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# Effect of Medium Chain Fatty Acid Supplementation on Nursery Pig Fecal Microbial Populations

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## Summary

A total of 360 pigs [DNA (Columbus, NE) 400 × 200; initially = 14.8 ± 0.15 lb] were used to evaluate the effects of dietary medium chain fatty acid (MCFA) addition on fecal microbial populations. Upon arrival at the nursery, pigs were randomized to pens (5 pigs per pen) and allowed a 6-d acclimation period, at which point pens of pigs were blocked by body weight (BW) and randomized to dietary treatment (9 pens per treatment). Medium chain fatty acids (Sigma Aldrich, St. Louis, MO) included hexanoic (C6), octanoic (C8), and decanoic (C10), and were guaranteed ≥ 98% purity. Treatment diets were formulated to meet or exceed NRC[3] requirements for 15- to 25-lb pigs. Fecal samples were collected from pigs fed control and 1.5% MCFA blend (1:1:1 ratio C6, C8, and C10) diets on d 0 and d 14 and analyzed using 16s rDNA sequencing. A total of 6 phyla were identified with ≥ 1% relative abundance for at least one of the treatment × day analysis combinations. The largest proportion of relative abundance on a phyla level on d 14 consisted of Firmicutes (54% control, 44% MCFA) and Bacteroidetes (32% control, 43% MCFA). A marginally significant treatment × day interaction was observed in the Proteobacteria phylum ($P = 0.080$), where relative abundance did not change over time in pigs fed the control diet ($P = 0.848$), whereas a marginally significant decrease over time was observed in pigs fed the MCFA diet ($P = 0.092$). There was no evidence of an effect of MCFA addition over time for the relative abundance of the remaining phyla (treatment × day, $P \geq 0.359$). The main effect of day indicated a significant increase over time in the Tenericutes phylum ($P = 0.008$) and a significant decrease over time in the Proteobacteria ($P = 0.017$) and Spirochaetes phyla ($P = 0.020$). A Firmicutes:Bacteroidetes ratio was calculated, and there was no evidence of a treatment × day interaction ($P = 0.338$) or day effect ($P = 0.211$). A total of 23 microbial families were detected at ≥ 1% relative abundance for at least one of the treatment × day analysis combinations. The families with the greatest relative abundance were Prevotellaceae, Ruminococcaceae, and S24-7 families, which had ≥ 10%

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[3]NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

relative abundance for at least one of the treatment × day analysis combinations. There was no evidence of an effect of MCFA addition over time on the relative abundance for any family (treatment × day, $P \geq 0.123$). For both treatments, a reduction over time was observed for Ruminococcaceae ($P = 0.048$), Lachnospiraceae ($P = 0.004$), Christensenellaceae ($P = 0.004$), Spirochaetaceae ($P = 0.029$), Bacteroidaceae ($P = 0.010$), and Succinivibrionaceae ($P = 0.029$) families. An increase in relative abundance over time was observed for the unclassified Clostridiales ($P = 0.019$), Clostridiaceae ($P < 0.001$), unclassified RF39 ($P = 0.008$), and Clostridiales; and other ($P < 0.001$) families. No evidence of a difference in alpha diversity was observed for either Chao1 (estimate of species richness) or observed operational taxonomic units (OTUs).

In summary, adding 1.5% MCFA blend in swine nursery diets did not appear to significantly alter the composition of fecal microbial populations compared to a control diet using 16s rDNA sequencing analysis. Changes in microbial populations were observed over time with both treatments. Further investigation into the mechanism by which MCFA addition benefits growth performance is necessary. Moreover, additional studies into understanding the interactions between MCFA and the gastrointestinal microbiome are warranted due to its well-known inactivation effects on selected microbes.

