BW).

151

152 *Total DNA sample extraction and electrophoresis protocols*

153 In order to observe the development and dynamic of bacterial communities, hard
154 faeces were collected from 5 rabbits per group at 35, 49, and 83 days of age, while
155 the caecal contents (n=5 per group) were taken during the slaughtering at 83 days of
156 age. Samples from the same group, the same collection site and day were pooled
157 together in sterilized polyethylene bags, and stored at -80°C until examination. DNA
158 extraction and denaturing gradient gel electrophoresis (DGGE) procedures
159 (Supplementary Material S1) were performed according to Ferrocino *et al.* (2015).

160

161 *16S rRNA amplicon target sequencing*

162 DNA, extracted directly from the faecal and caecal samples, was used to assess the
163 gut microbiota, and the V3-V4 region of the 16S rRNA gene was amplified according
164 to the Illumina sample preparation manual (Illumina Inc, San Diego, CA). To reduce
165 the inter-sample variability, the DNA of the 5 rabbits extracted from each treatment
166 was mixed, and an equimolar pool of the DNA was obtained prior to PCR
167 amplification. The PCR products were purified and tagged by using a Nextera XT
168 library preparation kit (Illumina Inc, San Diego, CA), according to the manufacturer's
169 instructions and sequenced with a MiSeq illumina, and 250 bp paired-end reads
170 were generated.

171

172 *Blood samples and antioxidant enzymes of the organs*

173 Blood, liver and kidney samples were collected from ten rabbits per group during the
174 slaughtering procedure. Serum biochemistry and electrophoresis was performed
175 according to Kovitvadhi *et al.* (2016). Liver and kidney samples were washed with

176 ice-cold 0.9% phosphate buffered saline, blotted dry and weighed. The tissues were
177 homogenized (10% w/v) in a potassium phosphate buffer solution (pH 7.4) for 30 s
178 and then centrifuged at 3000 rpm for 15 min at 4°C. The supernatant fraction was
179 collected and stored at -80°C for Superoxide dismutase (SOD), Glutathione
180 peroxidase (GSH-Px), Catalase (CAT) and Malondialdehyde (MDA) analyses by
181 means of spectrophotometric methods.

182

183 *Statistical analysis*

184 The statistical analyses were performed using an SPSS software package (version
185 17 for Windows, SPSS Inc., Chicago, IL, USA). Homogeneity of variance was tested
186 using Levene's test. **Data collected, except that of the gut microbiota, were tested by**
187 **one-way ANOVA, evaluating the effect of dietary BP inclusion by polynomial**
188 **contrasts.** Significance was accepted for $P < 0.05$. The similarity distance matrix
189 generated using Bionumerics version 5.1 software (Applied Maths, Sint Martens
190 Latem, Belgium) was used to build a nonmetric multidimensional scaling plot of
191 dissimilarity in which the Euclidean distance was adopted.

192

193 *Bioinformatics analysis*

194 Paired-end reads were analysed by using QIIME 1.9.0 Software and a recently
195 described pipeline (Ferrocino *et al.*, 2017). In order to avoid biases due to different
196 sequencing depths, all the samples were rarefied at 13 821 reads after raw read
197 quality filtering. Statistics and plotting were performed in R environment. Details are
198 provided as Supplementary Material S1. The 16S rRNA gene sequences are
199 available at the Sequence Read Archive of NCBI (National Center for Biotechnology
200 Information) (accession number SRP100668).

201

202 **Results**

203 *Digestibility and growth performance*

204 Table 2 reports the apparent digestibility coefficients of the nutrients. None of them
205 were affected by the inclusion level of BP except EE, which appears higher in rabbits
206 fed BP than BP0. Calculating the mean CP digestibility of the experimental diets
207 (67.1%), we found 10.47 % of DP level of BP, since no effect on CP digestibility were
208 registered and since the estimation was used for moderate incorporation (50, 100
209 and 150 g/kg). According to the mean of the four diets, the DE was estimated to 9.44
210 MJ/kg DM which corresponds to 2256 kcal.

211 During the trial no health problem was experienced and only two rabbits died (one
212 BP0 and one BP10). The initial BW ranged from 936 to 941 g, and the final BW
213 ranged from 3177 and 3208 g without significant differences among groups. Final
214 BW and ADG were not affected by the feeding treatments for all periods considered
215 (Table 3). ADFI and FCR showed a linear ($P<0.001$) decrease to BP with a minimum
216 observed for BP15 group from 49 to 83 days of age. During the whole period, ADFI
217 and FCR showed linear and quadratic effects to BP inclusion level with a minimum
218 corresponding to BP10 group ($P<0.001$).

219

220 *Caecal traits, blood parameters and oxidative status*

221 No significant effects related to BP inclusion level were observed for the caecal traits.
222 The inclusion level of BP did not affect the blood parameters (Table 4), except for the
223 total protein and beta 2 globulin concentration which showed a linear effect to BP
224 inclusion level. GSH-Px activity of the liver ($P<0.05$) and kidney ($P<0.001$) showed a
225 linear responses to BP with a maximum observed for BP15 group.

226

227 *Analysis of the gut microbiota*

228 The PCR-DGGE analysis presented in Figure 1 shows no clear separation of the
229 samples as affected by dietary incorporation. A few sub-clusters, with at least 2-4
230 samples in the same experimental group, were found to have a high percentage of
231 similarity (>60%). However, the caecal sample profiles were grouped together
232 (>70%). After sequencing, a total of 2 656 045 raw reads (2x250bp) were obtained.
233 After joint and quality filtering, a total of 1144 88 reads passed the filters applied by
234 QIIME, with an average value of 71511 reads/sample, and a sequence length of 433
235 bp (Table S2). The number of OTUs, the Good's estimated sample coverage (ESC),
236 the Chao1 and Shannon indices obtained for all the samples are reported in
237 Supplementary Table S2 and Supplementary Figure S1. The rarefaction analysis and
238 the estimated sample coverage indicated that there was satisfactory coverage for all
239 the samples (ESC average 85%). The trend of the rarefaction curves also confirmed
240 that bacterial richness was sampled (Supplementary Figure S1). Adonis and Anosim
241 statistical tests, based on the Weighted UniFrac distance matrix, showed significant
242 differences for the time, and when faeces and caecal samples were compared
243 ($P<0.001$), but no difference was observed when the different diets were compared.
244 The dietary treatment affects the microbiota composition (Figure 2). *Clostridium*,
245 *Oscillospira*, *Ruminococcus* and *Ruminococcaceae* were in particular found to be
246 characteristic of the BP groups ($P<0.01$), mainly in BP5 group. On the other hand,
247 *Lachnospiraceae* were found to be characteristic of the basal diet ($P<0.01$).
248 *Ruminococcus* and *Lachnospiraceae* were never found below 6% of the relative
249 abundance in any of the samples, a prevalence of *Ruminococcus* was observed in
250 BP5 (around 10%), while *Lachnospiraceae* were found to be characteristic of the

251 BP0 samples (6% of the relative abundance). *Ruminococcaceae*, *Oscillospira* and
252 *Clostridiales* were never found below 10%, 4% or 40% of the relative abundance,
253 respectively, and were always higher when affected by the inclusion of 5% BP.
254 Clear differences were observed when comparing fecal and caecal samples
255 ($P<0.001$). It was possible to observe a significant increase in *Ruminococcaceae* and
256 a reduction in *Clostridiales* (Figure 2). OTU co-occurrence was investigated (Figure
257 3) and *Clostridiales* and *Lachnospiraceae*, showed the highest number of negative
258 correlations, and a notable exclusion of *Ruminococcaceae* and *Enterococcaceae*
259 was observed. *Ruminococcaceae* co-occurred together with *Dorea*, *Coprococcus*
260 and *Clostridium*. Plotting the correlation between the OTUs and the predicted
261 pathways (Figure 4 and Supplementary Table S3), it appeared that
262 *Ruminococcaceae*, *Clostridium* and *Dorea* were mainly related to the lipid
263 metabolism (steroid biosynthesis and fatty acid (FA) elongation pathways)
264 (FDR<0.05). *Clostridiales* were mainly related to the ether lipid metabolism and α
265 linoleic acid metabolism (FDR<0.05) and *Ruminococcus* was found to be positively
266 related to galactose, starch and the sucrose metabolism (FDR<0.05).

267

268 **Discussion**

269 *Nutritive value of BP and digestibility trial*

270 Few information are available in bibliography about the chemical composition
271 of BP and about its suitability as a protein and fibre source. BP used in this trial
272 showed an interesting phenolic compound profile where chlorogenic acid and
273 delphinidin -3-galactoside were the most abundant phenolic acid and anthocyanin
274 respectively. BP is also high in protein (142 g/kg DM) and contained 626, 433 and
275 258 g/kg DM of NDF, ADF and ADL, respectively. References to the use of BP in

276 rabbit nutrition are not available in tables of ingredients (INRA, 2004). However, our
277 product remained close to the grape pomace (GP) mentioned in the INRA tables
278 (2004) and by Martens *et al.* (2002). The incorporation level of BP in the feed
279 increased especially the fiber fraction. In the contrary, CP remained similar among
280 the experimental feeds.

281 In the present trial, the BP inclusion level only improved the digestibility
282 coefficient of EE ($P<0.001$), compared to the BP0 group. The EE digestibility
283 coefficient is generally higher when the level of dietary fat is increased (Table 1), and
284 its value usually depends on the type of fat that is added (Pascual *et al.*, 2002). The
285 EE digestibility found here was affected by the increasing level of BP in the diet, and
286 depended on its unsaturation degree. In fact, this result is associated with a high EE
287 in BP (15.5% DM), and seems to be related to its richness in polyunsaturated FAs
288 (PUFA), especially α -linolenic acid (32.6 g/100 g of the total FAs; Dabbou *et al.*,
289 2017b), which are easier emulsified in the digestive tract than saturated FAs
290 (Pascual *et al.*, 2002). It is interesting to note that the BP groups, compared with
291 some raw materials used in rabbit feeds (soybean hulls and GP) with a similar NDF
292 content, showed higher NDF digestibility values (Gidenne *et al.*, 2010).

293 Our product presented a moderate energetic value than other by-products as GP (5.0
294 and 9.3 MJ/kg DM), dried beet pulp (10.3 MJ/kg DM) and dried citrus pulp (11.2
295 MJ/kg DM) (INRA, 2004; Martens *et al.*, 2002, Guemour *et al.*, 2010), since its
296 protein, ADF and ADL contents were respectively higher and lower than the above
297 mentioned by-products. Moreover, using a low incorporation level in the feed
298 probably avoid any major anti-nutritional effect from tannins or other components.
299 Villamide, (1996) and Villamide *et al.* (2001) showed that the incorporation of feed
300 ingredients at a low level leads to a lower precision in the estimated nutritive value,

301 particularly if measured on a low number of animals and on a short period. DP and
302 DE are applicable only in the tested range of incorporation ($\leq 150\text{g/kg}$) since we used
303 a low incorporation level in the feed.

304

305 *Growth performance*

306 It is important to highlight that diets were not exactly balanced due to the BP
307 inclusion level. This trial was designed not with the aim to evaluate the effects of BP
308 on rabbit growth performance but in order to supply more information about the
309 nutritive value of BP and to evaluate some valuable nutritional facts for further
310 experiments with balanced diets.

311 During the weaning period (35-49 d of age), ADFI and FCR did not differ significantly
312 between groups. However, during the finishing period and the whole period, the ADFI
313 and the FCR decreased linearly with the increase of BP incorporation rate.
314 Considering the whole period, FCR decreased by about 3.5% compared to BP0 (3.54
315 vs 3.41) at 5% of BP incorporation in the diet. This reduction was greater in the
316 animals fed the BP10 diet, and resulted in a 10% better efficiency than BP0. These
317 results could be related to the organic acids (such as malic, citric acid, and others)
318 contained in fruit pomaces that could improve flavour and palatability of the feed
319 mixture, and stimulate the secretion of gastric juice. On the basis of these results, it is
320 possible to state the 10% supplement of BP has led to the best results without
321 negative effect on growth performance. However, further experiments are necessary
322 to confirm the present results and try to determine the optimal incorporation rate for
323 the BP in balanced feeds.

324 Using other by-products, Guemour *et al.* (2010) did not report effect of GP in FCR
325 during the post weaning period showing a linear increase in FCR with 3 to 6 % GP

326 incorporation rate during the whole fattening period. Garcia *et al.* (1993) showed that
327 the moderate substitution of barley grain by sugar beet pulp (SBP) has little effect on
328 intake and growth performance in finishing rabbits while at high levels, SBP inclusion
329 (> 35%) severely impaired growth performance. Pieszka *et al.* (2017) demonstrated
330 that ADFI and FCR are influenced by the type of dry pomace fed to fattening pigs,
331 with lowest values being observed in apple and carrot fed groups. On the other hand,
332 Jurgoński *et al.* (2014) reported that the dietary addition of a polyphenol-rich extract
333 from blackcurrant pomace had no effect on the final body weight, ADG or ADFI of
334 rabbits fed standard and high fat diets.

335

336 *Caecal traits, blood analysis and oxidative status*

337 It should be pointed out that the caecal content was unaffected by the different BP
338 supplementation diets (Table 4). The values were similar to those reported by Rotolo
339 *et al.* (2014) in growing rabbits. The pH value of the caecum content was similar to
340 those obtained by Jurgoński *et al.* (2014), who studied the effect of a polyphenol-rich
341 extract, obtained from blackcurrant pomace, on growing rabbits. These authors
342 reported that the addition of the polyphenolic extract was associated with a decrease
343 in pH in the small intestine, but had no effect on the pH of the caecum.

344 The BP inclusion level did not influence the MDA values or CAT activities to any
345 great extent (Table 4). However, the highest GSH-Px level was recorded in the
346 kidney and liver samples obtained from the BP supplemented groups. GSH-Px
347 prevents tissues from suffering from oxidative damage and counteracts oxidative
348 stress (Choi *et al.*, 2010). An improved liver GSH-Px activity was also observed in
349 rabbits fed a diet supplemented with a grape seed and peel extracts (Choi *et al.*,
350 2010). Sgorlon *et al.* (2005) stated that the oxidative stress markers were significantly

351 increased for 0.03 and 0.15 (mg/kg) of grape polyphenol supplementation in New
352 Zealand White rabbit diets during a period of heat stress caused by the high
353 temperatures of the summer season. These results indicated that BP polyphenols act
354 as effective antioxidants and increase kidney and liver resistance to oxidative stress
355 through the activation of an antioxidant enzyme system.

356 Jankowski *et al.* (2016) revealed an improvement in the antioxidant status indicators,
357 including a decrease in the lipid peroxide levels, an increase in the antioxidant
358 capacity of the hydrophilic and lipophilic fractions of the blood plasma, and a
359 decrease in the concentration of the hepatic thiobarbituric acid reactive substances in
360 turkeys fed diets enriched with PUFA and fruit pomace as sources of polyphenols.

361

362 *Gut microbial community profile*

363 The microbiota of the digestive tract plays an important role in the development of gut
364 immunity and the prevention of pathogen overgrowth (Chung *et al.*, 2012). Hence, a
365 quick adaptation to reach an appropriate stable microbial ecosystem leads to health
366 conditions in rabbits (Combes *et al.*, 2013). Amplicon-based sequencing of 16S rRNA
367 and PCR-DGGE were performed on the faeces and caecal contents to examine in
368 order to establish the effects of the dietary supplementation on the intestinal
369 microbiota composition. No difference was observed, in terms of complexity between
370 samples, in the alpha diversity index or in the DGGE results.

