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1 Fecal microbiome and feed efficiency in pigs

2 Fecal microbial composition associated with variation in feed efficiency in pigs depends 3 on diet and sex[1]

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ABSTRACT

Dietary fiber content and composition affect microbial composition and activity in the gut, which in turn influence energetic contribution of fermentation products to the metabolic energy supply in pigs. This may affect feed efficiency (FE) in pigs. The present study investigated the relationship between the fecal microbial composition and FE in individual growing-finishing pigs. In addition, the effects of diet composition and sex on the fecal microbiome were studied. Fecal samples were collected of 154 grower-finisher pigs (three-way crossbreeds) the day before slaughter. Pigs were either fed a diet based on corn/soybean meal (CS) or a diet based on wheat/barley/by-products (WB). Fecal microbiome was characterized by 16S ribosomal DNA sequencing, clustered by operational taxonomic unit (OTU), and results were subjected to a discriminant approach combined with principal component analysis to discriminate diets, sexes and FE extreme groups (10 high and 10 low FE pigs for each diet by sex-combination). Pigs on different diets and males vs. females had a very distinct fecal microbiome, needing only two OTU for diet ($P = 0.020$) and 18 OTU for sex ($P = 0.040$) to separate the groups. The two most important OTU for diet, and the most important OTU for sex, were taxonomically classified as the same bacterium. In pigs fed the CS diet there was no significant association between FE and fecal microbiota composition based on OTU ($P > 0.05$), but in pigs fed the WB diet differences in FE were associated with 17 OTU in males ($P = 0.018$) and to 7 OTU in females ($P = 0.010$), with three OTU in common for both sexes. In conclusion, our results showed a diet and sex dependent relationship between FE and the fecal microbial composition at slaughter weight in grower-finisher pigs.

Keywords: feed efficiency, fecal microbiome, diet, sex, pig

INTRODUCTION

In pork production, feed efficiency (FE) is very important, as feed is the main component of the cost prize. The gut microbiota can play an important role in FE, as pigs do not produce digestive enzymes that allow them to digest the fiber fraction in the diet. Instead, they depend on microbiota residing in the gastrointestinal tract, in particular in the hindgut, to break down the dietary fiber in fermentation processes. VFA are resulting by-products of the fermentation activity of the microbiota and they serve, after absorption from the gut, as energy sources in systemic metabolism (Ingerslev et al., 2014). In pigs, efficiency of energy utilization is lower when energy comes from fiber instead of starch (Noblet and Le Goff, 2001). Thus, for improving FE in pigs low fiber, high starch diets have been favored (Zijlstra and Beltranena, 2013). However, dietary fiber has shown to reduce stereotypic behavior and aggression (Meunier-Salaün et al., 2001) and improve fecal consistency (Mateos et al., 2006; Wellock et al., 2008). Combined with the increasing competition of feed with human edible products for amongst others arable land (Van Kernebeek et al., 2016), this has caused the agricultural sector to move increasingly towards the formulation of diets with higher fiber contents. Therefore, the importance of intestinal microbiota and their fermentation activity in relation to FE in pigs is likely to increase.

The aim of this study was to investigate the association between FE and fecal microbial composition in commercial grower-finisher pigs. In addition, two factors affecting FE were investigated for their effect on the fecal microbiome: diet composition and sex.

MATERIALS AND METHODS

This study was carried out in strict accordance with the recommendations in the European Guidelines for accommodation and care of animals. The protocol was approved by the Animal Care and Use Committee of Schothorst Feed Research, The Netherlands (Protocol

64 Number: AVD 246002015120/132). The dataset is available on request from the corresponding
65 author.

66 ***Animals and experimental design***

67 Pigs used in this study originated from a three-way cross (Synthetic boar x (Large White
68 x Landrace)). Phenotypic data were available for 160 three-breed cross pigs, 81 males and 79
69 females, coming from 20 litters. All pigs were kept at the experimental facilities of Schothorst
70 Feed Research B.V. (Lelystad, The Netherlands) under commercial conditions. Up until the
71 start of the trial the animals were housed per litter and all animals were fed the same diet. The
72 pigs were put on test at 8 to 9 weeks of age (Day 0), in two groups of 80, and experimental
73 groups were set 13 weeks apart. Distribution was as follows: ten pigs per pen and eight pens
74 per compartment; one compartment was used per entrance date. Littermates were split
75 randomly over the two diets and sexes were housed in separate pens, resulting in two pens per
76 diet per sex per entrance date. All animals were used for the evaluation of the effects of diet
77 composition and sex on fecal microbiota composition, except for six animals of which no fecal
78 sample was obtained. The FE was defined as the ratio of body weight gain to cumulated feed
79 intake from start of the test until the day of slaughter. For evaluation of the effect of fecal
80 microbiota composition on FE the 25% pigs with the highest and the 25% with the lowest
81 individual FE per diet per sex (20 animals per combination) were used. Data of one animal
82 were excluded, since it had a very low feed intake and body weight gain during the second half
83 of the test. At the start of the experiment, the pigs had an average BW of 23.0 kg and were kept
84 in the facilities until they reached a live weight at slaughter of approximately 120 kg. Pigs were
85 allowed a minimal space of 1 m^2 per pig, and the pens were equipped with 60% concrete floor
86 and 40% slatted floor.

87 ***Feeding strategy***

88 Two different diets were studied, a diet based on corn/soybean meal (CS) as typically
89 fed to commercial grower-finisher pigs in The America's and a diet based on wheat/barley/by-
90 products (WB) as typically fed in Europe (Table 1). For both diets, the pigs were fed *ad libitum*
91 according to a three-phase feeding program. The first phase ($T_{starter}$) was from Day 0 to Day 25
92 on test and pigs were fed a starter diet. The second phase (T_{grower}) was from Day 26 to Day 67
93 on test and pigs were fed a grower diet. The third phase ($T_{finisher}$) was from Day 68 on test until
94 the pigs reached slaughter weight and they were fed a finisher diet. The diets were custom
95 made diets based on commonly used commercial diets and were formulated on a fixed ratio of
96 net energy to digestible lysine (NE:SID lysine). Each of the three phases had a different NE:SID
97 lysine, being 0.89 J/g at $T_{starter}$, 1.06 J/g at T_{grower} and 1.37 J/g at $T_{finisher}$. The increase of NE:SID
98 lysine in grower and finisher diets was mainly achieved by exchanging soybean meal with
99 corn, and peas with wheat for the CS and WB diets respectively. The experimental diets were
100 produced in the feed plant of ABZ Animal Nutrition, Leusden, The Netherlands.