## Introduction

Medium chain fatty acids (MCFA) have been widely discussed in recent years both in research settings and the popular press as an effective control strategy against porcine epidemic diarrhea virus (PEDV) in feed.[4,5] Evidence has also demonstrated that MCFA are active compounds against bacteria, parasites, and other viruses.[6] Therefore, MCFA are potent molecules that show significant potential to improve the safety of feed and feed ingredients by reducing bacterial and viral contamination. In addition to the economic benefits of reducing the risk of disease transmission, an evaluation into the impact of MCFA supplementation on growth performance in nursery pigs has recently demonstrated a substantial improvement in growth performance.[7] As a part of this experiment, series of fecal samples were collected to evaluate the impact of MCFA supplementation on the fecal microbial populations. Although reducing the bacterial and viral load within feed and/or feed ingredients would have a benefit for disease transmission risk, it is generally accepted that a diverse microbial population within the gastrointestinal tract of growing pigs is necessary for optimum performance. A reduction in the diversity of microbial populations by using compounds such as MCFA may potentially hinder the digestive efficiency, growth performance, and response to disease.

[4]Cochrane, R. A., M. Saensukjaroenphon, S. S. Dritz, J. C. Woodworth, A. R. Huss, C. R. Stark, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. F. Bai, Q. Chen, J. Zhang, P. C. Gauger, R. Main, and C. K. Jones. 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma. J. Anim. Sci. 94 (Suppl 2):50. doi:10.2527/msasas2016-107.

[5]Dee, S., C. Neill, A. Singrey, T. Clement, R. Cochrane, C. Jones, G. Patterson, G. Spronk, J. Christopher-Hennings, and E. Nelson. 2016. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. BMC Vet. Res. 12:51. doi:10.1186/s12917-016-0674-z.

[6]Zentek, J., S. Buchheit-Renko, F. Ferrara, W. Vahjen, A. G. Van Kessel, and R. Pieper. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim Health Res Rev. 12(1):83-93. doi: 10.1017/S1466252311000089.

[7]Gebhardt, J. T., K. A. Thomson, J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, and S. S. Dritz. 2017. Evaluation of medium chain fatty acids as a dietary additive in nursery pig diets. Kansas Agricultural Experiment Station Research Reports. 3(7). doi:10.4148/2378-5977.7463.

Therefore, the objective of this experiment was to characterize fecal microbial populations using 16s rDNA sequencing following the feeding of a 1:1:1 blend of hexanoic (C6), octanoic (C8), and decanoic (C10) acid at 1.5% inclusion in nursery pig diets compared to diets without added MCFA.

## Procedures

### *General*

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. Following arrival at the research facility, pigs were randomized to pens, allowed a 6-d acclimation period, and were fed a commercial starter pellet containing no feed-grade antimicrobials. Following acclimation, 360 pigs [DNA (Columbus, NE) 400 × 200; initial BW = 14.8 ± 0.15 lb] were blocked by BW and randomized to dietary treatment. Treatment diets were formulated to meet or exceed NRC[3] requirement estimates for 15- to 25-lb pigs. The dietary treatments from which fecal samples were collected were a control diet containing no added MCFA or a diet containing 1.5% of a 1:1:1 blend of hexanoic (C6), octanoic (C8), and decanoic (C10) acid. The MCFA blend was included in the diet at the expense of soybean oil. The C6, C8, and C10 were purchased individually (Sigma Aldrich, St. Louis, MO) and were guaranteed ≥ 98% purity. The 1:1:1 blend was made by combining each of the individual MCFA on an equal weight basis. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens had tri-bar floors and allowed 2.7 $ft^2$/pig. Complete diet samples were collected following feed manufacture using a feed probe from every fifth bag, subsampled, and submitted (Ward Laboratories, Inc., Kearney, NE) for analysis of dry matter (DM), crude protein (CP), crude fiber, calcium, phosphorous, and ether extract. In addition, MCFA concentration was evaluated at the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO).

Fecal samples were collected from one pig per pen within control and 1.5% MCFA blend (1:1:1 ratio C6, C8, and C10) treatments (9 pens/treatment) on d 0 and 14 of the study period. The same pig within each pen was sampled on both dates. Sterile cotton tipped applicators (Puritan Medical Products, Guilford, ME) were inserted into the rectum to stimulate defecation. Fecal samples were collected into clean, single use zipper storage bags and were then transferred into 3 mL snap-cap cryovials and stored at -112°F until shipment for analysis at the University of Nebraska-Lincoln for DNA extraction and bacterial community analysis.