371 The gut ecosystem of rabbits contains a wide variety of bacterial species that play an
372 important role in producing volatile FAs, as an energy source, amino acids (AA) and
373 vitamins by means of fermentation, whereas the major component of hard faeces
374 contains indigestible material with a short transit time (Michelland *et al.*, 2010). A
375 difference in the bacterial community was observed between the caecal content and

376 hard faeces on the basis of the microbiota and physiological activities as observed by
377 Michelland *et al.* (2010). The BP dietary supplementation increases the relative
378 abundance of *Clostridium*, *Oscillospira* and *Ruminococcus*. This increment has been
379 particularly important for the BP5 group. Moreover, their presence co-excluded the
380 presence of other OTUs. These results could be related to the BP composition. In
381 fact, fruit pomaces are characterized by a high content of fibre-polyphenol complexes
382 and polyphenols can exert both positive and negative effects on the properties of
383 dietary fibre in pomaces, including fibre influence on the composition of gut
384 microflora. *Oscillospira* in rabbit gut microbiota may be involved in the fermentation
385 process (Zeng *et al.*, 2015) while *Ruminococcus* produced propionate and butyrate
386 (Reichardt *et al.*, 2014), which play a protective role against different types of disease
387 (De Filippis *et al.*, 2015). Fruit pomaces led to a significant increase in butyric
388 proportion in the short-chain FAs profile in caecal digesta of turkeys Juskiwicz *et al.*
389 (2016). In the present trial, the dietary inclusion of BP has been characterized by the
390 dominance of *Clostridium*, which has recently been associated with elevated levels of
391 AA and phenolic compounds in the gut (Ponnusamy *et al.*, 2011). The main OTUs,
392 affected by the BP, were related to the putative genes involved in the lipid
393 metabolism, in particular steroid and FA biosynthesis. The presence of such long
394 FAs can be related to many health-related functions, such as anti-inflammatory
395 effects (Nguemeni *et al.*, 2013).

396

397 **Conclusion**

398 **The nutritive value obtained in the present study for BP appeared high if compared to**
399 **other by-products mentioned in the literature.** BP can be considered a good
400 candidate as alternative protein and fibre sources for the growing rabbits without

401 adverse effect on growth performance, caecal environmental condition and caecal
402 contents **in the tested range of incorporation (<150 g/kg)**. BP inclusion leads to a
403 modification of the gut microbiota, which in turn favors the development of several
404 taxa. Its use in rabbit nutrition could represent an opportunity to valorize agro-
405 industrial by-products. However, further researches are necessary to confirm the
406 present results and to determine the optimal inclusion level for BP in balanced diets.

407

408 **Conflict of interest statement**

409 The authors declare that there is no conflict of interest.

410

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414

415 **References**

416 Association of Official Analytical Chemists (AOAC) 2000. Official Method of Analysis,
417 16th edition. AOAC, Gaithersburg, MD, USA.

418 Association of Official Analytical Chemists (AOAC) 2003. Official Methods of Analysis
419 17th edition. AOAC, Gaithersburg, MD, USA.

420 Choi ChS, Chung H K, Choi M K and Kang M H 2010. Effects of grape pomace on
421 the antioxidant defense system in diet-induced hypercholesterolemic rabbits.
422 Nutrition Research and Practice 4, 114–120.

423 Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC,
424 Villablanca EJ, Wang S, Mora JR, Umesaki Y, Mathis D, Benoist C, Relman DA and

425 Kasper DL 2012. Gut immune maturation depends on colonization with a host
426 specific microbiota. *Cell* 149, 1578–1593.

427 Combes S, Fortun-Lamothe L, Cauquil L and Gidenne T 2013. Engineering the rabbit
428 digestive ecosystem to improve digestive health and efficacy. *Animal* 7, 1429–1439.

429 Dabbou S, Gasco L, Gai F, Zoccarato I, Rotolo L, Dabbou Fekih S, Brugiapaglia A,
430 Helal A N and Peiretti PG 2014. Dried artichoke bracts in rabbits nutrition: effects on
431 the carcass characteristics, meat quality and fatty-acid composition. *Animal* 8, 1547–
432 1553.

433 Dabbou S, Renna M, Lussiana C, Gai F, Rotolo L, Kovitvadhi A, Brugiapaglia A,
434 Helal AN, Schiavone A, Zoccarato I and Gasco L 2017a. Bilberry pomace in growing
435 rabbit diets: effects on quality traits of hind leg meat. *Italian Journal of Animal
436 Science* 16, 371–379.

437 Dabbou S, Gai F, Renna M, Rotolo L, Dabbou S, Lussiana C, Kovitvadhi A,
438 Brugiapaglia A, De Marco M, Helal AN, Zoccarato I and Gasco L 2017b. Inclusion of
439 bilberry pomace in rabbit diets: Effects on carcass characteristics and meat quality.
440 *Meat Science* 124, 77–83.

441 De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, Serrazanetti DI,
442 Di Cagno R, Ferrocino I, Lazzi C, Turrone S, Cocolin L, Brigidi P, Neviani E, Gobbetti
443 M, O’Toole PW and Ercolini D 2015. High-level adherence to a Mediterranean diet
444 beneficially impacts the gut microbiota and associated metabolome. *Gut Microbiota*
445 0, 1–10.

446 EGRAN (European Group on Rabbit Nutrition) 2001. Technical note: attempts to
447 harmonize chemical analyses of feeds and faeces, for rabbit feed evaluation. *World
448 Rabbit Science* 9, 57–64.

449 Ferrocino I, Di Cagno R, De Angelis M, Turrone S, Vannini L, Bancalari E, Rantsiou
450 K, Cardinali G, Neviani E and Cocolin L 2015. Fecal microbiota in healthy subjects
451 following omnivore, vegetarian and vegan diets: culturable populations and rRNA
452 DGGE Profiling. PLoS ONE 10, 1–16.

453 Ferrocino I, Bellio A, Romano A, Macori G, Rantsiou K, Decastelli L and Cocolin L
454 2017. RNA-based amplicon sequencing reveals microbiota development during
455 ripening of artisanal versus industrial lard d'Arnad. Applied and Environmental
456 Microbiology 83, 1–14. doi:10.1128/AEM.00983-17.

457 Garciaz G, Galvez JF and JC de Blas 1993. Effect of substitution of sugarbeet pulp
458 for barley in diets for finishing rabbits on growth performance and on energy and
459 nitrogen efficiency. Journal of Animal Science 71, 1823–1830.

460 Gidenne T, Carabaño R, García J and De Blas JC 2010. Fibre Digestion. In The
461 nutrition of the rabbits. (ed. De Blas J C and J. Wiseman), 2nd Edition. pp. 66–82.
462 CABI International, Wallingford. UK.

463 Guemour D, Bannelier C, Dellal A, Gidenne T 2010. Nutritive value of sun-dried
464 grape pomace, incorporated at a low level in complete feed for the rabbit bred under
465 Magrebian conditions. World Rabbit Science 18, 17–25.

466 INRA 2004. Tables de composition et de valeur nutritive des matières premières
467 destinées aux animaux d'élevage: porc, volailles, bovins, ovins, caprins, lapins,
468 chevaux, poissons. In: Sauvant D., Perez J.M., Tran G. (eds), 2^{ème} Edition. INRA
469 Editions, Paris, 301p.

470 Jankowski J, Juśkiewicz J, Zduńczyk P, Kosmala M, Zieliński H, Antoszkiewicz Z,
471 Zduńczyk Z 2016. Antioxidant status of blood and liver of turkeys fed diets enriched
472 with polyunsaturated fatty acids and fruit pomaces as a source of polyphenols. Polish
473 Journal of Veterinary Sciences 19, 89–98.

474 Jurgoński A, Juśkiewicz J, Zduńczyk Z, Matusevicius P and Kołodziejczyk K 2014.
475 Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and
476 serum lipid changes induced by a high-fat diet in rabbits. *European Journal of*
477 *Nutrition* 53, 1603–1613.

478 Jusiewicz J, Jankowski J, Kosmala M, Zdunczyk Z, Slominski BA and Zdunczyk P
479 2016. The effects of dietary dried fruit pomaces on growth performance and
480 gastrointestinal biochemistry of turkey poults. *Journal of Animal Physiology and*
481 *Animal Nutrition* 100, 967–976.

482 Khanal RC, Howard LR, Wilkes SE, Rogers TJ and Prior RL 2012. Effect of dietary
483 blueberry pomace on selected metabolic factors associated with high fructose
484 feeding in growing Sprague-dawley rats. *Journal of Medicinal Food* 15, 802–810.

485 Kovitvadhi A, Gai F, Dabbou S, Ferrocino I, Rotolo L, Falzone M Vignolini C,
486 Gennero MS, Bergagna S, Dezzutto D, Barbero R, Nebbia P, Rosati S, Cocolin L,
487 Zoccarato I and Gasco L 2016. Rabbit dietary supplementation with pale purple
488 coneflower. 2. Effects on the performances, bacterial community, blood parameters
489 and immunity of growing rabbits. *Animal* 10, 1110–1117.

490 Martens L, Perez JM, Villamide M, Cervera C, Gidenne T, Xiccato G 2002. Nutritive
491 value of raw materials for rabbits: EGRAN tables 2002. *World Rabbit Science* 10,
492 157–166.

493 Michelland RJ, Combes S, Monteils V, Cauquil L, Gidenne T and Fortun-Lamothe L
494 2010. Molecular analysis of the bacterial community in digestive tract of rabbit.
495 *Anaerobe* 16, 61–65.

496 Nguemeni C, Gouix E, Bourourou M, Heurteaux C and Blondeau N 2013. Alpha-
497 linolenic acid: A promising nutraceutical for the prevention of stroke. *Pharma*
498 *Nutrition* 1, 1–8.

499 Pascual JJ, Motta W, Cervera C, Quevedo F, Blas E, Fernández-Carmona J 2002.
500 Effect of dietary energy source on the performance and perirenal fat thickness
501 evolution of primiparous rabbit does. *Animal Science* 75, 267–279.

502 Pieszka M, Szczurek P, Bederska-Łojewska D, Migdał W, Pieszka M, Gogol P and
503 Jagusiak W 2017. The effect of dietary supplementation with dried fruit and vegetable
504 pomaces on production parameters and meat quality in fattening pigs. *Meat Science*
505 126, 1–10.

506 Ponnusamy K, Choi JN, Kim J, Lee SY and Lee CH 2011. Microbial community and
507 metabolomic comparison of irritable bowel syndrome faeces. *Journal of Medical*
508 *Microbiology* 60, 817–827.

509 Puupponen-Pimiä R, Nohynek L, Hartmann-Schmidlin S, M. Kähkönen, Heinonen M,
510 Määttä-Riihinen K and KM Oksman-Caldentey 2005. Berry phenolics selectively
511 inhibit the growth of intestinal pathogens. *Journal of Applied Microbiology*, 98, 991–
512 1000.

513 Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, Flint
514 HJ and Louis P 2014. Phylogenetic distribution of three pathways for propionate
515 production within the human gut microbiota. *The ISME Journal* 8,1323–1335.

516 Rotolo L, Gai F, Peiretti P G, Ortoffi M, Zoccarato I and Gasco L 2014. Live yeast
517 (*Saccharomyces cerevisiae* var. *boulardii*) supplementation in fattening rabbit diet:
518 Effect on productive performance and meat quality. *Livestock Science* 162, 178–184.

519 Saura-Calixto F 2011. Dietary fiber as a carrier of antioxidants: an essential
520 physiological function. *Journal of Agricultural and Food Chemistry* 59, 43–49.

521 Sgorlon S, Stradaoli G, Stefanon B, Altimer G and Della Loggia R 2005. Dietary
522 grape polyphenols modulate oxidative stress in ageing rabbits. *Italian Journal of*
523 *Animal Science* 4, 541–543.

524 Silva VK, Morita VD and Boleli IC 2013. Effect of pectin extracted from citrus pulp on
525 digesta characteristics and nutrient digestibility in broilers chickens. *Revista Brasileira*
526 *de Zootecnia* 42, 575–583.

527 Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral
528 detergent fibre, and non starch polysaccharides in relation to animal nutrition. *Journal*
529 *of Dairy Science* 74, 3583–3591.

530 Villamide MJ. 1996. Methods of energy evaluation of feed ingredients for rabbits and
531 their accuracy. *Animal Feed Science and Technology* 57, 211–223.

532 Villamide MJ, Martens L, Cervera C, Perez JM and Xiccato G 2001. A critical
533 approach of the calculation procedures to be used in digestibility determination of
534 feed ingredients for rabbits. *World Rabbit Science* 9, 19–25.

535 Zeng B, Han S, Wang P, Wen B, Jian W, Guo W, Yu Z, Du D, Fu X, Kong F, Yang M,
536 Si X, Zhao J and Li Y 2015. The bacterial communities associated with fecal types
537 and body weight of rex rabbits. *Scientific Reports* 5, 9342.

538

539 **Table 1**

540 Proximate composition of the bilberry pomace and the experimental diets of the
 541 rabbits (modified from Dabbou *et al.*, 2017b).

Experimental diets (% as fed)	BP	BP0	BP5	BP10	BP15
Basal mixture*		98.0	93.0	88.0	83.0
Bilberry Pomace		.	5.0	10.0	15.0
Vitamin mineral premix [#]		1.5	1.5	1.5	1.5
Bicalcium phosphate		0.5	0.5	0.5	0.5
Proximate composition					
(% DM, unless otherwise stated)					
DM	94.4	88.2	88.2	88.0	88.5
Ash	1.8	7.5	7.5	7.1	7.2
CP	14.2	17.7	17.7	17.5	17.6
EE	15.5	2.6	3.3	3.9	4.2
NDF	62.6	36.8	37.2	39.1	40.8
ADF	43.3	19.8	20.8	22.0	23.3
ADL ¹	25.8	3.5	4.6	5.6	6.8
Gross Energy (MJ/kg DM)	22.7	17.9	18.1	18.4	18.6

542 Abbreviations: BP= Bilberry pomace; BP0= Bilberry pomace 0%; BP5= Bilberry
 543 pomace 5%; BP10= Bilberry pomace 10%; BP15= Bilberry pomace 15%; DM= Dry
 544 Matter; CP= Crude Protein; EE= Ether Extract; NDF= Neutral Detergent Fibre; ADF=
 545 Acid Detergent Fibre; ADL= Acid Detergent Lignin.

546 *Containing (% fresh matter): alfalfa meal 30, wheat bran 20, barley 17, dried beet
 547 pulp 15, soybean meal 11.5, molasses 2, wheat straw 2 and soybean oil 0.5.

548 #Vitamin A200 U, α -tocopherylacetate 16 mg, Niacin 72 mg, Vitamin B6 16 mg,
549 Choline 0.48 mg, DL-methionin 600 mg, Ca 500 mg, P 920 mg, K 500 mg, Na 1 g,
550 Mg60 mg, Mn 17 mg, Cu0.6 mg per kg of diet.

551 ¹Analysed with the modified Van Soest procedure adapted to tannins content with
552 sodium bisulfate.

553 **Table 2**

554 Feed intake and nutrient digestibility coefficients of the rabbits fed the experimental
555 diets.

	BP0	BP5	BP10	BP15	SEM	<i>P</i>	<i>P</i>
						Linear effect	Quadratic effect
Feed intake (g/day)	140	137	139	140	2.0	0.846	0.618
DM (%)	60.9	60.3	63.3	61.5	0.54	0.334	0.509
OM (%)	59.6	59.0	61.5	59.8	0.56	0.551	0.590
CP (%)	67.7	67.2	66.8	66.8	0.50	0.488	0.801
EE (%)	74.4 ^B	80.5 ^A	81.4 ^A	81.9 ^A	0.62	<0.001	0.001
NDF (%)	30.4	30.9	31.4	31.4	0.72	0.626	0.857
ADF (%)	21.8	22.8	23.2	23.9	0.95	0.459	0.917
Gross Energy (MJ/kg)	60.0	59.5	59.8	59.8	0.49	0.963	0.947
Nutritive value							
Digestible protein (g/kg DM)	122.3	121.3	119.2	119.9			
Digestible energy (MJ/kg DM)	10.96	11.02	11.27	11.37			

556 ^{A-B}Different superscript letters indicate significant differences ($P<0.001$).

557 Abbreviations: BP0= Bilberry pomace 0%; BP5= Bilberry pomace 5%; BP10=
558 Bilberry pomace 10%; BP15= Bilberry pomace 15%; DM= Dry Matter; OM= Organic
559 Matter; CP= Crude Protein; EE= Ether Extract; NDF= Neutral Detergent Fibre; ADF=
560 Acid Detergent Fibre.

