Dietary micro-fibrillated cellulose improves gut health and piglet performance at weaning

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Research Article

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Abstract

Background: Weaning is an abrupt and stressful event for piglets, characterized by reduced feed intake, low weight- gain, indigestion, and diarrhea. In addition, during this period, piglets face different stressors and possible infections, making it very urgent to develop alternative feeding strategies to prevent these clinical problems. Dietary fiber (DF) supplementation is one of the strategies to prevent on-farm infections, which has the capability to improve gut health and piglet performance. Among the beneficial DFs, micro-fibrillated cellulose (MFC), is a new-generation plant-derived innovative feed ingredient; it originates from sugar-beet pulp and has a hyper-branched structure with the ability to form shear-thinning hydrogel and a high water-binding capacity. We therefore aimed to determine the effects of MFC supplementation on performance of piglets before and after weaning. We included 45 sows and their piglets in this trial and monitored the results until piglets were seven weeks of age. Sows received 75 g during gestation and 100 g MFC during lactation. Pre-weaning and post-weaning piglets' diets contained 1% of MFC for creep feed and 2% as post-weaning feed.

Results: Piglets supplemented with MFC had higher body weight and average daily growth (ADG) at pre- and post-weaning than did control piglets. MFC supplementation also increased fecal total volatile fatty acids (VFA), especially butyrate content, and reduced diarrhea incidence post-weaning. Ruminococcus, Roseburia, Intestinibacter, and Oribacterium genera were highly abundant in MFC piglets, whereas, Escherichia_Shigella, Campylobacter, and Parabacteroides were higher in control piglets.

Conclusion: MFC-supplemented piglets exhibited superior intestinal health and production performance after weaning, perhaps due to changes in their intestinal development, and improved intestinal permeability attributable to the MFC supplementation to piglets at different stages until seven weeks of age. In addition, VFA, specially butyrate, may play a crucial role in growth of mucosa and in enterocyte differentiation, and may improve barrier function. MFC supplementation stimulated growth of butyrate- producing bacteria and reduced pathogenic bacteria. It is evident that supplementation of MFC in feed to young piglets can improve growth performance and volatile fatty-acid content and reduce post-weaning diarrhea.

Background

Weaning piglets undergo weaning stress caused by the immaturity of their digestive and immune systems by changes in their environment and feed. This results in low feed intake, minimal weight gain, and diarrhea [1]. In modern pig production, various feeding strategies have been practiced to reduce diarrhea incidence at weaning, and to improve production performance. Feeding strategies include the use of prebiotics, probiotics, fatty acids, organic acids, essential oils, and dietary fibers [2, 3]. Like other feeding strategies, supplementation of dietary fiber (DF) is one of the feeding regimes implemented at various stages of pig production [2]. Dietary fiber plays a crucial role in maintaining diversified gut microbiota and of human and animal gut health [4]. Adding a high-fiber diet can surge the activity of fibrolytic bacteria in the large intestine of growing pigs [5], and a high volume of cellulolytic bacteria favors the establishment and development of some beneficial bacteria, meanwhile, reducing the harmful ones, which is advantageous to gut health and seeming to have a prebiotic effect [6]. In addition, cellulolytic bacteria produce short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate[7]. These SCFA produced in the large intestine are estimated to contribute 5% to 15% of the total caloric requirements of humans [8] while providing approximately 24% of the energy for body thermoregulation in pigs [9]. Approximately 15% of the maintenance energy requirement of growing pigs and 30% in gestating sows comes from SCFA in the large intestine [10]. Moreover, SCFAs, especially butyrate, originating from DF fermentation, demonstrate numerous health benefits, including action as as the main energy source for colonocytes, influencing immune system regulation, and reducing inflammation [11] . Moreover, DF leads to increased abundance of Lactobacilli and reduces coliform abundance in the small intestine[12]. And in addition, inclusion of moderate amounts of insoluble fiber sources in diets for young pigs, ones with compromised hygienic and health status, may reduce the incidence of post-weaning diarrhea (PWD) in the first 2 weeks [13]. DF supplementation can alleviate weaning stress in piglets by improving bacterial diversity and rapidly stabilizing the gut microbial community [14], helping the piglets' gut environment in the delicate phase of weaning. Sugar beet pulp (SBP), a pectin-rich fiber, contains nitrogen-free leachate, crude protein (CP), and high-quality crude fiber(CF) including a higher quantity of l-arabinose polymer [15]. Due to its highly soluble fiber content, SBP is easily digested in the porcine gut[16]. Micro-fibrillated cellulose (MFC), a new-generation plant-derived innovative feed ingredient, originated from sugar beet pulp with a hyper-branched structure consisting of more than 90% dry matter[17] . It has the ability to form shear-thinning hydrogel with high water-binding capacity[18]. In addition, cellulose hydrogel is widely applied for tissue regeneration of bone, cartilage, and neural tissues because of its biocompatibility [19].

Gut microbiota play a crucial role in piglet health and nutrition [20]. Its microbial composition can be modulated by various factors such as the maternal microbiota [21], piglet age, health status, environmental factors, growth promoter [22], and feeding regime [23]. Among

feeding regimes, fiber supplementation plays a significant role in gut microbiota development and in improved intestinal integrity [24]. Researchers exploring the relationship between dietary fiber and pig gut microbiota found changes in feed efficiency with fiber supplementation [25]. Moreover, a diet lacking in fiber is associated with the impaired intestinal-barrier function of colonic mucosa and with higher pathogen susceptibility [26]. Researchers have revealed that fibers such as SBP supplemented to piglets increase the weight of the large intestine [27] and reduce fecal Enterococcus spp [28]. In addition, they increase the abundance of Lactobacillus and inhibit the colonization of coliform bacteria [29], which, in weaned piglets, leads to reduced postweaning diarrhea incidence [30].

We hypothesized that MFC supplementation to sows and piglets improves piglet performance at birth, weaning, and post-weaning, in regard to body growth, microbiota modulation, intestinal SCFA increase, reduced diarrhea incidence and mortality.

Material And Methods

The experiment was carried out on a commercial farm in Kouvola, Finland, from February to April, 2021 (Animal ethics permission from Project Authorisation Board; ESAVI/2325/04.10.07/2017 with modification ESAVI/17315/2020).

Sow Selection

Our study's 45 multiparous sows (Topigs Norsvin (TN 70) were balanced based on parity between treatment groups, and were selected based on the principle of first come, first farrowing. During gestation, these sows were housed loose in groups of 10-15 in pens equipped with individual feeding stalls. From the last five weeks of farrowing, they were fed with a standard gestation diet (Hankkija Oy, Hyvinkää, Finland) differing only in that 24 treatment sows received, per day, 75 g MFC (Hankkija). After farrowing, both the control and treatment sows received a standard lactation diet (Hankkija) (Table S1) differing only in supplementation of 100 gm MFC (Hankkija Oy, Hyvinkää, Finland, Table S2) per day /treatment sow until weaning (21 days).

Pre-weaning piglet Feeding

On the one hand, 50% of the MFC-treated sows received MFC (1%) -treated creep feed (Hankkija) (Table S3), and the remaining litters received only control creep feed. On the other hand, 50% of the litters from the CONTROl sows received MFC (1%) -treated creep feed, and the remaining litters received only control creep feed (Fig. 1).

Post-Weaning Piglet Feeding

At weaning, after 21± 1 days of lactation, 530 piglets from four pre-weaning piglet groups (MM = 155, MC = 135, CM = 109, and CC = 131) were included in the post-weaning feeding regime. All the four pre-weaning piglet groups were divided equally to produce eight treatment groups: CCC, CCM, CMC, CMM, MCC, MCM, MMC, and MMM. Whereas four treatment groups (CCM=56, CMM=54, MCM=61, MMM=77) received MFC (2%) -treated post-weaning feed (Hankkija) (Table S3), the remaining four groups (CCC=63, CMC=48, MCC=58, MMC=74) received control post-weaning feed (Fig. 1). We followed the eight post-weaning treatment groups until seven weeks of age.

Parameters and Measurements

We measured the body weight of these piglets at birth, at weaning (three weeks of age), and at post-weaning (seven weeks of age). Similarly, we calculated the piglets' diarrhea incidence, mortality rate, and volatile fatty acids (VFA) at weaning and at post-weaning. At 24 h after birth, for management reasons, some piglets were allowed to move to other sows between days 2 to 21, their ear tags were removed, and the piglets excluded from the study.

Collection of fecal samples and diarrhea evaluation

Fresh fecal samples we collected from each sow's rectum into sterile plastic bags (CON = 21, MFC = 24). We also collected piglets' fecal samples at weaning (MM = 76, MC = 66, CM = 62, and CC = 67) and post-weaning (CCC = 36, CCM = 31, CMC = 29, CMM = 32, MCC = 33, MCM = 33, MMC = 36, and MMM = 40) into sterile bags and stored them at –80°C for further analysis. During collection of fecal

samples, if the feces were liquid and not formed, the sample was considered diarrhea, and was counted in calculation of diarrhea incidence.

