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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

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

Animals and experimental design

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

Feeding strategy

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

Measurements and sampling

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

Statistical analysis

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

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

Taxonomy

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

RESULTS

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

Differences between diets

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

Differences between sexes

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

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

Differences between feed efficiency extremes

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

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

DISCUSSION

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

Diets

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

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

Sexes

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

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

Feed efficiencies

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

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

Implications

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

CONCLUSION

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

composition by means of nutrition (e.g. by use of specific additives such as pro- and prebiotics) in order to improve FE of grower-finisher pigs.

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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

[1]Supplied per kilogram of feed: 2500 IU of vitamin A, 500 IU of vitamin D3, and 5 IU of vitamin E (Mervit AD3E; PreMervo, Utrecht, the Netherlands).

[2]Supplied per kilogram of feed: 12 mg of Fe (ferrous sulfate), 10 mg of Mn (manganous oxide), 0.04 mg of Co 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 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 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, 0.2 mg of vitamin K3, and 40 mg of choline (Mervit Sporavit; PreMervo).

[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 mg of Mn (manganous oxide), 62 mg of Zn (zinc oxide), 0.04 mg of Co (cobalt oxide), 0.4 mg of I (potassium 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 of vitamin B2, 6 mg of pantothenate, 30 mg of niacin, 0.02 mg of vitamin B12, and 0.752 mg of vitamin K3 (Mervit START M220; PreMervo, Utrecht, the Netherlands).

[4]Based on chemical composition, digestibility, and energy values for pigs from the Centraal Veevoeder Bureau 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

		Percentage of total sequences	
OTU ID	Classification	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

	FE groups			*P*-value	
Item	Low	High	SEM	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

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

		Percentage of total sequences	
OTU ID	Classification	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

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

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%)