### *16S rDNA Analysis of Fecal Samples*

DNA was extracted using the manufacturer's protocol for Mag-Bind Soil DNA 96 Kit (Omega Bio-tek, Inc., Norcross, GA, USA) with the following modifications: precipitation of nucleic acids was done by using sodium acetate, isopropanol, and ethyl alcohol. Each sample tube received 0.1× volumes of 10 mM sodium acetate, which were vortexed and later incubated on ice for 5 min. Subsequently, 1 mL of ice-cold isopropanol was added and samples were incubated at -112°F overnight to precipitate the DNA. The following day, samples were centrifuged at 39°F for 15 min at 16,000 × g. The supernatants of the resulting samples were discarded and the nucleic acid pellet

was washed with 0.5 mL of ice-cold 70% ethyl alcohol. The samples were centrifuged for 2 min at 13,000 × g, the residual supernatant was discarded, and the nucleic acid pellet was air dried for 3 min. The nucleic acid pellet was dissolved in a 0.45 mL of Tris [tris(hydroxymethyl)aminomethane; 10 mM, pH 8] and incubated for 1 hr at 39°F. For further purification of dissolved nucleic acids, the KingFisher (ThermoFisher Scientific) robot was used with reagents from the Mag-Bind Soil DNA 96 Kit. The resulting DNA was used for the tag-sequencing of the V4 region of 16S rDNA using the universal bacterial primers described previously.[8] A 20 μL PCR reaction contained 1× TerraTM PCR Direct Polymerase Mix, 0.5 μL Terra polymerase, 20 mM of each primer, and 20-50 ng of DNA. The cycling conditions for PCR were the same as previously described.[9] The PCR product size was confirmed by agarose gel electrophoresis. Normalization of the amplified PCR products were performed with Just-a-PlateTM 96 PCR Purification & Normalization kit (Charm Biotech, CA, USA) according to the manufacturer's protocol. Following normalization, 10 μL from each sample were pooled and concentrated using Nucleospin Gel & PCR Cleanup kit (MACHEREY-NAGEL Gmbh & Co. KG, Duren, Germany) and was eluted using 20 μL of elution buffer. This pooled and purified sample was analyzed in an Agilent 2100 bioanalyzer (Agilent Scientific Instruments, CA, USA) using Agilent High Sensitivity DNA Kit (Agilent Technologies, Inc. Waldbronn, Germany) to ensure the quality and quantity of the targeted V4 region of 16S rDNA. The concentration of the DNA library was determined using the DeNovix QFX Fluorometer (DeNovix Inc., Wilmington, DE, USA) and using DeNovix dsDNA Fluorescence Quantification Assay (DeNovix Inc., Wilmington, DE, USA). The resulting 16S rDNA libraries were sequenced using the Illumina MiSeq platform utilizing the 2 × 250 paired end sequencing strategy using a MiSeq Reagent Kit V3 (Illumina Inc., San Diego, CA, USA).

Data processing was performed on a custom pipeline utilizing several publicly available software tools. The paired-end reads were assembled into contiguous sets of overlapping clones (contigs) after quality filtering using MOTHUR v.1.38.1 as previously described.[10] Operational taxonomic units (OTUs) were generated from the quality filtered sequences using the UPARSE pipeline (USEARCH v7.0.1090)[11] at a threshold of 97% identity. Chimeric sequences were removed using the ChimeraSlayer gold.fa as the reference database using UCHIME.[12] OTUs were aligned against the v128 (SILVA) database and mismatched sequences were discarded. A phylogenetic tree was

[8]Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol. 79(17):5112-5120. doi: 10.1128/AEM.01043-13.
[9]Paz, H.A., C. L. Anderson, M. J. Muller, P. J. Kononoff, and S. C. Fernando. 2016. Rumen bacterial community composition in Holstein and Jersey cows is different under same dietary condition and is not affected by sampling method. Front Microbiol. 7:1206. doi: 10.3389/fmicb.2016.01206.
[10]Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75:7537–7541. doi:10.1128/AEM.01541-09.
[11]Edgar, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods. 10:996–998. doi:10.1038/nmeth.2604.
[12]Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27:2194–200. doi:10.1093/bioinformatics/btr381.