101 ***Measurements and sampling***

102 The experimental facilities of Schothorst Feed Research B.V. were equipped with
103 IVOG feeding stations (INSENTEC, Marknesse, The Netherlands) that register individual feed
104 intake of group housed animals. All animals had ear tags with unique incremental numbering,
105 therefore, individual feed intake records were available for all pigs for each day on test.
106 Animals were weighted at Day 0, Day 56 and at the end of the test. At the end of the feeding
107 trial (one day before slaughter), individual fecal samples were collected directly at defecation
108 by hand, with gloves, mixed in the glove and put in small tubes. The samples were immediately
109 frozen in liquid nitrogen and stored at -80°C. The ADFI was calculated as the cumulated
110 individual feed intake records throughout the trial divided by the length of the trial. The ADG
111 was calculated as the difference between BW measurements divided by the duration of the trial.

Fecal microbiota analysis

Fecal samples were used for ribosomal 16S DNA gene sequencing and analysis. Bead beating lyzed the microbial cells and the DNA was purified using the ZR-96 Soil Microbe DNA kit (Zymo Research, Irvine, CA) according to the manufacturer description (Frese et al., 2015). The V3-V4 region was amplified from purified genomic DNA with the primers F343 (CTTTCCCTACACGACGCTCTTCCGATCTTACGGRAGGCAGCAG) and R784 (GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT) using 30 amplification cycles with an annealing temperature of 65 °C (an amplicon of 510 bp, although length varies depending on the organisms). Full length reads of the V3-V4 region were obtained using Illumina Miseq 250-bp paired end reads. Single multiplexing was performed using in house 6 bp index, which were added to R784 during a second PCR with 12 cycles using forward primer (AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGAC) and reverse primer (CAAGCAGAAGACGGCATACGAGAT-index-GTGACTGGAGTTCAGACGTGT). The resulting PCR products were purified and loaded onto the Illumina MiSeq cartridge according to the manufacturer instructions. The quality of the run was checked internally using PhiX control as recommended by manufacturer, and then each pair-end sequence was assigned to its sample with the help of the previously integrated index. Each pair-end sequence was assembled using Flash software (Magoč and Salzberg, 2011) using at least a 10bp-overlap between the forward and reverse sequences, allowing 10% of mismatch (Lluch et al., 2015). The absence of contamination was checked with a negative control during the PCR (water as template). The quality of the stitching procedure was controlled using 4 bacterial samples that are run routinely in the sequencing facility in parallel to the current samples.

135 ***Statistical analysis***

136 The resulting sequences of the 154 samples were clustered with Usearch (Edgar, 2010)
137 using the Uparse pipeline (Edgar, 2013) to create operational taxonomic units (OTU). The OTU
138 table of abundance was analyzed by discriminant analysis using principal components (DAPC)
139 (Jombart et al., 2010), to test the association of OTU abundance with a number of factors.
140 Number of dimensions to be included in further analyses was chosen based on stability of the
141 results, determined by adding increasingly more dimensions. In case the stability test gave a
142 range of dimensions, a threshold value of 99% of the original variance was used to decide the
143 number of dimensions. The OTU were sorted based on their contribution to the separation of
144 tested factors in the discriminant analysis, which echoes the weight of each OTU in separating
145 the groups. Using this order, increasingly more OTU were added to separate the groups, until
146 the separation reached significance at $P < 0.05$. The built-in a-score method of the DAPC was
147 used to determine the statistical significance of the separation based on a permutation test.
148 Briefly, 1000 simulations with randomized group labels were performed to evaluate if the
149 discriminant analysis could separate the samples in any of those random configurations. The
150 a-score obtained with the true groups was compared to the distribution of the a-scores obtained
151 with the 1000 simulations to determine if the separation was due to chance (Jombart et al.,
152 2010). The method was repeated to test separation for diet, sex, and FE groups, and to test
153 association of phylum, class and genera abundance (based on OTU taxonomy) with these
154 factors. To test whether the results extrapolated to the whole dataset, the OTU relevant for
155 separating the FE groups were used for partial least squares regression (PLSr)(Mevik and
156 Wehrens, 2007) on all animals within the groups and not only the FE extreme pigs. The number
157 of components kept was based on the lowest root-mean-squares error of prediction after leave-
158 one-out cross validation.

159 After rarefying the data (McMurdie and Holmes, 2013), Bray-Curtis distances between
160 diet, sex and FE extreme groups were calculated using a maximum of 200 iterations for diet
161 and sex and 100 iterations for FE groups and tested with ADONIS for significance (Oksanen
162 et al., 2017). Shannon Index, Simpson diversity index and chao1 richness estimator were
163 calculated using the vegan package (Oksanen et al., 2017). Significance of difference in the
164 diversity estimates between the diets, sexes and FE groups was determined using a generalized
165 linear model (SAS 9.3; SAS Inst. Inc., Cary, NC) with diet, sex and FE groups as fixed effect.
166 Significance of difference in ADG, ADFI and FE between the high and low FE groups was
167 determined using a mixed model (SAS 9.3; SAS Inst. Inc., Cary, NC) with animal as
168 experimental unit, FE groups and pen as fixed effect, and BW at start of the test as co-variable.
169 For the least squares means calculations BW at start of the test was fixed at 22 kg.

170 ***Taxonomy***

171 To investigate biological functionality of differences between groups, the taxonomy
172 was determined for each OTU by the SILVA Incremental Aligner (SINA) software (Pruesse et
173 al., 2012), which aligns the OTU with the rRNA gene databases provided by the SILVA
174 ribosomal RNA project (Quast et al., 2013). Default SINA settings were used to assign the
175 taxonomy of each OTU, with the minimum identity with query sequence set at 0.97 and number
176 of neighbors per query sequence set at ten. Group level information within genera classification
177 was deleted. In addition, OTU found by DAPC analysis were blasted against the NCBI 16S
178 ribosomal RNA sequences (Bacteria and Archeae) database using BLASTn (McGinnis and
179 Madden, 2004) to determine the bacteria with closest sequence similarity. Default
180 megaBLAST settings were used.

181 **RESULTS**

182 Within the DAPC analysis it is not possible to account for the pen effect directly.
183 However, when doing a DAPC analysis for all the piglets across all the pens, the cohoused
184 piglets did not group together (results not shown).