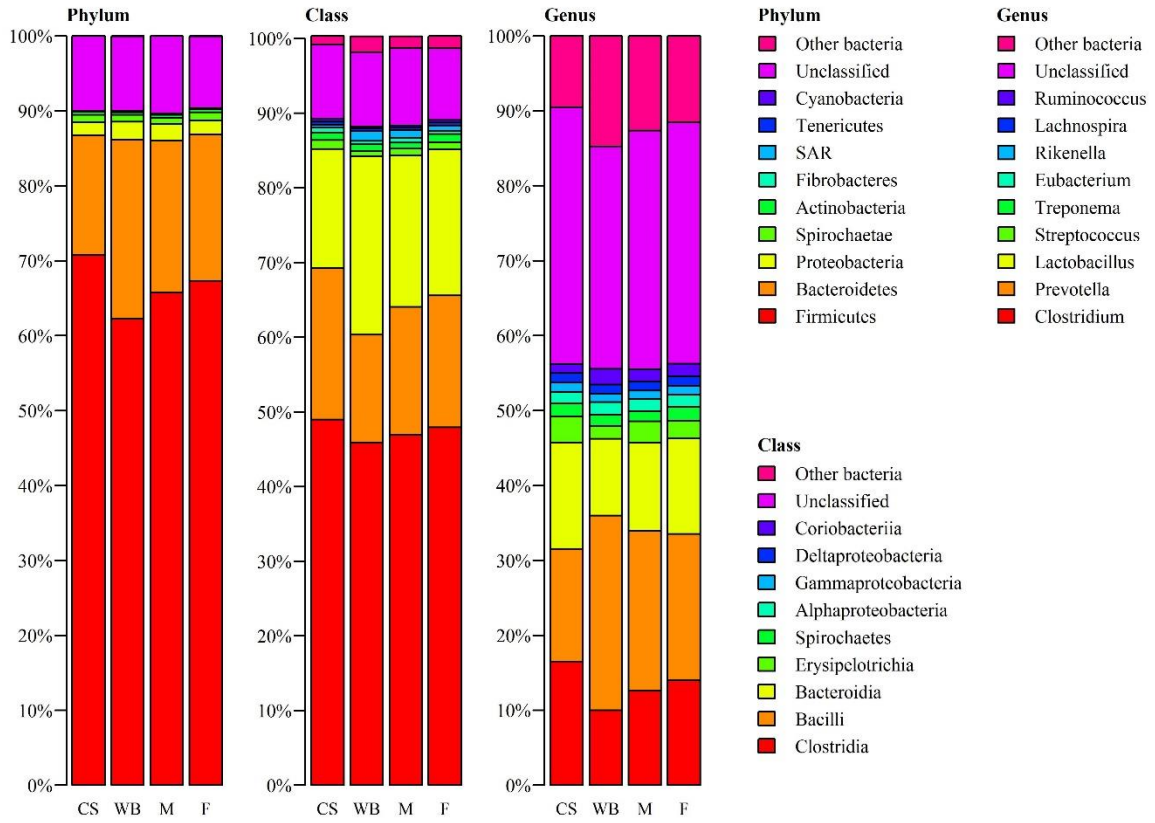
549

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

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

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

Figure 4. Measured versus predicted feed efficiency by partial least squares regression based on significant operational taxonomic units found by discriminant analysis of principal components. A) Male pigs fed a wheat/barley/by-products diet. $R^2 = 0.14$. B) Female pigs fed a wheat/barley/by-products diet. $R^2 = 0.11$. Green = pigs in high feed efficiency group, purple = pigs in low feed efficiency group.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Other bacteria