Volatile fatty acid (VFA) analysis

VFA of piglets' feces at weaning and post-weaning we measured by ultra-performance liquid chromatography (UPLC). A fecal sample's aliquot of 0.3 g was homogenized in 1.2 mL of distilled water and centrifuged at 18 000 x g for 10 min at 4°C. The resulting supernatant we filtered twice, first through a 0.45-µm syringe filter and then with a 0.22-µm syringe filter. The filtrate was stored at −20°C until analysis of VFA. Then VFA content of the fecal samples of the piglets was determined by the method described by Puhakka et al [31]

Microbial characterization

We conducted DNA extraction by taking 250 mg of feces from each sample using the DNeasy PowerSoil Pro Kit (Qiagen, ct. no. 47014, Hilden, Germany) according to manufacturer's instructions (Qiagen). Then we quantified the yields and purity of the extracted DNA with Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). After that, we executed the 16S PCR amplification and sequencing with primer modifications by the method described by Pereira et al [32]. After the amplification of V3-V4 16s region, primers 341F_1–4 (CCTACGGGNGGCWGCAG) and 785R_1–4 (GACTACHVGGGTATCTAATCC) were added to the 5´ ends with partial Illumina TruSeq adapter sequences (Table S4). Amplification of the PCR and Miseq sequencing we carried out by the method of Pereira et al[32]. The whole process was executed at the DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki. We used, to pre-process the FASTQ files produced following Miseq sequencing steps, the Dada2 R package (https://benjjneb.github.io/dada2/tutorial_1_8.html). We truncated the forward reads at position 295, and at 160 for the reverse reads. To learn the error rate, we used the DADA2 algorithm followed by the dereplication step, which combines all the identical sequencing reads into unique sequences. Then dereplicated data were used to apply the algorithm of the core sample inference. In the following steps, to get the denoised sequences, we then merged the forward and the reverse reads by overlapping 20 bases where they were identical in the overlapped position. After constructing the sequence table, the dada method performed chimera removal. After tracking reads through pipeline taxonomy were assigned to the sequences by using Silva reference database. Then we organized the taxonomy for downstream analysis.

Statistical analysis

We used Stata 17.0 (Stata MP/17 for Windows; Stata Corp., College Station, TX, USA) software for data analysis. In the descriptive statistics, we expressed data as Least-Square Means ± SEM after running ANOVA. Significance level was stated as $p < 0.05$.
We used sow-level data to study the association between farrowing and piglets' birth data. Similarly, separate data served for study of the association between piglets' variables from birth to seven weeks of age. After seven weeks of age, sow-level data were merged with the individual piglet's data to produce a new dataset to study the associations between sow and piglet variables. At first, univariate analyses explanatory variables were performed for building the statistical models. In the full model, a stepwise backward elimination procedure was applied with the inclusion of explanatory variables with $p \leq 0.2$. For the microbiome analysis, we used the "Mare" package [33] in R software (4.2.0). In downstream analysis, we measured the relative abundance (phylum to genus) by using "GroupTest" function; alpha and beta diversity were estimated using the R vegan package. Heatmap visualized the associations between microbiota and clinical variables using the R function of mare package "CorrelationMap," which implements Spearman's correlation, and performed statistical tests at the different taxa levels. We estimated the Linear discriminant analysis (LDA) Effect Size (LEfSe) using a galaxy computational tool (http://huttenhower.sph.harvard.edu/galaxy), following a metagenomic biomarker-discovery approach [34]. It performed differential abundance testing using the Kruskal-Wallis rank sum test between groups, and calculated the effect size at the 2.0 LDA score threshold. To determine the functional potential of the microbiota between groups, we used the Namco webtool (https://exbio.wzw.tum.de/namco/ , accessed on 13th May 2022), which adapts the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2)[35] approach. It performs differential analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG), number of enzymes classification, different Kyoto orthology (KO), and pathways by Aldex2[36].

Results

Effects of MFC on piglet body weight

At weaning, at three weeks of age, body weight for piglet groups CM and MM was higher than for CC groups, and the MC group had lower body weight than that of CC piglets (Fig. 2). Post-weaning, at seven weeks of age, body weight of MMM, MCM, CMM, CCM, MMC, and CMC piglets were higher than that of CCC piglets, whereas no significance difference emerged between CCC and MCC piglets (Fig. 2).

Effects of MFC on piglet ADG

In the case of ADG, CM and MM showed higher body growth than did CC groups, and the MC group lower body growth than did CC piglets during weaning at three weeks of age (Table1). Post-weaning, at seven weeks of age, MMM, MCM, CMM, CCM, MMC, and CMC piglets had higher ADG than did CCC piglets, whereas, no significance difference in ADG appeared between CCC, and MCC piglets (Table 1).

Volatile fatty acids

At weaning, no significant differences were observable on total VFA, butyric acid, isobutyric acid, 2-mebutyric acid, 3-mebutyric acid, pentanoic acid, and hexanoic acid, whereas propionic acid tended to be higher in MFC piglets than in control piglets (Table 2). At post-weaning, seven weeks of age, total VFA level tended to be higher in MFC-treated piglets than in control piglets, but butyric and hexanoic acids were higher in MFC-treated piglets than in control piglets (Table 2). No significant differences were were evident for acetic acid, propionic acid, isobutyric acid, 2-mebutyric acid, 3-mebutyric acid, or pentanoic acid (Table 2).

Diarrhea incidence

We observed no significance differences in diarrhea incidence before weaning among the four treatment groups that received control (CC, and MC) or MFC (CM, and MM) ($p > 0.05$). Interestingly, at seven weeks of age, diarrhea incidence tended to be lower in the CCM, CMM, and MMM groups, whereas the MCM group had lower diarrhea incidence than the CCC group. The remaining MMC, MCC, and CMC group showed no significant differences with CCC group. Overall, piglets that received MFC (MMM, MCM, CMM, and CCM, $p < 0.05$) had lower diarrhea incidence than those receiving the control diet (MMC, MCC, CMC, and CCC) (Fig. 3).

Piglet mortality

We observed no differences in piglet mortality at three weeks of age. At seven weeks (of age), no significance difference occurs between MFC (MMM, MCM, CMM, and CCM) and the control diet (MMC, MCC, CMC, and CCC).

Microbiome results

Alpha and beta diversity

We estimated alpha diversity, where no differences were observed between groups (Fig. 4.). We measured the beta diversity (PcoA) with bray curtis distance. We didn't find any differences between control and MFC groups (Fig. 4).

Microbial composition

Effects of MFC on Fecal Microbiota

At phylum level, relative abundances of Epsilonbacteraeota were significantly higher in the MFC group compared to control group, whereas, Proteobacteria tended to be higher in the control group (Fig. 5). At class level, higher abundances of Campylobacteria and Gammaproteobacteria classes occurred in the control group.

In addition, Clostridia and Bacilli tended to be higher in the MFC and control group respectively (Fig. 5). Campylobacteriales was significantly higher in the control group. Clostridiales and Lactobacillales occurred in MFC, and in the control group respectively (Fig. 5). As for the control group, at family level, Campylobacteraceae, Tannerellaceae, Family_XIII, and Muribaculaceae were higher, and Streptococcaceaeand Rikenellaceae families tended to be higher than the MFC group, whereas, Lachnospiraceae family abundances tended to be higher in the MFC group (Fig. 5, Table S5). At genus level, *Ruminococcus.2, Ruminococcaceae.UCG.014, Intestinibacter, Roseburia,* and *Oribacterium* genera were highly abundant in the MFC group. While, *Campylobacter,* and *Parabacteroides* genera were abundant and *Streptococcus,* and Rikenellaceae.RC9.gut.roup tended to be abundant in the control group compared to MFC group (Fig. 6, Table S5). To further examine the differential abundant taxa, a LEFSe analysis with LDA threshold >2.0 was carried at phylum to genus level. Lefse analysis identified 28 taxa as a potential biomarker for the control group and MFC group; 15 taxa were unique to the MFC group, and 13 to the control group based on LDA score (Fig. 7). At class level, Gammaproteobacteria was highly enriched in the control group. In addition, *Betaproteobacteriales and Enterobacteriales were enriched in the control group at order level. Bacteroidaceae, Burkholderiaceae, Enterobacteriaceae, Helicobacteraceae, and Tannerellaceae families were more enriched in the control group than in the enriched Coriobacteriaceae family in MFC group. At genus level, Allisonella, Collinsella, Intestinibacter, Lachnoclostridium_12, Lachnospira*, the Lachnospiraceae_NK3A20_group, *Oribacterium, Prevotella_7, Pseudobutyrivibrio, Roseburia*, Ruminococcaceae_UCG_014, and *Ruminococcus_2* were highly enriched in the MFC group, whereas, enrichment of Bacteroides*, Helicobacter,* Lachnospiraceae_UCG_003, *Parabacteroides*, Prevotellaceae

_UCG_001, *Sutterella*, and *Escherichia_Shigella* genera occurred in the control group (Fig. 7). We found some similarities between Mare and Lefse analysis in differential taxa abundance at class, order, family, and genus level (**Table** 3). The Gammaproteobacteria class and Tannerellaceae family were enriched in the control group in both analyses, whereas at class level, Ruminococcus.2, Roseburia, Intestinibacter, Oribacterium, and Ruminococcaceae.UCG.014 genera were abundant in the MFC group. In PICRUSt2 functional analysis, we observed 247 enzymatic function, 627 KEGG orthology, and 98 molecular pathways were significant between groups at OUT level. Top 20 pathways three analysis are visualized (supplementary figure 1).

Correlations between microbial population and performance parameter.

Clostridium.sensu.stricto.1,Roseburia, Agathobacter, and *Prevotella.9* were positively correlated with ADG and body weight at seven weeks of age. In addition, all the above genera except *Clostridium.sensu. stricto.1* were positively correlated with Butyric acid (Fig. 8). However, *Ruminococcaceae.UCG.014, Ruminococcaceae.NK4A21, Ruminococcaceae.UCG.002, Rikenellaceae.RC9.gut.group, Prevotellaceae.UCG.003, Coprococcus.1*, and Lactobacillus were negatively correlated with ADG and body weight at seven weeks of age. Agathobacter*, Prevotella.9, Anaerovibrio, Faecalibacterium, Lachnospiraceae.UCG.001, Alloprevotella, Blautia, Prevotella.2*, and Dorea genera were negatively correlated with IsoButyric acid, 2-MeButyric acid, and 3-MeButyric acid. *Ruminococcaceae.UCG.014, Ruminococcaceae.NK4A21, Ruminococcaceae.UCG.002, and Rikenellaceae.RC9.gut.group* were positively correlated with IsoButyric acid, 2-MeButyric acid, and 3-MeButyric acid (Fig. 8).

Discussion

In our study, at post weaning, MFC supplementation reduced PWD incidence in piglets, raised the abundance of butyrate-producing beneficial bacteria, and reduced in abundance pathogenic bacteria. In addition, piglets supplemented with MFC had higher body growth at 3 weeks and at 7 weeks of age than did control piglets. Moreover, MFC supplementation tended to raise total VFA and raise butyric acid and hexanoic acid in the feces of post-weaning piglets.