561

562 **Table 3**

563 Growth performance of the rabbits fed the experimental diets.

	BP0	BP5	BP10	BP15	SEM	<i>P</i> Linear effect	<i>P</i> Quadratic effect
Number of rabbits	35	36	35	36			
Body weight (g)							
At 35 days of age	937	941	938	936	2.80	0.790	0.650
At 49 days of age	1642	1667	1638	1667	8.38	0.544	0.896
At 83 days of age	3179	3187	3177	3208	15.86	0.598	0.718
Average daily feed intake (g/d)							
35-49 day of age	122	120	121	122	0.94	0.900	0.415
49-83 day of age	180 ^a	174 ^{ab}	173 ^{ab}	168 ^b	1.52	0.008	0.950
35-83 day of age	165 ^A	159 ^B	148 ^C	156 ^B	1.16	<0.001	<0.001
Average daily gain (g/d)							
35-49 day of age	50.1	51.8	49.6	52.6	0.52	0.406	0.638
49-83 day of age	44.3	45.3	44.8	44.9	0.44	0.754	0.584
35-83 day of age	46.7	46.8	46.6	47.3	0.31	0.545	0.641
Feed conversion ratio							
35-49 day of age	2.45	2.32	2.48	2.32	0.03	0.595	0.949
49-83 day of age	4.06 ^A	3.86 ^B	3.87 ^B	3.74 ^B	0.02	<0.001	0.353
35-83 day of age	3.54 ^A	3.41 ^B	3.17 ^D	3.30 ^C	0.02	<0.001	<0.001

564 ^{a-b}Different superscript letters indicate significant differences ($P<0.05$); ^{A-D}Different565 superscript letters indicate significant differences ($P<0.01$).

566 Abbreviations: BP0= Bilberry pomace 0%; BP5= Bilberry pomace 5%; BP10=

567 Bilberry pomace 10%; BP15= Bilberry pomace 15%

568

570 Caecal traits, blood parameters and oxidative status of the rabbit organs.

	BP0	BP5	BP10	BP15	SEM	<i>P</i> Linear effect	<i>P</i> Quadratic effect
Full caecum (%BW)	4.9	5.2	4.8	4.6	0.12	0.224	0.415
Empty caecum (%BW)	1.7	1.7	1.5	1.5	0.04	0.123	0.672
Caecal content (%BW)	3.2	3.5	3.2	3.1	0.09	0.599	0.276
Caecal pH	6.9	6.8	6.9	6.9	0.03	0.989	0.215
Total protein (g/dl)	5.4 ^{ab}	6.0 ^a	5.1 ^{ab}	3.8 ^b	0.31	0.038	0.113
Albumin (g/dl)	4.4	4.0	3.4	3.5	0.23	0.114	0.503
Uric Acid (mg/dl)	1.1	0.4	1.0	0.9	0.13	0.878	0.295
Creatinine (mg/dl)	1.1	1.1	1.10	1.1	0.06	0.800	0.620
Triglycerides (mg/dl)	60.9	41.9	44.2	52.3	4.09	0.525	0.108
Cholesterol (mg/dl)	36.9	40.6	37.9	33.7	2.40	0.584	0.435
AST (U/l)	39.1	42.3	57.5	48.6	5.31	0.377	0.586
ALT (U/l)	47.3	47.4	43.6	39.2	2.93	0.312	0.711
ALP (U/l)	163.1	183.7	169.7	162.6	6.88	0.809	0.338
GGT (U/l)	13.9	14.1	14.2	17.4	0.64	0.062	0.225
Urea (mg/dl)	22.4	20.3	20.1	18.6	1.23	0.316	0.902
BUN (mg/dl)	13.0	13.5	15.0	14.4	0.45	0.182	0.514
Alpha-1 globulin (g/dl)	0.1	0.1	0.1	0.1	0.01	0.203	0.818
Alpha-2 globulin (g/dl)	0.1	0.1	0.1	0.1	0.01	0.225	1.000
Beta-1 globulin (g/dl)	0.2	0.2	0.1	0.1	0.01	0.259	0.597
Beta-2 globulin (g/dl)	0.3 ^a	0.3 ^{ab}	0.2 ^b	0.2 ^{ab}	0.02	0.018	0.216
Gamma (g/dl)	0.7	0.7	0.5	0.6	0.05	0.153	0.626
Liver							
GSH-Px (µmole gsh/min/mg protein)	378.7 ^b	429.2 ^{ab}	436.0 ^{ab}	487.8 ^a	15.05	0.012	0.996
MDA (µmole/mg protein)	2.6	2.7	2.4	2.4	0.10	0.356	0.691
Catalase (µmol H ₂ O ₂ degraded /min/mg protein)	448.2	588.0	362.6	626.4	52.37	0.503	0.546
Kidney							
GSH-Px (µmole gsh/min/mg protein)	151.4 ^C	174.4 ^{AB}	219.8 ^B	278.5 ^A	13.68	<0.001	0.376
MDA (µmole/mg protein)	3.7	3.7	3.7	3.6	0.04	0.640	0.675
Catalase (µmol H ₂ O ₂ degraded /min/mg protein)	240.4	223.6	209.9	178.9	16.15	0.196	0.832

571 ^{a-b}Different superscript letters indicate significant differences ($P<0.05$); ^{A-C}Different
572 superscript letters indicate significant differences ($P<0.01$).

573 Abbreviations: BP0=Bilberry pomace 0%; BP5= Bilberry pomace 5%; BP10= Bilberry
574 pomace 10%; BP15=Bilberry pomace 15%; BP0= Bilberry pomace 0%; BP5=
575 Bilberry pomace 5%; BP10=Bilberry pomace 10%; BP15= Bilberry pomace 15%;
576 BW: body weight; AST=aspartate aminotransferase; ALT= alanine aminotransferase;
577 ALP=Alkaline phosphatase; GGT: gamma glutamyltransferase; BUN=blood urea
578 nitrogen; GSH-Px=Glutathione peroxidase; MDA=Malondialdehyde.

579 **Figure 1.** Cluster analysis of the denaturing gradient gel electrophoresis profile of the
580 bacterial communities in the hard faeces (F) and caecal content (C) of the rabbits fed
581 with 0, 5, 10 and 15% bilberry pomace (BP) levels sampled at 35, 49, and 83 days of
582 age; Five replicates per group.

583

584 **Figure 2.** Incidence of the major taxonomic groups detected by means of 16S
585 amplicon target sequencing. Only Operational Taxonomic Units (OTUs) with an
586 incidence above 0.2% in at least 2 samples are shown. The samples are labelled
587 according to the bilberry pomace dietary supplementation levels at 0, 5, 10 and 15%
588 (BP0, BP5, BP10 and BP15), to the type (hard faeces (F) and caecal content (C))
589 and to the days of age (35, 49 and 83).

590

591 **Figure 3.** Significant co-occurrence and co-exclusion relationships between the
592 bacterial Operational Taxonomic Units (OTUs). Spearman's rank correlation matrix of
593 the OTUs with > 0.2% abundance in at least 2 samples. Close correlations are
594 indicated with large circles, whereas weak correlations are indicated by/with small
595 circles. The colour of the scale bar denotes the nature of the correlation, with 1
596 indicating a perfectly positive correlation (dark blue) and -1 indicating a perfectly
597 negative correlation (dark red). Only significant correlations (False Discovery Rate <
598 0.05) are shown.

599

600 **Figure 4.** Heat plot showing Spearman's correlations between the Operational
601 Taxonomic Units (OTUs) occurring/ at 0.2% in at least 2 samples and the predicted
602 metabolic pathways, related to the Amino Acid Metabolism (red), Biosynthesis of
603 Other Secondary Metabolites (green), the Carbohydrate Metabolism (blue), Energy

604 Metabolism (orange), Lipid Metabolism (black) and the Metabolism of the Cofactors
605 and Vitamins (yellow). The Rows and columns are clustered according to Ward
606 linkage hierarchical clustering.

