RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: Assessing the role of medium chain fatty acids as an alternative to medically important antibiotic **NPB #17-049**

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Industry Summary:

Research has demonstrated that medium chain fatty acids (MCFA) can serve as reduction strategies for bacterial and viral pathogens in animal feed and ingredients. However, it is unknown how the type or level of MCFA impact bacteria growth. Furthermore, it is not known if MCFA can serve as an antibiotic alternative to therapeutic doses of antibiotic used in the swine industry. The objective of this study was to assess the role of medium chain fatty acids as an alternative to medically important antibiotics by 1) evaluating fatty acid concentrations of commercially-available or based MCFA products and evaluation of bactericidal activity through the development of a low-cost benchtop model and 2) compare the efficacy of MCFA vs. therapeutic chlortetracycline supplementation in feed for disease-challenged pigs.

From the first objective, it was determined that the MIC of MCFA varied among bacteria species. The lowest MIC of the MCFA was 0.43% of a 1:1:1 blend of C6:0, C8:0, and C10:0 for *Campylobacte*r coli, 0.25% C12:0 for *Clostridium* perfringens, 0.60% 1:1:1 blend for generic *Escherichia* coli, 0.53% C6:0 for ETEC, and 0.40% C6:0 for *Salmonella* Typhimurium. It was also noted that the commercially based product containing higher concentrations of C6:0 or C8:0 had lower MIC in gram negative bacteria.

For the second objective, 100 entire male pigs (initially 14.1 ± 1.6 lb BW and weaned at 22 days of age) were used in a 29-day disease challenge study. Pigs were allowed 5 acclimation days, followed by 2 days of disease challenge with enterotoxigenic β-hemolytic Escherichia. coli, serotype O149:K91: K88 (ETEC). After the challenge, pigs were allotted to a diet with 1 of 5 treatments: 1) Control with no additives, 2) 400 g/ton CTC (Chlortet 200G, Eco Animal Health, London, United Kingdom), 3) 1.08% of a 1:1:1 blend of C6:0, C8:0, and C10:0 (Nuscience Group, Drongen, Belgium), 4) 3.93% developmental Product A (Nuscience Group, Drongen, Belgium), and 5) 1.04% developmental Product B (Kemin Industries, Des Moines, IA). Treatments 3, 4, and 5 were included at rates to derive a 1% MCFA concentration in finished feed. Pigs were fed treatment diets for 14 days following the disease challenge to mimic a therapeutic dose of CTC and fed a common diet from d 14 to

21. There was no effect of dietary treatment on growth performance form d 0 to 7 or d 14 to 21. From d 7 to 14, pigs fed diets supplemented with CTC, 1:1:1 blend, or Product B had improved F:G compared to those fed the control diet.
Pigs fed diets with CTC had increased fecal shedding of ETEC from d 7 to 14, while those fed diets with Product B having less fecal ETEC shedding on d 1 compared to d 14. While other disease markers, such as fecal score, plasma urea nitrogen, and haptoglobin, decreased with time, they were not affected by dietary treatment.

Overall, the inhibitory efficacy of MCFA varies among bacteria species. This suggest that MCFA mixtures may provide a wider spectrum of bacterial control. It was also noted that supplementing ETEC-challenged nursery pigs with MCFA-based dietary treatments led to similar growth performance as a therapeutic dose of 400 g/ton of CTC. As commercial products containing MCFA become available for livestock, it is important to consider the interaction between MCFA chain length and concentration on the potential to effectively mitigate various feed-based bacteria.

Keywords: Medium chain fatty acids, minimum inhibitory concentration, bacteria, chlortetracycline, enterotoxigenic E. coli

Scientific Abstract

Research has demonstrated that medium chain fatty acids (MCFA) can serve as reduction strategies for bacterial and viral pathogens in animal feed and ingredients. However, it is unknown how the type or level of MCFA impact bacteria growth. Furthermore, it is not known if MCFA can serve as an antibiotic alternative to therapeutic doses of antibiotic used in the swine industry. The objective of this study was to assess the role of medium chain fatty acids as an alternative to medically important antibiotics by 1) evaluating fatty acid concentrations of commercially-available or based MCFA products and evaluation of bactericidal activity through the development of a low-cost benchtop model and 2) compare the efficacy of MCFA vs. therapeutic chlortetracycline supplementation in feed for disease-challenged pigs.

From the first objective, it was determined that the MIC of MCFA varied among bacteria species. The lowest MIC of the MCFA was 0.43% of a 1:1:1 blend of C6:0, C8:0, and C10:0 for *Campylobacte*r coli, 0.25% C12:0 for *Clostridium* perfringens, 0.60% 1:1:1 blend for generic *Escherichia* coli, 0.53% C6:0 for ETEC, and 0.40% C6:0 for *Salmonella* Typhimurium. It was also noted that the commercially based product containing higher concentrations of C6:0 or C8:0 had lower MIC in gram negative bacteria.