As a management practice, providing creep feed is relevant to lactating piglets, because rooting and grazing behaviors are observable in wild pigs between 2 to 4 weeks of age [37, 38]. A fibrous diet may play a needed role in preparing piglets for their post-weaning period by early development of their gastrointestinal tract. This may improve feed intake after weaning due to adjustability of the diet, resulting in maintenance of good intestinal health [37]. In our study, lactating piglets who had MFC effects from their mother and received MFC through creep feed had higher ADG than did control piglets. This might be due to changes in their intestinal development,

improved intestinal permeability [37], and stimulation to VFA production by supplementation with cellulose [39]. We found propionic acid tending to be higher in the MFC group, which may be an indication of stimulation of the microbial fermentation [39]. Supplementation of dietary fiber in suckling piglets could affect their post-weaning performance by developing and stimulating their milk-oriented gut microbial population towards being fibrolytic-oriented, which would shift the microbiota that adapt the metabolic trait of breaking down (the) complex polysaccharides [40, 41]. Dietary fiber in piglets promotes bacterial fermentation and production of VFA in the large intestine[42] .

Here, total VFA tended to be higher in MFC-fed post-weaning piglets. This VFA plays a crucial role in maintaining colonic health and acts as a key indicator of microbial fermentation in the intestine [43] .We found, at post-weaning, that the butyrate level of piglets which received MFC was significantly higher than that of control piglets. Butyrate plays a vital role in energy metabolism in the gut and in improving mucosal immunity [44]. In addition, butyrate can improve gut barrier function, the first-line defense against gut pathogens [45], and can assist in maintaining a physical barrier by stimulating the goblet cells, followed by mucus secretion [46]. Butyrate, as a fuel for enterocytes, has the capacity to stimulate the growth of mucosa and stimulate cell differentiation, and also to improve barrier function [47] [48]. Lower gut integrity, on the other hand, can lead to increased permeability to pathogens and to toxic metabolites [47].

At post-weaning, piglets experience many types of physiological and environmental stress, which persist during the first weeks after weaning due to the changes in their diet, high feed consumption, and changes in intestinal function followed by reduced feed consumption, low weight gain, indigestion, and diarrhea [49, 50]. Dietary inclusion of insoluble fiber is shown to reduce piglets' post-weaning diarrhea [51]. In our study, its incidence was reduced when MFC was supplemented to these post-weaning piglets. Dietary fiber, especially cellulose material, may block the attachment of gut pathogens, which will reduce their ability to stay in the gut , hence, promoting their expulsion with the chyme and thereby reducing diarrhea incidence [52] . Dietary fiber may mitigate the gut microbial dysbiosis at weaning by creating an ambient environment for the growth of beneficial bacteria which reduce enterotoxigenic pathogenic bacteria, for example *E. coli* [39, 53] Moreover, it may encourage the functional maturation and growth of the gastrointestinal tract [49]. Due to its higher water-holding capacity, it may change the gastric transit, through altering the rate of gastric emptying or altering gut motility in favor of gastrointestinal-tract (GIT) development [54].

This development of GIT, especially in the large intestine, might reduce diarrhea incidence by means of its high water-resorption capacity [37]. In addition, fibers like MFC may increase ileal nitrogen losses, which would facilitate the starch and protein as substrate for the gut microbiota; this is beneficial for the host [39, 55]. We found that dietary inclusion of MFC after weaning reduced the incidence of PWD in post-weaning piglets. In In other studies, supplementation of 1.5% cellulose reduced the incidence of PWD in post-weaning piglets [56, 57]. Another crucial mechanism by which MFC reduces PWD by MFC is by its ability to form extremely shear-thinning hydrogel with high water-binding capacity, and zero-shear viscosity. Which may facilitate tissue regeneration in injured intestinal layers, as cellulosic hydrogel does in regeneration of bone, cartilage, and neural tissues [19]. Moreover, the end- product of MFC is glucose, which is beneficial for cell growth [58].

At three weeks and seven weeks of age, piglets which received 2% MFC had significantly higher body weight than did control piglets. Several mechanisms may be responsible for this result. Firstly, this might be due to less energy loss by reduced diarrhea incidence; hence this energy could improve piglet body weight [59]. Another mechanism might be attributed to the high water-holding capacity of MFC, which results in an increased size of their digestive organs [60].

Similarly, higher ADG was achieved by supplementation of 2% MFC to piglets after weaning, at 3 weeks, and up until 7 weeks of age. Our findings corroborate the findings of Pascoal et al [51], who found higher ADG of post-weaning piglets by supplementation with 1.5% cellulose. These kinds of performance were also reported by other authors [61, 62], who observed improvements in weight gain and intestinal health by the inclusion in the diet of purified cellulose. Although Högberg et al [60] attributed the improvement in weight gain to the increased size of the digestive organs. Gerritsen et al [49] suggest that this growth might be due to the better gut environment, resulting in high enzyme activity and microbiota modulation. Piglets supplemented with 6% fibers of SBP origin did not affect ADG at their post weaning, but 5% fibers of SBP origin might be able to raise ADG [63]. Supplementation with purified cellulose has been responsible for an increased ratio between villus height and crypt depth, including decreased cox-2 which overcomes mucosal injury followed by piglets' improved intestinal health [64]. In addition, Hanczakowska et al [62] demonstrated that a small amount of insoluble non-starch polysaccharide (iNSP) could improve piglets' health and performance by making changes in the gut morphology and gut pH, and by lowering the growth of pathogenic microbes.

It is well known that gut microbiota homeostasis plays a crucial role in the host's gut health and immune organ maturation [65]. Gut microbial dysbiosis in piglets is related to many enteric diseases [66], but stability of the gut microbiota depends on feed supplementation, on the types of bacterial species, their abundance, and their interactions within the microbial community [67]. Effects of feed supplementation, especially dietary fiber are receiving increasing attention due to their positive effects on enzymatic activities and the gut microbiota [49, 68]. In our study, MFC supplementation after weaning reduced the Epsilonbacteraeota phylum formed by reclassification of the Epsilonproteobacteria and Desulfurellales [69], widely known for containing several pathogenic genera, such as Helicobacter, Arcobacter and Campylobacter. However, MFC supplementation likely suppressed the abundance of Proteobacteria, and improved the abundance of Firmicutes. Member organisms of the Proteobacteria are gram-negative pathogenic bacteria including Escherichia, Salmonella, and Vibrio regarded as an indicator of gut dysbiosis [70], gut inflammation, and gut diseases [59]. This might be an explanation for the lower post- weaning diarrhea incidence in our MFC-supplemented piglets. Members of the Firmicutes phylum are (known for being) butyrate producers, and this might have played a beneficial role in MFC piglets. Our study's Campylobacteria and Gammaproteobacteria were suppressed by the supplementation of MFC. LEFSe analysis also revealed that MFC supplementation suppresses the enrichment of Gammaproteobacteria, Enterobacteriales, Enterobacteriaceae, and Escherichia_Shigella, a class to genus cluster. This taxonomic cluster is well known for its pathogenicity, as a few members of the Gammaproteobacteria , such as Escherichia cause post- weaning diarrhea in piglets [71]. Some other genera are also associated with diarrhea in piglets, for example, *Campylobacter* [72]. We established that MFC supplementation reduced the relative abundance of Campylobacteria, Campylobacterales, Campylobacteraceae, and *Campylobacter* at a respective class, order, family, and genus level. *Escherichia* and *Campylobacter* can be regarded as etiological agents of post-weaning diarrhea [71, 73]; therefore MFC supplementation may be involved in lowering diarrhea incidence in the post-weaning piglets. Relative abundance of another pathogenic family, Tannerellaceae, was inhibited by the MFC supplementation. In human beings, organisms under Tannerellaceae families are associated with oral infections called periodontitis [74]. Lachnospiraceae was likely higher in the MFC group. Members of this family associated with fiber degradation include cellulose and hemicellulose [75]. At genus level, *Ruminococcus.2, Ruminococcaceae.UCG.014, Roseburia, Oribacterium* were elevated by MFC supplementation. Among them, *Ruminococcus* may degrade complex polysaccharides and produce butyrate [59]. In addition, *Roseburia* is well known for its beneficial effects on butyrate production, and for stimulating the growth of beneficial bacteria and inhibiting the proliferation of pathogenic bacteria. Moreover, *Oribacterium* is also associated with butyrate production, and linked with improved gut health and homeostasis [76]. Increased abundances of these butyrate genera: *Ruminococcus, Roseburia, and Oribacterium* in the MFC group may be connected with the increased butyrate production by MFC supplementation. The MFC supplementation may therefore promote not only ADG by providing energy for colonocytes but also may improve gut health by exerting anti-inflammatory action [59]. A study conducted by Ju et al [77] revealed *Parabacteroides* to be associated with reduced body weight and ADG. In our study, the *Parabacteroides* genus was higher in the control group, whereas body weight and ADG were lower than in the MFC-supplemented piglets. This might explain why MFC piglets showed the dominant growth performance. Some of the *Streptococcus* genera of the Streptococcaceae family are less abundant in pigs with high feed efficiency[78]. In addition, a major pathogen, *Streptococcus suis* is linked with high mortality and low performance in pigs [79]. In our study, MFC supplementation suppressed the growth of *Streptococcus*, which might have led to the higher body growth. We have some limitations in this study. We included the samples of CCC and RRR piglets for volatile fatty-acid determination and microbial sequencing. Because of this, we observed that piglets belonging to CCC and MMM groups showed inferior and superior growth performance. Another limitation of our study was to include only 12 to 14 piglets per sow, and to exclude those piglets transferred to nursing sows'. Sows were hyper-prolific and therefore unable to produce enough milk to feed all their piglets.

