

Evaluating Medium Chain Fatty Acids as an Alternative to Chlortetracycline in Nursery Pig Diets¹

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Summary

An experiment was conducted to evaluate medium chain fatty acids (MCFA) as a potential alternative to chlortetracycline (CTC) in nursery pigs. One hundred entire male pigs (initially 14.1 ± 1.6 lb body weight (BW) and weaned at 22 d of age) were used in a 29-d disease challenge study. Pigs were allowed 5 acclimation days after weaning, followed by 2 d of disease challenge with Enterotoxigenic β -hemolytic *Escherichia coli* (ETEC), serotype O149:K91:K88. 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, USA). 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 evidence of difference ($P > 0.10$) of dietary treatment on growth performance from d 0 to 7 or d 14 to 21. From d 7 to 14, pigs fed diets with added 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 intermediate. A treatment \times 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 d 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. In conclusion, 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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Introduction

There is increasing consumer and regulatory pressure to reduce feed-based antibiotic use in food animals.^{5,6} As stewards of animal health, pork producers are challenged to reduce their reliance on antimicrobials, particularly when pigs are faced with a disease challenge. Antibiotics, such as chlortetracycline (CTC), are highly effective at reducing mortality and morbidity of nursery pigs challenged by disease, and their removal from the diet leaves pork producers concerned about both profitability and animal well-being.^{7,8} 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.⁹ Even with new federal 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.⁸ 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.^{10,11} A 2% and 1% inclusion of a 1:1:1 ratio of C6:0, C8:0, and C10:0 as well as 0.66% of the C6:0, C8:0, and C10:0 individually, led to a reduction of detectable PEDV RNA and prevented infection within a swine bioassay.¹² This demonstrates the potential for MCFA to work both in vivo and in vitro. 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 in inoculated feed ingredients.¹⁰ More recently, minimum inhibitory concentrations (MIC) of MCFA against *Campylobacter coli*, *Clostridium perfringens*, generic *Escherichia coli*, Enterotoxigenic *E. coli*, and *Salmonella* Typhimurium were established.¹² However, it was determined that MIC of MCFA varied among bacterial species.¹² The ability of MCFA to mitigate bacterial species, including Enterotoxigenic *E. coli*, and serve as an antimicrobial replacement in swine diets is unknown. Therefore, the objective of this study was to compare the efficacy of MCFA vs. a therapeutic dose of chlortetracycline in feed for Enterotoxigenic *E. coli*-challenged pigs.

Procedures

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

⁵Centers for Disease Control. 2013. Antibiotic resistance threats in the United States. Electronic. <http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf>.

⁶Landers et al. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Public Health Rep. 127(1):4-22. doi: 10.1177/003335491212700103.

⁷Thacker. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. J Anim Sci Biotechnol. 4(1):1. doi:10.1186/2049-1891-4-35.

⁸Fouhse et al. 2016. The role of gut microbiota in the health and disease of pigs. Animal Frontiers. 6(3):30-36. doi: 10.2527/af.2016-0031.

⁹Apley et al. 2012. Use estimates of in-feed antimicrobials in swine production in the United States. Foodborne Pathog. Dis. 9(3):272-279. doi: 10.1089/fpd.2011.0983.

¹⁰Cochrane et al. 2016. Evaluating chemical mitigation of *Salmonella* Typhimurium in animal feed ingredients. J Food Prot. 79(4):672-676.

¹¹Dee et al. 2016. Modeling the transboundary risk of feed ingredients contaminated with porcine epidemic diarrhea virus. BMC Vet. Rsrch. 12:51-63. doi: /10.1186/s12917-016-0674-z.

¹²Cochrane et al. 2018. Young scholar presentation: A review of medium chain fatty acids and their recent role in feed safety. J. Anim. Sci. 96 (E2):55. doi:10.1093/jas/sky073.103.

Animals and Housing

A total of 100 male pigs (Large White × Landrace (initially 6.4 ± 0.72 kg)) were weaned at an average of 22 d of age and 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 ETEC. Pens were equipped with a 5-hole, dry self-feeder, and a pan waterer to provide *ad libitum* access to feed and water. All pigs were allowed 5 d of acclimation on a corn and soybean meal diet (d -7 to -2; Table 1). On d -2, pigs were weighed, and randomly allotted to dietary treatments based on BW with 5 pigs per pen and 4 pens per treatment. Blood was collected from 2 randomly selected pigs per pen to establish baseline blood concentrations for plasma urea nitrogen, C-reactive protein, and haptoglobin.

Infection Procedures

All pigs were orally inoculated with ETEC according to Heo et al. (2009) on d -2 and -1. Briefly, a strain of Enterotoxigenic β -hemolytic *E. coli*, serotype O149:K91:K88 (variants STa and STb), was grown, selected, and incubated.¹³ The resultant pellet was suspended, placed into gelatin capsules, and held on dry ice until use. On days -2 and -1, each pig received two capsules of inoculum, for a total of 1600 μ L. Enterotoxigenic β -hemolytic *E. coli* concentration of the capsules on d -2 was 2.56×10^9 CFU/mL and on d -1 was 8.80×10^8 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, USA). 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, 1,570 kcal/lb digestible energy, 0.85% calcium, and no added zinc oxide or antibiotics. Diet samples were collected and analyzed for dry matter (DM), crude protein (CP), crude fiber, ether extract, Ca, and P by Agrifood Technology (Bibra Lake, Western Australia) (Table 2). 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).