For the second objective, 100 entire male pigs (initially 14.1 ± 1.6 lb BW and weaned at 22 days of age) were used in a 29-day disease challenge study. Pigs were allowed 5 acclimation days, followed by 2 days of disease challenge with enterotoxigenic β-hemolytic Escherichia. coli, serotype O149:K91: K88 (ETEC). After the challenge, pigs were allotted to a diet with 1 of 5 treatments: 1) Control with no additives, 2) 400 g/ton CTC (Chlortet 200G, Eco Animal Health, London, United Kingdom), 3) 1.08% of a 1:1:1 blend of C6:0, C8:0, and C10:0 (Nuscience Group, Drongen, Belgium), 4) 3.93% developmental Product A (Nuscience Group, Drongen, Belgium), and 5) 1.04% developmental Product B (Kemin Industries, Des Moines, IA). Treatments 3, 4, and 5 were included at rates to derive a 1% MCFA concentration in finished feed. Pigs were fed treatment diets for 14 days following the disease challenge to mimic a therapeutic dose of CTC and fed a common diet from d 14 to 21. There was no difference ($P > 0.10$) of dietary treatment on growth performance form d 0 to 7 or d 14 to 21. From d 7 to 14, pigs fed diets supplemented with CTC, 1:1:1 blend, or Product B had improved ($P < 0.05$) F:G compared to those fed the control diet, with pigs fed diets with Product A being intermediate. A treatment × day interaction for the ETEC fecal shedding was observed ($P < 0.05$), which was driven by pigs fed diets with CTC having decreased ($P < 0.05$) fecal shedding on d 7 than 14, while those fed diets with Product B having greater ($P < 0.05$) fecal ETEC shedding on d 1 than d 14. While other disease markers, such as fecal score, plasma urea

nitrogen, and haptoglobin, decreased ($P < 0.05$) with time, they were not affected ($P > 0.05$) by dietary treatment.

Overall, the inhibitory efficacy of MCFA varies among bacteria species. This suggest that MCFA mixtures may provide a wider spectrum of bacterial control. It was also noted that supplementing ETEC-challenged nursery pigs with MCFA-based dietary treatments led to similar growth performance as a therapeutic dose of 400 g/ton of CTC. As commercial products containing MCFA become available for livestock, it is important to consider the interaction between MCFA chain length and concentration on the potential to effectively mitigate various feed-based bacteria.

Introduction

There is increasing consumer and regulatory pressure to reduce feed-based antibiotic use in food animals (Center for Disease Control, 2013; Landers et al., 2012). As stewards of animal health, pork producers are challenged to reduce their reliance on antimicrobials, particularly when pigs are faced with a disease challenge. There is concern regarding the use of CTC which represents 61% of the volume of highly-important antibiotics and 42% of the total antibiotic use in swine feed (Apley et al., 2012). Even with these regulations in place, the future potential use of antibiotics in feed is unknown. Thus, pork producers are looking for alternatives to medically-important antibiotics, particularly those used therapeutically at weaning to maintain animal health. Several classes of feed additives have antimicrobial properties, including probiotics, prebiotics, enzymes, acidifiers, plant extracts, and nutraceuticals (Thacker, 2013). One such alternative includes medium chain fatty acids (MCFA), specifically C6:0, C8:0, and C10:0. These MCFA have recently demonstrated mitigation potential against PEDV and bacteria (Cochrane et al., 2016; Dee et al., 2016; Cochrane et al., 2017;). A 2%, and 1% inclusion of a 1:1:1 ratio of C6:0, C8:0, and C10:0 as well as the inclusion of 0.66% of the C6:0, C8:0, and C10:0 individually, led to a reduction of detectable PEDV RNA and prevent infection within a swine bioassay (Cochrane et al., 2018). The same effect has also been noted in bacterial species as a 2% inclusion of the 1:1:1 blend utilized by Cochrane et al., (2016) led to a 2-log reduction of Salmonella Typhimurium inoculated feed ingredients. This demonstrates the potential for MCFA to serve as a potential antibiotic alternative. However, there is a lack of information regarding which specific MCFA is the most effective, whether combinations of different MCFA exhibit additive effects, and what the optimal level of MCFA is that will impact various bacteria associated with animal production. This can be determined utilizing a minimum inhibitory concentration (MIC) benchtop assay, which identifies the lowest concentration of a treatment that prevents visible growth of a bacterium. Once the MIC values are determined, the information can then be used to develop an inclusion level for the MCFA to serve as an antibiotic alternative. Therefore, the objective of this study was to assess the role of medium chain fatty acids as an alternative to medically important antibiotics by 1) evaluating fatty acid concentrations of commercially-available or based MCFA products and evaluation of bactericidal activity through the development of a low-cost benchtop model and 2) compare the efficacy of MCFA vs. therapeutic chlortetracycline supplementation in feed for disease-challenged pigs.

Objective

Primary objective: Assess the role of medium chain fatty acids as an alternative to medically important antibiotics.

Sub-objectives to complete the primary objective:

1. Evaluate fatty acid concentration of commercially-available products and evaluation of bactericidal activity through the development of a low-cost benchtop model.
2. Compare the efficacy of MCFA vs. therapeutic chlortetracycline supplementation in feed for disease-challenged pigs.

Materials and Methods

Sub-objective 1

Bacterial inoculum

Bacterial strains of generic *Escherichia* coli (*E*. coli) ATCC 25922, Enterotoxigenic *Escherichia* coli (ETEC) 3030-2, and *Salmonella enterica* serotype Typhimurium (*S*. Typhimurium) ATCC 14028 were grown using Luria Bertani, *Campylobacter* coli (*C*. coli) 7A #2016-1 using Mueller-Hinton, and *Clostridium* perfringens (*C.* perfringens) 4026 using anaerobic Brain Heart Infusion broth medium at 37°C for 24 h. For *E*. coli, ETEC, *S.* Typhimurium, and *C.* coli, 1 ml of bacterial inoculum was serially diluted using 9 ml of PBS to achieve one concentrations (10^5 CFU/ml) for each bacterial strain. For *Clostridium* perfringens, the bacterial concentration was adjusted to 0.5 McFarland Standards using fresh Brain Heart Infusion broth medium per Clinical and Laboratory Standards Institute recommendations (2013).