185 ***Differences between diets***

186 Between the two diet, differences in the relative abundance of the 9 major phyla, classes
187 and genera for both diets were observed (Fig. 1). This was reflected in the Bray-Curtis distances
188 at phylum and OTU level (Fig. 2), which were significantly different (P < 0.001), but not on
189 class and genera level. The DAPC analysis gave a clear separation in fecal microbiota
190 composition between the two diets based on phyla, classes, genera and OTU (Fig. 3) (P <
191 0.001). The separation was based on 3, 4, 10 and 55 dimensions for phyla, classes, genera and
192 OTU respectively, which represented at least 99% of the original variance in microbiota
193 composition. Keeping the two phyla (Bacteroidetes and Proteobacteria), three classes
194 (Gammaproteobacteria, Spirochaetes, and Bacteroidia), two genera (Ruminococcus and
195 Blautia) and two OTU (OTU 33 and OTU 16) with the highest contribution to the separation
196 was sufficient to discriminate pigs on different diets. Blasting the sequence of the two most
197 contributing OTU to NCBI gave a 95% identity with 99% query coverage with the bacterium
198 *Butyricicoccus pullicaecorum*. The second most important OTU resulted in the same
199 bacterium, with 96% identity and 99% query coverage. This difference between the diets,
200 however, was not depicted in the measures for diversity. The CS diet had a higher Shannon
201 index than the WB diet (P = 0.021), but the Simpson Index and the chao1 Index were similar
202 for both diets.

203 ***Differences between sexes***

204 In contrast to the diets, the overview of the relative abundance of the 9 major phyla,
205 classes and genera (Fig. 1) does not indicate obvious differences between the sexes. This is

206 reflected by the results of the Bray-Curtis distances, which were only significant at OTU level
207 (P = 0.037) (Fig. 2). The DAPC analysis gave somewhat similar results, as it indicated no
208 separation between the two sexes based on phyla (seven dimensions) and needed 22 out of 45
209 classes to reach a significant difference between the male and female pigs using 16 dimensions
210 and 100.0% of the original variance. However, there was a highly significant distinction for
211 sex based on genera (P = 0.003) and OTU (P = 0.001) (Fig. 3), based on 38 and 60 dimensions
212 (100.0% and 99.2% of the original variance) respectively. There were 6 genera and 18 OTU
213 required to reach a significant separation between sexes. For nine out of those 18 OTU it was
214 possible to reliably assign the genus, for eight it was possible to reliably assign the family, and
215 for one OTU it was not possible to assign any taxonomy (Table 2). The main class differing
216 between the sexes was Methanobacteria and the main genera differing was Bifidobacterium.
217 The most important OTU for sex separation was the same as for diet, which was associated
218 with *Butyricicoccus pullicaecorum*. There was no difference in any of the diversity indexes
219 between the sexes.

220 ***Differences between feed efficiency extremes***

221 As there was a strong effect of diet and sex on the fecal, the dataset was split in four
222 groups to estimate the association between FE and microbiome within diet by sex combination.
223 There was a 0.062 to 0.078 g/g difference between the FE groups in FE (Table 3) and there
224 was no pen effect in any of the groups.

225 There was no difference in diversity index between the high and low FE animals in any
226 of the diet by sex combinations. In addition, there were only significant Bray-Curtis distances
227 at OTU level for the pigs fed the WB diet (Fig. 2). Compared to the diet and sex analyses, the
228 separation between the FE groups using the DAPC analysis was not as clear (Fig. 3). At phylum
229 level, only the male pigs fed a WB diet could be separated using five dimension (100.0% of
230 the original variance). Two phyla were necessary for significant separation, Actinobacteria and

231 Proteobacteria, which were both highest in the high FE pigs. Also at class level the male pigs
232 fed a WB diet could be significantly separated, based on five dimensions explaining 99.7% of
233 the original variance. Gammaproteobacteria was the first out of the nine contributing classes
234 used for the separation. In addition, the male pigs fed a CS diet could be significantly separated
235 (P = 0.008) and there were16 classes used for the separation. At genera level the analysis only
236 showed significant separation between high and low female pigs fed the CS diet (P = 0.009)
237 and male pigs fed the WB diet (P = 0.038). Four dimensions were used, explaining 98.7% and
238 98.3% of the original variance respectively, and keeping only two genera was sufficient for the
239 separation in the female pigs. These genera were Prevotella and Streptococcus. There were 11
240 genera needed for the separation in the male pigs with the main genera being Roseburia.

241 In the pigs fed the CS diet, there was no significant separation for either of the sexes
242 when using OTU, based on eight dimensions for male animals and three for females, explaining
243 96.7% and 83.2% of the original variance, respectively. In the pigs fed the WB diet, when five
244 dimensions were used (82.7% of the original variance), the low FE (P = 0.016), but not the
245 high FE (P = 0.690), could be identified in the pool of males. In the females fed the WB diet,
246 the high FE pigs were identified (P=0.016), but not the low FE animals (P = 0.094), based on
247 five dimensions (87.8% of the original variance). In total, 17 OTU were necessary to
248 discriminate the low FE male pigs (P = 0.018) (Table 4) and seven OTU to distinguish the high
249 FE female pigs (P = 0.010) fed a WB diet (Table 5). Putting these OTU in PLSr resulted in an
250 R^2 of 0.14 (2 components) and 0.11 (3 components) for male and female pigs fed the WB diet
251 respectively (Fig. 4). Three of the OTU significant for discriminating high and low FE pigs
252 were common for the male and female pigs. Strikingly, the effects of OTU 4 and 2 had different
253 directions in male and female pigs, as higher abundance was associated with high FE in males
254 and low FE in females.

255 ## DISCUSSION

256 The aim of this study was to investigate the association between FE and the composition
257 of the fecal microbiome in commercial grower-finisher pigs. In the present experiment the fecal
258 microbiome was used as an indicator for the microbiome in the gastro-intestinal tract during
259 the whole grower-finisher period. However, extrapolation of results of the microbial
260 composition in the feces to other compartments of the gastro-intestinal tract might not be valid.
261 Microbial composition in the ileum, cecum, and colon differs, with the ileal intestinal
262 microbiome being most different from that in other compartments (Looft et al., 2014).
263 Moreover, microbial composition in digesta in the lumen of the gut is different from the mucosa
264 associated microbiota (Looft et al., 2014). It is also questionable whether the fecal samples,
265 taken at the end of the grower-finisher period, are representative for the whole grower finisher
266 period, as the microbial composition in the feces might change with age (Kim et al., 2011). As
267 the microbial composition at the start and at other time points of the experiment was not
268 measured, the age at which differences in the microbiome for the tested effects appear are
269 unknown. Nevertheless, the fecal microbiome seems most similar to both luminal and mucosal
270 microbiome in the mid-colon (Looft et al., 2014) and is most similar for pigs aged 10 and 13
271 weeks, and for pigs aged 16, 19 and 22 weeks (Kim et al., 2011). Therefore, when interpreting
272 our results in terms of relationships between microbial composition and performance of the
273 pig, it should be considered that the fecal microbiome measured in the present study is likely
274 most representative for the microbial composition in the colon, in particular during the second
275 part of the growth trajectory considered.