Conclusions

In our study, piglets supplemented with MFC had higher body weight and ADG than did control piglets, both pre- and post-weaning. MFC supplementation led to raised total VFA and butyrate content, and reduced diarrhea incidence in post-weaning piglets. These beneficial effects may be due to the changes in the intestinal development and the improved intestinal permeability attributable to MFC supplementation to piglets at different stages of early development until seven weeks of age. In addition, MFC supplementation reduced the abundance of pathogenic bacteria and raised the abundance of butyrate-producing beneficial bacteria. Considering these results, it is promising that in piglets, MFC supplementation may be a potential feeding strategy to improve growth performance, microbial modulation, and volatile fatty acid content and to reduce post-weaning diarrhea.

Declarations

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References

1. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. Journal of animal science and biotechnology. 2013;4(1):1-4.

2. Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends in Microbiology. 2017;25(10):851-73.

3. Hasan S, Saha S, Junnikkala S, Orro T, Peltoniemi O, Oliviero C. Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota. animal. 2019;13(8):1599-606.

4. Jha R, Rossnagel B, Pieper R, Van Kessel A, Leterme P. Barley and oat cultivars with diverse carbohydrate composition alter ileal and total tract nutrient digestibility and fermentation metabolites in weaned piglets. animal. 2010;4(5):724-31.

5. Metzler B, Mosenthin RJ. A review of interactions between dietary fiber and the gastrointestinal microbiota and their consequences on intestinal phosphorus metabolism in growing pigs. Asian-Aust. J. Anim. Sci. 2008;21(4):603-15.

6. Williams BA, Verstegen MW, Tamminga S. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. Nutrition Research Reviews. 2001;14(2):207-28.

7. Shang Q, Liu S, Liu H, Mahfuz S, Piao X. Impact of sugar beet pulp and wheat bran on serum biochemical profile, inflammatory responses and gut microbiota in sows during late gestation and lactation. Journal of Animal Science and Biotechnology. 2021;12(1):1-14.

8. Bergman E. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological reviews.1990;70(2):567-90.

9. Yen J, Nienaber J, Hill D, Pond WJ. Potential contribution of absorbed volatile fatty acids to whole-animal energy requirement in conscious swine. Journal of Animal Science. 1991;69(5):2001-12.

10. Varel V, Yen JT. Microbial perspective on fiber utilization by swine. Journal of Animal Science. 1997;75(10):2715-22.

11. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. 2016;165(6):1332-45.

12. Kreuzer M, Machmüller A, Gerdemann M, Hanneken H, Wittmann M. Reduction of gaseous nitrogen loss from pig manure using feeds rich in easily-fermentable non-starch polysaccharides. Animal Feed Science and Technology.1998;73(1-2):1-19.

13. Molist F, Van Oostrum M, Pérez J, Mateos G, Nyachoti C, Van Der Aar PJ, et al. Relevance of functional properties of dietary fibre in diets for weanling pigs. Animal Feed Science and Technology. 2014;189:1-10.

14. Konstantinov SR, Zhu W-Y, Williams BA, Tamminga S, de Vos WM, Akkermans AD. Effect of fermentable carbohydrates on piglet faecal bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA. FEMS microbiology ecology. 2003;43(2):225-35.

15. Wu R, Zhang H, Zeng X, Zhang J, Xiong H. L-Arabinose and oligosaccharides production from sugar beet pulp by xylanase and acid hydrolysis. African Journal of Biotechnology. 2011;10(10):1907-12.

16. Von Heimendahl E, Breves G, Abel H. Fiber-related digestive processes in three different breeds of pigs. Journal of animal science. 2010;88(3):972-81.

17. Serpa A, Velásquez-Cock J, Gañán P, Castro C, Vélez L, Zuluaga R. Vegetable nanocellulose in food science: A review. Food Hydrocolloids. 2016;57:178-86.

18. Ciolacu DE, Nicu R, Ciolacu F. Cellulose-based hydrogels as sustained drug-delivery systems. Materials. 2020;13(22):5270.

19. Leone G, Fini M, Torricelli P, Giardino R, Barbucci R. An amidated carboxymethylcellulose hydrogel for cartilage regeneration. Journal of Materials Science: Materials in Medicine. 2008;19(8):2873-80.

20. Le Sciellour M, Labussière E, Zemb O, Renaudeau D. Effect of dietary fiber content on nutrient digestibility and fecal microbiota composition in growing-finishing pigs. PLoS One. 2018;13(10):e0206159.

21. Chen X, Xu J, Ren E, Su Y, Zhu W. Co-occurrence of early gut colonization in neonatal piglets with microbiota in the maternal and surrounding delivery environments. Anaerobe. 2018;49:30-40.

22. Holman DB, Chénier MR. Temporal changes and the effect of subtherapeutic concentrations of antibiotics in the gut microbiota of swine. FEMS microbiology ecology. 2014;90(3):599-608.

23. Le Floc'h N, Knudsen C, Gidenne T, Montagne L, Merlot E, Zemb O. Impact of feed restriction on health, digestion and faecal microbiota of growing pigs housed in good or poor hygiene conditions. Animal. 2014;8(10):1632-42.

24. Heinritz SN, Weiss E, Eklund M, Aumiller T, Louis S, Rings A, et al. Intestinal microbiota and microbial metabolites are changed in a pig model fed a high-fat/low-fiber or a low-fat/high-fiber diet. PloS one. 2016;11(4):e0154329.

25. Verschuren LM, Calus MP, Jansman AJ, Bergsma R, Knol EF, Gilbert H, et al. Fecal microbial composition associated with variation in feed efficiency in pigs depends on diet and sex. Journal of animal science. 2018;96(4):1405-18.

26. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. Cell. 2016;167(5):1339-53. e21.

27. Lizardo R, Aumaître A. Non-starch polysaccharides of sugar-beet pulp improve the adaptation to the starter diet, growth and digestive process of the weaned pig. Cahiers Options Méditerranéennes. 2001;54:185-9.

28. Hermes R, Molist F, Ywazaki M, de Segura AG, Gasa J, Torrallardona D, et al. Effects of type of cereal and fibre level on growth and parameters of the gastrointestinal tract in young pigs. Livestock Science. 2010;133(1-3):225-8.

29. Laitat M, Antoine N, Cabaraux J-F, Cassart D, Mainil J, Moula N, et al. Influence of sugar beet pulp on feeding behavior, growth performance, carcass quality and gut health of fattening pigs. Biotechnologie, Agronomie, Société et Environnement. 2015;19(1):20-31.

30. Yan C, Kim H, Hong J, Lee J, Han Y, Jin Y, et al. Effect of dietary sugar beet pulp supplementation on growth performance, nutrient digestibility, fecal microflora, blood profiles and diarrhea incidence in weaning pigs. Journal of animal science and technology. 2017;59(1):1-8.

31. Puhakka L, Jaakkola S, Simpura I, Kokkonen T, Vanhatalo A. Effects of replacing rapeseed meal with fava bean at 2 concentrate crude protein levels on feed intake, nutrient digestion, and milk production in cows fed grass silage–based diets. Journal of dairy science. 2016;99(10):7993-8006.

32. Pereira PA, Aho VT, Paulin L, Pekkonen E, Auvinen P, Scheperjans F. Oral and nasal microbiota in Parkinson's disease. Parkinsonism & related disorders. 2017;38:61-7.

33. Korpela K. Mare: microbiota analysis in R easily. R package version. 2016;1:10.5281.

34. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome biology. 2011;12(6):1-18.

35. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nature Biotechnology. 2020;38(6):685-8.

36. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PloS one. 2013;8(7):e67019.

37. van Hees H, Maes D, Millet S, den Hartog L, van Kempen T, Janssens G. Fibre supplementation to pre-weaning piglet diets did not improve the resilience towards a post-weaning enterotoxigenic E. coli challenge. Journal of Animal Physiology and Animal Nutrition. 2021;105(2):260-71.

38. Petersen V. The development of feeding and investigatory behaviour in free-ranging domestic pigs during their first 18 weeks of life. Applied Animal Behaviour Science. 1994;42(2):87-98.

39. Van Hees H, Davids M, Maes D, Millet S, Possemiers S, Den Hartog L, et al. Dietary fibre enrichment of supplemental feed modulates the development of the intestinal tract in suckling piglets. Journal of animal science and biotechnology. 2019;10(1):1-11.

40. Choudhury R, Middelkoop A, Boekhorst J, Gerrits W, Kemp B, Bolhuis J, et al. Early life feeding accelerates gut microbiome maturation and suppresses acute post-weaning stress in piglets. Environmental Microbiology. 2021;23(11):7201-13.

41. Frese SA, Parker K, Calvert CC, Mills DA. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome. 2015;3(1):1-10.

42. Metzler-Zebeli BU, Zijlstra RT, Mosenthin R, Gänzle MG. Dietary calcium phosphate content and oat β-glucan influence gastrointestinal microbiota, butyrate-producing bacteria and butyrate fermentation in weaned pigs. FEMS microbiology ecology. 2011;75(3):402-13.

43. Mu C, Zhang L, He X, Smidt H, Zhu W. Dietary fibres modulate the composition and activity of butyrate-producing bacteria in the large intestine of suckling piglets. Antonie Van Leeuwenhoek. 2017;110(5):687.

44. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489(7415):242-9.

45. Zheng L, Kelly CJ, Battista KD, Schaefer R, Lanis JM, Alexeev EE, et al. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor–dependent repression of claudin-2. The Journal of Immunology. 2017;199(8):2976-84.

46. Wrzosek L, Miquel S, Noordine M-L, Bouet S, Chevalier-Curt MJ, Robert V, et al. Bacteroides thetaiotaomicron and Faecalibacterium prausnitzii influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC biology. 2013;11(1):1-13.

47. Scheppach W. Effects of short chain fatty acids on gut morphology and function. Gut. 1994;35(1 Suppl):S35-S8.

48. Le Gall M, Gallois M, Seve B, Louveau I, Holst JJ, Oswald IP, et al. Comparative effect of orally administered sodium butyrate before or after weaning on growth and several indices of gastrointestinal biology of piglets. British Journal of Nutrition. 2009;102(9):1285-96.