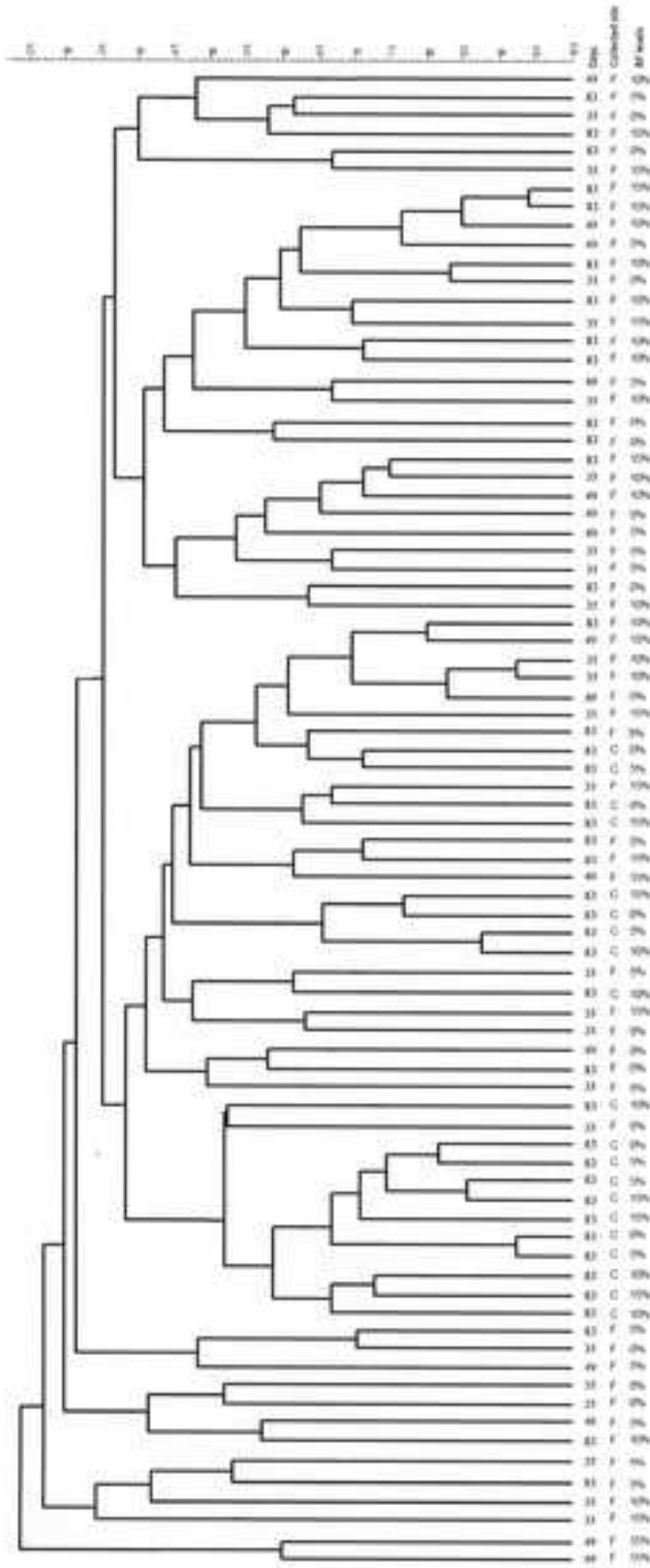


Figure 2

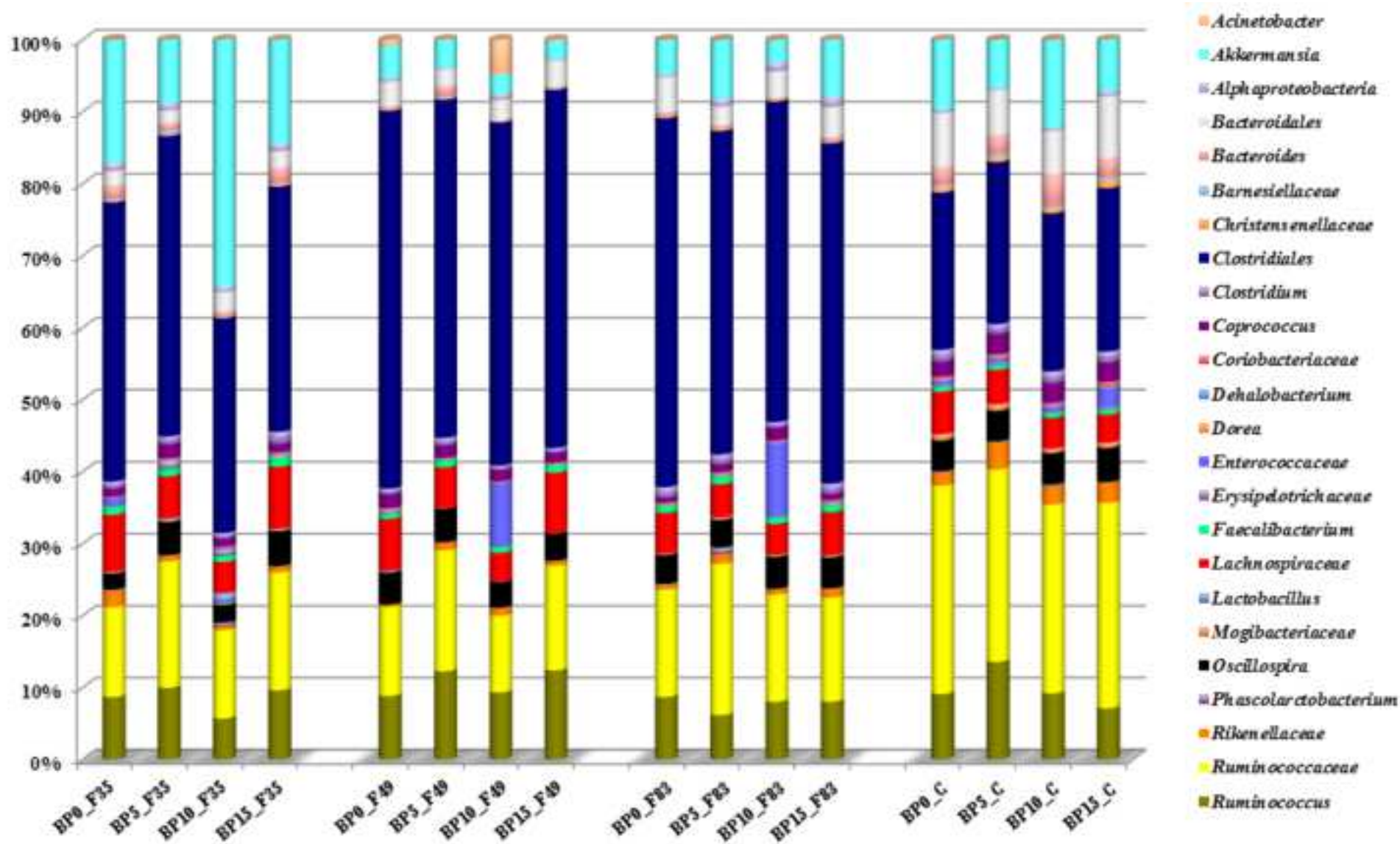
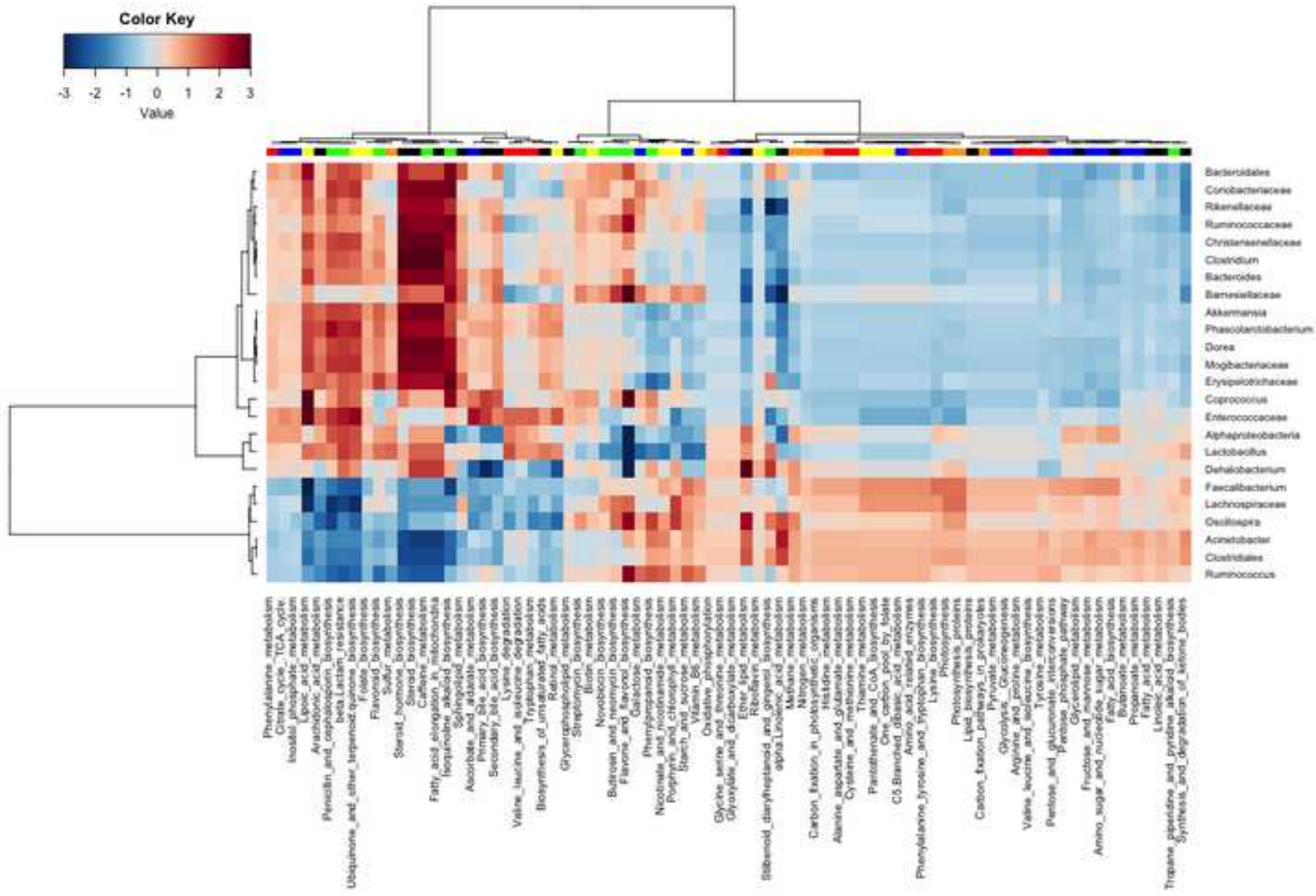
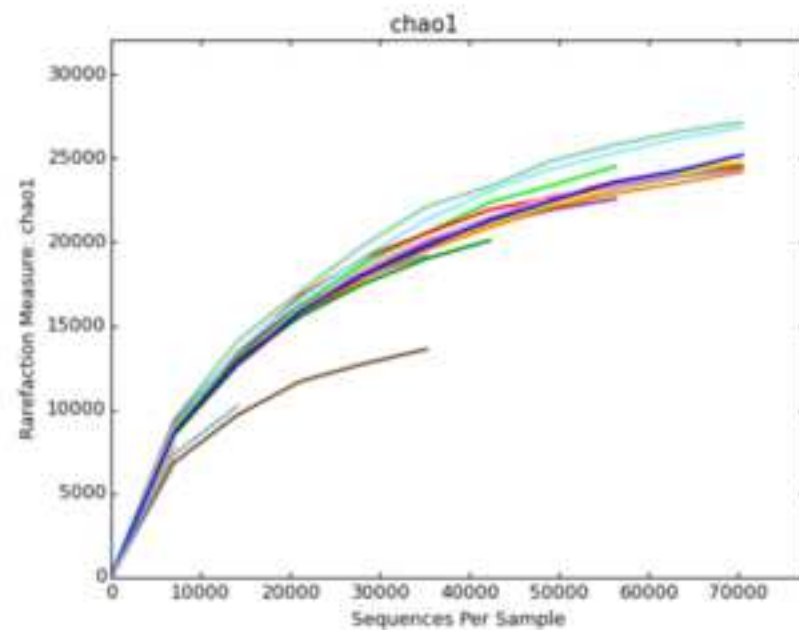
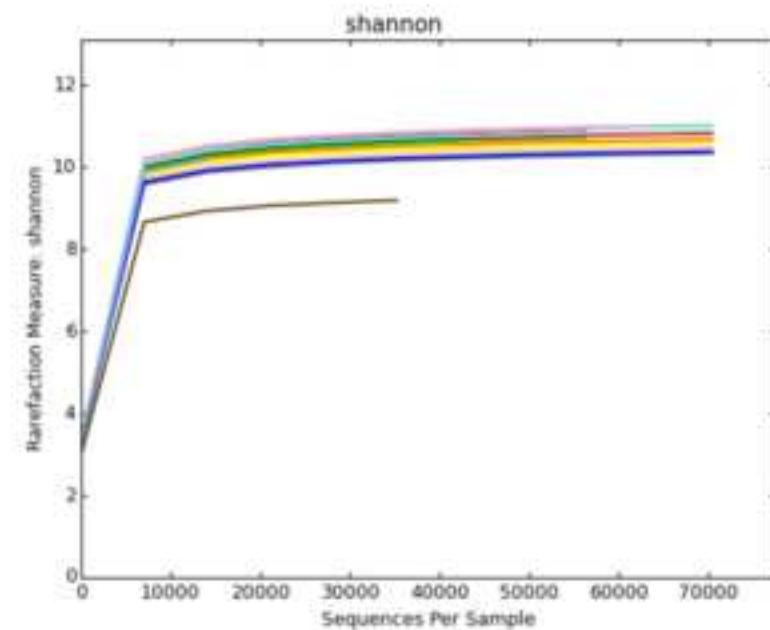
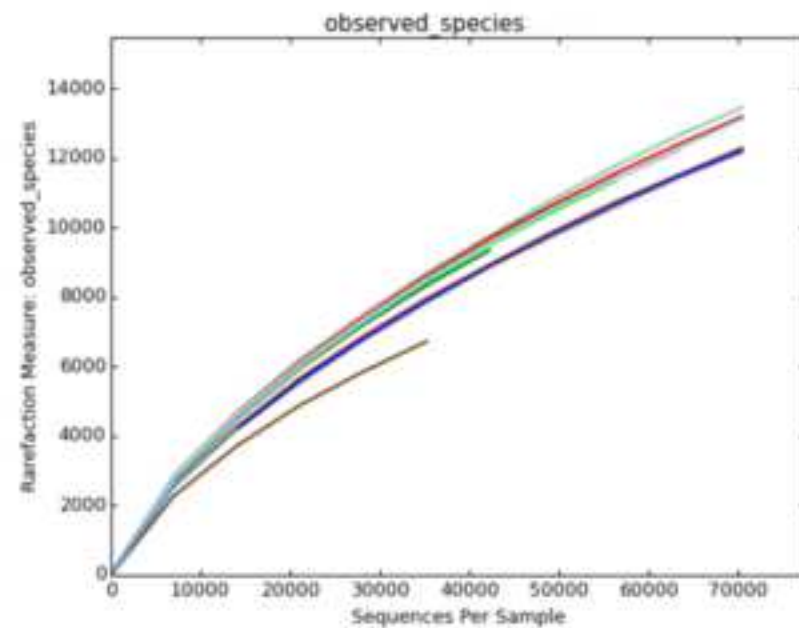
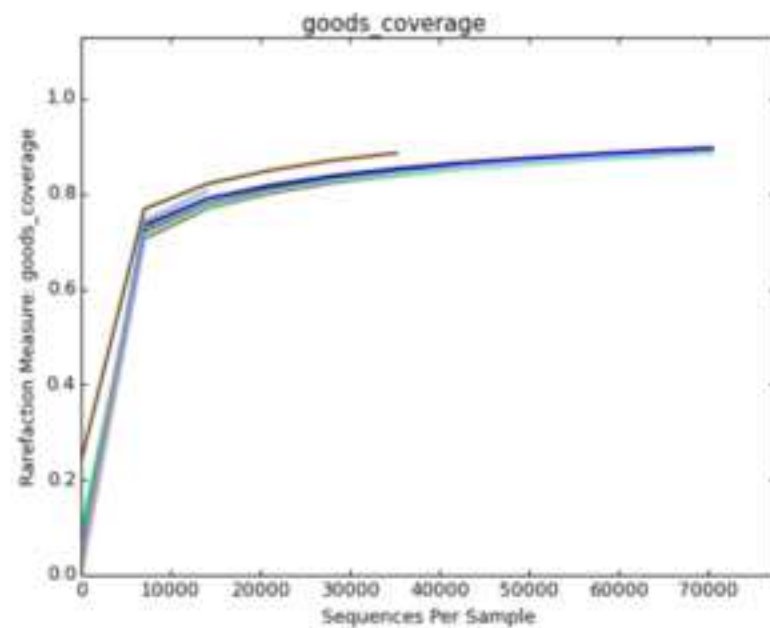
[Click here to download Figure Figure 2.tif](#)

Figure 4





- BP0_C
- BP0_F35
- BP0_F49
- BP0_F83
- BP5_C
- BP5_F35
- BP5_F49
- BP5_F83
- BP10_C
- BP10_F35
- BP10_F49
- BP10_F83
- BP15_C
- BP15_F35
- BP15_F49
- BP15_F83

Bilberry pomace in rabbit nutrition: effects on growth performance, apparent digestibility, caecal traits, bacterial community and antioxidant status

S. Dabbou, I. Ferrocino, A. Kovitvadi, S. Dabbou, S. Bergagna, D. Dezzuto, A. Schiavone, L. Coccolin, F. Gai, V. Santoro, L. Gasco

Samples extraction

Samples weighing 1 g were extracted with 10 mL of 80:20 (v/v) methanol:water with formic acid (1%) using a ultrasonic water-bath (KNF Italia, Cernusco Sul Naviglio, Italy) for 30 min and centrifuged for 15 min at 4000g (Borges et al 2009). The pellets were re-extracted three times and supernatants were pooled and then evaporated under vacuum at room temperature using a Speedvac (SC210A; Savant Instruments, Farmingdale, NY, USA). The residues were resuspended in 10mL of methanol/water (50:50, v/v) with formic acid (0.1%).

All the extracts were characterized by HPLC-DAD-ESI-HRMS in positive and negative ion mode. On the basis of the information obtained from the UV-VIS spectrum and of the accurate mass of precursor ions and tandem MS experiments, the main polyphenols were identified and quantified.

Determination of main polyphenols

The instrument setup for the polyphenols (phenolic acids, flavanols, flavonols and anthocyanins) analyses consisted of a Dionex Ultimate 3000 HPLC system equipped with a DAD detector (Thermo Scientific Surveyor), coupled with a high resolution mass spectrometer LTQ-Orbitrap (Thermo Scientific, Bremen, Germany) through an ESI interface operating in both positive and negative ion mode.

Two different instrumental conditions were adopted in order to analyze anthocyanins and other flavonoid compounds.

For anthocyanin compounds a RP C18 column (Varian Pursuit C18 150 × 2.0 mm, 3 μm particle size, Agilent, Milan; Italy) at 200 μL/min flow rate was used. The elution solvents adopted were formic acid 5% in methanol (B) and in water (A). The gradient profile was 0-6 min from 10 to 15% of B, 6-12 min from 15 to 25% B, 12-16 min from 25 to 30% B, an isocratic step to 30% of solvent B for 14 minutes, and finally 20-32 min from 30 to 100% B. The tuning parameters used for the ESI source were: capillary temperature 270°C, flow rate of sheath gas and auxiliary gas set at 35.0 and 15.0 arbitrary units, capillary voltage 8.0 V, source voltage 4.5 kV and tube lens 65 V. Full scan spectra were acquired in positive ion mode in the range 250-1000 m/z with the resolution of 30,000 (FWHM). MSⁿ spectra were acquired in the range between ion trap cut-off and precursor ion m/z values.

For other polyphenol compounds (phenolic acids, flavanols, flavonols) a biphenylic stationary phase (Pinnacle DB BiPh 150 × 2.1 mm, 3 μm particle size, Resteck, Milan, Italy) at 200 μL/min flow rate was used. The elution solvent adopted were methanol (B) and ammonium acetate 5mM (A). The gradient profile was 0-3 min to 2% of B, 3-60 min from 2 to 62% B, 60-65 min from 62 to 100% B.

The tuning parameters used for the ESI source were: capillary temperature 270°C, flow rate of sheath gas and auxiliary gas set at 35.0 and 15.0 arbitrary units, capillary voltage 16.0 V, source voltage 3.5 kV and tube lens 55 V. Full scan spectra were acquired in negative ion mode in the range 100-1000 m/z with the resolution of 30,000 (FWHM). MSⁿ spectra were acquired in the range between ion trap cut-off and precursor ion m/z values.

Reference

Borges G, Degeneve A, Mullen W, Crozier A. 2009. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *Journal of Agricultural and Food Chemistry* 58, 3901–3909.

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Total DNA sample extraction and electrophoresis protocols

Ten g of each samples was mixed with 20 ml of quarter-strength Ringer's solution (Oxoid, Milan, Italy) for 2 minutes in a stomacher (LAB Blender 400, PBI, Italy; stomacher bags: Sto-circul-bag, PBI, Italy) at room temperature. Two ml of the dilution was collected and centrifuged at maximum speed for 30 s. The pellets were then used for DNA extraction. The adopted protocols were those outlined in the manufacturer's instructions for the Powersoil DNA kit (MO-BIO, Carlsbad, CA). The DNA solution was incubated at 37°C for 30 minutes, with one µl of RNase (Promega, Milan, Italy), and stored at -20°C. DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Milan, Italy) and was standardized at 100 ng/µl. Two primers, 338F-GC and 518R, were used to amplify the variable V3 region of the 16S rRNA gene (Muyzer et al., 1993).

Microbial diversity analysis Bioinformatics and statistical analysis.

Paired-end reads were first merged using the FLASH software (34), with default parameters (min overlap 10bp max overlap 65 bp). Joint reads were further quality filtered (Phred < Q20) using the QIIME 1.9.0 software (Caporaso et al., 2010) through a `multiple_split_libraries_fastq.py` script. The USEARCH version 8.1 software (Edgar et al., 2011) was adopted for chimera filtering, using the 16S reference databases version 9 (RDP Classifier training database). OTUs were picked at 99% of similarity by means of UCLUST clustering methods (Edgar, 2010), and representative sequences of each cluster were used to assign taxonomy using the Greengenes 16S rRNA gene database, version 2013, by means of the RDP Classifier, with a minimum confidence score of 0.80 (Wang et al., 2007). Statistics and plotting were carried out Alpha diversity indices were calculated using the diversity function of the vegan package (Dixon, 2003) in the R environment (www.r-project.org). Alpha diversity was evaluated through QIIME to generate rarefaction curves, Good's coverage, Chao1 richness and Shannon diversity indices. In order to avoid biases due to different sequencing depths, all samples were rarefied at 13821 reads after raw read quality filtering. Weighted UniFrac distance matrices and an OTU table were used to perform ADONIS and ANOSIM statistical test with 999 permutations in R environment. A filtered OTU table was generated at 0.2% abundance in at least 2 samples using QIIME. The OTU table displays the higher taxonomy resolution that was reached by the 16S data. The statistical test Kruskal-Wallis and Mann-Whitney tests were used to find significant differences in microbial taxa abundance, to the dietary supplementation. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013) that predicts abundances of gene families based on 16S-based structure of the microbiota was used to investigate the functional profiles in microbial communities. For the analysis with PICRUSt, the pick OTUs module was performed at 97% identity in a closed reference way using the Green genes database (V.05/2013) in QIIME. Data were normalised for 16S rRNA gene copy numbers, and the metagenomes were predicted. From the inferred metagenomes, KEGG Orthologs (KO) were identified. Spearman correlation coefficients were calculated between the OTU abundance data matrix and the inferred metagenomes through the `psych` function of R, and were then plotted through the `made 4` function of R. OTU table were used for

cooccurrence/coexclusion analysis, carried out by using the psych package of R. The correlation matrix was plotted by using the corrplot package of R. Correction of p values for multiple testing (Benjamini and Hochberg, 1995) was performed when necessary.

References

- Benjamini Y and Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of The Royal Statistical Society Series B Statistical Methodology* 57, 289–300.
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich K, Gordon JI, Huttley G, Kelley ST, Knights D, Jeremy E, Ley RE, Lozupone C, Mcdonald D, Muegge BD, Reeder J, Sevinsky JR, Turnbaugh PJ and Walters W 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335–336.
- Dixon P. 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14, 927–930.
- Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461.
- Edgar RC, Haas BJ, Clemente JC, Quince C and Knight R 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27, 2194–200.
- Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes J, Clemente JC, Burkepille DE, Vega Thurber RL, Knight R, Beiko RG and Huttenhower C 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology* 31, 814–21.
- Muyzer G, De Waal EC and Uitterlinden A G 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59, 695–700.
- Wang Q, Garrity G M, Tiedje J M and Cole JR 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied Environmental Microbiology* 73, 5261–5267.