generated using high quality aligned sequences within MOTHUR v.1.38.1 using the Clearcut algorithm.[13] Taxonomies to the identified OTUs were assigned using QIIME v.1.9.1 pipeline[14] with the Greengenes reference database (gg_13_5_otus). OTUs representing Archaea and Cyanobacteria were removed as Cyanobacterial reads may be a result of contamination of plant chloroplast[15] and the archaea sequences may be biased as the primers used are not designed to universally amplify all archaea. Alpha diversity matrices (Chao1 and Observed OTUs) were calculated using the QIIME v.1.9.1 pipeline. The rarefaction of the OTU table was performed using QIIME v.1.9.1 with the lowest number of reads.[12] For the experiment, 29,663 was used as the lowest depth. The difference in bacterial communities (beta-diversity) among transplanted and control pigs was determined using the QIIME v.1.9.1 pipeline using distance matrices (weighted UniFrac, unweighted UniFrac and Bray Curtis) from the rarefied-OTU table.

### *Statistical Analysis*

Fecal microbial population relative abundance was analyzed using a linear mixed model (PROC GLIMMIX; version 9.4; SAS Institute, Cary, NC) with individual pig as the experimental unit representing the single sample collected from each pen on a given sampling day. Relative abundance represents the proportion of total reads for a specific sample that were classified into the designated microbial phyla or family, therefore, they were analyzed using a binomial distribution. The sum of all values for phyla or family within a treatment × day combination equals 1. Day of analysis, treatment, and the associated interaction were included in the statistical model as fixed effects. Degrees of freedom were approximated using the Kenward-Roger approach. A Tukey-Kramer multiple comparison adjustment was applied to the statistical analysis to set the experiment-wise type 1 error rate at $\alpha = 0.05$. A repeated measure statement was used to account for repeated sampling of the same set of pigs on both d 0 and d 14. Means separation was performed using the LINES option to perform pairwise comparisons. The Kruskal-Wallis test was performed on the Chao1 and observed OTUs to see the bacterial richness difference among the treatments with open source statistical software R[16] (Kruskal.test function). All results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

## Results and Discussion

Analysis of manufactured diets (Table 2) resulted in values consistent with formulation. Results of growth performance have been previously reported.[7]

[13]Sheneman, L., J. Evans, and J. A. Foster. 2006. Clearcut: a fast implementation of relaxed neighbor joining. Bioinformatics. 22:2823–4. doi:10.1093/bioinformatics/btl478.

[14]Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods. 7:335–6. doi:10.1038/nmeth.f.303.

[15]Giovannoni, S. J., S. Turner, G. J. Olsen, S. Barns, D. J. Lane, and N. R. Pace. 1988. Evolutionary relationships among cyanobacteria and green chloroplasts. J. Bacteriol. 170:3584–92. doi:10.1128/JB.170.8.3584-3592.1988.

[16]R Core Team. 2013. R Foundation for Statistical Computing, Vienna, Austria.

The largest proportion of relative abundance on a phyla level on d 14 consisted of Firmicutes (54% control, 44% MCFA) and Bacteroidetes (32% control, 43% MCFA; Figure 1). A total of 6 phyla were found to be ≥ 1% relative abundance for at least one of the treatment × day analysis combinations. Phyla and families with low (< 1%) relative abundance were excluded from the results. A marginally significant treatment × day interaction was observed in the Proteobacteria phylum ($P = 0.080$), where relative abundance over time did not change in pigs fed the control diet ($P = 0.848$), whereas a marginally significant decrease over time was observed in pigs fed the 1.5% MCFA blend diet ($P = 0.092$). There was no evidence of a difference in the effect of MCFA addition over time for the relative abundance of the remaining phyla (treatment × day, $P \geq 0.359$).