Experiment 1 MIC Determination of MCFA

For *E*. coli, ETEC, *S.* Typhimurium, and *C.* coli the compounds tested were C6:0, C8:0, C10:0, and a 1:1:1 blend of C6:0, C8:0, and C10:0. For *C.* perfringens, the compounds tested were C6:0, C8:0, C10:0, and C12:0. The MIC were determined by the micro-broth dilution method as per Clinical Laboratory Standards Institute CLSI guidelines (2013) in E. coli, *ETEC*, *S.* Typhimurium, and *C.* coli from 0.1% until an MIC was established, with a maximum tested level of 1.0%. The MIC was also determined using the same method for *C*. perfringens, with a maximum tested level of 2.0%. There were three replications per product and bacteria combination.

Experiment 2 MCFA Profiles and MIC Determination of Commercially-Based Products

The fatty acid profile of 21 commercially-based products was analyzed, with an emphasis on the MCFA concentration. The 24 products were, 1.) Product A, 2.) Product B 3.) Product C, 4.) Product D, 5.) Product E, 6.) Product F, 7.) Product G, 8.) Product H, 9.) Product I, 10.) Product J, 11.) Product K, 12.) Product L, 13.) Product M, 14.) Product N, 15.) Product O, 16.) Product P, 17.) Product Q, 18.) Product R, 19.) Coconut Oil, 20.) Palm Oil, and 21.) Palm Kernel Oil. Samples were analyzed according to procedures outlined by Sukhija and Palmquist (1988). From this analysis, Product A, B, G, H, and a commodity fat source (coconut oil) were selected as having representative MCFA profiles for use in MIC assays. The profiles were selected based on products having the highest concentrations of C6:0 and C8:0 within the fatty acid profile and coconut oil because of its natural source of MCFA and medium chain triglycerides. The MIC were determined as described in Exp. 1 in *E*. coli, *ETEC*, *S.* Typhimurium, and *C.* coli from 0.1% until an MIC was established, with a maximum tested level of 5.0%. There were three replications per product and bacteria combination.

Experiment 3 Quantification of Enterotoxigenic Escherichia coli-inoculated feed after treatment with two commercially-based MCFA-containing products

Based on their lower MIC compared to other products tested in Exp. 2, Products A and B were selected as treatments to determine their reduction capacity in swine feed inoculated with ETEC. The strain of *ETEC* was first made resistant to 50 µl/ml nalidixic acid (Nal^R) antibiotic before being used for inoculation. A complete swine diet was either left un-inoculated and untreated, or mixed with 0.00, 0.25, 0.50, 1.00, or 2.00% Product A or B and inoculated with *ETEC*. For inoculation, 1 g of each feed sample was mixed with 1 ml of Nal^R *ETEC* at one of two concentrations (10^6 or 10^2 CFU per g of feed) of bacteria. The higher concentration was utilized for quantification of *ETEC* and the lower for detection. The 10 treatments were: 1.) control feed with no bacteria; 2). Control feed inoculated with bacteria and no addition of an additive; 3.) 0.25% Product A; 4.) 0.5%, Product A; 5.) 1.0%, Product A; 6). 2%. Product A; 7.) 0.5% Product B; 8.) 1.0% Product B; 9.) 2.0% Product B; and 10.) 4.0% Product B. The levels for each product were selected based on the results of Exp. 2. Product A was tested at a lower inclusion level in the feed because of the lower MIC value established in Exp. 2. Product B

was then tested at higher inclusion levels because of the higher MIC value that was established in Exp. 2. It was also determined that treatment 1 was confirmed to be negative of ETEC and was not included in the statistical model.
Samples were incubated at 37°C for 24 h. Then, 1 g of the incubated feed containing bacterial inoculum was suspended in 9 ml of PBS, serially diluted, and plated onto MacConkey agar containing nalidixic acid. The plates were incubated at 37°C for 24 h for bacterial enumeration using a standard plate count for viable cells. There were three replications per product and bacteria combination.

Statistical Analysis

Data from each MIC experiment were analyzed as a completely randomized design using PROC GLIMMIX in SAS to evaluate the effect of each treatment within each bacterium. If the MIC value was greater than the detection limit of the analysis, the next logical inclusion level (increase in 0.1% inclusion) was utilized for the statistical analysis. For Exp. 3, the PROC GLIMMIX procedure of SAS was utilized to evaluate linear and quadratic contrasts of increasing product levels. The coefficients for the unequally spaced linear and quadratic contrasts utilized in Exp. 3 were derived using the PROC IML procedure in SAS. In all experiments, results for treatment criteria were considered significant at $P \leq 0.05$.

Sub-objective 2

This study was approved by the Animal Ethics Committee of Murdoch University, Murdoch Western Australia (R2969/17).

Animals and Housing

A total of 100 entire male pigs (Large White × Landrace: initially 6.4 ± 0.72 kg weaned at an average of 22 days of age) were used in a 29-d disease challenge study to evaluate MCFA as a potential alternative to CTC. Pigs were obtained from a commercial operation on the day of weaning and transported to the Murdoch University research facility. Upon arrival, pigs were weighed, allotted to pens based on body weight, and fecal rectal swabs were collected for baseline levels of β-hemolytic Escherichia. Coli (ETEC). Pens were equipped with a 5-hole, dry self-feeder, and a pan waterer to provide ad libitum access to feed and water. Pigs were allowed 5 days of acclimation on a corn and soybean meal common diet (d -7 to -2; Table 6). On d -2, pigs were weighed, and randomly allotted to dietary treatments based on BW with 5 pigs per pen and 4 pens per treatments. Blood was collected from 2 random pigs per pen to establish baseline blood metabolites for plasma urea nitrogen, C-reactive protein, and haptoglobin.