Table S1 Profile of main polyphenols of bilberry pomace (mg/100g freeze dried weight)*

Compound	Quantity
Chlorogenic acid	192.80
Catechin	11.03
Epicatechin	12.93
Quercetin	13.70
Iperoside	28.69
Rutine	4.54
Kaempferol-3- rutinoside	0.04
Delphinidin -3-galactoside	104.50
Cyanidin-3-glucoside	86.87
Total	455.1

* Mean values of three replicates

Table S2 Sample description, statistics of the sequencing bioinformatic pipeline and alpha- diversity index.

Sample_ID	days	diet (%BP)	sample type	Total pairs sequence	Combined pairs	Percent combined	Clean reads	PD_ whole_ tree	chao1	observed_species	shannon	Estimated sample coverage
BP15_F0	35	15	feces	44925	37264	82.95%	18405	135,26	11170,93	4964	10,27	88,93
BP10_F0	35	10	feces	120010	90000	74.99%	39245	164,06	13896,63	7173	9,21	89,25
BP5_F0	35	5	feces	385076	305080	79.23%	136831	286,94	27424,72	17648	10,71	93,55
BP0_F0	35	0	feces	319583	253076	79.19%	112143	265,83	27237,38	15985	10,46	92,30
BP15_F21	49	15	feces	321163	240884	75.00%	91973	247,91	26040,01	14309	10,53	91,15
BP10_F21	49	10	feces	301195	243862	80.96%	105947	273,12	26060,03	15390	10,40	92,13
BP5_F21	49	5	feces	261440	201681	77.14%	85406	263,23	27824,32	14692	10,92	90,04
BP0_F21	49	0	feces	255057	207396	81.31%	95763	255,17	25360,59	14631	10,73	91,64
BP15_F89	83	15	feces	251888	207562	82.40%	98937	288,27	28643,98	16415	11,08	90,81
BP10_F89	83	10	feces	196291	153927	78.42%	59722	219,05	24877,44	11752	10,73	88,09
BP5_F89	83	5	feces	177917	141619	79.60%	63452	234,52	24027,42	12227	10,99	88,69
BP0_F89	83	0	feces	142582	113548	79.64%	48743	208,65	20855,53	10182	10,72	87,36
BP15_C	83	15	caecal	37747	30542	80.91%	13821	134,34	9995,80	4269	10,22	89,88
BP10_C	83	10	caecal	130782	99448	76.04%	38687	201,83	19642,82	9100	10,64	85,24
BP5_C	83	5	caecal	183549	141723	77.21%	57606	230,97	22700,16	11704	10,87	88,07
BP0_C	83	0	caecal	243352	188433	77.43%	77507	259,61	24896,93	13924	10,84	89,90

Table S3 Correlation between OTUs and inferred KEGG pathways.
Table display the FDR value of the correlation only value < 0.05 are
highlight.

otu	Alanine_aspartate_and_glutamate_metabolism
Ruminococcus	0,311
Ruminococcaceae	0,080
Rikenellaceae	0,528
Phascolarctobacterium	0,974
Oscillospira	0,333
Mogibacteriaceae	0,060
Lactobacillus	0,213
Lachnospiraceae	0,094
Faecalibacterium	0,295
Erysipelotrichaceae	0,974
Enterococcaceae	0,837
Dorea	0,259
Dehalobacterium	0,557
Coriobacteriaceae	0,050
Coproccoccus	0,438
Clostridium	0,019
Clostridiales	0,128
Christensenellaceae	0,004
Barnesiellaceae	0,704
Bacteroides	0,192
Bacteroidales	0,047
Alphaproteobacteria	0,322
Akkermansia	0,664
Acinetobacter	0,429

otu	alpha-Linolenic_acid_metabolism
Ruminococcus	0,374
Ruminococcaceae	0,030
Rikenellaceae	0,128
Phascolarctobacterium	0,478
Oscillospira	0,549
Mogibacteriaceae	0,007
Lactobacillus	0,295
Lachnospiraceae	0,196
Faecalibacterium	0,485
Erysipelotrichaceae	0,812
Enterococcaceae	0,782
Dorea	0,092
Dehalobacterium	0,625
Coriobacteriaceae	0,037
Coprococcus	0,572
Clostridium	0,002
Clostridiales	0,008
Christensenellaceae	0,000
Barnesiellaceae	0,217
Bacteroides	0,019
Bacteroidales	0,102
Alphaproteobacteria	0,587
Akkermansia	0,107
Acinetobacter	0,053

otu	Amino_acid_related_enzymes
Ruminococcus	0,289
Ruminococcaceae	0,094
Rikenellaceae	0,499
Phascolarctobacterium	1,000
Oscillospira	0,350
Mogibacteriaceae	0,058
Lactobacillus	0,240
Lachnospiraceae	0,066
Faecalibacterium	0,254
Erysipelotrichaceae	0,983
Enterococcaceae	0,725
Dorea	0,269
Dehalobacterium	0,528
Coriobacteriaceae	0,046
Coprococcus	0,431
Clostridium	0,019
Clostridiales	0,122
Christensenellaceae	0,004
Barnesiellaceae	0,762
Bacteroides	0,204
Bacteroidales	0,053
Alphaproteobacteria	0,362
Akkermansia	0,656
Acinetobacter	0,442

otu	Amino_sugar_and_nucleotide_sugar_metabolism
Ruminococcus	0,374
Ruminococcaceae	0,070
Rikenellaceae	0,451
Phascolarctobacterium	0,991
Oscillospira	0,362
Mogibacteriaceae	0,047
Lactobacillus	0,188
Lachnospiraceae	0,097
Faecalibacterium	0,249
Erysipelotrichaceae	0,897
Enterococcaceae	0,888
Dorea	0,217
Dehalobacterium	0,602
Coriobacteriaceae	0,040
Coprococcus	0,399
Clostridium	0,015
Clostridiales	0,105
Christensenellaceae	0,003
Barnesiellaceae	0,633
Bacteroides	0,141
Bacteroidales	0,041
Alphaproteobacteria	0,249
Akkermansia	0,633
Acinetobacter	0,402

otu	Arachidonic_acid_metabolism
Ruminococcus	0,983
Ruminococcaceae	0,213
Rikenellaceae	0,820
Phascolarctobacterium	0,144
Oscillospira	0,110
Mogibacteriaceae	0,721
Lactobacillus	0,083
Lachnospiraceae	0,322
Faecalibacterium	0,729
Erysipelotrichaceae	0,393
Enterococcaceae	0,656
Dorea	0,991
Dehalobacterium	0,471
Coriobacteriaceae	0,097
Coprococcus	0,587
Clostridium	0,161
Clostridiales	0,897
Christensenellaceae	0,080
Barnesiellaceae	0,721
Bacteroides	0,696
Bacteroidales	0,097
Alphaproteobacteria	0,264
Akkermansia	0,192
Acinetobacter	0,660

otu	Arginine_and_proline_metabolism
Ruminococcus	0,316
Ruminococcaceae	0,062
Rikenellaceae	0,464
Phascolarctobacterium	0,983
Oscillospira	0,322
Mogibacteriaceae	0,057
Lactobacillus	0,176
Lachnospiraceae	0,092
Faecalibacterium	0,322
Erysipelotrichaceae	0,983
Enterococcaceae	0,888
Dorea	0,269
Dehalobacterium	0,506
Coriobacteriaceae	0,036
Coprococcus	0,451
Clostridium	0,015
Clostridiales	0,141
Christensenellaceae	0,003
Barnesiellaceae	0,688
Bacteroides	0,213
Bacteroidales	0,050
Alphaproteobacteria	0,345
Akkermansia	0,713
Acinetobacter	0,473

otu	Ascorbate_and_aldarate_metabolism
Ruminococcus	0,520
Ruminococcaceae	0,119
Rikenellaceae	0,696
Phascolarctobacterium	0,380
Oscillospira	0,147
Mogibacteriaceae	0,322
Lactobacillus	0,144
Lachnospiraceae	0,289
Faecalibacterium	0,721
Erysipelotrichaceae	0,721
Enterococcaceae	0,457
Dorea	0,641
Dehalobacterium	0,274
Coriobacteriaceae	0,053
Coprococcus	0,745
Clostridium	0,041
Clostridiales	0,762
Christensenellaceae	0,027
Barnesiellaceae	0,721
Bacteroides	0,535
Bacteroidales	0,090
Alphaproteobacteria	0,412
Akkermansia	0,602
Acinetobacter	0,894

otu	beta-Lactam_resistance	otu
Ruminococcus	0,729	Ruminococcus
Ruminococcaceae	0,295	Ruminococcaceae
Rikenellaceae	0,940	Rikenellaceae
Phascolarctobacterium	0,080	Phascolarctobacterium
Oscillospira	0,141	Oscillospira
Mogibacteriaceae	0,931	Mogibacteriaceae
Lactobacillus	0,050	Lactobacillus
Lachnospiraceae	0,499	Lachnospiraceae
Faecalibacterium	0,974	Faecalibacterium
Erysipelotrichaceae	0,368	Erysipelotrichaceae
Enterococcaceae	0,464	Enterococcaceae
Dorea	0,664	Dorea
Dehalobacterium	0,762	Dehalobacterium
Coriobacteriaceae	0,208	Coriobacteriaceae
Coprococcus	0,795	Coprococcus
Clostridium	0,311	Clostridium
Clostridiales	0,633	Clostridiales
Christensenellaceae	0,200	Christensenellaceae
Barnesiellaceae	0,762	Barnesiellaceae
Bacteroides	0,871	Bacteroides
Bacteroidales	0,176	Bacteroidales
Alphaproteobacteria	0,176	Alphaproteobacteria
Akkermansia	0,083	Akkermansia
Acinetobacter	0,505	Acinetobacter

Biosynthesis_of_unsaturated_fatty_acids	otu	Biotin_metabolism
0,846	Ruminococcus	0,438
0,125	Ruminococcaceae	0,200
0,633	Rikenellaceae	0,721
0,295	Phascolarctobacterium	0,737
0,147	Oscillospira	0,368
0,405	Mogibacteriaceae	0,244
0,078	Lactobacillus	0,235
0,200	Lachnospiraceae	0,125
0,602	Faecalibacterium	0,572
0,506	Erysipelotrichaceae	0,762
0,753	Enterococcaceae	0,880
0,770	Dorea	0,594
0,374	Dehalobacterium	0,602
0,041	Coriobacteriaceae	0,122
0,535	Coprococcus	0,680
0,090	Clostridium	0,074
0,829	Clostridiales	0,451
0,026	Christensenellaceae	0,030
0,713	Barnesiellaceae	0,940
0,641	Bacteroides	0,431
0,090	Bacteroidales	0,122
0,305	Alphaproteobacteria	0,322
0,305	Akkermansia	0,888
0,777	Acinetobacter	0,850

otu	Butanoate_metabolism	otu
Ruminococcus	0,350	Ruminococcus
Ruminococcaceae	0,074	Ruminococcaceae
Rikenellaceae	0,513	Rikenellaceae
Phascolarctobacterium	0,922	Phascolarctobacterium
Oscillospira	0,295	Oscillospira
Mogibacteriaceae	0,080	Mogibacteriaceae
Lactobacillus	0,188	Lactobacillus
Lachnospiraceae	0,128	Lachnospiraceae
Faecalibacterium	0,399	Faecalibacterium
Erysipelotrichaceae	0,983	Erysipelotrichaceae
Enterococcaceae	0,996	Enterococcaceae
Dorea	0,339	Dorea
Dehalobacterium	0,587	Dehalobacterium
Coriobacteriaceae	0,064	Coriobacteriaceae
Coprococcus	0,520	Coprococcus
Clostridium	0,018	Clostridium
Clostridiales	0,154	Clostridiales
Christensenellaceae	0,006	Christensenellaceae
Barnesiellaceae	0,633	Barnesiellaceae
Bacteroides	0,180	Bacteroides
Bacteroidales	0,053	Bacteroidales
Alphaproteobacteria	0,316	Alphaproteobacteria
Akkermansia	0,704	Akkermansia
Acinetobacter	0,402	Acinetobacter

Butirosin_and_neomycin_biosynthesis	otu
0,327	Ruminococcus
0,345	Ruminococcaceae
0,729	Rikenellaceae
0,795	Phascolarctobacterium
0,499	Oscillospira
0,235	Mogibacteriaceae
0,368	Lactobacillus
0,050	Lachnospiraceae
0,387	Faecalibacterium
0,672	Erysipelotrichaceae
0,829	Enterococcaceae
0,557	Dorea
0,451	Dehalobacterium
0,085	Coriobacteriaceae
0,672	Coproccoccus
0,102	Clostridium
0,458	Clostridiales
0,037	Christensenellaceae
0,787	Barnesiellaceae
0,572	Bacteroides
0,128	Bacteroidales
0,492	Alphaproteobacteria
0,897	Akkermansia
0,929	Acinetobacter

C5-Branched_dibasic_acid_metabolism	otu
0,289	Ruminococcus
0,094	Ruminococcaceae
0,499	Rikenellaceae
1,000	Phascolarctobacterium
0,350	Oscillospira
0,058	Mogibacteriaceae
0,240	Lactobacillus
0,066	Lachnospiraceae
0,254	Faecalibacterium
0,983	Erysipelotrichaceae
0,725	Enterococcaceae
0,269	Dorea
0,528	Dehalobacterium
0,046	Coriobacteriaceae
0,431	Coprococcus
0,019	Clostridium
0,122	Clostridiales
0,004	Christensenellaceae
0,762	Barnesiellaceae
0,204	Bacteroides
0,053	Bacteroidales
0,362	Alphaproteobacteria
0,656	Akkermansia
0,442	Acinetobacter