The main effect of day indicated a significant increase over time in the Tenericutes phylum ($P = 0.008$; 2.6% on d 0, 5.0% on d 14; Figure 2). A marginally significant increase over time was observed in the Firmicutes ($P = 0.066$; 43.0% on d 0, 49.0% on d 14) and Actinobacteria phyla ($P = 0.063$, 2.9% on d 0, 5.5% on d 14). A significant decrease over time was observed for the Proteobacteria phylum ($P = 0.017$; 1.9% on d 0, 0.6% on d 14) and Spirochaetes phylum ($P = 0.020$; 4.0% on d 0, 1.6% on d 14). A Firmicutes:Bacteroidetes ratio was calculated, and there was no evidence of a treatment × day interaction ($P = 0.338$) or day effect ($P = 0.211$).

A total of 23 families were found to be ≥ 1% relative abundance for at least one of the treatment × day of analysis combinations. The families with the greatest relative abundance were Prevotellaceae, Ruminococcaceae, and S24-7, which each had ≥ 10% relative abundance for at least one of the treatment × day analysis combinations. There was no evidence of a difference in the effect of MCFA addition over time for the relative abundance for any family (treatment × day, $P \geq 0.123$). A reduction over time was observed for Ruminococcaceae ($P = 0.048$; 16.5% d 0, 13.3% d 14), Lachnospiraceae ($P = 0.004$; 6.6% d 0, 3.9% d 14), Christensenellaceae ($P = 0.004$; 4.5% d 0, 0.6% d 14), Spirochaetaceae ($P = 0.029$; 3.6% d 0, 1.5% d 14), Bacteroidaceae ($P = 0.010$; 2.3% d 0, 0.1% d 14), and Succinivibrionaceae ($P = 0.029$; 1.0% d 0, 0.1% d 14), and marginally significant evidence of a decrease over time for p-2534-18B5 ($P = 0.095$; 1.0% d 0, 0.2% d 14).

An increase in relative abundance over time was observed for the unclassified Clostridiales ($P = 0.019$; 5.2% d 0, 7.4% d 14), Clostridiaceae ($P < 0.001$; 1.3% d 0, 6.9% d 14), unclassified RF39 ($P = 0.008$; 2.6% d 0, 5.0% d 14), and Clostridiales; other ($P < 0.001$; 0.1% d 0, 1.5% d 14) families, and marginally significant evidence of an increase over time ($P = 0.084$) was observed for the Veillonellaceae family (4.3% d 0, 6.3% d 14). No evidence of a difference in alpha diversity (Chao1; all pairwise comparisons, $P = 1.000$) or observed OTU's (all pairwise comparisons, $P = 1.000$) was observed.

In summary, changes over time appeared to be the primary driving factor in the shift of microbiome populations, whereas little evidence was detected to suggest addition of a 1.5% MCFA blend in swine nursery diets altered fecal microbiota. Using MCFA to control both bacteria and viruses in feed has been documented; however, MCFA do not appear to significantly alter fecal microbial populations using the methods and under the conditions of this study. It is possible that significant changes associated with added

MCFA may be detected in the fecal microbiota when fed for longer than 14 d. Additionally, further characterization of the microbiome, using metagenomic sequencing or the evaluation of non-bacterial microbial composition, may reveal a more meaningful impact of MCFA on the gut microbiome. Nonetheless, the current study suggests that the positive impact associated with feeding MCFA in swine nursery diets is not directly related to substantial changes in the fecal microbial populations. Further investigations into the role of added dietary MCFA on both growth performance and gastrointestinal bacterial flora are warranted.

**Table 1. Diet composition (as-fed basis)[1]**

| Item | Phase 1 |
|---|---|
| Ingredient, % | |
| Corn | 54.92 |
| Soybean meal, 46.5% crude protein | 26.38 |
| Whey powder | 10.00 |
| Soybean oil | 1.50 |
| Calcium carbonate | 0.95 |
| Monocalcium phosphate, 21% P | 1.30 |
| Salt | 0.60 |
| Crystalline amino acids[2] | 1.15 |
| Vitamins and trace minerals | 0.40 |
| Phytase[3] | 0.07 |
| Zinc oxide | 0.25 |
| HP 300[4] | 2.50 |
| Hexanoic acid[5] | +/- |
| Octanoic acid[5] | +/- |
| Decanoic acid[5] | +/- |
| Total | 100 |
| | |
| Calculated analysis[6] | |
| Standardized ileal digestible (SID) amino acids, % | |
| Lysine | 1.35 |
| Isoleucine:lysine | 56 |
| Leucine:lysine | 111 |
| Methionine:lysine | 37.4 |
| Methionine and cysteine:lysine | 58.2 |
| Threonine:lysine | 63.0 |
| Tryptophan:lysine | 20.1 |
| Valine:lysine | 70.3 |
| Total lysine, % | 1.48 |
| Metabolizable energy (ME), kcal/lb | 1,519 |
| Net energy (NE), kcal/lb | 1,135 |
| SID lysine:ME, g/Mcal | 4.03 |
| SID lysine:NE, g/Mcal | 5.40 |
| Crude protein, % | 20.6 |
| Calcium, % | 0.75 |
| Phosphorus, % | 0.68 |
| Standardized total track digestible phosphorus, % | 0.57 |