276 ***Diets***

277 From literature it is well known that diet composition affects the microbial composition
278 in the gastrointestinal tract (Bauer et al., 2006) and the current study confirms these
279 observations. Worldwide there are two mainstream diets fed to grower-finishers based on the

280 availability of main ingredients: a diet based on corn and soybean meal as is common in North
281 and South America, and a diet based on wheat, barley, and by-products from the agro-food
282 sector as is common in Europe and parts of China. Both diets are used to grow pigs as fast and
283 cost-efficient as possible, even though the ingredient composition is rather different. The diets
284 studied differed mostly in dietary fiber content and composition. The main fiber components
285 in wheat, barley and corn are arabinoxylans, β-glucans and cellulose, whereas in soybean meal
286 the fiber mainly contains pectic substances in the form of rhamnogalacturonan (Choct, 1997).
287 This is reflected in the observed differences in microbiome between the two diets in the current
288 study, as *Butyricicoccus pullicaecorum*, comprising the two OTU with highest abundance in
289 the CS diet, is highly efficient in fermenting starch (Eeckhaut et al., 2008). This most likely
290 relates to the high starch content in the CS diet. Additionally, the third most important OTU
291 was found to be *Blautia wexlera*, and had the highest abundancy in the WB diet. This bacterium
292 mainly ferments arabinose, glucose, mannose and xylose (Liu et al., 2008), which relates to the
293 high arabinoxylans content of the WB diet. So the most contributing OTU to discriminate pigs
294 on the different diets resemble the source of dietary fiber.

295 ***Sexes***

296 Our results are in accordance with a recent study of Xiao et al. (2016), which also
297 showed a difference between male and female finisher pigs in fecal microbial composition.
298 Both studies found differences in bacteria belonging to the Prevotella and Ruminococcus
299 genus. Previously, most of the research in pigs investigated changes in intestinal microbiota
300 related to digestive problems and diarrhea post-weaning in weaners (Konstantinov et al., 2006;
301 Pajarillo et al., 2014). These studies in weaners did not find a sex effect on the microbiome
302 (Mach et al., 2015). Sex steroids hormones might partially explain this, as levels of some sex
303 steroids hormones rapidly increase at onset of puberty (Camous et al., 1985; Zamaratskaia et
304 al., 2004). In mice, gonadectomy of males and females resulted in a change in microbial

305 composition of the feces, but testosterone treatment of the castrated males resulted in a
306 microbiome similar to that of intact males (Org et al., 2016). Metabolism residues of sex
307 steroids hormones are excreted through bile into the lumen of the small intestine (Goymann,
308 2012), resulting in different bile composition between sexes (Org et al., 2016). Mainly the
309 Firmicutes, Proteobacteria and Actinobacteria can metabolize and degrade steroid hormones
310 (García-Gómez et al., 2012), which is reflected in the difference in OTU between the sexes in
311 our study, where 11 out of the 18 OTU belonged to the Firmicutes phyla. Other pathways
312 through which sex steroid hormones might influence microbiota are the mucosal immune
313 activation (Sankaran-Walters et al., 2013) and expression of steroid receptors (Menon et al.,
314 2013). The observed limited effect of sex on microbial composition in the feces of weaners and
315 the substantial effect at slaughter age is likely because sex steroid hormones only start to play
316 a large role in finisher pigs.

317 ***Feed efficiencies***

318 There are several ways via which the intestinal microbiota could influence FE of pigs,
319 including competition between the host and the microbiota for nutrients in the small intestine
320 and activation of the immune system through stimulation of the development of the mucus
321 layer, epithelial cells, and lamina propria (Dibner and Richards, 2005). The latter could
322 possibly induce changes in nutrient partitioning between utilization for immune system
323 functioning and for deposition e.g. in muscle protein, but this is likely to be primarily a juvenile
324 phenomenon (Dibner and Richards, 2005). In addition, quantitative production of VFA by
325 intestinal microbiota can relate to FE. Approximately 68% of the gross energy in fermentable
326 carbohydrates can be transformed into VFA (Williams et al., 2001). The VFA composition
327 depends amongst others on the composition of the substrates, microbial composition and
328 activity, and absorption of the VFA across the large intestinal wall (Williams et al., 2001).
329 Butyrate is the preferred energy source for colonocytes, 76% of the mucosal absorbed butyrate

330 is metabolized in these cells (Herrmann et al., 2011). Once absorbed across the intestinal wall
331 the VFA are available as precursor and energy substrate in organs and tissues in the body.
332 Propionate is a precursor for glucose and is almost fully extracted by the liver (Ingerslev et al.,
333 2014), whereas acetate and butyrate are used for Acetyl-CoA production. Next to being direct
334 energy substrates, VFA are also involved as regulators in fatty acid, glucose and cholesterol
335 metabolism (den Besten et al., 2013). Therefore, the microbiota might influence FE by the
336 amount and composition of VFA produced.

337 There was a significant relationship between microbiome and FE in pigs fed the WB
338 diet, but there was no significant relationship in pigs fed the CS diet on OTU level. The fiber
339 level in the diets might explain this difference. When assuming the VFA production to
340 contribute to the FE of the pigs, the difference in performance between the high and low FE
341 pigs due to microbial composition differences is expected to be more pronounced at a higher
342 content of fermentation substrate in the diet. As the finisher WB diet contained 2.8 times more
343 crude fiber than the CS diet, there was more substrate available for fermentation in the WB
344 diet. Consequently, in our study the amount of substrate available might not have been
345 sufficient to detect a relationship between microbiome and FE in the pigs fed the CS diet,
346 whereas it was sufficient in the pigs fed the WB diet.

347 In male pigs fed the WB diet, the most contributing OTU to separate the FE groups was
348 taxonomically classified as *Lactobacillus*, the high FE group having a higher abundance of this
349 OTU. In contradiction to our results, Vigors et al. (2016) only showed a difference in
350 *Lactobacilli* spp. in the cecum, and not in the colon, between divergent groups in residual feed
351 intake in pigs. Nevertheless, the direction of the effect was similar in both studies, with an
352 increase in *Lactobacillus* having a positive effect on FE. The species related to this OTU only
353 produce D- and L-lactate (Roos et al., 2005; Slavica et al., 2015). In contrast, in the female pigs
354 fed the WB diet, the same OTU was higher in the low FE group, but the difference was smaller

355 between the FE groups in the female pigs. In accordance with the results of McCormack et al.
356 (2017), the *Clostridium* abundancy in feces was important to distinguish between the high and
357 low FE pigs. However, this was only the case in the male pigs fed the WB diet, and the two
358 OTU classified as *Clostridium* had opposite effects. In addition, the other five genera important
359 for distinguishing pigs divergent in residual feed intake discovered by McCormack et al. (2017)
360 were not found in our study. An explanation may lie in the difference between the diets of the
361 studies. Everything considered, the microbiota associated with FE in grower-finisher pigs
362 might consist of several crucial species and other species only relevant in certain situations e.g.
363 when certain diets are fed.