Infection Procedures

All pigs were oral inoculated with ETEC according to Heo et al., (2009) on d -2 and d -1. Briefly, a strain of enterotoxigenic β-hemolytic E. coli, serotype O149:K91:K88 (variants STa and STb), was grown, selected, and incubated. The resultant pellet was suspended, placed into gelatin capsules, and held on dry ice until use. On day -2 and -1, each pig received two capsules of inoculum, for a total of 1600 μL. Enterotoxigenic β-hemolytic E. coli concentration of the capsules on d -2 was 2.56×109 CFU/mL, and on d -1 was 8.80×108 CFU/mL.

Experimental Design and Treatment Diets

On d 0, the common diet used during acclimation was changed to include one of the following treatments: 1) no additives (control); 2) 400 g/ton CTC (Chlortet 200G, Eco Animal Health, London, United Kingdom),; 3) 1.08% of a 1:1:1 blend of C6:0, C8:0, and C10:0 (contained 32.7% C6:0, 33.7% C8:0, 33.3% C10:0 Nuscience Group, Drongen, Belgium); 4) 3.93% developmental Product A (contained 2.95% C6:0, 12.3% C8:0, 10.1% C10:0; Nuscience Group, Drongen, Belgium), and 5) 1.04% developmental Product B (contained 3.9% C6:0, 54.2% C8:0, 38.5% C10:0; Kemin Industries, Des Moines, IA). Treatments 3, 4, and 5 were included at rates to

derive a 1% MCFA concentration in finished feed. Dietary treatments were fed for 14 d, then pigs were fed a common commercial pelleted diet (Farmyard Pig Weaner, Weston Milling, Perth Western Australia) from d 14 to 21. The commercial pelleted diet contained 20% CP, 1.2% Lysine, 14.5 MJ/kg digestible energy, 0.85% calcium, and no added zinc oxide or antibiotics. Diet samples were collected and analyzed for DM, CP, crude fiber, EE, Ca, and P by Agrifood technology (Bibra Lake Western Australia) (Table 7). Chlortetracycline levels in the feed were analyzed at Symbio Laboratories (Sydney, Australia). Diets were also analyzed for MCFA concentration by Fatty Acid Methyl Estes Gas Chromatography at the Department of Primary Industries (Wagga Wagga, New South Wales Australia).

Clinical Disease Characterization

Pigs and feeders were weighed on d -2, 0, 7, and 14 of the trial to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). Pigs were evaluated daily for fecal scores using the following systems: 1) firm, well-formed feces, 2) soft formed feces, 3) soft and loose shape, or 4) watery liquid consistency. In accordance with animal ethics application (R2969/17), if a pig exhibited a diarrhea score of 4 for 48 hours, it was treated with Moxylan (Amoxycillin, Jurox, Rutherford, New South Wales Australia). Each pig treated received three doses of the Moxylan. One pig in each of the control, 1:1:1 MCFA blend, and Product A groups was treated, while 2 pigs were treated in the CTC and Product B groups. Fecal shedding of ETEC was evaluated according to Heo et al., (2009) by fecal swabs collected on d -7, -2, 0, 1, 3, 7, and 14. Swabs were plated using a 5-zone streaking method, incubated overnight, and scored from 0 to 5 with 0 representing no growth and 5 representing growth out to the fifth section. Blood samples were collected on d -2, 7, and 14 from 2 pigs per pen according to Stensland et al., (2015). Briefly, samples were collected via jugular vein puncture into a lithium heparin tube. Tubes were centrifuged at 3000 × g for 10 min at room temperature, plasma collected, and stored at -20°C until analyzed for plasma urea nitrogen (PUN), haptoglobin, and C-reactive protein (C-RP). The PUN was determined using a Beckman Coulter/Olympus Reagent Kit (OSR6134) and haptoglobin by In-House Method NTM-62 (Eckersall et la., 1991). Both PUN and Haptoglobin analysis were performed on an Olympus Clinical Chemistry Analyzer. The PUN and Haptoglobin were analyzed by Animal Health Labs (Department of Primary Industries and Regional Development, South Perth, Western Australia). C-reactive protein was analyzed using a DuoSet ELISA (R&D systems for Porcine C-Reactive Protein/CRP cat No: DY2648) and analyzed at Murdoch University (Murdoch Western Australia).

Statistical Analysis

Data was analyzed as a completely randomized design with pens randomly allotted to treatment based on BW. Pen was considered the experimental unit. Fecal scores and enterotoxigenic β-hemolytic E. coli fecal shedding scores were analyzed as repeated measures across day. Unequal spaced analysis days for enterotoxigenic β-hemolytic E. coli fecal shedding scores were accounted for within the statistical model. All possible pairwise comparisons were protected by the Tukey-Kramer adjustment. Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant from $P > 0.05$ to $P \leq 0.10$. Data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC).