[1]Diets were fed from approximately 15- to 24-lb body weight.
[2]Crystalline amino acids including L-lysine HCl, DL-methionine, L-threonine, L-tryptophan, and L-valine.
[3]HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided an estimated release of 0.12% STTD P.
[4]HP300 (Hamlet Protein, Findlay, OH).
[5]Sigma Aldrich (St. Louis, MO), guaranteed ≥ 98% purity added at the expense of soybean oil.
[6]NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

**Table 2. Analyzed diet composition (as-fed basis)**

| Analyzed composition, %[1] | Control | 1.5% MCFA blend[2] |
|---|---|---|
| Dry matter | 89.15 | 88.58 |
| Crude protein | 20.90 | 20.45 |
| Acid detergent fiber | 3.90 | 2.30 |
| Ether extract | 4.40 | 2.15 |
| Calcium | 0.91 | 0.95 |
| Phosphorus | 0.76 | 0.71 |
| Hexanoic acid | 0.02 | 0.47 |
| Octanoic acid | 0.01 | 0.54 |
| Decanoic acid | 0.01 | 0.66 |
| Total MCFA[3] | 0.03 | 1.68 |

[1]Complete diet samples were collected following feed manufacture using a feed probe to create a composite sample, subsample, and submit to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis and the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for medium chain fatty acid (MCFA) analysis performed in duplicate. Reported values are the average of duplicate analysis.
[2]Sigma Aldrich (St. Louis, MO). MCFA blend consisted of a 1:1:1 blend of C6, C8, and C10.
[3]Sum of analyzed C6, C8, and C10 medium chain fatty acids.