Caffeine_metabolism	otu	Carbon_fixation_in_photosynthetic_organisms
0,721	Ruminococcus	0,311
0,633	Ruminococcaceae	0,080
0,713	Rikenellaceae	0,528
0,044	Phascolarctobacterium	0,974
0,196	Oscillospira	0,333
0,549	Mogibacteriaceae	0,060
0,068	Lactobacillus	0,213
0,300	Lachnospiraceae	0,094
0,721	Faecalibacterium	0,295
0,217	Erysipelotrichaceae	0,974
0,944	Enterococcaceae	0,837
0,322	Dorea	0,259
0,837	Dehalobacterium	0,557
0,322	Coriobacteriaceae	0,050
0,649	Coprococcus	0,438
0,957	Clostridium	0,019
0,295	Clostridiales	0,128
0,471	Christensenellaceae	0,004
0,905	Barnesiellaceae	0,704
0,528	Bacteroides	0,192
0,172	Bacteroidales	0,047
0,249	Alphaproteobacteria	0,322
0,005	Akkermansia	0,664
0,168	Acinetobacter	0,429

otu	Carbon_fixation_pathways_in_prokaryotes
Ruminococcus	0,316
Ruminococcaceae	0,062
Rikenellaceae	0,464
Phascolarctobacterium	0,983
Oscillospira	0,322
Mogibacteriaceae	0,057
Lactobacillus	0,176
Lachnospiraceae	0,092
Faecalibacterium	0,322
Erysipelotrichaceae	0,983
Enterococcaceae	0,888
Dorea	0,269
Dehalobacterium	0,506
Coriobacteriaceae	0,036
Coproccoccus	0,451
Clostridium	0,015
Clostridiales	0,141
Christensenellaceae	0,003
Barnesiellaceae	0,688
Bacteroides	0,213
Bacteroidales	0,050
Alphaproteobacteria	0,345
Akkermansia	0,713
Acinetobacter	0,473

otu	Citrate_cycle_(TCA_cycle)
Ruminococcus	0,664
Ruminococcaceae	0,144
Rikenellaceae	0,745
Phascolarctobacterium	0,528
Oscillospira	0,249
Mogibacteriaceae	0,305
Lactobacillus	0,144
Lachnospiraceae	0,172
Faecalibacterium	0,641
Erysipelotrichaceae	0,721
Enterococcaceae	0,712
Dorea	0,610
Dehalobacterium	0,564
Coriobacteriaceae	0,097
Coprococcus	0,579
Clostridium	0,083
Clostridiales	0,602
Christensenellaceae	0,039
Barnesiellaceae	0,787
Bacteroides	0,485
Bacteroidales	0,107
Alphaproteobacteria	0,249
Akkermansia	0,610
Acinetobacter	0,987

otu	Cysteine_and_methionine_metabolism
Ruminococcus	0,311
Ruminococcaceae	0,080
Rikenellaceae	0,528
Phascolarctobacterium	0,974
Oscillospira	0,333
Mogibacteriaceae	0,060
Lactobacillus	0,213
Lachnospiraceae	0,094
Faecalibacterium	0,295
Erysipelotrichaceae	0,974
Enterococcaceae	0,837
Dorea	0,259
Dehalobacterium	0,557
Coriobacteriaceae	0,050
Coprococcus	0,438
Clostridium	0,019
Clostridiales	0,128
Christensenellaceae	0,004
Barnesiellaceae	0,704
Bacteroides	0,192
Bacteroidales	0,047
Alphaproteobacteria	0,322
Akkermansia	0,664
Acinetobacter	0,429

otu	Ether_lipid_metabolism	otu
Ruminococcus	0,594	Ruminococcus
Ruminococcaceae	0,031	Ruminococcaceae
Rikenellaceae	0,158	Rikenellaceae
Phascolarctobacterium	0,602	Phascolarctobacterium
Oscillospira	0,664	Oscillospira
Mogibacteriaceae	0,022	Mogibacteriaceae
Lactobacillus	0,097	Lactobacillus
Lachnospiraceae	0,134	Lachnospiraceae
Faecalibacterium	0,311	Faecalibacterium
Erysipelotrichaceae	0,991	Erysipelotrichaceae
Enterococcaceae	0,733	Enterococcaceae
Dorea	0,158	Dorea
Dehalobacterium	0,880	Dehalobacterium
Coriobacteriaceae	0,040	Coriobacteriaceae
Coprococcus	0,418	Coprococcus
Clostridium	0,006	Clostridium
Clostridiales	0,013	Clostridiales
Christensenellaceae	0,001	Christensenellaceae
Barnesiellaceae	0,311	Barnesiellaceae
Bacteroides	0,024	Bacteroides
Bacteroidales	0,043	Bacteroidales
Alphaproteobacteria	0,204	Alphaproteobacteria
Akkermansia	0,295	Akkermansia
Acinetobacter	0,168	Acinetobacter

Fatty_acid_biosynthesis	otu	Fatty_acid_elongation
0,374	Ruminococcus	0,721
0,070	Ruminococcaceae	0,033
0,451	Rikenellaceae	0,713
0,991	Phascolarctobacterium	0,044
0,362	Oscillospira	0,196
0,047	Mogibacteriaceae	0,549
0,188	Lactobacillus	0,068
0,097	Lachnospiraceae	0,300
0,249	Faecalibacterium	0,721
0,897	Erysipelotrichaceae	0,217
0,888	Enterococcaceae	0,944
0,217	Dorea	0,022
0,602	Dehalobacterium	0,837
0,040	Coriobacteriaceae	0,322
0,399	Coprococcus	0,649
0,015	Clostridium	0,037
0,105	Clostridiales	0,295
0,003	Christensenellaceae	0,471
0,633	Barnesiellaceae	0,905
0,141	Bacteroides	0,528
0,041	Bacteroidales	0,172
0,249	Alphaproteobacteria	0,249
0,633	Akkermansia	0,005
0,402	Acinetobacter	0,168

otu	Fatty_acid_metabolism	otu
Ruminococcus	0,356	Ruminococcus
Ruminococcaceae	0,057	Ruminococcaceae
Rikenellaceae	0,451	Rikenellaceae
Phascolarctobacterium	0,931	Phascolarctobacterium
Oscillospira	0,284	Oscillospira
Mogibacteriaceae	0,076	Mogibacteriaceae
Lactobacillus	0,154	Lactobacillus
Lachnospiraceae	0,125	Lachnospiraceae
Faecalibacterium	0,431	Faecalibacterium
Erysipelotrichaceae	0,940	Erysipelotrichaceae
Enterococcaceae	0,953	Enterococcaceae
Dorea	0,350	Dorea
Dehalobacterium	0,535	Dehalobacterium
Coriobacteriaceae	0,047	Coriobacteriaceae
Coprococcus	0,535	Coprococcus
Clostridium	0,014	Clostridium
Clostridiales	0,169	Clostridiales
Christensenellaceae	0,004	Christensenellaceae
Barnesiellaceae	0,617	Barnesiellaceae
Bacteroides	0,200	Bacteroides
Bacteroidales	0,057	Bacteroidales
Alphaproteobacteria	0,339	Alphaproteobacteria
Akkermansia	0,753	Akkermansia
Acinetobacter	0,445	Acinetobacter

Flavone_and_flavonol_biosynthesis	otu
0,035	Ruminococcus
0,594	Ruminococcaceae
0,957	Rikenellaceae
0,991	Phascolarctobacterium
0,688	Oscillospira
0,161	Mogibacteriaceae
0,846	Lactobacillus
0,046	Lachnospiraceae
0,412	Faecalibacterium
1,000	Erysipelotrichaceae
0,948	Enterococcaceae
0,485	Dorea
0,176	Dehalobacterium
0,099	Coriobacteriaceae
0,729	Coprococcus
0,094	Clostridium
0,405	Clostridiales
0,090	Christensenellaceae
0,438	Barnesiellaceae
0,880	Bacteroides
0,172	Bacteroidales
0,863	Alphaproteobacteria
0,680	Akkermansia
0,374	Acinetobacter

Flavonoid_biosynthesis	otu	Folate_biosynthesis	otu
0,829	Ruminococcus	0,795	Ruminococcus
0,264	Ruminococcaceae	0,180	Ruminococcaceae
0,633	Rikenellaceae	0,721	Rikenellaceae
0,204	Phascolarctobacterium	0,405	Phascolarctobacterium
0,200	Oscillospira	0,231	Oscillospira
0,610	Mogibacteriaceae	0,431	Mogibacteriaceae
0,076	Lactobacillus	0,113	Lactobacillus
0,125	Lachnospiraceae	0,141	Lachnospiraceae
0,438	Faecalibacterium	0,656	Faecalibacterium
0,305	Erysipelotrichaceae	0,499	Erysipelotrichaceae
0,880	Enterococcaceae	0,845	Enterococcaceae
0,991	Dorea	0,820	Dorea
0,549	Dehalobacterium	0,513	Dehalobacterium
0,053	Coriobacteriaceae	0,080	Coriobacteriaceae
0,458	Coprococcus	0,587	Coprococcus
0,217	Clostridium	0,134	Clostridium
0,897	Clostridiales	0,787	Clostridiales
0,070	Christensenellaceae	0,047	Christensenellaceae
0,787	Barnesiellaceae	0,863	Barnesiellaceae
0,688	Bacteroides	0,688	Bacteroides
0,090	Bacteroidales	0,128	Bacteroidales
0,249	Alphaproteobacteria	0,305	Alphaproteobacteria
0,158	Akkermansia	0,350	Akkermansia
0,628	Acinetobacter	0,768	Acinetobacter

Fructose_and_mannose_metabolism	otu
0,333	Ruminococcus
0,062	Ruminococcaceae
0,438	Rikenellaceae
0,974	Phascolarctobacterium
0,327	Oscillospira
0,040	Mogibacteriaceae
0,196	Lactobacillus
0,119	Lachnospiraceae
0,254	Faecalibacterium
0,846	Erysipelotrichaceae
0,974	Enterococcaceae
0,204	Dorea
0,520	Dehalobacterium
0,034	Coriobacteriaceae
0,438	Coprococcus
0,010	Clostridium
0,099	Clostridiales
0,003	Christensenellaceae
0,587	Barnesiellaceae
0,125	Bacteroides
0,035	Bacteroidales
0,279	Alphaproteobacteria
0,579	Akkermansia
0,326	Acinetobacter

Galactose_metabolism	otu	Glycerolipid_metabolism
0,015	Ruminococcus	0,380
0,226	Ruminococcaceae	0,053
0,880	Rikenellaceae	0,393
0,914	Phascolarctobacterium	1,000
0,405	Oscillospira	0,350
0,134	Mogibacteriaceae	0,044
0,464	Lactobacillus	0,154
0,134	Lachnospiraceae	0,094
0,418	Faecalibacterium	0,274
0,837	Erysipelotrichaceae	0,940
0,948	Enterococcaceae	0,940
0,305	Dorea	0,226
0,528	Dehalobacterium	0,549
0,158	Coriobacteriaceae	0,028
0,641	Coprococcus	0,412
0,043	Clostridium	0,012
0,269	Clostridiales	0,116
0,025	Christensenellaceae	0,002
0,914	Barnesiellaceae	0,617
0,339	Bacteroides	0,158
0,110	Bacteroidales	0,044
0,431	Alphaproteobacteria	0,269
0,579	Akkermansia	0,680
0,535	Acinetobacter	0,445

otu	Glycerophospholipid_metabolism	otu
Ruminococcus	0,418	Ruminococcus
Ruminococcaceae	0,144	Ruminococcaceae
Rikenellaceae	0,688	Rikenellaceae
Phascolarctobacterium	0,770	Phascolarctobacterium
Oscillospira	0,339	Oscillospira
Mogibacteriaceae	0,188	Mogibacteriaceae
Lactobacillus	0,204	Lactobacillus
Lachnospiraceae	0,147	Lachnospiraceae
Faecalibacterium	0,549	Faecalibacterium
Erysipelotrichaceae	0,846	Erysipelotrichaceae
Enterococcaceae	0,845	Enterococcaceae
Dorea	0,506	Dorea
Dehalobacterium	0,594	Dehalobacterium
Coriobacteriaceae	0,102	Coriobacteriaceae
Coprococcus	0,649	Coprococcus
Clostridium	0,050	Clostridium
Clostridiales	0,374	Clostridiales
Christensenellaceae	0,019	Christensenellaceae
Barnesiellaceae	0,837	Barnesiellaceae
Bacteroides	0,362	Bacteroides
Bacteroidales	0,097	Bacteroidales
Alphaproteobacteria	0,295	Alphaproteobacteria
Akkermansia	0,974	Akkermansia
Acinetobacter	0,743	Acinetobacter

Glycine_serine_and_threonine_metabolism	otu
0,399	Ruminococcus
0,064	Ruminococcaceae
0,464	Rikenellaceae
0,948	Phascolarctobacterium
0,316	Oscillospira
0,087	Mogibacteriaceae
0,147	Lactobacillus
0,102	Lachnospiraceae
0,425	Faecalibacterium
0,888	Erysipelotrichaceae
0,961	Enterococcaceae
0,368	Dorea
0,617	Dehalobacterium
0,055	Coriobacteriaceae
0,492	Coprococcus
0,020	Clostridium
0,176	Clostridiales
0,005	Christensenellaceae
0,664	Barnesiellaceae
0,222	Bacteroides
0,066	Bacteroidales
0,305	Alphaproteobacteria
0,812	Akkermansia
0,535	Acinetobacter

Glycolysis__Gluconeogenesis	otu
0,316	Ruminococcus
0,062	Ruminococcaceae
0,464	Rikenellaceae
0,983	Phascolarctobacterium
0,322	Oscillospira
0,057	Mogibacteriaceae
0,176	Lactobacillus
0,092	Lachnospiraceae
0,322	Faecalibacterium
0,983	Erysipelotrichaceae
0,888	Enterococcaceae
0,269	Dorea
0,506	Dehalobacterium
0,036	Coriobacteriaceae
0,451	Coprococcus
0,015	Clostridium
0,141	Clostridiales
0,003	Christensenellaceae
0,688	Barnesiellaceae
0,213	Bacteroides
0,050	Bacteroidales
0,345	Alphaproteobacteria
0,713	Akkermansia
0,473	Acinetobacter

Glyoxylate_and_dicarboxylate_metabolism	otu
0,399	Ruminococcus
0,064	Ruminococcaceae
0,464	Rikenellaceae
0,948	Phascolarctobacterium
0,316	Oscillospira
0,087	Mogibacteriaceae
0,147	Lactobacillus
0,102	Lachnospiraceae
0,425	Faecalibacterium
0,888	Erysipelotrichaceae
0,961	Enterococcaceae
0,368	Dorea
0,617	Dehalobacterium
0,055	Coriobacteriaceae
0,492	Coprococcus
0,020	Clostridium
0,176	Clostridiales
0,005	Christensenellaceae
0,664	Barnesiellaceae
0,222	Bacteroides
0,066	Bacteroidales
0,305	Alphaproteobacteria
0,812	Akkermansia
0,535	Acinetobacter

Histidine_metabolism	otu	Inositol_phosphate_metabolism
0,311	Ruminococcus	0,656
0,080	Ruminococcaceae	0,134
0,528	Rikenellaceae	0,704
0,974	Phascolarctobacterium	0,520
0,333	Oscillospira	0,213
0,060	Mogibacteriaceae	0,295
0,213	Lactobacillus	0,151
0,094	Lachnospiraceae	0,158
0,295	Faecalibacterium	0,617
0,974	Erysipelotrichaceae	0,737
0,837	Enterococcaceae	0,757
0,259	Dorea	0,633
0,557	Dehalobacterium	0,557
0,050	Coriobacteriaceae	0,102
0,438	Coprococcus	0,557
0,019	Clostridium	0,072
0,128	Clostridiales	0,579
0,004	Christensenellaceae	0,031
0,704	Barnesiellaceae	0,762
0,192	Bacteroides	0,451
0,047	Bacteroidales	0,125
0,322	Alphaproteobacteria	0,244
0,664	Akkermansia	0,633
0,429	Acinetobacter	0,978

otu	Isoquinoline_alkaloid_biosynthesis
Ruminococcus	0,803
Ruminococcaceae	0,672
Rikenellaceae	0,812
Phascolarctobacterium	0,110
Oscillospira	0,231
Mogibacteriaceae	0,633
Lactobacillus	0,172
Lachnospiraceae	0,300
Faecalibacterium	0,940
Erysipelotrichaceae	0,137
Enterococcaceae	0,858
Dorea	0,458
Dehalobacterium	0,656
Coriobacteriaceae	0,339
Coproccoccus	0,957
Clostridium	0,506
Clostridiales	0,464
Christensenellaceae	0,333
Barnesiellaceae	0,656
Bacteroides	0,704
Bacteroidales	0,249
Alphaproteobacteria	0,579
Akkermansia	0,102
Acinetobacter	0,331

otu	Linoleic_acid_metabolism	otu
Ruminococcus	0,322	Ruminococcus
Ruminococcaceae	0,047	Ruminococcaceae
Rikenellaceae	0,431	Rikenellaceae
Phascolarctobacterium	1,000	Phascolarctobacterium
Oscillospira	0,316	Oscillospira
Mogibacteriaceae	0,053	Mogibacteriaceae
Lactobacillus	0,144	Lactobacillus
Lachnospiraceae	0,119	Lachnospiraceae
Faecalibacterium	0,380	Faecalibacterium
Erysipelotrichaceae	0,966	Erysipelotrichaceae
Enterococcaceae	0,987	Enterococcaceae
Dorea	0,279	Dorea
Dehalobacterium	0,506	Dehalobacterium
Coriobacteriaceae	0,031	Coriobacteriaceae
Coprococcus	0,520	Coprococcus
Clostridium	0,010	Clostridium
Clostridiales	0,131	Clostridiales
Christensenellaceae	0,002	Christensenellaceae
Barnesiellaceae	0,602	Barnesiellaceae
Bacteroides	0,188	Bacteroides
Bacteroidales	0,040	Bacteroidales
Alphaproteobacteria	0,356	Alphaproteobacteria
Akkermansia	0,688	Akkermansia
Acinetobacter	0,383	Acinetobacter