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Cyanobacteria
Tenericutes
SAR
Fibrobacteres
Actinobacteria
Spirochaetae
Proteobacteria
Bacteroidetes
Firmicutes
Genus
Other bacteria
Unclassified
Ruminococcus
Lachnospira
Rikenella
Eubacterium
Treponema
Streptococcus
Lactobacillus
Prevotella
Clostridium
Class
Other bacteria
Unclassified
Coriobacteriia
Deltaproteobacteria
Gammaproteobacteria
Alphaproteobacteria
Spirochaetes
Erysipelotrichia
Bacteroidia
Bacilli
Clostridia

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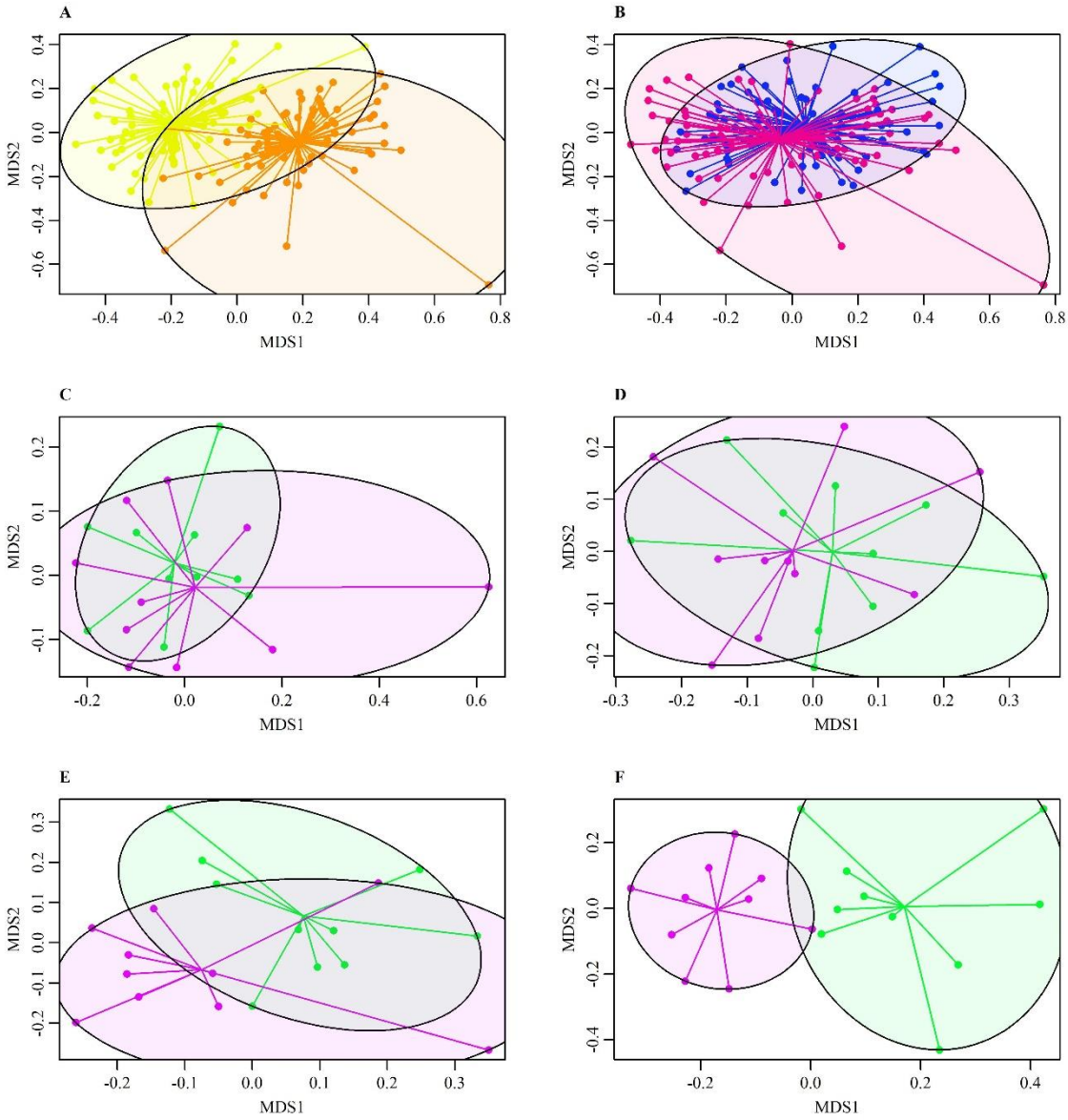