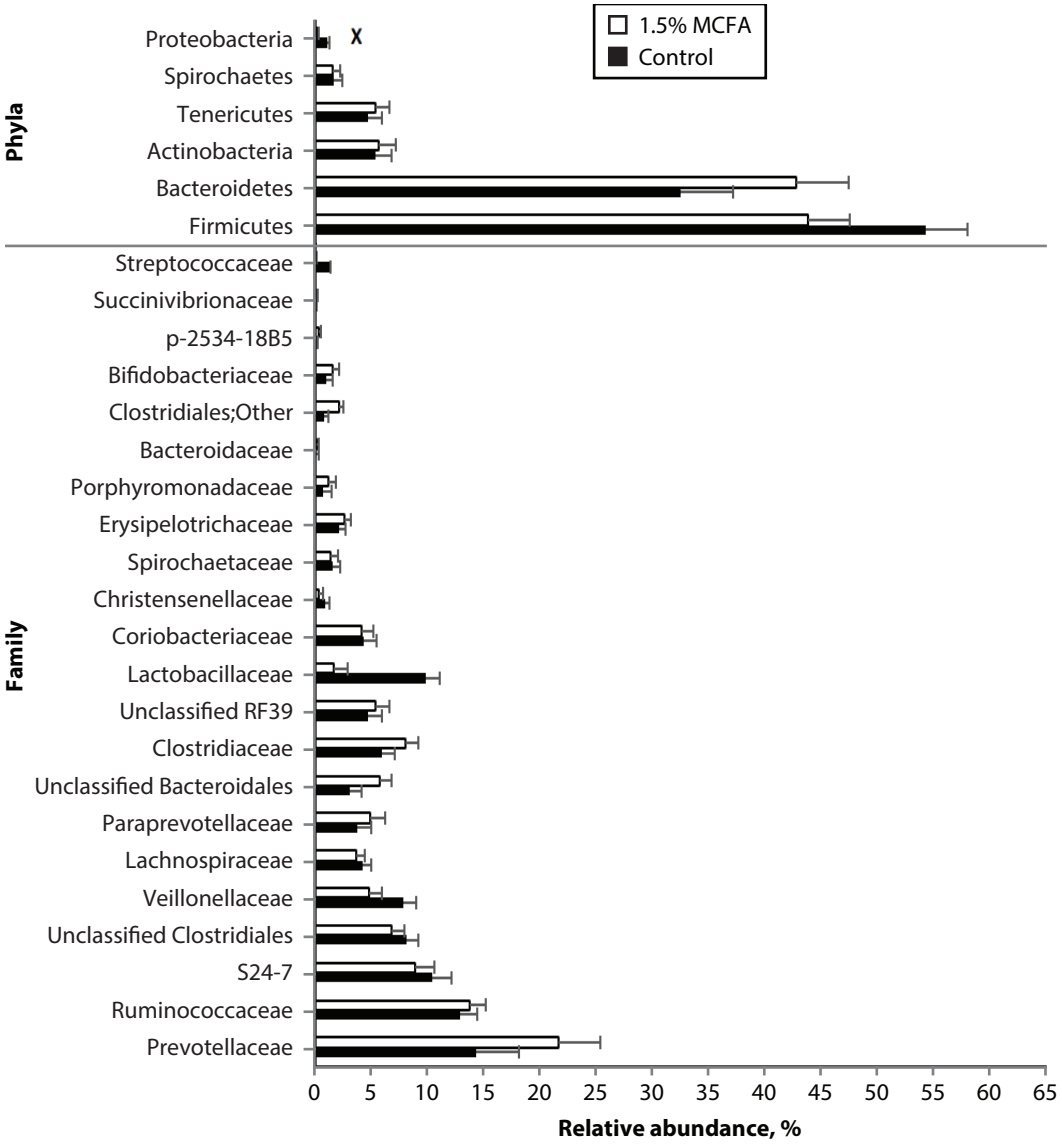


**Figure 1. Relative abundance of microbial phyla and families, d 14. Treatment diets were fed to a total of 360 pigs [DNA 400 × 200 (Columbus, NE); initial BW = 14.8 ± 0.15 lb] for 14 d with 5 pigs per pen and 9 pens per treatment. A total of 18 pigs were randomly selected for sampling of fecal material for characterization of fecal microbial populations. Pens of pigs assigned to be fed the control diet and pens assigned to be fed 1.5% medium chain fatty acid (MCFA) blend [1:1:1 blend of C6, C8, and C10; Sigma Aldrich (St. Louis, MO)] diet with 9 pigs sampled per treatment. Sampling of the same set of pigs occurred on d 0 and d 14. Samples were analyzed using 16s rDNA sequencing. Phyla and families lacking at least one treatment × day combination having a relative abundance of ≤ 1% are not shown.**

[x]Treatment × day interaction, $P = 0.080$; no evidence of a difference over time for pigs fed control diets ($P = 0.848$), whereas a marginally decrease over time was observed in pigs fed the 1.5% MCFA blend diet ($P = 0.092$). All other treatment × day, $P \geq 0.123$.