49. Gerritsen R, van Der Aar P, Molist F. Insoluble nonstarch polysaccharides in diets for weaned piglets. Journal of Animal Science. 2012;90(suppl_4):318-20.

50. Lallès J-P, Bosi P, Smidt H, Stokes CR. Weaning—a challenge to gut physiologists. Livestock Science. 2007;108(1-3):82-93.

51. Pascoal LAF, Thomaz MC, Watanabe PH, Ruiz UdS, Ezequiel JMB, Amorim AB, et al. Fiber sources in diets for newly weaned piglets. Revista Brasileira de Zootecnia. 2012;41:636-42.

52. Schley P, Field C. The immune-enhancing effects of dietary fibres and prebiotics. British Journal of Nutrition. 2002;87(S2):S221-S30.

53. Schokker D, Fledderus J, Jansen R, Vastenhouw SA, de Bree FM, Smits MA, et al. Supplementation of fructooligosaccharides to suckling piglets affects intestinal microbiota colonization and immune development. Journal of animal science. 2018;96(6):2139-53.

54. Molist F, Van Oostrum M, Pérez J, Mateos G, Nyachoti C, Van Der Aar P. Relevance of functional properties of dietary fibre in diets for weanling pigs. Animal Feed Science and Technology. 2014;189:1-10.

55. Schulze H, Van Leeuwen P, Verstegen M, Van den Berg J. Dietary level and source of neutral detergent fiber and ileal endogenous nitrogen flow in pigs. Journal of Animal Science. 1995;73(2):441-8.

56. Pascoal LAF, Thomaz MC, Watanabe PH, Ruiz UdS, Ezequiel JMB, Amorim AB, et al. Fiber sources in diets for newly weaned piglets. Revista Brasileira de Zootecnia. 2012;41(3):636-42.

57. Flis M, Sobotka W, Antoszkiewicz Z. Fiber substrates in the nutrition of weaned piglets–a review. Annals of Animal Science. 2017;17(3):627-44.

58. Sannino A, Demitri C, Madaghiele M. Biodegradable cellulose-based hydrogels: design and applications. Materials. 2009;2(2):353-73.

59. Uddin MK, Hasan S, Mahmud M, Peltoniemi O, Oliviero C. In-feed supplementation of resin acid-enriched composition modulates gut microbiota, improves growth performance, and reduces post-weaning diarrhea and gut inflammation in piglets. Animals. 2021;11(9):2511.

60. Högberg A, Lindberg JE. Influence of cereal non-starch polysaccharides and enzyme supplementation on digestion site and gut environment in weaned piglets. Animal Feed Science and Technology. 2004;116(1-2):113-28.

61. Han Y, Han K, Lee J. Effects of insoluble dietary fiber supplementation on performance and nutrient digestibility of weanling pigs. Journal of Animal Science and Technology. 2005;47(4):565-72.

62. Hanczakowska E, Swiatkiewicz M, Bialecka A. Pure cellulose as feed supplement for piglets. Medycyna Wet. 2008;64:45-8.

63. Jeaurond E, Rademacher M, Pluske J, Zhu C, De Lange C. Impact of feeding fermentable proteins and carbohydrates on growth performance, gut health and gastrointestinal function of newly weaned pigs. Canadian Journal of Animal Science. 2008;88(2):271-81.

64. Cho HM, Kim E, Wickramasuriya SS, Shin TK, Heo JM. Growth and gut performance of young pigs in response to different dietary cellulose concentration and rearing condition. Asian-Australasian Journal of Animal Sciences. 2021.

65. Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. Nature Reviews Microbiology. 2017;15(10):630-8.

66. Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends in microbiology. 2017;25(10):851-73.

67. Coyte KZ, Schluter J, Foster KR. The ecology of the microbiome: networks, competition, and stability. Science. 2015;350(6261):663-6.

68. Molist F, Manzanilla EG, Pérez JF, Nyachoti CM. Coarse, but not finely ground, dietary fibre increases intestinal Firmicutes: Bacteroidetes ratio and reduces diarrhoea induced by experimental infection in piglets. British Journal of Nutrition. 2012;108(1):9-15.

69. Waite DW, Vanwonterghem I, Rinke C, Parks DH, Zhang Y, Takai K, et al. Comparative genomic analysis of the class Epsilonproteobacteria and proposed reclassification to Epsilonbacteraeota (phyl. nov.). Frontiers in microbiology. 2017;8:682.

70. Shin N-R, Whon TW, Bae J-W. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends in biotechnology. 2015;33(9):496-503.

71. Luppi A, Gibellini M, Gin T, Vangroenweghe F, Vandenbroucke V, Bauerfeind R, et al. Prevalence of virulence factors in enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhoea in Europe. Porcine Health Management. 2016;2(1):1-6.

72. Cremonesi P, Biscarini F, Castiglioni B, Sgoifo CA, Compiani R, Moroni P. Gut microbiome modifications over time when removing in-feed antibiotics from the prophylaxis of post-weaning diarrhea in piglets. PloS one. 2022;17(3):e0262199.

73. Adhikari B, Kim SW, Kwon YM. Characterization of microbiota associated with digesta and mucosa in different regions of gastrointestinal tract of nursery pigs. International journal of molecular sciences. 2019;20(7):1630.

74. Jensen A, Ladegaard Grønkjær L, Holmstrup P, Vilstrup H, Kilian M. Unique subgingival microbiota associated with periodontitis in cirrhosis patients. Scientific reports. 2018;8(1):1-16.

75. Biddle A, Stewart L, Blanchard J, Leschine S. Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. Diversity. 2013;5(3):627-40.

76. McCormack UM, Curião T, Wilkinson T, Metzler-Zebeli BU, Reyer H, Ryan T, et al. Fecal microbiota transplantation in gestating sows and neonatal offspring alters lifetime intestinal microbiota and growth in offspring. MSystems. 2018;3(3):e00134-17.

77. Oh JK, Chae JP, Pajarillo EAB, Kim SH, Kwak MJ, Eun JS, et al. Association between the body weight of growing pigs and the functional capacity of their gut microbiota. Animal Science Journal. 2020;91(1):e13418.

78. McCormack UM, Curião T, Buzoianu SG, Prieto ML, Ryan T, Varley P, et al. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. Applied and environmental microbiology. 2017;83(15):e00380-17.

79. Shao Y, Zhou J, Xiong X, Zou L, Kong X, Tan B, et al. Differences in gut microbial and serum biochemical indices between sows with different productive capacities during perinatal period. Frontiers in microbiology. 2020;10:3047.

Tables

Table 1: piglet growth at weaning and post-weaning

Treatment	Treatment	ADG	P-value
Creep feed treatment	CC	307±0.11	
	MC	293±0.11	0.06
	CM	326±0.13	0.02
	MM	333±0.11	<0.01
Post-weaning feed treatment piglets	CCC	261±0.36	
	CMC	305±0.42	<0.01
	MCC	252±0.38	0.35
	MMC	291±0.31	<0.01
	CCM	282±0.29	0.03
	CMM	300±0.38	<0.01
	MCM	285±0.3	0.013
	MMM	307±0.29	<0.01

Table 2. Effects of MFC supplementation on fecal volatile fatty acids content of pre-weaning and post-weaning piglets

VFA, mg/kg	Preweaning			Post-weaning		
	Control (55)	MFC (64)	P-value	Control (29)	MFC (34)	P-value
Total VFA	4128.9±300.96	4344.15±246.31	0.58	6022.18±255.55	6552.09±244.8	**0.14**
Acetic acid	1600.61±86.33	1700.94±81.71	0.4	3036.9±116.14	3097.91±96.35	0.68
Propionic acid	933.93±59.97	1066.04±69.76	0.16	1537.35±75.87	1661.2±89.23	0.30
Butyric acid	650.37±92.15	592.76±58.89	0.59	984.19±62.95	1287.13±76.63	**<0.01**
IsoButyric acid	202.5±17.8	204.19±14.08	0.94	81.76±9.28	92.16±6.72	0.35
2-MeButyric acid	170.29±15.19	171.72±12.32	0.94	50.07±7.28	57.63±5.53	0.40
3-MeButyric acid	240.45±21.49	246.75±18.4	0.82	70.25±9.27	78.71±6.37	0.44
Pentanoic acid	284.05±26.08	311.56±21.17	0.41	211.29±14.82	212.92±9.9	0.92
Hexanoic acid	46.69±3.48	50.19±4.08	0.52	50.36±5.55	64.42±4.76	0.057

Table 3. Comparison of significant taxa based on relative abundance in Mare package, and estimated effect size(LDA score) between control and MFC group.