364 ***Implications***

365 Results of the present study suggest possibilities to improve FE of grower-finisher pigs
366 by altering microbial composition in the distal part of the intestinal tract. Modification of diet
367 composition might be an option to change microbiota composition, e.g. by changing fiber
368 source or inclusion level, or by including specific additives such as probiotics, prebiotics,
369 organic and inorganic acids, and essential oils (De Lange et al., 2010). In summary, FE might
370 be improved by changing the nutrition of pigs partly through resulting changes in microbiota
371 composition.

372 **CONCLUSION**

373 There is a sex dependent relationship between the fecal microbial composition and FE
374 in grower-finisher pigs fed a WB diet, having a higher concentration of dietary fiber than a CS
375 diet. The exact interplay between the fecal microbial composition, composition and
376 concentration of fiber, and production of VFA by intestinal microbiota remains to be
377 determined. Furthermore, results on the relationship between microbiota composition in the
378 digestive tract and FE remain to be confirmed in more and larger scale studies. Results of the
379 present experiment suggest that there are possibilities to modify the intestinal microbial

380 composition by means of nutrition (e.g. by use of specific additives such as pro- and prebiotics)

381 in order to improve FE of grower-finisher pigs.

LITERATURE CITED

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509

510

511 TABLES AND FIGURES

512 Table 1. Ingredient and calculated nutrient composition of the diets, as-fed basis

	Starter (d 0 to 25)		Grower (d 26 to 67)		Finisher (d 68 to end)	
Item	CS	WB	CS	WB	CS	WB
Ingredient, g/kg						
Corn	647.1	-	698.4	-	755.1	-
Corn gluten	18.1	-	25.0	50.0	50.0	50.0
Soybean meal	240.5	100.0	180.5	21.5	98.3	-
Soybean hull	-	-	-	14.3	-	50.0
Soybean oil	-	25.0	-	0.3	-	-
Barley	-	200.0	-	100.0	-	150.0
Wheat	-	321.9	-	400.0	-	350.0
Wheat middlings	-	-	-	50.0	-	125.0
Rapeseed meal	-	63.0	-	80.0	-	100.0
Sunflower meal	-	80.0	-	80.0	-	21.9
Palmkernel meal	-	-	-	50.0	-	50.0
Palm oil	5.0	17.3	5.0	16.0	5.0	5.0
Peas	-	120.0	-	29.4	-	-
Sugarcane molasses	40.0	30.0	50.0	50.0	50.0	50.0
Animal fat	-	-	-	27.5	-	29.4
Monocalcium phosphate	6.7	5.3	2.0	-	0.7	-
Salt	2.7	2.1	2.4	1.8	1.8	2.1
Calcium carbonate	11.6	10.9	9.4	8.9	9.9	4.0
Sodium bicarbonate	-	1.1	1.0	1.0	3.4	-
Phytase	5.0	5.0	5.0	5.0	5.0	1.9
L-Lysine HCl	-	3.8	-	4.3	-	-
DL-Methionine	-	1.3	-	0.7	-	-
L-Threonine	-	1.7	-	1.6	-	-
Lysine + Thrypophan	7.7	4.3	8.2	3.6	9.1	-
Lysine HC	3.0	-	2.7	-	2.3	4.0
Methionine HC	2.8	-	2.5	-	1.5	0.3
Threonine HC	3.8	-	3.9	-	3.8	2.4
Valine	-	1.4	-	-	-	-
Vitamin premix[1]	0.1	0.1				
Vitamin-trace mineral premix 1[2]	0.1	0.1				
Vitamin-trace mineral premix 2[3]	0.4	0.4	0.4	0.4	0.4	0.4
Nutrient composition, g/kg[4]						
NE, MJ/kg	9.9	9.9	10.1	9.7	10.3	9.3
Moisture	127	126	130	126	130	129
Ash	51	52	42	47	38	42
Crude protein	182	190	159	166	128	147
Crude fat	34	58	35	64	36	57

Crude fibre	24	45	24	60	25	71
Starch	437	360	471	335	512	334
Sugar	44	50	46	58	42	59
NSP	135	170	130	216	126	246
Ca	6.9	6.9	5.2	5.5	5.0	3.8
P	4.8	5.5	3.6	4.7	3.2	4.7
SID Lys	11.1	11.1	9.5	9.1	7.5	6.8
SID Met + Cys	6.6	6.6	5.9	5.6	4.6	4.6
SID Thr	7.1	7.1	6.3	6.0	5.2	4.7
SID Trp	2.1	2.1	1.8	1.7	1.4	1.3

513 [1]Supplied per kilogram of feed: 2500 IU of vitamin A, 500 IU of vitamin D3, and 5 IU of vitamin E (Mervit
514 AD3E; PreMervo, Utrecht, the Netherlands).
515 [2]Supplied per kilogram of feed: 12 mg of Fe (ferrous sulfate), 10 mg of Mn (manganous oxide), 0.04 mg of Co
516 cobalt oxide), 0.12 g of Ca, 0.0501 g of P, 0.04 mg of I (potassium iodide), 1000 IU of vitamin A, 100 IU of
517 vitamin D3, 5 IU of vitamin E, 0.4 mg of vitamin B1, 0.8 mg of vitamin B2, 2 mg of pantothenic acid, 4 mg of
518 niacine, 0.4 mg of vitamin B6, 0.2 mg of folate, 0.003 mg of vitamin B12, 10 mg of vitamin C, 0.01 mg of biotine,
519 0.2 mg of vitamin K3, and 40 mg of choline (Mervit Sporavit; PreMervo).
520 [3]Supplied per kilogram of premix: 0.4 g of Ca, 15 mg of Cu (copper sulfate)0, 80 mg of Fe (ferrous sulfate), 24
521 mg of Mn (manganous oxide), 62 mg of Zn (zinc oxide), 0.04 mg of Co (cobalt oxide), 0.4 mg of I (potassium
522 iodide), 0.2 mg of Se (sodium selenite), 7500 IU of vitamin A, 1500 IU of vitamin D3, 25 IU of vitamin E, 4 mg
523 of vitamin B2, 6 mg of pantothenate, 30 mg of niacin, 0.02 mg of vitamin B12, and 0.752 mg of vitamin K3
524 (Mervit START M220; PreMervo, Utrecht, the Netherlands).
525 [4]Based on chemical composition, digestibility, and energy values for pigs from the Centraal Veevoeder Bureau
526 livestock feed table (CVB, 2011).