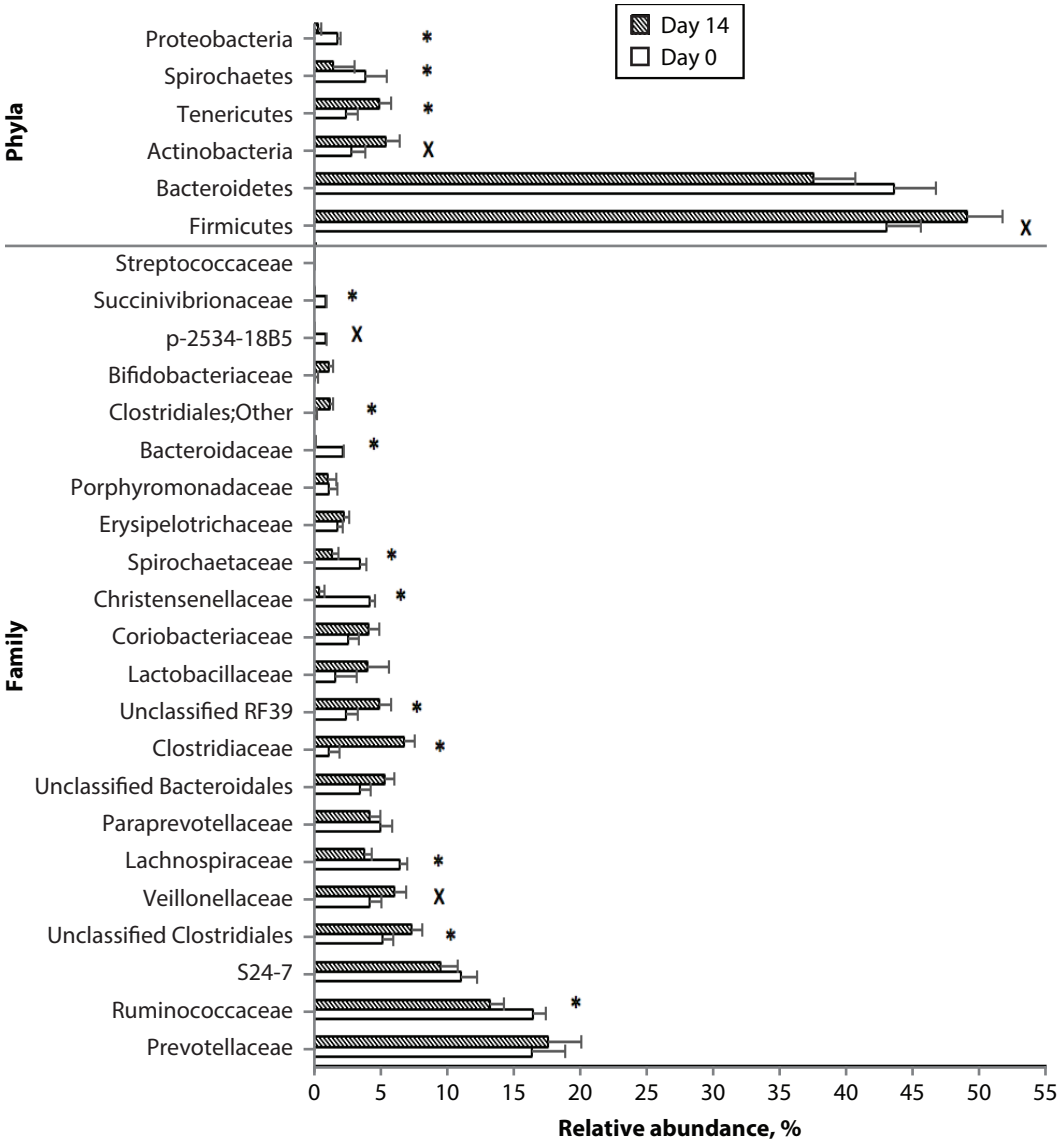


**Figure 2. Relative abundance of microbial phyla and families by day. Treatment diets were fed to a total of 360 pigs [DNA 400 × 200 (Columbus, NE); initially = 14.8 ± 0.15 lb] for 14 d with 5 pigs per pen and 9 pens per treatment. A total of 18 pigs were randomly selected for sampling of fecal material for characterization of fecal microbial populations. Pens of pigs assigned to be fed the control diet and pens assigned to be fed 1.5% medium chain fatty acid (MCFA) blend [1:1:1 blend of C6, C8, and C10; Sigma Aldrich (St. Louis, MO)] diet with 9 pigs sampled per treatment. Sampling of the same set of pigs occurred on d 0 and d 14. Samples were analyzed using 16s rDNA sequencing. Microbial phyla and families lacking at least one treatment × day combination having a relative abundance of ≤ 1% are not shown.**

*Main effect of day, $P < 0.05$.

xMain effect of day, $0.05 < P \leq 0.10$.