Lipid_biosynthesis_proteins	otu	Lipoic_acid_metabolism
0,316	Ruminococcus	0,897
0,062	Ruminococcaceae	0,289
0,464	Rikenellaceae	0,931
0,983	Phascolarctobacterium	0,119
0,322	Oscillospira	0,169
0,057	Mogibacteriaceae	0,957
0,176	Lactobacillus	0,074
0,092	Lachnospiraceae	0,557
0,322	Faecalibacterium	0,812
0,983	Erysipelotrichaceae	0,425
0,888	Enterococcaceae	0,300
0,269	Dorea	0,713
0,506	Dehalobacterium	0,572
0,036	Coriobacteriaceae	0,213
0,451	Coprococcus	0,966
0,015	Clostridium	0,259
0,141	Clostridiales	0,528
0,003	Christensenellaceae	0,192
0,688	Barnesiellaceae	0,974
0,213	Bacteroides	0,880
0,050	Bacteroidales	0,231
0,345	Alphaproteobacteria	0,327
0,713	Akkermansia	0,122
0,473	Acinetobacter	0,491

otu	Lysine_biosynthesis	otu	Lysine_degradation
Ruminococcus	0,350	Ruminococcus	0,696
Ruminococcaceae	0,083	Ruminococcaceae	0,041
Rikenellaceae	0,425	Rikenellaceae	0,542
Phascolarctobacterium	0,983	Phascolarctobacterium	0,499
Oscillospira	0,380	Oscillospira	0,154
Mogibacteriaceae	0,046	Mogibacteriaceae	0,176
Lactobacillus	0,213	Lactobacillus	0,060
Lachnospiraceae	0,068	Lachnospiraceae	0,254
Faecalibacterium	0,213	Faecalibacterium	0,549
Erysipelotrichaceae	0,940	Erysipelotrichaceae	0,787
Enterococcaceae	0,774	Enterococcaceae	0,633
Dorea	0,226	Dorea	0,444
Dehalobacterium	0,572	Dehalobacterium	0,451
Coriobacteriaceae	0,036	Coriobacteriaceae	0,030
Coprococcus	0,393	Coprococcus	0,492
Clostridium	0,016	Clostridium	0,023
Clostridiales	0,099	Clostridiales	0,444
Christensenellaceae	0,003	Christensenellaceae	0,007
Barnesiellaceae	0,688	Barnesiellaceae	0,478
Bacteroides	0,151	Bacteroides	0,322
Bacteroidales	0,047	Bacteroidales	0,046
Alphaproteobacteria	0,284	Alphaproteobacteria	0,217
Akkermansia	0,625	Akkermansia	0,680
Acinetobacter	0,415	Acinetobacter	0,768

otu	Methane_metabolism	otu
Ruminococcus	0,269	Ruminococcus
Ruminococcaceae	0,105	Ruminococcaceae
Rikenellaceae	0,572	Rikenellaceae
Phascolarctobacterium	0,922	Phascolarctobacterium
Oscillospira	0,458	Oscillospira
Mogibacteriaceae	0,053	Mogibacteriaceae
Lactobacillus	0,269	Lactobacillus
Lachnospiraceae	0,110	Lachnospiraceae
Faecalibacterium	0,274	Faecalibacterium
Erysipelotrichaceae	0,787	Erysipelotrichaceae
Enterococcaceae	0,957	Enterococcaceae
Dorea	0,196	Dorea
Dehalobacterium	0,696	Dehalobacterium
Coriobacteriaceae	0,076	Coriobacteriaceae
Coprococcus	0,492	Coprococcus
Clostridium	0,017	Clostridium
Clostridiales	0,099	Clostridiales
Christensenellaceae	0,008	Christensenellaceae
Barnesiellaceae	0,753	Barnesiellaceae
Bacteroides	0,141	Bacteroides
Bacteroidales	0,053	Bacteroidales
Alphaproteobacteria	0,259	Alphaproteobacteria
Akkermansia	0,471	Akkermansia
Acinetobacter	0,334	Acinetobacter

Nicotinate_and_nicotinamide_metabolism	otu
0,144	Ruminococcus
0,141	Ruminococcaceae
0,713	Rikenellaceae
0,940	Phascolarctobacterium
0,425	Oscillospira
0,050	Mogibacteriaceae
0,412	Lactobacillus
0,134	Lachnospiraceae
0,289	Faecalibacterium
0,656	Erysipelotrichaceae
0,957	Enterococcaceae
0,176	Dorea
0,535	Dehalobacterium
0,094	Coriobacteriaceae
0,542	Coprococcus
0,023	Clostridium
0,102	Clostridiales
0,012	Christensenellaceae
0,880	Barnesiellaceae
0,188	Bacteroides
0,053	Bacteroidales
0,431	Alphaproteobacteria
0,380	Akkermansia
0,253	Acinetobacter

Nitrogen_metabolism	otu	Novobiocin_biosynthesis
0,345	Ruminococcus	0,374
0,094	Ruminococcaceae	0,254
0,549	Rikenellaceae	0,696
0,905	Phascolarctobacterium	0,745
0,300	Oscillospira	0,339
0,085	Mogibacteriaceae	0,204
0,226	Lactobacillus	0,362
0,099	Lachnospiraceae	0,076
0,339	Faecalibacterium	0,387
1,000	Erysipelotrichaceae	0,854
0,871	Enterococcaceae	0,845
0,327	Dorea	0,513
0,587	Dehalobacterium	0,557
0,072	Coriobacteriaceae	0,131
0,451	Coprococcus	0,513
0,024	Clostridium	0,080
0,165	Clostridiales	0,350
0,006	Christensenellaceae	0,029
0,721	Barnesiellaceae	0,991
0,204	Bacteroides	0,362
0,066	Bacteroidales	0,128
0,305	Alphaproteobacteria	0,374
0,729	Akkermansia	0,957
0,495	Acinetobacter	0,785

otu	One_carbon_pool_by_folate	otu
Ruminococcus	0,289	Ruminococcus
Ruminococcaceae	0,094	Ruminococcaceae
Rikenellaceae	0,499	Rikenellaceae
Phascolarctobacterium	1,000	Phascolarctobacterium
Oscillospira	0,350	Oscillospira
Mogibacteriaceae	0,058	Mogibacteriaceae
Lactobacillus	0,240	Lactobacillus
Lachnospiraceae	0,066	Lachnospiraceae
Faecalibacterium	0,254	Faecalibacterium
Erysipelotrichaceae	0,983	Erysipelotrichaceae
Enterococcaceae	0,725	Enterococcaceae
Dorea	0,269	Dorea
Dehalobacterium	0,528	Dehalobacterium
Coriobacteriaceae	0,046	Coriobacteriaceae
Coprococcus	0,431	Coprococcus
Clostridium	0,019	Clostridium
Clostridiales	0,122	Clostridiales
Christensenellaceae	0,004	Christensenellaceae
Barnesiellaceae	0,762	Barnesiellaceae
Bacteroides	0,204	Bacteroides
Bacteroidales	0,053	Bacteroidales
Alphaproteobacteria	0,362	Alphaproteobacteria
Akkermansia	0,656	Akkermansia
Acinetobacter	0,442	Acinetobacter

Oxidative_phosphorylation	otu	Pantothenate_and_CoA_biosynthesis
0,399	Ruminococcus	0,289
0,064	Ruminococcaceae	0,094
0,464	Rikenellaceae	0,499
0,948	Phascolarctobacterium	1,000
0,316	Oscillospira	0,350
0,087	Mogibacteriaceae	0,058
0,147	Lactobacillus	0,240
0,102	Lachnospiraceae	0,066
0,425	Faecalibacterium	0,254
0,888	Erysipelotrichaceae	0,983
0,961	Enterococcaceae	0,725
0,368	Dorea	0,269
0,617	Dehalobacterium	0,528
0,055	Coriobacteriaceae	0,046
0,492	Coprococcus	0,431
0,020	Clostridium	0,019
0,176	Clostridiales	0,122
0,005	Christensenellaceae	0,004
0,664	Barnesiellaceae	0,762
0,222	Bacteroides	0,204
0,066	Bacteroidales	0,053
0,305	Alphaproteobacteria	0,362
0,812	Akkermansia	0,656
0,535	Acinetobacter	0,442

otu	Penicillin_and_cephalosporin_biosynthesis
Ruminococcus	0,803
Ruminococcaceae	0,339
Rikenellaceae	0,922
Phascolarctobacterium	0,047
Oscillospira	0,083
Mogibacteriaceae	0,940
Lactobacillus	0,087
Lachnospiraceae	0,549
Faecalibacterium	0,863
Erysipelotrichaceae	0,464
Enterococcaceae	0,524
Dorea	0,721
Dehalobacterium	0,602
Coriobacteriaceae	0,226
Coprococcus	0,688
Clostridium	0,333
Clostridiales	0,594
Christensenellaceae	0,184
Barnesiellaceae	0,778
Bacteroides	0,880
Bacteroidales	0,161
Alphaproteobacteria	0,188
Akkermansia	0,083
Acinetobacter	0,498

otu	Pentose_and_glucuronate_interconversions
Ruminococcus	0,279
Ruminococcaceae	0,078
Rikenellaceae	0,520
Phascolarctobacterium	0,957
Oscillospira	0,339
Mogibacteriaceae	0,055
Lactobacillus	0,213
Lachnospiraceae	0,097
Faecalibacterium	0,289
Erysipelotrichaceae	0,966
Enterococcaceae	0,897
Dorea	0,231
Dehalobacterium	0,438
Coriobacteriaceae	0,033
Coprococcus	0,444
Clostridium	0,017
Clostridiales	0,154
Christensenellaceae	0,003
Barnesiellaceae	0,753
Bacteroides	0,240
Bacteroidales	0,044
Alphaproteobacteria	0,374
Akkermansia	0,704
Acinetobacter	0,487

otu	Pentose_phosphate_pathway
Ruminococcus	0,380
Ruminococcaceae	0,053
Rikenellaceae	0,393
Phascolarctobacterium	1,000
Oscillospira	0,350
Mogibacteriaceae	0,044
Lactobacillus	0,154
Lachnospiraceae	0,094
Faecalibacterium	0,274
Erysipelotrichaceae	0,940
Enterococcaceae	0,940
Dorea	0,226
Dehalobacterium	0,549
Coriobacteriaceae	0,028
Coprococcus	0,412
Clostridium	0,012
Clostridiales	0,116
Christensenellaceae	0,002
Barnesiellaceae	0,617
Bacteroides	0,158
Bacteroidales	0,044
Alphaproteobacteria	0,269
Akkermansia	0,680
Acinetobacter	0,445

otu	Phenylalanine_metabolism	otu
Ruminococcus	0,680	Ruminococcus
Ruminococcaceae	0,161	Ruminococcaceae
Rikenellaceae	0,656	Rikenellaceae
Phascolarctobacterium	0,471	Phascolarctobacterium
Oscillospira	0,235	Oscillospira
Mogibacteriaceae	0,362	Mogibacteriaceae
Lactobacillus	0,134	Lactobacillus
Lachnospiraceae	0,188	Lachnospiraceae
Faecalibacterium	0,617	Faecalibacterium
Erysipelotrichaceae	0,625	Erysipelotrichaceae
Enterococcaceae	0,774	Enterococcaceae
Dorea	0,721	Dorea
Dehalobacterium	0,579	Dehalobacterium
Coriobacteriaceae	0,099	Coriobacteriaceae
Coprococcus	0,579	Coprococcus
Clostridium	0,080	Clostridium
Clostridiales	0,656	Clostridiales
Christensenellaceae	0,027	Christensenellaceae
Barnesiellaceae	0,803	Barnesiellaceae
Bacteroides	0,464	Bacteroides
Bacteroidales	0,119	Bacteroidales
Alphaproteobacteria	0,244	Alphaproteobacteria
Akkermansia	0,579	Akkermansia
Acinetobacter	0,872	Acinetobacter

Phenylalanine_tyrosine_and_tryptophan_biosynthesis	otu
0,289	Ruminococcus
0,094	Ruminococcaceae
0,499	Rikenellaceae
1,000	Phascolarctobacterium
0,350	Oscillospira
0,058	Mogibacteriaceae
0,240	Lactobacillus
0,066	Lachnospiraceae
0,254	Faecalibacterium
0,983	Erysipelotrichaceae
0,725	Enterococcaceae
0,269	Dorea
0,528	Dehalobacterium
0,046	Coriobacteriaceae
0,431	Coprococcus
0,019	Clostridium
0,122	Clostridiales
0,004	Christensenellaceae
0,762	Barnesiellaceae
0,204	Bacteroides
0,053	Bacteroidales
0,362	Alphaproteobacteria
0,656	Akkermansia
0,442	Acinetobacter

Phenylpropanoid_biosynthesis	otu	Photosynthesis
0,122	Ruminococcus	0,374
0,125	Ruminococcaceae	0,085
0,649	Rikenellaceae	0,380
0,778	Phascolarctobacterium	0,922
0,572	Oscillospira	0,431
0,044	Mogibacteriaceae	0,049
0,380	Lactobacillus	0,192
0,154	Lachnospiraceae	0,049
0,380	Faecalibacterium	0,217
0,535	Erysipelotrichaceae	0,948
0,803	Enterococcaceae	0,700
0,180	Dorea	0,259
0,672	Dehalobacterium	0,625
0,119	Coriobacteriaceae	0,034
0,729	Coproccoccus	0,412
0,017	Clostridium	0,017
0,092	Clostridiales	0,092
0,015	Christensenellaceae	0,004
0,914	Barnesiellaceae	0,704
0,180	Bacteroides	0,158
0,092	Bacteroidales	0,050
0,393	Alphaproteobacteria	0,300
0,289	Akkermansia	0,649
0,213	Acinetobacter	0,422

otu	Photosynthesis_proteins	otu
Ruminococcus	0,374	Ruminococcus
Ruminococcaceae	0,085	Ruminococcaceae
Rikenellaceae	0,380	Rikenellaceae
Phascolarctobacterium	0,922	Phascolarctobacterium
Oscillospira	0,431	Oscillospira
Mogibacteriaceae	0,049	Mogibacteriaceae
Lactobacillus	0,192	Lactobacillus
Lachnospiraceae	0,049	Lachnospiraceae
Faecalibacterium	0,217	Faecalibacterium
Erysipelotrichaceae	0,948	Erysipelotrichaceae
Enterococcaceae	0,700	Enterococcaceae
Dorea	0,259	Dorea
Dehalobacterium	0,625	Dehalobacterium
Coriobacteriaceae	0,034	Coriobacteriaceae
Coprococcus	0,412	Coprococcus
Clostridium	0,017	Clostridium
Clostridiales	0,092	Clostridiales
Christensenellaceae	0,004	Christensenellaceae
Barnesiellaceae	0,704	Barnesiellaceae
Bacteroides	0,158	Bacteroides
Bacteroidales	0,050	Bacteroidales
Alphaproteobacteria	0,300	Alphaproteobacteria
Akkermansia	0,649	Akkermansia
Acinetobacter	0,422	Acinetobacter

Porphyrin_and_chlorophyll_metabolism	otu
0,274	Ruminococcus
0,192	Ruminococcaceae
0,564	Rikenellaceae
0,871	Phascolarctobacterium
0,579	Oscillospira
0,116	Mogibacteriaceae
0,274	Lactobacillus
0,031	Lachnospiraceae
0,333	Faecalibacterium
0,680	Erysipelotrichaceae
0,602	Enterococcaceae
0,412	Dorea
0,672	Dehalobacterium
0,072	Coriobacteriaceae
0,594	Coprococcus
0,049	Clostridium
0,161	Clostridiales
0,018	Christensenellaceae
0,974	Barnesiellaceae
0,339	Bacteroides
0,078	Bacteroidales
0,492	Alphaproteobacteria
0,737	Akkermansia
0,528	Acinetobacter