Results and Discussion (see attached tables)

Sub-objective 1

Experiment 1

The MIC of each MCFA in C. coli, C. perfringens, E. coli, ETEC, and S. Typhimurium, are presented in Table 1. The MIC for C. coli was lower ($P < 0.05$) in C6:0, C8:0, or the MCFA blend than in C10:0. In C. perfringens,

the longer chain fatty acids were more effective with C12:0 and C10:0 providing the lowest ($P < 0.05$) MIC results with C12 being the most effective ($P < 0.05$) overall. Within generic E. coli, the 1:1:1 MCFA blend of C6:0, C8:0, and C10:0 provided the lowest ($P < 0.05$) MIC value followed by C6:0 and C8:0. Within Enterotoxigenic E. coli, C6:0 had a lower ($P < 0.05$) MIC than C8:0, which was still lower ($P < 0.05$) than either C10:0 or the MCFA blend, which were greater than the maximum tested value of 1%. In Salmonella Typhimurium, C6:0 resulted in an MIC similar ($P > 0.05$) to C8:0. However, C6 did differ ($P < 0.05$) from the blend. Again, no MIC was determined for C10:0 within S. Typhimurium.

Experiment 2

The fatty acid profile varied widely in the 21 commercially-based products (Table 2). Based on these analysis, Product A, B, F, G, and coconut oil were selected as candidate products for MIC determination in gram negative bacteria due to their high concentrations of C6:0 and C8:0. In C. coli, the MIC for Product B was lower ($P < 0.05$) than either Product F or G, with Product A being intermediate (Table 3). Product A and B had lower ($P < 0.05$) MIC in generic E. coli, ETEC, and Salmonella Typhimurium than other tested products. The MIC for coconut oil was not detected in any bacteria as it was greater than the maximum tested level of 5.0%.

Experiment 3
Due to their efficacy in the MIC determination, Product A and B were selected as treatments to determine their effect on detectable or quantifiable ETEC in feed. In the higher concentration of bacteria, Product A resulted in a linear decrease (linear, $P < 0.05$) in the number of quantifiable bacteria (Table 4). For Product B, as the inclusion level increased, the number of quantifiable bacteria quadratically decreased ($P < 0.05$). In the lower concentration of bacteria, Product A again resulted in a linear decrease (linear, $P < 0.05$) in the number of quantifiable bacteria (Table 5). However, in Product B no linear or quadratic response was observed ($P > 0.10$).

Sub-objective 2

Dietary treatment did not impact ($P > 0.10$) body weight, ADG, ADFI, and F:G from d 0 to 7 (Table 8). From d 7 to 14, pigs fed diets supplemented with CTC, 1:1:1 blend, or Product B all had greater ($P < 0.05$) F:G than pigs fed the control diet, with pigs fed Product A being intermediate ($P > 0.10$). This led to pigs being fed CTC or Product B having improved ($P < 0.05$) F:G during the entire treatment phase, from d 0 to 14. This effect continued after treatment diets ended, where pigs fed diets containing Product B had marginally significant greater ($P < 0.10$) F:G than those fed the control diet from d 0 to 21.

A treatment × day interaction for the ETEC fecal shedding was observed ($P < 0.05$; Table 9). This was driven by pigs fed diets with CTC having decreased ($P < 0.05$) fecal shedding on d 7 than 14, while those fed diets with Product B having greater ($P < 0.05$) fecal ETEC shedding on d 1 than d 14. While other disease markers, fecal score, PUN, and haptoglobin, decreased ($P < 0.05$) with time, there was no evidence for ($P > 0.10$) effects of dietary treatment or the interaction between treatment and time. A decrease ($P < 0.05$) in fecal scores (2.6, 1.9, and 1.4) was notated as time increased on d 0, 3, and 7 respectively with no further reduction beyond d 6. A decrease ($P < 0.05$) in PUN (2.8 to 2.2) and haptoglobin (0.7 to 0.1) were noted from on d -2 to 14 respectively. No evidence for C-RP was observed in the experiment ($P > 0.10$).

Conclusion

In summary MCFA mixtures may provide a wider spectrum of bacterial control. As commercial products containing MCFA become available for livestock, it is important to consider the interaction between MCFA chain length and concentration on the potential to effectively mitigate various feed-based bacteria. Supplementing ETEC-challenged nursery pigs with MCFA-based dietary treatments led to similar growth performance as a therapeutic dose of 400 g/ton of CTC. Further research is needed to confirm the mode of

action, most effective MCFA or combination, and effective dose of medium chain fatty acids in ETEC-challenged pigs.

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I. Tables

Table 1. Minimum inhibitory concentration of medium chain fatty acids in generic *Escherichia* coli, Enterotoxigenic *Escherichia* coli (ETEC), *Salmonella enterica* serotype Typhimurium, and *Clostridium* perfringens[1]

Item	MIC, %	SEM	*P*-Value
Campylobacter coli		0.047	0.0004
C6:0	0.50[b]		
C8:0	0.47[b]		
C10:0	0.90[a]		
1:1:1 Blend	0.43[b]		
Clostridium perfringens		0.030	< 0.0001
C6:0	1.65[a]		
C8:0	0.85[b]		
C10:0	0.70[c]		
C12:0	0.25[d]		
Generic *E*. coli		0.014	<.0001
C6:0	0.70[a]		
C8:0	0.85[b]		
C10:0[2]	> 1.00[c]		
1:1:1 Blend	0.60[d]		
Enterotoxigenic *E. coli*		0.024	<.0001
C6:0	0.53[c]		
C8:0	0.67[b]		
C10:0[2]	> 1.00[a]		
1:1:1 Blend[2]	> 1.00[a]		
Salmonella Typhimurium		0.050	<.0001
C6:0	0.40[c]		
C8:0	0.50[cb]		
C10:0[2]	> 1.00[a]		
1:1:1 Blend	0.60[b]		

[1] Minimum inhibitory concentration for C6:0, C8:0, C10:0, and a 1:1:1 blend of C6:0, C8:0, and C10:0 were tested in E. coli, ETEC, S. Typhimurium, and C. coli using a 96 well microtiter plate with a concentration of 10^5 CFU/ml for each bacterial strain. For C. perfringens, the compounds tested were C6:0, C8:0, C10:0, and C12:0 utilizing a 96 well microtiter plate with a concentration of 0.5 McFarland Standards for each well. Each value is represented by an N=3.