527 Table 2. Abundancy and taxonomy (genus level) of the operational taxonomic units (OTU) in

528 order of statistical contribution to the separation between sexes

OTU ID	Classification	Percentage of total sequences	
		Boar	Gilt
OTU16	Unclassified Ruminococcaceae[1]	0.57	0.38
OTU35	Unclassified Ruminococcaceae[1]	0.84	0.76
OTU12472	*Clostridium*	0.77	0.77
OTU373	*Subdoligranulum*	0.16	0.23
OTU191	Unclassified[2]	0.05	0.14
OTU174	Unclassified Bacteroidales[1]	0.05	0.15
OTU22	*Roseburia*	0.26	0.33
OTU71	*Ruminococcus*	0.15	0.21
OTU33	Unclassified Ruminococcaceae[1]	0.28	0.27
OTU19	*Coprococcus*	0.34	0.35
OTU136	*Prevotella*	0.06	0.13
OTU29	Unclassified Succinivibrionaceae[1]	0.28	0.32
OTU20	*Ruminococcus*	0.39	0.42
OTU8	*Turicibacter*	0.88	0.91
OTU38	Unclassified Prevotellaceae[1]	0.44	0.42
OTU127	Unclassified Prevotellaceae[1]	0.20	0.13
OTU1050	Unclassified Prevotellaceae[1]	0.42	0.32
OTU44	*Ruminococcus*	0.32	0.19

529 [1]Reliable depth of taxonomy is limited to family level (query sequence identical for at least 95%)

530 [2]No taxonomic classification available (query sequence identical for at least 95%)

531

532 Table 3. Least squares means of the high and low feed efficiency (FE) groups during the
533 experimental period (overall mean BW at start = 22 kg, overall mean BW at end = 121 kg) per
534 diet by sex combination

Item	FE groups		SEM	*P*-value	
	Low	High		BW start	FE group
CSM[1]					
ADG, g/d	894	1028	24	0.255	0.001
ADFI, kg/d	2.28	2.19	0.07	0.123	0.357
FE, g/g	0.39	0.47	0.01	0.057	<0.001
CSF[1]					
ADG, g/d	909	1045	25	0.004	0.001
ADFI, kg/d	2.41	2.38	0.06	0.001	0.724
FE, g/g	0.38	0.44	0.00	0.243	<0.001
WBM[1]					
ADG, g/d	899	1016	23	0.008	0.001
ADFI, kg/d	2.27	2.18	0.06	0.003	0.274
FE, g/g	0.40	0.47	0.00	0.051	<0.001
WBF[1]					
ADG, g/d	931	992	27	0.499	0.120
ADFI, kg/d	2.60	2.27	0.06	0.305	0.002
FE, g/g	0.36	0.44	0.00	0.471	<0.001

535 [1]CSM = male pigs fed a corn/soybean meal diet

536 [2]CSF = female pigs fed a corn/soybean meal diet

537 [3]WBM = male pigs fed a wheat/barley/by-products diet

538 [4]WBF = female pigs fed a wheat/barley/by-products diet

539

540 Table 4. Abundancy and taxonomy (genus level) of the operational taxonomic units (OTU) in
541 order of statistical contribution to the separation between high and low feed efficient (FE) boars
542 fed a wheat/barley/by-product diet

OTU ID	Classification	Percentage of total sequences	
		Low FE	High FE
OTU4	*Lactobacillus*	1.75	4.36
OTU24	*Roseburia*	0.23	1.36
OTU2	Unclassified Peptostreptococcaceae[1]	4.59	5.10
OTU12	Unclassified Prevotellaceae[1]	0.89	1.34
OTU3	*Lactobacillus*	1.71	1.60
OTU244	*Prevotella*	3.96	2.60
OTU5	*Streptococcus*	1.80	2.41
OTU8955	*Roseburia*	0.01	0.45
OTU1050	Unclassified Prevotellaceae[1]	0.95	0.16
OTU9	*Prevotella*	6.43	4.73
OTU3132	*Roseburia*	0.03	0.43
OTU1	*Clostridium*	8.96	7.28
OTU22	*Roseburia*	0.29	0.67
OTU12472	*Clostridium*	0.47	0.95
OTU41	Unclassified Prevotellaceae[1]	1.82	1.34
OTU180	*Ruminococcus*	0.07	0.29
OTU13	*Roseburia*	3.27	2.50

543 [1]Reliable depth of taxonomy is limited to family level (query sequence identical for at least 95%)

544

545 Table 5. Abundancy and taxonomy (genus level) of the operational taxonomic units (OTU) in
546 order of statistical contribution to the separation between high and low feed efficient (FE) gilts
547 fed a wheat/barley/by-product diet

		Percentage of total sequences	
OTU ID	Classification	Low FE	High FE
OTU2	Unclassified Peptostreptococcaceae[1]	5.35	5.27
OTU10	*Prevotella*	0.65	2.00
OTU55	*Ruminococcus*	0.24	0.82
OTU13	*Roseburia*	2.80	1.76
OTU4	*Lactobacillus*	4.25	3.14
OTU49	*Prevotella*	0.97	0.32
OTU6	*Lactobacillus*	4.21	2.45

548 [1]Reliable depth of taxonomy is limited to family level (query sequence identical for at least 95%)