Taxa	Relative abundance -Mare package		LDA Score	
	CCC	MMM	CCC	MMM
Phylum	Epsilonbacteraeota			
Class	Campylobacteria			
	Gammaproteobacteria		Gammaproteobacteria	
Order	Campylobacterales		Betaproteobacteriales	
			Enterobacteriales	
Family	Campylobacteraceae		Enterobacteriaceae	
	Tannerellaceae		Tannerellaceae	
	Family_XIII		Burkholderiaceae	
	Muribaculaceae		Helicobacteraceae	
			Bacteroidaceae	
Genus	Campylobacter	Ruminococcus.2	Bacteroides	Ruminococcus_2
	Parabacteroides	Intestinibacter	Helicobacter	Intestinibacter
		Roseburia	Escherichia_Shigella	Roseburia
			Prevotellaceae_UCG_001	Pseudobutyrivibrio
		Ruminococcaceae.UCG.014	Sutterella	Ruminococcaceae_UCG_014
		Oribacterium	Parabacteroides	Oribacterium
				Lachnoclostridium_12
			Lachnospiraceae_UCG_003	Lachnospira
				Lachnospiraceae_NK3A20_group
				Prevotella_7
				Allisonella
				Collinsella

Figures

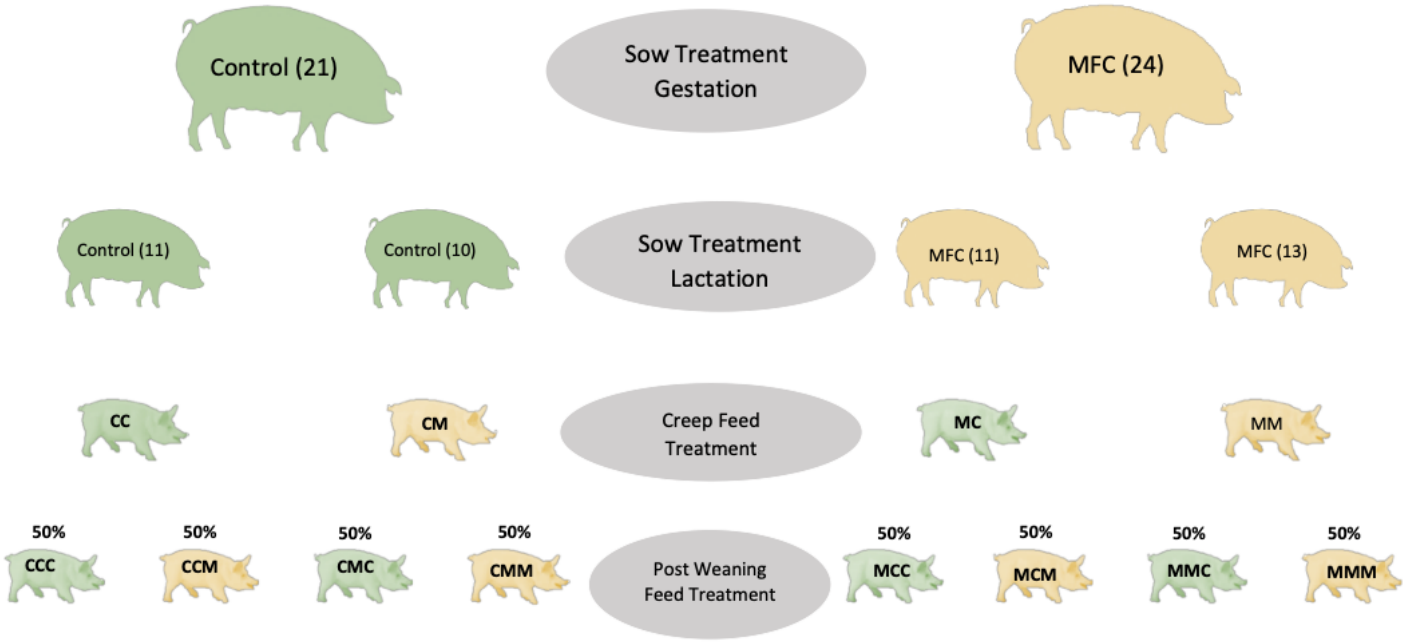

Figure 1

Schematic diagram of sow and piglet feeding plan

MFC= Sows fed micro fibrillated cellulose; MM=sow MFC, piglets fed MFC creep feed: MC=sow MFC, piglets fed control creep feed: CM=sow control, piglets fed MFC creep feed; and CC=sow control, piglets fed control creep feed.

MMM= sow MFC, piglets fed MFC creep feed, post-weaning piglets MFC feed; MMC= sow MFC, piglets fed MFC creep feed, post-weaning piglets control feed; MCM=sow MFC, piglets fed control creep feed, post-weaning MFC feed; MCC=sow sow MFC, piglets fed control creep feed, post-weaning control feed; CMM=sow control feed, piglets fed MFC creep feed, piglets post-weaning MFC feed; CMC=sow control feed, piglets fed MFC creep feed; piglets post-weaning control feed; CCM=sow control feed, piglets fed control creep feed, piglets post-weaning MFC feed; CCC=sow control feed, piglets fed control creep feed, piglets post-weaning control feed.

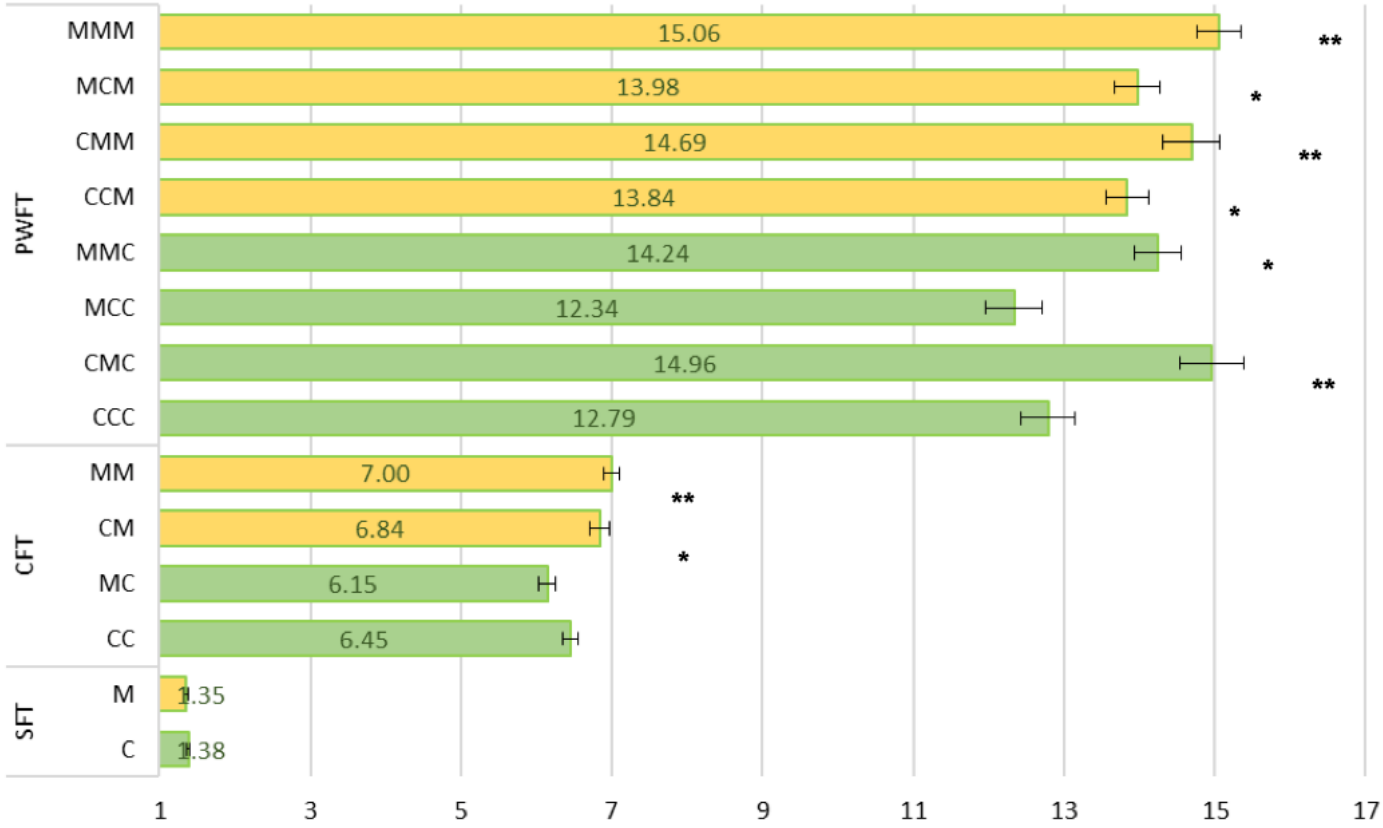

Figure 2

Body weights of piglets at different ages. OK.

MFC= Sows fed micro-fibrillated cellulose; MM=sow MFC, piglets fed MFC creep feed: MC=sow MFC, piglets fed control creep feed: CM=sow control, piglets fed MFC creep feeding: and CC=sow control, piglets fed control creep feed. C=Control feed of sows: M=MFC feed of sows; CFT=Creep feed treatment piglets: PWFT=Post-weaning feed treatment piglets

MMM= sow MFC, piglets fed MFC creep feed, post-weaning piglets MFC feeding; MMC= sow MFC, piglets fed MFC creep feed, post-weaning piglets control feed; MCM=sow MFC, piglets fed control creep feed, post-weaning MFC feed; MCC=sow MFC, piglets fed control creep feed, post-weaning control feed; CMM=sow control feed, piglets fed MFC creep feed, piglets post-weaning MFC feed; CMC=sow control feed, piglets fed MFC creep feed; piglets post-weaning control feed; CCM=sow control feed, piglets fed control creep feeding, piglets post-weaning MFC feed; CCC=sow control feed, piglets fed control creep feed, piglets post-weaning control feed.

* $P < 0.05$, and ** $P < 0.01$

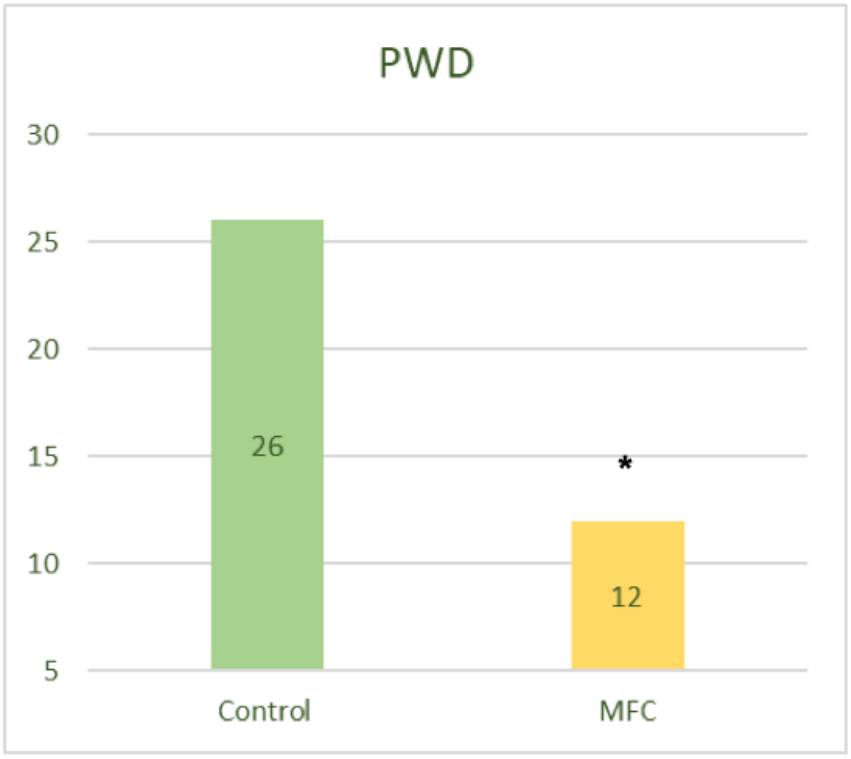

Figure 3

Piglet diarrhea cases at seven weeks of age (PWD), in piglets MFC after weaning. * p < 0.05.