[2] Minimum inhibitory concentration was above the tested detection limit and therefore the next logical inclusion level (increase in 0.1% inclusion) was utilized for the statistical analysis.

[abcd] Means within a bacterial species lacking a common superscript differ ($P < 0.05$).

Table 2. Medium chain fatty acid profiles for the tested products (mg/g).

Item	Total analyzed fatty acids	C6:0	C8:0	C10:0	C12:0
Product A[1]	294.58	29.53	123.20	101.43	40.23
Product B[2]	1092.66	43.12	610.28	436.50	2.15
Product C[1]	123.07	12.35	51.42	42.28	16.85
Product D[3]	303.36	8.43	103.64	88.92	86.81
Product E[3]	369.33	9.02	123.38	105.61	111.06
Product F[3]	603.77	27.37	248.7	206.41	120.18
Product G[3]	494.34	0.98	227.13	188.00	74.50
Product H[4]	362.92	0.09	1.32	1.16	359.47
Product I[5]	349.54	2.19	159.32	131.10	56.71
Product J[5]	101.32	0.00	41.42	34.03	25.70
Product K[5]	402.37	0.20	128.21	99.30	122.71
Product L[7]	983.16	0.02	0.02	0.04	0.19
Product M[7]	520.80	3.78	40.87	31.21	227.83
Product N[5]	158.76	1.8	69.72	57.91	19.36
Product O[5]	145.57	1.74	68.08	56.43	18.56
Product P[5]	317.48	4.78	151.41	129.46	31.33
Product Q[5]	2.78	0.00	0.02	2.60	0.00
Product R[5]	314.01	0.69	101.44	83.01	90.15
Coconut Oil[6]	894.09	6.82	72.07	53.74	409.62
Palm Oil[6]	894.34	0.00	0.51	0.22	2.35
Palm Kernel Oil[6]	918.84	2.83	37.86	33.21	418.05

[1] Nuscience Group, Ghent (Drongen), Belgium
[2] Kemin Industries, Des Moines, IA.
[3] PMI Nutritional Additives, Arden Hills, Minnesota, USA
[4] Framelco, Raamsdonksveer, Netherlands
[5] Nutreco, Amersfoort, Netherlands
[6] ADM, Chicago, Illinois
[7] Cargill, Minneapolis, MN

Table 3. Minimum inhibitory concentration of commercially - based medium chain fatty acid based products in generic *Escherichia* coli, Enterotoxigenic *Escherichia* coli (ETEC), and *Salmonella enterica* serotype Typhimurium[1]

Item	MIC, %	SEM	*P*-Value
Campylobacter coli		0.629	0.0026
Product A[2]	1.20[cd]		
Product B[3]	0.33[d]		
Product F[2]	2.75[bc]		
Product G[2]	3.33[ab]		
Coconut oil[4,5]	> 5.0[a]		
Generic *E. coli*		0.424	<.0001
Product A[2]	0.37[c]		
Product B[3]	1.20[c]		
Product F[2]	3.33[b]		
Product G[2]	4.17[ab]		
Coconut oil[4,5]	> 5.0[a]		
Enterotoxigenic *E. coli*		0.309	< .0001
Product A[2]	0.33[c]		
Product B[3]	1.30[c]		
Product F[2]	3.83[b]		
Product G[2]	4.33[ab]		
Coconut oil[4,5]	> 5.0[a]		
Salmonella Typhimurium		0.308	<.0001
Product A[2]	0.47[c]		
Product B[3]	1.30[c]		
Product F[2]	3.83[b]		
Product G[2]	4.33[ab]		
Coconut oil[4,5]	> 5.0[a]		

[1] Minimum inhibitory concentration for products (Product A, B, F, G, and Coconut oil were tested in *E*. coli, *ETEC*, *S*. Typhimurium, and C. coli using a 96 well microtiter plate with a concentration of 10^5 CFU/ml for each bacterial strain. Each value is represented by an N=3.

[2] Nuscience Group, Ghent (Drongen), Belgium

[3] Kemin Industries, Des Moines, IA.

[4] ADM, Chicago, Illinois

[5] Minimum inhibitory concentration was above the tested detection limit and therefore the next logical inclusion level (increase in 0.1% inclusion) was utilized for the statistical analysis.

[abcd] Means within a bacteria species lacking a common superscript differ ($P < 0.05$).

Table 4. Effects of commercially-based products containing medium chain fatty acids on the growth of 10^6 CFU/g feed Enterotoxigenic Escherichia coli (ETEC)[1]

Item	Log CFU/g	SEM	Linear	Quadratic
Product A[2]		0.011	<.0001	0.9641
0.00%	5.44			
0.25%	5.37			
0.50%	5.24			
1.00%	5.15			
2.00%	4.81			
Product B[3]		0.017	<.0001	<.0001
0.00%	5.44			
0.50%	5.19			
1.00%	5.14			
2.00%	4.71			
4.00%	3.49			

[1] Product A and B were tested in a concentration of 10^6 CFU/g of feed ETEC in a complete swine diet in order to determine the growth of that bacteria using MacConkey agar containing nalidixic acid for bacterial enumeration
[2] Nuscience Group, Ghent (Drongen), Belgium
[3] Kemin Industries, Des Moines, IA.