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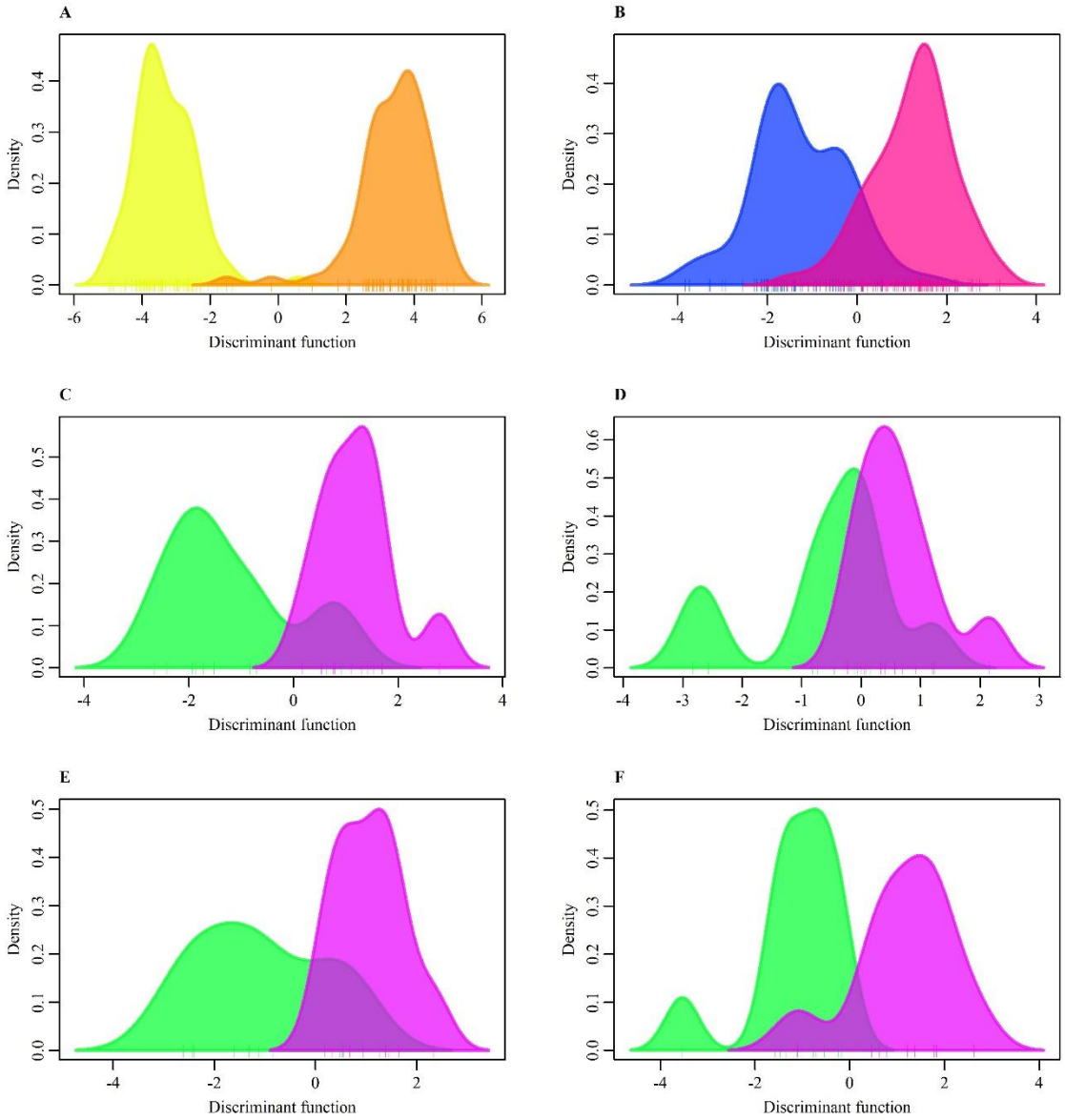
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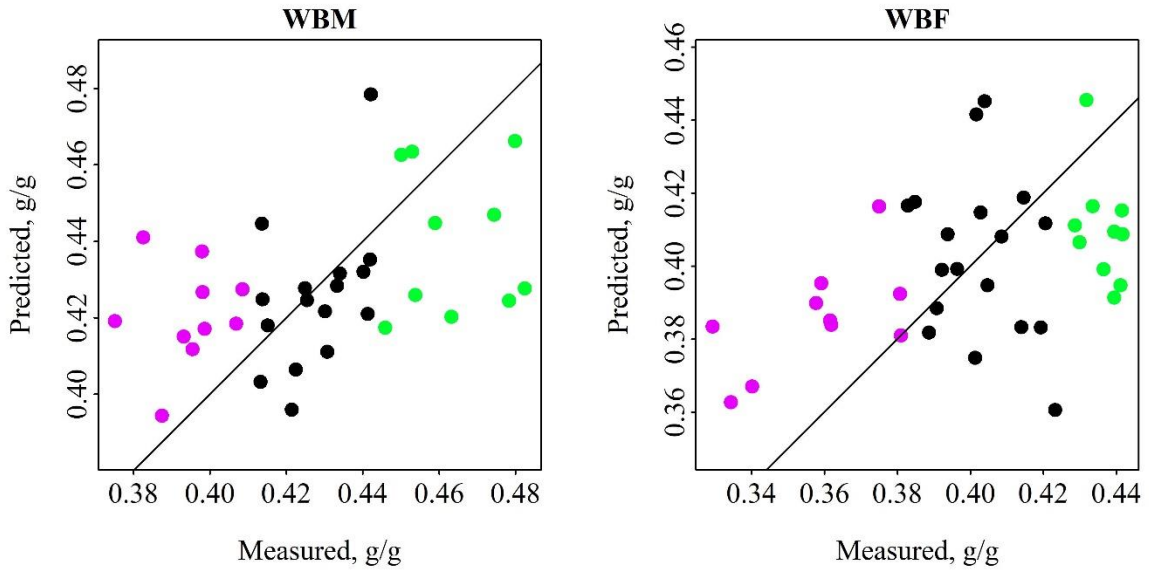
WBM
Predicted, g/g
0.40
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0.48
Measured, g/g
WBF
Predicted, g/g
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0.38
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Measured, g/g

575