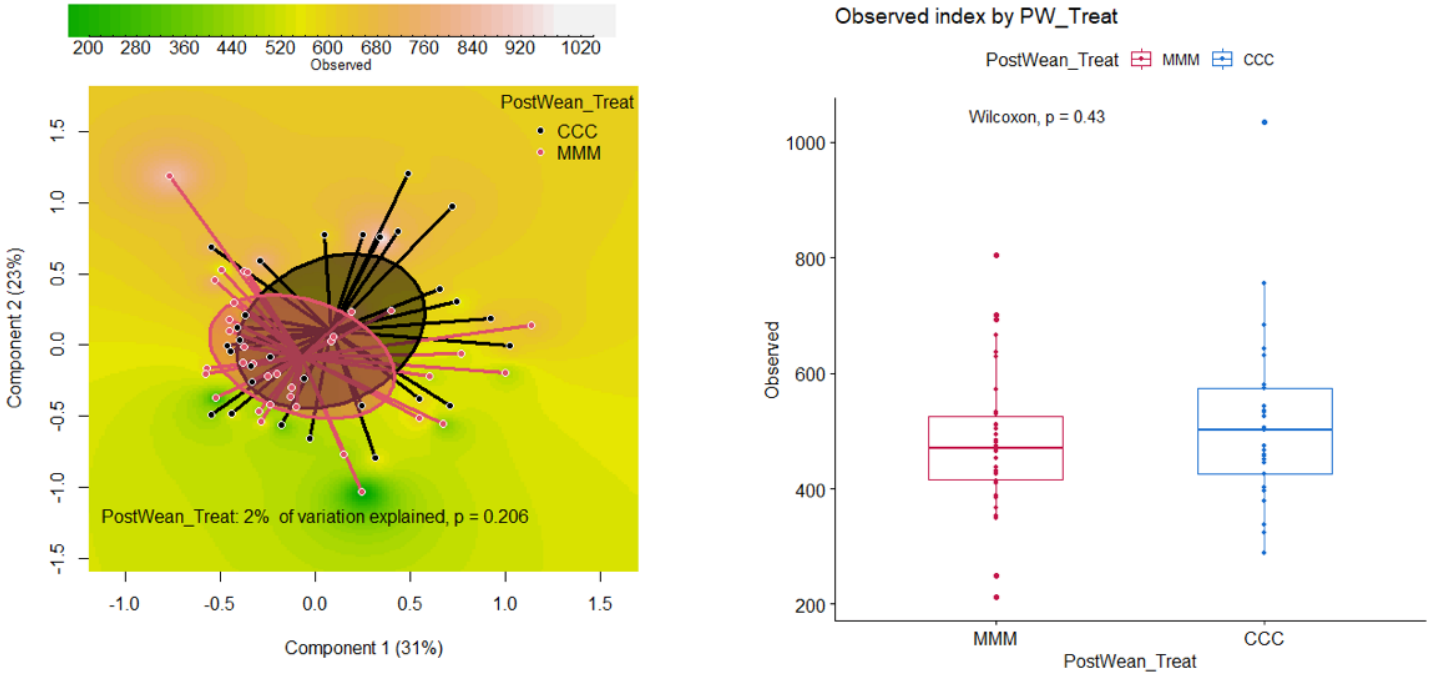

Figure 4

Diversity of the gut microbiota of control and MFC piglets. A) Principal coordinates analysis (PCoA) plot, and B) alpha diversity (observed index)

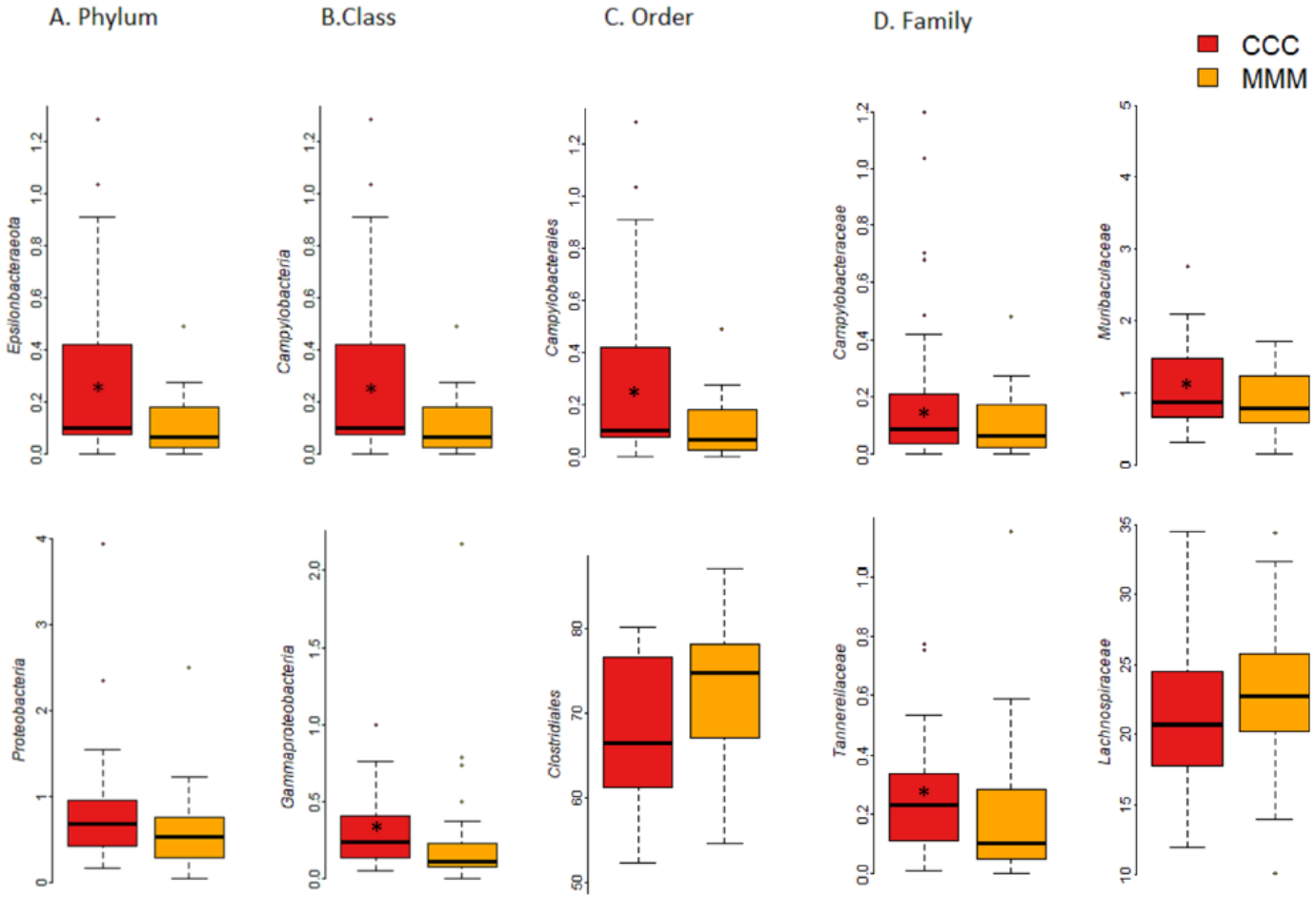

Figure 5

Relative abundances of microbiota between groups at different taxa. A)Phylum; B) Class; C) Order; D) family. *p<0.05