Primary_bile_acid_biosynthesis	otu	Propanoate_metabolism
0,374	Ruminococcus	0,316
0,213	Ruminococcaceae	0,062
0,803	Rikenellaceae	0,492
0,356	Phascolarctobacterium	0,991
0,184	Oscillospira	0,327
0,362	Mogibacteriaceae	0,057
0,226	Lactobacillus	0,176
0,305	Lachnospiraceae	0,122
0,672	Faecalibacterium	0,350
0,721	Erysipelotrichaceae	0,991
0,546	Enterococcaceae	0,961
0,696	Dorea	0,269
0,204	Dehalobacterium	0,557
0,060	Coriobacteriaceae	0,044
0,837	Coprococcus	0,506
0,068	Clostridium	0,013
0,854	Clostridiales	0,119
0,028	Christensenellaceae	0,004
0,940	Barnesiellaceae	0,617
0,688	Bacteroides	0,169
0,094	Bacteroidales	0,037
0,549	Alphaproteobacteria	0,333
0,594	Akkermansia	0,641
0,996	Acinetobacter	0,343

otu	Pyruvate_metabolism	otu	Retinol_metabolism
Ruminococcus	0,316	Ruminococcus	0,729
Ruminococcaceae	0,062	Ruminococcaceae	0,102
Rikenellaceae	0,464	Rikenellaceae	0,602
Phascolarctobacterium	0,983	Phascolarctobacterium	0,274
Oscillospira	0,322	Oscillospira	0,110
Mogibacteriaceae	0,057	Mogibacteriaceae	0,345
Lactobacillus	0,176	Lactobacillus	0,087
Lachnospiraceae	0,092	Lachnospiraceae	0,274
Faecalibacterium	0,322	Faecalibacterium	0,617
Erysipelotrichaceae	0,983	Erysipelotrichaceae	0,594
Enterococcaceae	0,888	Enterococcaceae	0,594
Dorea	0,269	Dorea	0,721
Dehalobacterium	0,506	Dehalobacterium	0,254
Coriobacteriaceae	0,036	Coriobacteriaceae	0,029
Coprococcus	0,451	Coprococcus	0,625
Clostridium	0,015	Clostridium	0,053
Clostridiales	0,141	Clostridiales	0,795
Christensenellaceae	0,003	Christensenellaceae	0,019
Barnesiellaceae	0,688	Barnesiellaceae	0,617
Bacteroides	0,213	Bacteroides	0,564
Bacteroidales	0,050	Bacteroidales	0,068
Alphaproteobacteria	0,345	Alphaproteobacteria	0,374
Akkermansia	0,713	Akkermansia	0,387
Acinetobacter	0,473	Acinetobacter	0,996

otu	Riboflavin_metabolism	otu
Ruminococcus	0,444	Ruminococcus
Ruminococcaceae	0,083	Ruminococcaceae
Rikenellaceae	0,520	Rikenellaceae
Phascolarctobacterium	0,888	Phascolarctobacterium
Oscillospira	0,327	Oscillospira
Mogibacteriaceae	0,125	Mogibacteriaceae
Lactobacillus	0,151	Lactobacillus
Lachnospiraceae	0,119	Lachnospiraceae
Faecalibacterium	0,499	Faecalibacterium
Erysipelotrichaceae	0,846	Erysipelotrichaceae
Enterococcaceae	0,957	Enterococcaceae
Dorea	0,431	Dorea
Dehalobacterium	0,656	Dehalobacterium
Coriobacteriaceae	0,074	Coriobacteriaceae
Coprococcus	0,528	Coprococcus
Clostridium	0,031	Clostridium
Clostridiales	0,235	Clostridiales
Christensenellaceae	0,009	Christensenellaceae
Barnesiellaceae	0,704	Barnesiellaceae
Bacteroides	0,259	Bacteroides
Bacteroidales	0,076	Bacteroidales
Alphaproteobacteria	0,295	Alphaproteobacteria
Akkermansia	0,905	Akkermansia
Acinetobacter	0,616	Acinetobacter

Secondary_bile_acid_biosynthesis	otu
0,506	Ruminococcus
0,316	Ruminococcaceae
0,803	Rikenellaceae
0,305	Phascolarctobacterium
0,226	Oscillospira
0,535	Mogibacteriaceae
0,217	Lactobacillus
0,208	Lachnospiraceae
0,672	Faecalibacterium
0,492	Erysipelotrichaceae
0,700	Enterococcaceae
0,888	Dorea
0,254	Dehalobacterium
0,072	Coriobacteriaceae
0,795	Coprococcus
0,134	Clostridium
0,957	Clostridiales
0,052	Christensenellaceae
0,897	Barnesiellaceae
0,871	Bacteroides
0,137	Bacteroidales
0,549	Alphaproteobacteria
0,380	Akkermansia
0,680	Acinetobacter

Sphingolipid_metabolism	otu	Starch_and_sucrose_metabolism
0,625	Ruminococcus	0,034
0,249	Ruminococcaceae	0,172
0,737	Rikenellaceae	0,641
0,485	Phascolarctobacterium	0,931
0,316	Oscillospira	0,444
0,458	Mogibacteriaceae	0,053
0,154	Lactobacillus	0,438
0,083	Lachnospiraceae	0,070
0,649	Faecalibacterium	0,217
0,399	Erysipelotrichaceae	0,846
0,970	Enterococcaceae	0,770
0,863	Dorea	0,204
0,438	Dehalobacterium	0,438
0,072	Coriobacteriaceae	0,062
0,688	Coprococcus	0,557
0,151	Clostridium	0,019
0,837	Clostridiales	0,116
0,057	Christensenellaceae	0,008
0,905	Barnesiellaceae	0,966
0,880	Bacteroides	0,222
0,161	Bacteroidales	0,058
0,471	Alphaproteobacteria	0,492
0,362	Akkermansia	0,431
0,701	Acinetobacter	0,320

otu	Steroid_biosynthesis	otu
Ruminococcus	0,721	Ruminococcus
Ruminococcaceae	0,033	Ruminococcaceae
Rikenellaceae	0,713	Rikenellaceae
Phascolarctobacterium	0,044	Phascolarctobacterium
Oscillospira	0,196	Oscillospira
Mogibacteriaceae	0,549	Mogibacteriaceae
Lactobacillus	0,068	Lactobacillus
Lachnospiraceae	0,300	Lachnospiraceae
Faecalibacterium	0,721	Faecalibacterium
Erysipelotrichaceae	0,217	Erysipelotrichaceae
Enterococcaceae	0,944	Enterococcaceae
Dorea	0,022	Dorea
Dehalobacterium	0,837	Dehalobacterium
Coriobacteriaceae	0,322	Coriobacteriaceae
Coprococcus	0,649	Coprococcus
Clostridium	0,017	Clostridium
Clostridiales	0,295	Clostridiales
Christensenellaceae	0,471	Christensenellaceae
Barnesiellaceae	0,905	Barnesiellaceae
Bacteroides	0,528	Bacteroides
Bacteroidales	0,172	Bacteroidales
Alphaproteobacteria	0,249	Alphaproteobacteria
Akkermansia	0,005	Akkermansia
Acinetobacter	0,168	Acinetobacter

Steroid_hormone_biosynthesis	otu
0,778	Ruminococcus
0,704	Ruminococcaceae
0,721	Rikenellaceae
0,033	Phascolarctobacterium
0,154	Oscillospira
0,549	Mogibacteriaceae
0,113	Lactobacillus
0,311	Lachnospiraceae
0,770	Faecalibacterium
0,213	Erysipelotrichaceae
0,996	Enterococcaceae
0,333	Dorea
0,641	Dehalobacterium
0,305	Coriobacteriaceae
0,737	Coprococcus
0,871	Clostridium
0,289	Clostridiales
0,458	Christensenellaceae
0,863	Barnesiellaceae
0,535	Bacteroides
0,217	Bacteroidales
0,350	Alphaproteobacteria
0,008	Akkermansia
0,211	Acinetobacter

Stilbenoid_diarylheptanoid_and_gingerol_biosynthesis
0,688
0,030
0,076
0,729
0,478
0,068
0,076
0,110
0,557
0,458
0,598
0,425
0,846
0,029
0,535
0,006
0,064
0,000
0,305
0,070
0,131
0,418
0,557
0,463

otu	Streptomycin_biosynthesis
Ruminococcus	0,399
Ruminococcaceae	0,249
Rikenellaceae	0,829
Phascolarctobacterium	0,721
Oscillospira	0,438
Mogibacteriaceae	0,249
Lactobacillus	0,269
Lachnospiraceae	0,144
Faecalibacterium	0,549
Erysipelotrichaceae	0,795
Enterococcaceae	0,824
Dorea	0,513
Dehalobacterium	0,535
Coriobacteriaceae	0,107
Coproccoccus	0,696
Clostridium	0,094
Clostridiales	0,499
Christensenellaceae	0,041
Barnesiellaceae	0,966
Bacteroides	0,506
Bacteroidales	0,094
Alphaproteobacteria	0,356
Akkermansia	0,871
Acinetobacter	0,876

otu	Sulfur_metabolism	otu
Ruminococcus	0,888	Ruminococcus
Ruminococcaceae	0,116	Ruminococcaceae
Rikenellaceae	0,572	Rikenellaceae
Phascolarctobacterium	0,412	hascolarctobacteriur
Oscillospira	0,213	Oscillospira
Mogibacteriaceae	0,368	Mogibacteriaceae
Lactobacillus	0,070	Lactobacillus
Lachnospiraceae	0,144	Lachnospiraceae
Faecalibacterium	0,572	Faecalibacterium
Erysipelotrichaceae	0,478	Erysipelotrichaceae
Enterococcaceae	0,829	Enterococcaceae
Dorea	0,704	Dorea
Dehalobacterium	0,535	Dehalobacterium
Coriobacteriaceae	0,047	Coriobacteriaceae
Coprococcus	0,485	Coprococcus
Clostridium	0,080	Clostridium
Clostridiales	0,696	Clostridiales
Christensenellaceae	0,027	Christensenellaceae
Barnesiellaceae	0,713	Barnesiellaceae
Bacteroides	0,535	Bacteroides
Bacteroidales	0,092	Bacteroidales
Alphaproteobacteria	0,240	Alphaproteobacteria
Akkermansia	0,387	Akkermansia
Acinetobacter	0,794	Acinetobacter

Synthesis_and_degradation_of_ketone_bodies	otu
0,431	Ruminococcus
0,028	Ruminococcaceae
0,244	Rikenellaceae
0,931	Phascolarctobacterium
0,345	Oscillospira
0,026	Mogibacteriaceae
0,105	Lactobacillus
0,105	Lachnospiraceae
0,295	Faecalibacterium
0,922	Erysipelotrichaceae
0,978	Enterococcaceae
0,231	Dorea
0,542	Dehalobacterium
0,015	Coriobacteriaceae
0,444	Coprococcus
0,007	Clostridium
0,068	Clostridiales
0,001	Christensenellaceae
0,451	Barnesiellaceae
0,102	Bacteroides
0,030	Bacteroidales
0,311	Alphaproteobacteria
0,617	Akkermansia
0,266	Acinetobacter

Thiamine_metabolism	otu
0,289	Ruminococcus
0,094	Ruminococcaceae
0,499	Rikenellaceae
1,000	Phascolarctobacterium
0,350	Oscillospira
0,058	Mogibacteriaceae
0,240	Lactobacillus
0,066	Lachnospiraceae
0,254	Faecalibacterium
0,983	Erysipelotrichaceae
0,725	Enterococcaceae
0,269	Dorea
0,528	Dehalobacterium
0,046	Coriobacteriaceae
0,431	Coprococcus
0,019	Clostridium
0,122	Clostridiales
0,004	Christensenellaceae
0,762	Barnesiellaceae
0,204	Bacteroides
0,053	Bacteroidales
0,362	Alphaproteobacteria
0,656	Akkermansia
0,442	Acinetobacter

Tropane_piperidine_and_pyridine_alkaloid_biosynthesis
0,362
0,053
0,444
0,983
0,350
0,062
0,137
0,097
0,374
0,914
0,927
0,295
0,587
0,037
0,478
0,015
0,137
0,003
0,649
0,208
0,047
0,322
0,745
0,466

otu	Tryptophan_metabolism
Ruminococcus	0,656
Ruminococcaceae	0,076
Rikenellaceae	0,696
Phascolarctobacterium	0,399
Oscillospira	0,137
Mogibacteriaceae	0,244
Lactobacillus	0,094
Lachnospiraceae	0,279
Faecalibacterium	0,610
Erysipelotrichaceae	0,803
Enterococcaceae	0,625
Dorea	0,549
Dehalobacterium	0,380
Coriobacteriaceae	0,049
Coprococcus	0,549
Clostridium	0,049
Clostridiales	0,602
Christensenellaceae	0,015
Barnesiellaceae	0,602
Bacteroides	0,471
Bacteroidales	0,066
Alphaproteobacteria	0,269
Akkermansia	0,549
Acinetobacter	0,894

otu	Tyrosine_metabolism	otu
Ruminococcus	0,279	Ruminococcus
Ruminococcaceae	0,055	Ruminococcaceae
Rikenellaceae	0,451	Rikenellaceae
Phascolarctobacterium	0,966	Phascolarctobacterium
Oscillospira	0,289	Oscillospira
Mogibacteriaceae	0,049	Mogibacteriaceae
Lactobacillus	0,184	Lactobacillus
Lachnospiraceae	0,113	Lachnospiraceae
Faecalibacterium	0,327	Faecalibacterium
Erysipelotrichaceae	0,966	Erysipelotrichaceae
Enterococcaceae	0,974	Enterococcaceae
Dorea	0,254	Dorea
Dehalobacterium	0,431	Dehalobacterium
Coriobacteriaceae	0,030	Coriobacteriaceae
Coprococcus	0,492	Coprococcus
Clostridium	0,010	Clostridium
Clostridiales	0,134	Clostridiales
Christensenellaceae	0,002	Christensenellaceae
Barnesiellaceae	0,641	Barnesiellaceae
Bacteroides	0,192	Bacteroides
Bacteroidales	0,043	Bacteroidales
Alphaproteobacteria	0,380	Alphaproteobacteria
Akkermansia	0,656	Akkermansia
Acinetobacter	0,389	Acinetobacter

Ubiquinone_and_other_terpenoid-quinone_biosynthesis
0,820
0,284
0,983
0,070
0,107
0,922
0,062
0,564
0,991
0,399
0,418
0,729
0,696
0,226
0,762
0,269
0,602
0,188
0,762
0,846
0,165
0,184
0,105
0,505

otu	Valine_leucine_and_isoleucine_biosynthesis
Ruminococcus	0,316
Ruminococcaceae	0,062
Rikenellaceae	0,464
Phascolarctobacterium	0,983
Oscillospira	0,322
Mogibacteriaceae	0,057
Lactobacillus	0,176
Lachnospiraceae	0,092
Faecalibacterium	0,322
Erysipelotrichaceae	0,983
Enterococcaceae	0,888
Dorea	0,269
Dehalobacterium	0,506
Coriobacteriaceae	0,036
Coprococcus	0,451
Clostridium	0,015
Clostridiales	0,141
Christensenellaceae	0,003
Barnesiellaceae	0,688
Bacteroides	0,213
Bacteroidales	0,050
Alphaproteobacteria	0,345
Akkermansia	0,713
Acinetobacter	0,473

otu	Valine_leucine_and_isoleucine_degradation
Ruminococcus	0,594
Ruminococcaceae	0,060
Rikenellaceae	0,610
Phascolarctobacterium	0,625
Oscillospira	0,213
Mogibacteriaceae	0,172
Lactobacillus	0,094
Lachnospiraceae	0,217
Faecalibacterium	0,610
Erysipelotrichaceae	0,829
Enterococcaceae	0,640
Dorea	0,464
Dehalobacterium	0,520
Coriobacteriaceae	0,053
Coprococcus	0,587
Clostridium	0,029
Clostridiales	0,399
Christensenellaceae	0,013
Barnesiellaceae	0,564
Bacteroides	0,327
Bacteroidales	0,060
Alphaproteobacteria	0,244
Akkermansia	0,803
Acinetobacter	0,672

otu	Vitamin_B6_metabolism
Ruminococcus	0,184
Ruminococcaceae	0,200
Rikenellaceae	0,753
Phascolarctobacterium	0,914
Oscillospira	0,380
Mogibacteriaceae	0,092
Lactobacillus	0,425
Lachnospiraceae	0,083
Faecalibacterium	0,269
Erysipelotrichaceae	0,922
Enterococcaceae	0,782
Dorea	0,264
Dehalobacterium	0,438
Coriobacteriaceae	0,083
Coprococcus	0,499
Clostridium	0,039
Clostridiales	0,200
Christensenellaceae	0,012
Barnesiellaceae	0,983
Bacteroides	0,311
Bacteroidales	0,070
Alphaproteobacteria	0,458
Akkermansia	0,633
Acinetobacter	0,513