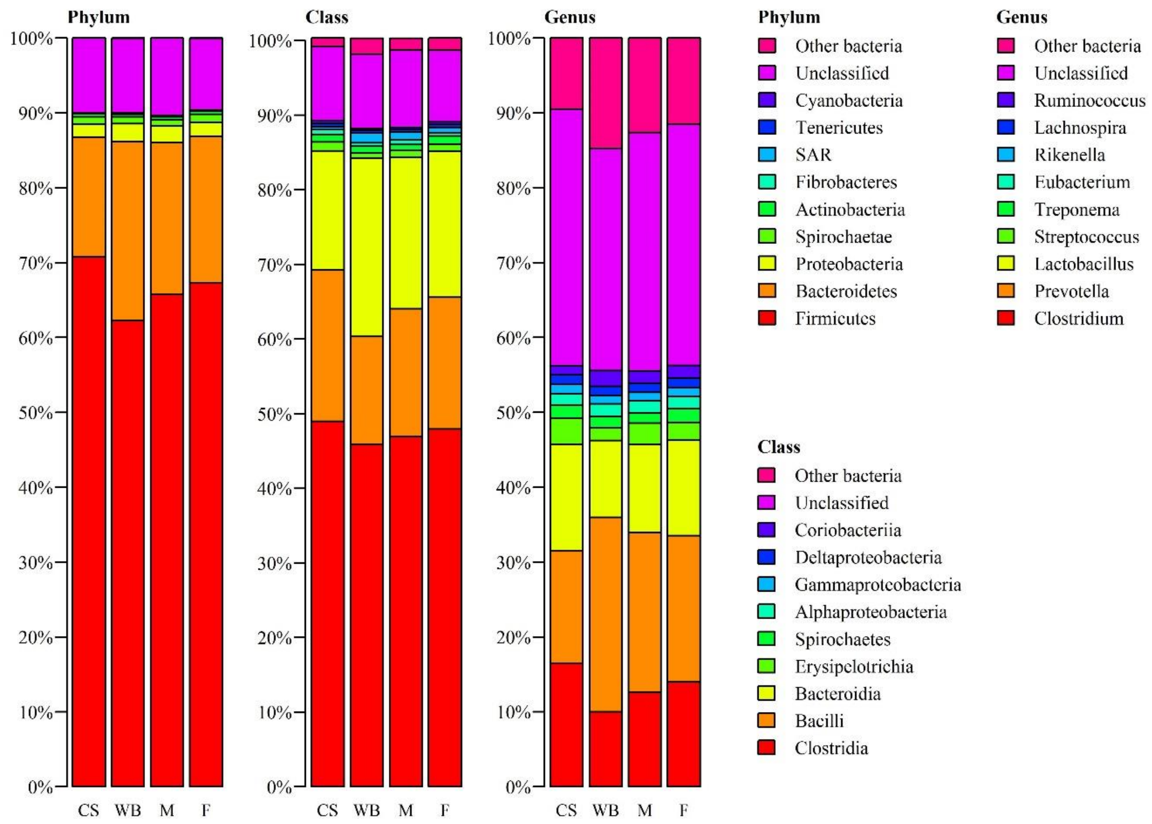
549

550 Figure 1. Relative abundance of 9 major bacterial phyla, classes and genera in the feces male
551 (M) and female (F) of pigs fed a corn/soybean meal diet (CS) or a wheat/barley/by-products
552 diet (WB). Data are mean percentage of total identified sequences.

553 Figure 2. Bray-Curtis distances for tested groups based on operational taxonomic units. A)
554 Diet. Yellow = corn/soybean meal diet, orange = wheat/barley/by-products diet. B) Sex. Blue
555 = male pigs, pink = female pigs. C-F) Feed efficiency. Green = high feed efficiency, purple =
556 low feed efficiency. C) Male pigs fed a corn/soybean meal diet, D) Female pigs fed a
557 corn/soybean meal diet, E) Male pigs fed a wheat/barley/by-products diet, F) Female pigs fed
558 a wheat/barley/by-products diet.

559 Figure 3. Gaussian kernel density estimation of the discriminant function as result of the
560 discriminant analysis of principle components for tested groups based on operational
561 taxonomic units. A) Diet. Yellow = corn/soybean meal diet, orange = wheat/barley/by-products
562 diet. B) Sex. Blue = male pigs, pink = female pigs. C-F) Feed efficiency. Green = high feed
563 efficiency, purple = low feed efficiency. C) Male pigs fed a corn/soybean meal diet, D) Female
564 pigs fed a corn/soybean meal diet, E) Male pigs fed a wheat/barley/by-products diet, F) Female
565 pigs fed a wheat/barley/by-products diet.

566 Figure 4. Measured versus predicted feed efficiency by partial least squares regression based
567 on significant operational taxonomic units found by discriminant analysis of principal
568 components. A) Male pigs fed a wheat/barley/by-products diet. $R^2 = 0.14$. B) Female pigs fed
569 a wheat/barley/by-products diet. $R^2 = 0.11$. Green = pigs in high feed efficiency group, purple
570 = pigs in low feed efficiency group.



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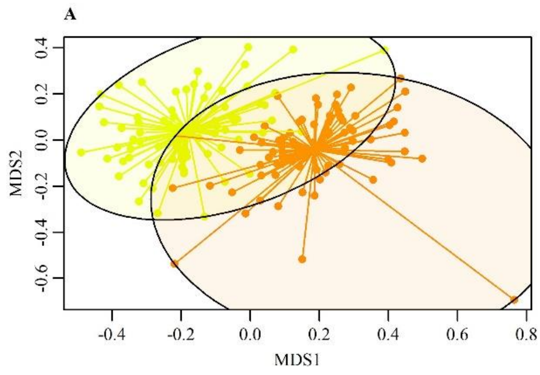