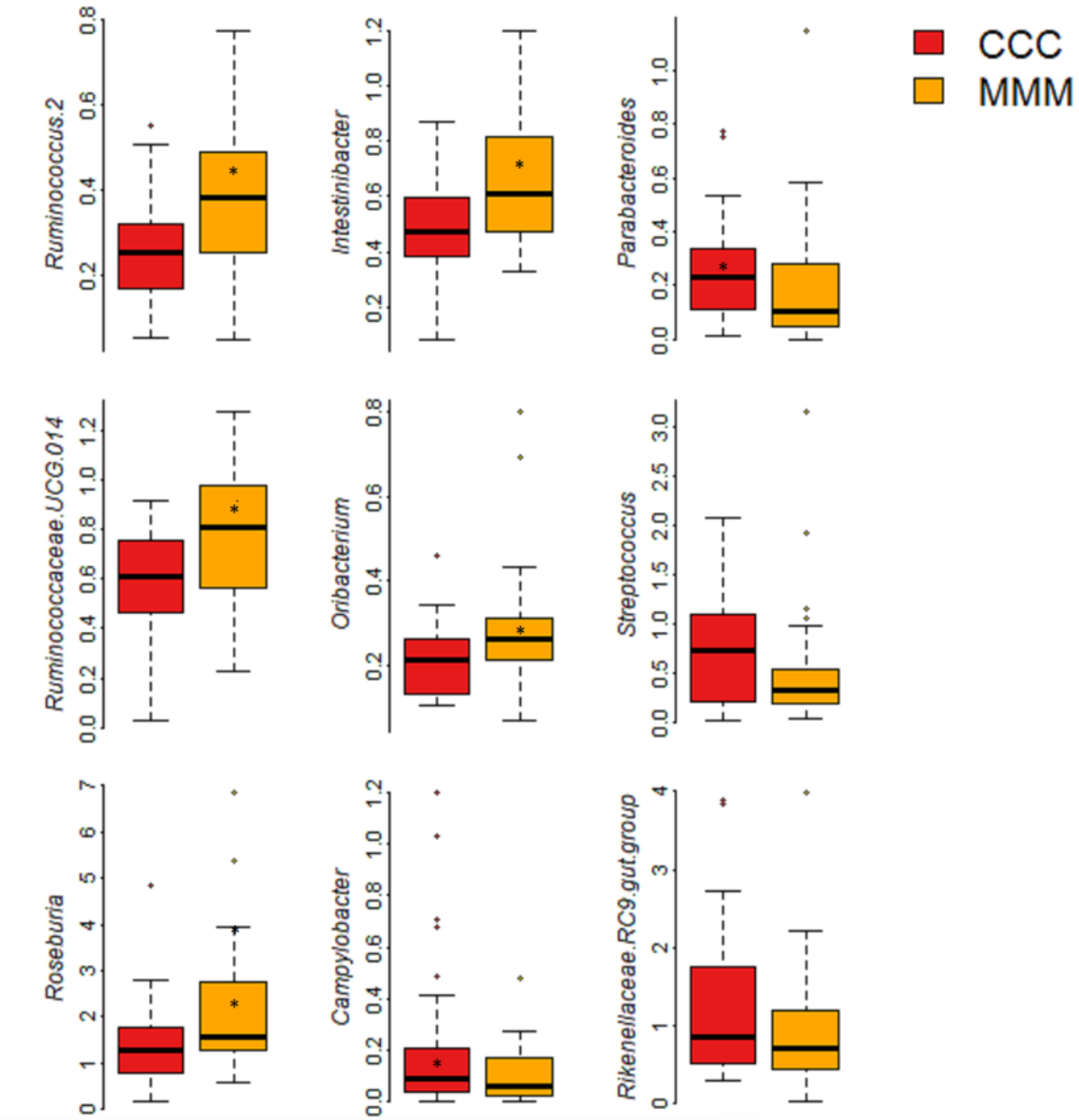

Figure 6

Relative abundances of microbiota between groups at genus level. *p<0.05

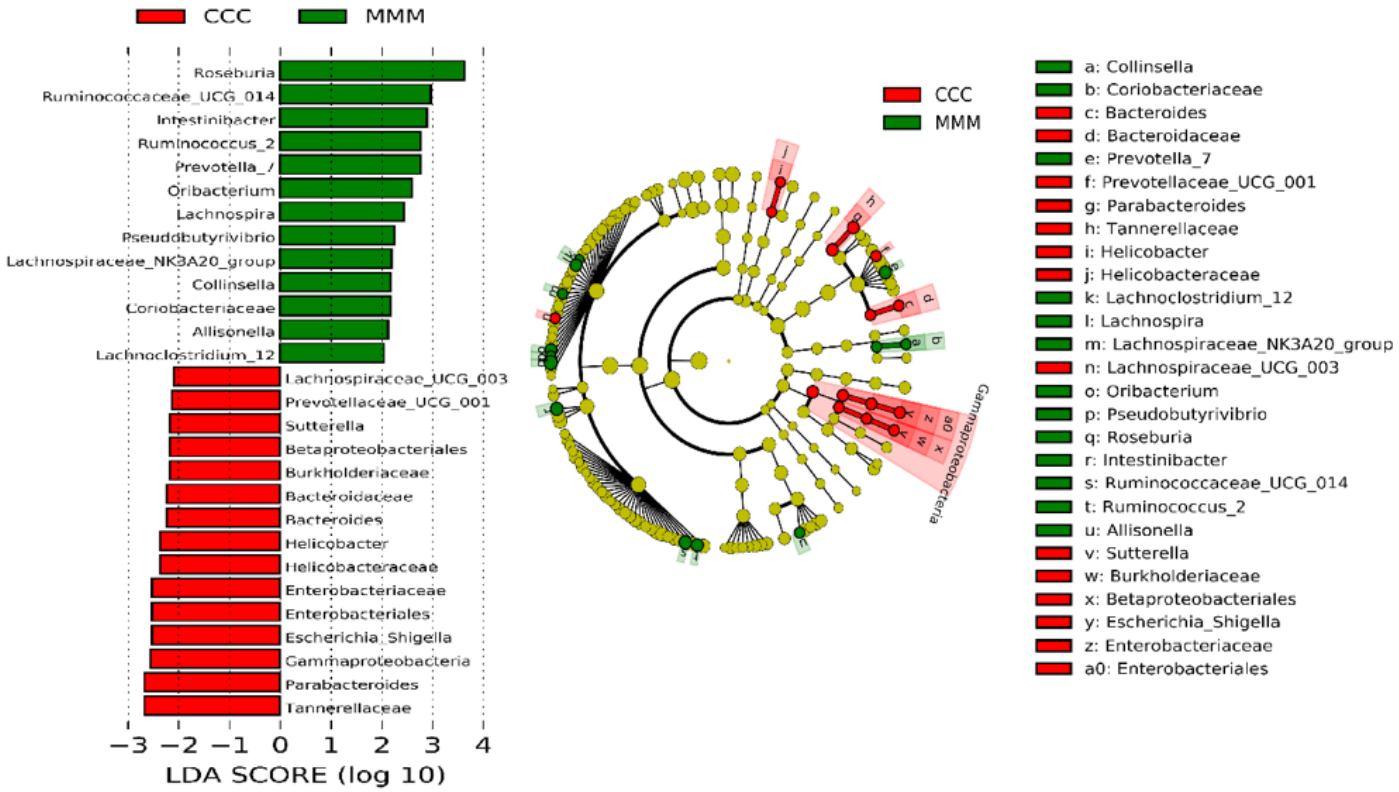

Figure 7

Linear discriminant analysis (LDA) effect size (LEfSe) of gut microbiota between control (CCC) and treatment (MMM) groups. A) bar plot showing the differentially abundant taxa between groups, and (B) cladogram showing differences in abundant taxa between groups. LDA score threshold was >2.0 .

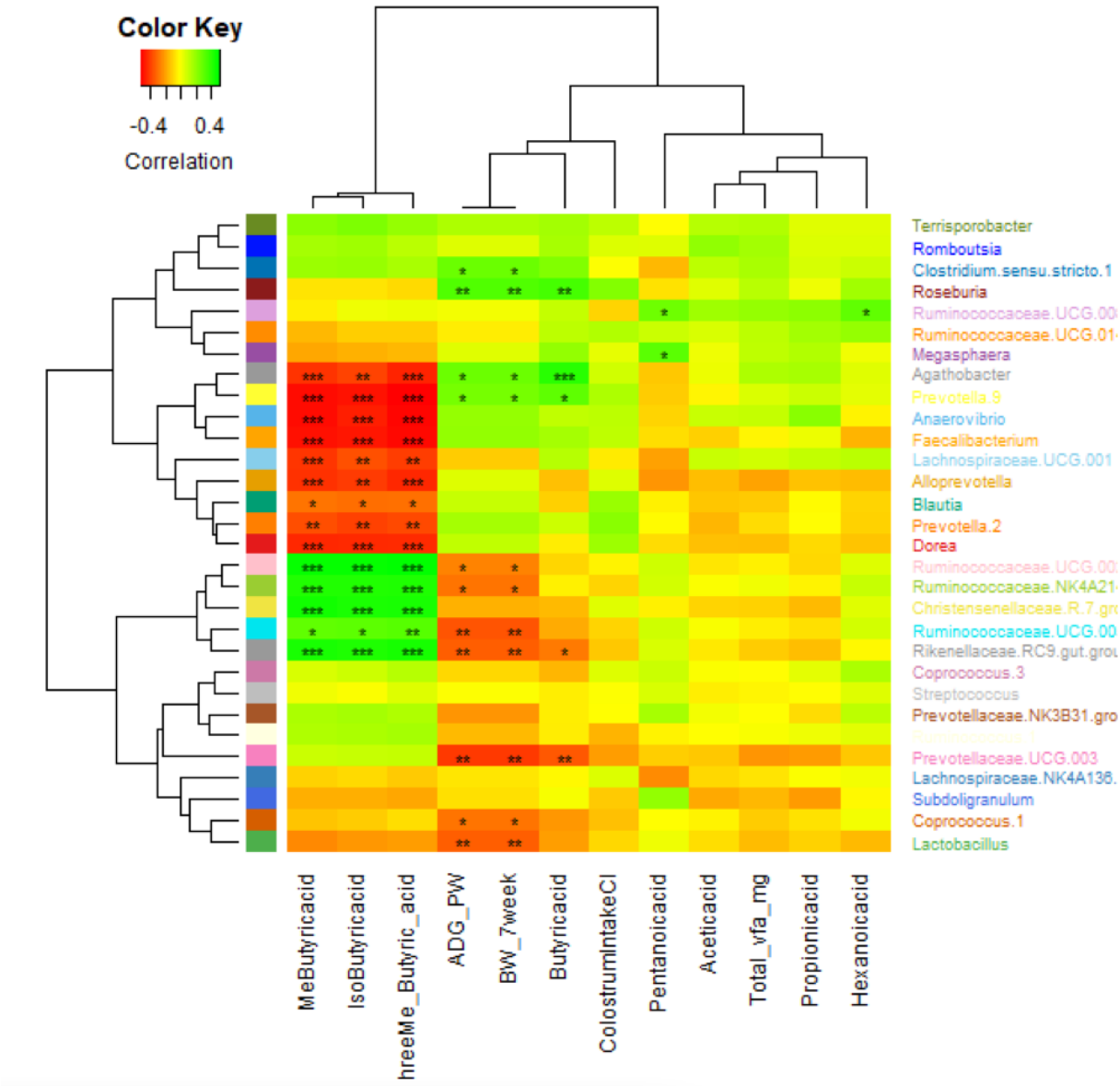

Figure 8

Correlations between microbial population at genus level, performance parameters and fecal VFA. *p<0.05

Supplementary Files

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- Additionalfile6FigureS1.png