Table 5. Effects of commercially-based products containing medium chain fatty acids on the growth of 10^2 CFU/g feed Enterotoxigenic Escherichia coli (ETEC)[1]

Item	Log CFU/g	SEM	Linear	Quadratic
Product A[2]		0.007	0.0060	0.1180
0.00%	2.95			
0.25%	2.93			
0.50%	2.93			
1.00%	2.95			
2.00%	2.91			
Product B[3]		0.012	0.1041	0.1579
0.00%	2.95			
0.50%	2.90			
1.00%	2.93			
2.00%	2.91			
4.00%	2.91			

[1] Product A and B were tested in a concentration of 10^2 CFU/g of feed ETEC in a complete swine diet in order to determine the growth of that bacteria using MacConkey agar containing nalidixic acid for bacterial enumeration
[2] Nuscience Group, Ghent (Drongen), Belgium
[3] Kemin Industries, Des Moines, IA.

Table 6. Formulated composition of the basal diet (as-fed basis)[1]

Item	Control
Ingredient, %	
Corn	55.00
Soybean meal (48%)	21.87
Fish Meal	4.00
HP 300	5.0
Whey	10.00
Soybean oil	1.00
Monocalcium phosphate	1.20
Limestone	1.23
Sodium chloride	0.60
L-Lys-HCl	0.35
DL-Met	0.18
L-Thr	0.18
L-Trp	0.03
L-Val	0.05
Trace mineral and vitamin premix[2]	0.10
Total	100.00
Calculated analysis	
Standard ileal digestible (SID) amino acids, %	
Lys	1.35
Ile:lys	59
Leu:lys	119
Met:lys	37
Met & Cys:lys	58
Thr:lys	65
Trp:lys	19
Val:lys	68
SID lysine:ME, g/Mcal	4.43
ME, kcal/lb	1,532
Total lysine, %	1.50
CP, %	22.1
Ca, %	0.79
P, %	0.75
Available P, %	0.47

[1] The basal diet was fed to all pigs from d -7 to 0 and for the control group of pigs. The basal diet was also used for each treatment diet in which corn was replaced with the respective treatments. The CTC treatment was included at 400g/ton, 1:1:1 MCFA blend at 1.1%, Product A at 3.9%, and Product B at 1.0%. In each instance, the same percentage of corn was removed and replaced with the addition of the treatments. The 1:1:1 MCFA blend, Product A, and Product b were included to reach a total MCFA inclusion level of 1.0%

[2] BJ Grower (Biojohn Pty Ltd, Perth, WA, Australia) Provided the following nutrients (per kg of premix) Vitamins: A 5300 IU, D3 1000 IU, E 46.67g, K 1.33 g, B1 1.33 g, B2 3.33 g, Niacin 16.67 g, B5 28.72 g, B6 1.67 g, folic acid 0.67 g, B12 13.33 mg, and biotin 66.67 mg. Minerals: Co 0.33 g (as cobalt sulfate), Cu 13.33 g (as copper sulfate), iodine 0.67 g (as potassium iodine), iron 40 g (as ferrous sulfate), Mn 26.67 g (as manganous oxide), Se 0.2 g (as sodium selenite), Se inorganic 0.07g (as selenosource), Se organic 0.13 g (as selenosource), and Zn 66.67 g (as zinc sulphate).

Table 7. Analyzed diet composition (as-fed basis)[1]

Analyzed composition, %	Control	CTC[2]	1:1:1 MCFA Blend[3]	Product A[4]	Product B[5]
DM	91.3	91.3	91.0	90.0	90.7
CP	21.6	21.8	20	22.1	22.2
Crude Fiber	1.9	2.1	1.7	1.6	1.9
Total Fat	3.4	3.4	3.4	3.2	3.5
Ca	0.93	1.50	0.87	0.86	1.10
P	0.76	0.72	0.74	0.79	0.74
CTC	0.00	0.04	0.00	0.00	0.00
C6:0	0.01	0.01	0.28	0.09	0.01
C8:0	0.02	0.01	0.33	0.38	0.44
C10:0	0.03	0.03	0.30	0.32	0.35
Total MCFA[6]	0.06	0.05	0.91	0.79	0.80

[1] Complete diet samples were collected following feed manufacture, subsampled, and submitted to Agrifood Technology (Bibra Lake Western Australia for proximate analysis. The samples were also analyzed for MCFA concentration at the Department of Primary Industries (Wagga Wagga New South Wales Australia)

[2] Formulated to contain the regulatory limit of chlortetracycline (400g/ton). Analyzed value for the CTC diet was 356 g/ton or 0.0356%.

[3] 1:1:1 ratio of C6:0, C8:0, and C10:0 formulated to contain 1% of MCFA in the complete diet. Each fatty acid supplied from Nuscience Group, Ghent (Drongen), Belgium.

[4] Formulated to contain 1% of MCFA in the complete diet (Nuscience Group, Ghent (Drongen), Belgium).

[5] Formulated to contain 1% of MCFA in the complete diet (Kemin Industries, Des Moines, IA).

[6] Sum of analyzed C6, C8, and C10 medium chain fatty acids.