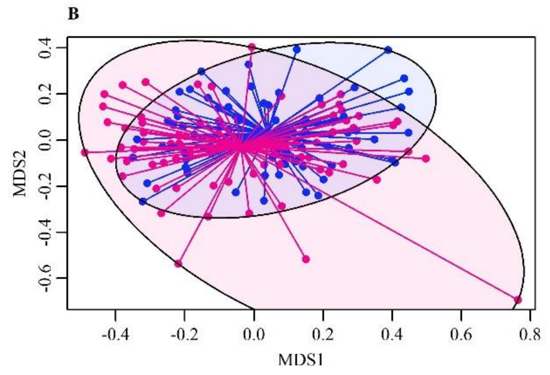

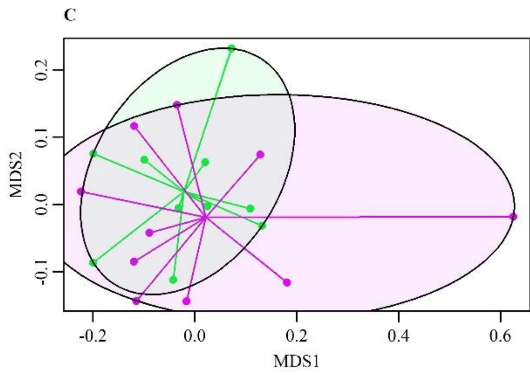

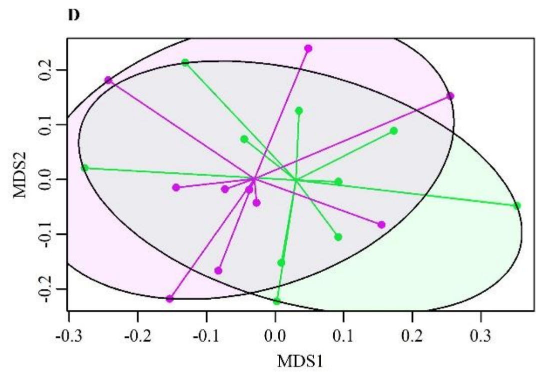

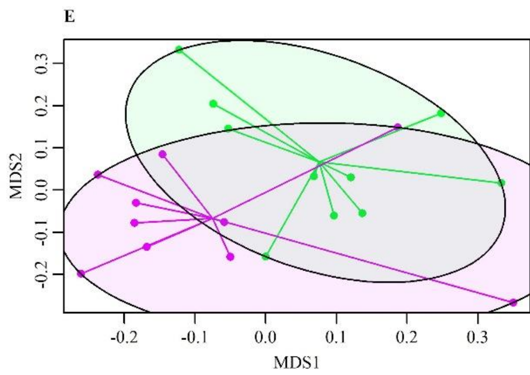

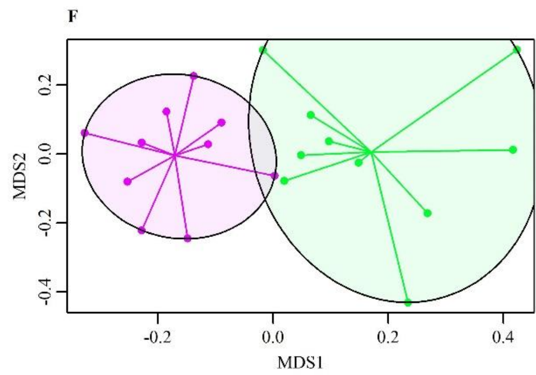

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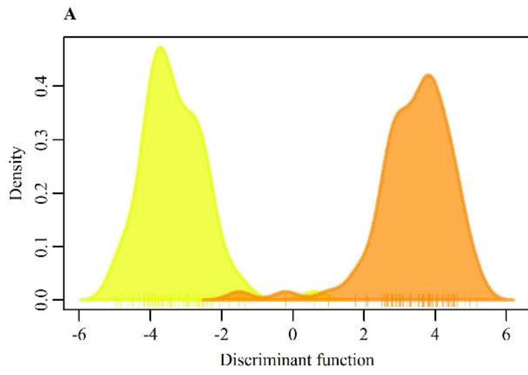

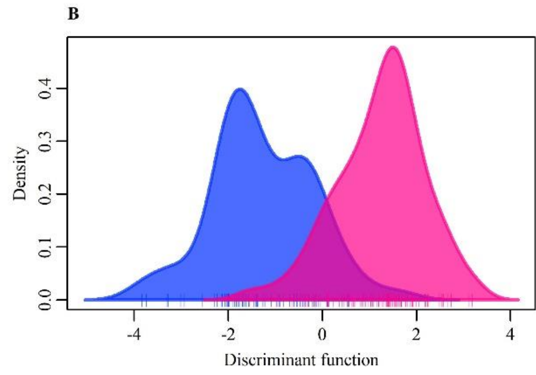

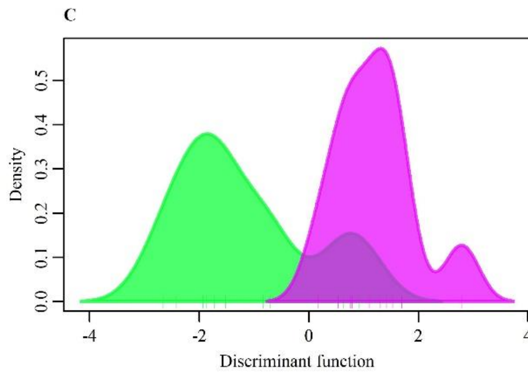

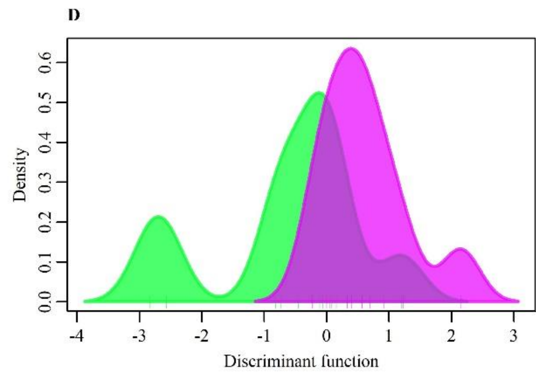

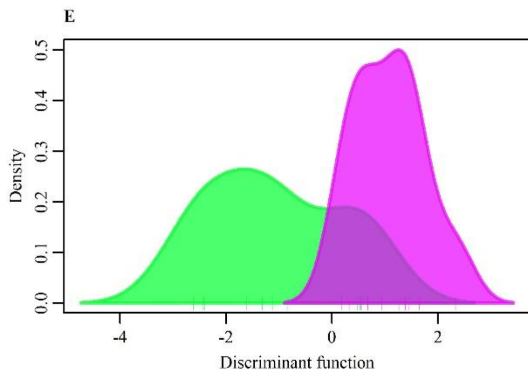

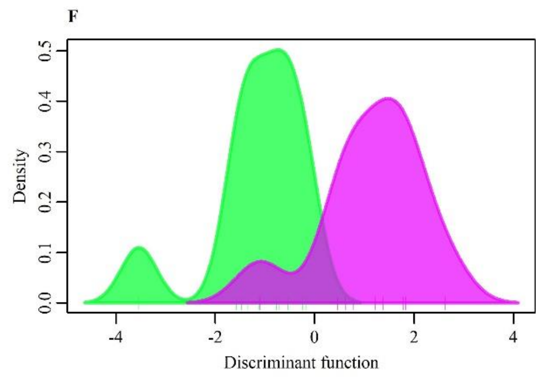

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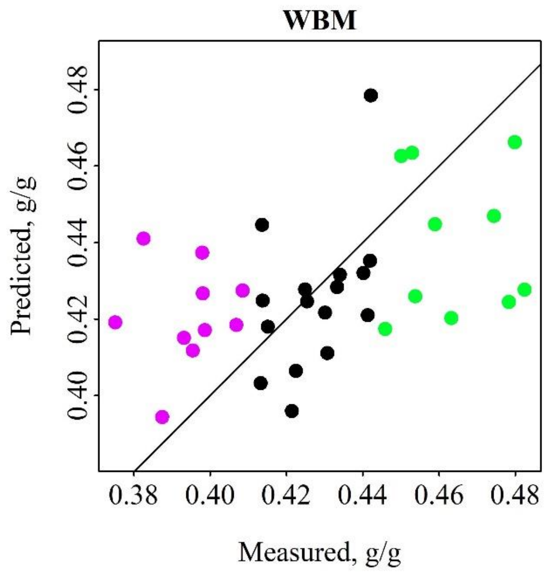

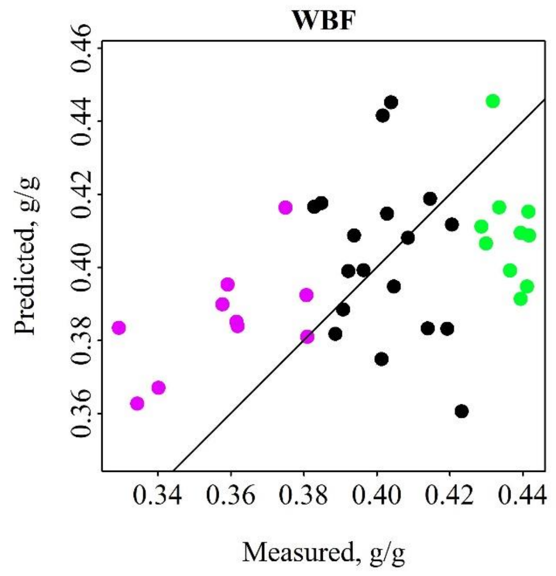

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