Table 8. Effects of Chlortetracycline and MCFA Treatments on Nursery Pig Performance.[1]

Item;	Control	CTC	1:1:1 MCFA Blend[2]	Product A[3]	Product B[4]	SEM	*P* =
BW, lb[5]							
d 0	16.4	16.1	16.4	16.4	16.1	0.447	0.9669
d 7	23.2	23.1	22.7	23.3	21.7	0.845	0.6756
d 14	31.9	33.0	32.5	31.5	30.8	1.045	0.6251
d 21	41.1	41.9	42.3	41.6	40.3	1.151	0.7690
d 0 to 7							
ADG, lb[6]	0.97	0.99	0.90	0.98	0.80	0.064	0.2456
ADFI, lb[7]	0.96	1.02	0.96	0.99	0.81	0.075	0.3380
F:G[8]	1.00	1.03	1.07	1.01	1.00	0.035	0.6118
d 7 to 14							
ADG, lb	1.24	1.43	1.41	1.18	1.30	0.070	0.0848
ADFI, lb	1.94	1.67	1.84	1.64	1.56	0.101	0.0740
F:G	1.58^{a}	1.18^{b}	1.31^{b}	1.39^{ab}	1.20^{b}	0.052	0.0004
d 14 to 21							
ADG, lb	1.31	1.26	1.40	1.44	1.35	0.076	0.5341
ADFI, lb	1.89	1.95	1.99	2.00	1.94	0.076	0.8753
F:G	1.46	1.57	1.42	1.39	1.43	0.053	0.2323
d 0 to 14							
ADG, lb	1.11	1.21	1.15	1.08	1.05	0.054	0.3153
ADFI, lb	1.46	1.34	1.40	1.31	1.18	0.078	0.1746
F:G	1.33^{a}	1.11^{b}	1.21^{ab}	1.22^{ab}	1.12^{b}	0.035	0.0043
d 0 to 21							
ADG, lb	1.17	1.23	1.24	1.20	1.15	0.044	0.5065
ADFI, lb	1.60	1.55	1.60	1.55	1.43	0.066	0.3408
F:G	1.37^{x}	1.26^{xy}	1.29^{xy}	1.29^{xy}	1.24^{y}	0.017	0.0582

[1] A total 100 entire male pigs (Large White × Landrace: initially 14.1 ± 1.6 lbs weaned at an average of 22 days of age) were used in a 29 - day disease challenge study to evaluate MCFA as a potential antibiotic alternative to CTC. The pigs were acclimated for 6 days (d -7 to -2) before receiving 2 capsules of ETEC inoculum each on d -2 and -1 for a total of 4 capsules. During the acclimation phase and inoculation phase, pigs received a basal diet. Treatment diets were then fed from d 0 to 14 and then placed onto a commercial pelleted diet for the final 7 grow out days.

[2] 1:1:1 ratio of C6:0, C8:0, and C10:0. Each fatty acid supplied from Nuscience Group, Ghent (Drongen), Belgium.

[3] Nuscience Group, Ghent (Drongen), Belgium.

[4] Kemin Industries, Des Moines, IA.

[5] Body weight.

[6] Average Daily Gain.

[7] Average Daily feed intake.

[8] Feed to Gain ratio.

[ab] Means within a row lacking a common superscript differ ($P < 0.05$). All possible pairwise comparisons were protected by the Tukey-Kramer adjustment.

[xy] Means within a row lacking a common superscript differ ($P \leq 0.10$). All possible pairwise comparisons were protected by the Tukey-Kramer adjustment.

Table 9. Interactive means of Treatments × Day on Enterotoxigenic E. coli fecal shedding. [1]

Item;	Control	CTC	1:1:1 MCFA Blend[2]	Product A[3]	Product B[4]	SEM	*P* =
Pre-inoculation[5]						0.3020	<.0001
d -7	0.00	0.25	0.15	0.20	0.00		
Inoculation[6]							
d -2	0.10	0.10	0.00	0.05	0.20		
Treatment phase							
d 0	0.65^{abcde}	1.00^{abcde}	1.10^{abcde}	1.25^{abcde}	1.05^{abcde}		
d 1	0.75^{abcde}	1.30^{abcde}	1.05^{abcde}	1.55^{abcde}	1.80^{ac}		
d 3	0.90^{abcde}	0.45^{abcde}	1.25^{abcde}	0.35^{abcde}	0.95^{abcde}		
d 7	0.80^{abcde}	0.10^{bcd}	0.10^{abcde}	0.25^{abcde}	1.25^{abcde}		
d 14	0.90^{abcde}	1.40^{ae}	0.70^{abcde}	0.70^{abcde}	0.05^{bde}		

[1] Fecal rectal swabs were collected on each pig on d -7, -2, 0, 1, 3, 7, and 14 by inserting a cotton swab into the anus of the pig. The swabs were then plated using a 5-zone streaking method in which each swab was streaked onto zone 1 of the plate. A wire loop was then utilized to streak from zone 1 to zone 5. The wire loop was sanitized before moving to the next zone. Plates were incubated overnight at 37°C. The plates were scored on a scale of 0 to 5 according to the number of sections containing viable hemolytic E. coli where 0 was no growth and 5 was growth out to the fifth section. Statistical analysis was only completed on samples taken during the treatment phase. Means presented are the interaction of treatment and day.
[2]1:1:1 ratio of C6:0, C8:0, and C10:0. Each fatty acid supplied from Nuscience Group, Ghent (Drongen), Belgium.
[3] Nuscience Group, Ghent (Drongen), Belgium.
[4] Kemin Industries, Des Moines, IA.
[5] Baseline levels were taken for each pig on the day of arrival.
[6] Baseline levels were taken for each pig prior to receiving the ETEC inoculum.
[abcde]Means within a row and column lacking a common superscript differ ($P < 0.05$). All possible pairwise comparisons were protected by the Tukey-Kramer adjustment.