¹³Heo et al. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidences of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*. J. Anim. Sci. 87:2833-2843. doi: 10.2527/jas.2008-1274.

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 (F:G). 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 h, it was treated with Moxylan (Amoxicillin, 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.¹³ Blood samples were collected on d -2, 7, and 14 from 2 pigs per pen according to Stensland et al. (2015).¹⁴ Briefly, samples were collected via jugular vein puncture into a lithium heparin tube. Tubes were centrifuged at $3000 \times g$ 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-62.¹⁵ 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 were 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

Dietary treatment did not impact ($P > 0.10$) body weight, ADG, ADFI, and F/G from d 0 to 7 (Table 3). From d 7 to 14, pigs fed diets supplemented with CTC, 1:1:1 blend, or Product B all had improved ($P < 0.05$) F/G compared with pigs fed the control diet, with pigs fed Product A intermediate ($P > 0.10$). This led to pigs fed CTC or Product

¹⁴Stensland et al. 2015. A comparison of diets supplemented with a feed additive containing organic acids, cinnamaldehyde and a permeabilizing complex, or zinc oxide, on post-weaning diarrhea, selected bacterial populations, blood measures and performance in weaned pigs experimentally infected with enterotoxigenic *E. coli*. *Animals (Basel)*. 5(4):1147-1168. doi: 10.3390/ani5040403.

¹⁵Eckersall et al. 1991. An automated biochemical assay for haptoglobin: Prevention of interference from albumin. *Comp Haematol Int*. 9: 117–124 doi: 10.1007/BF02600369

B having improved ($P < 0.05$) F/G during the entire treatment phase (d 0 to 14). This effect continued after treatment diets ended, where pigs previously fed diets containing Product B had marginally significant improved ($P < 0.10$) F/G compared with those fed the control diet from d 0 to 21.

A treatment \times day interaction for the ETEC fecal shedding was observed ($P < 0.05$; Table 4). This was driven by pigs fed diets with CTC having decreased ($P < 0.05$) fecal shedding on d 7 compared with 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. Decreases ($P < 0.05$) in PUN (2.8 to 2.2 mmol/L) and haptoglobin (0.7 to 0.1 mg/dL) were noted from on d -2 to 14 respectively. No evidence for C-RP was observed in the experiment ($P > 0.10$).

In conclusion, supplementing ETEC-challenged nursery pigs with MCFA-based dietary treatments led to a similar improvement in F/G 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 MCFA in ETEC-challenged pigs.

Table 1. 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

continued

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

Item	Control
Calculated analysis	
Standardized ileal digestible (SID) amino acids, %	
Lys	1.35
Ile:lys	59
Leu:lys	119
Met:lys	37
Met and 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
Crude protein, %	22.1
Ca, %	0.79
P, %	0.75
Available P, %	0.47

¹The 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 400 g/ton, 1:1:1 medium chain fatty acids (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%.

²BJ Grower (Biojohn Pty Ltd, Perth, Western Australia, Australia). Provided the following nutrients (per kg of premix) Vitamins: A 5300 IU, D3 1000 IU, E 46.67 g, 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, Diamond V, Cedar Rapids, IA), Se organic 0.13 g (as SelenoSource, Diamond V, Cedar Rapids, IA), and Zn 66.67 g (as zinc sulfate).

Table 2. Analyzed diet composition (as-fed basis)¹

Analyzed composition, %	Control	CTC ²	1:1:1 MCFA		
			blend ³	Product A ⁴	Product B ⁵
Dry matter	91.3	91.3	91.0	90.0	90.7
Crude protein	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

¹Complete 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 medium chain fatty acids (MCFA) concentration at the Department of Primary Industries (Wagga Wagga, New South Wales, Australia).

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

³1:1:1 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.

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

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

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

Table 3. Effects of chlortetracycline and medium chain fatty acids (MCFA) treatments on nursery pig performance¹

Item	Control	CTC	1:1:1 MCFA			SEM	P =
			blend ²	Product A ³	Product B ⁴		
BW, lb							
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	0.97	0.99	0.90	0.98	0.80	0.064	0.2456
ADFI, lb	0.96	1.02	0.96	0.99	0.81	0.075	0.3380
F:G	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

¹A total of 100 male pigs (Large White × Landrace; initially 14.1 ± 1.6 lb weaned at an average of 22 days of age) were used in a 29 - day disease challenge study to evaluate medium chain fatty acids (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 pigs were placed onto a commercial pelleted diet for the final 7 grow out days.

²1:1:1 ratio of C6:0, C8:0, and C10:0. Each fatty acid supplied from Nuscience Group, Ghent (Drongen), Belgium.

³Nuscience Group, Ghent (Drongen), Belgium.

⁴Kemin Industries, Des Moines, IA, USA.

^{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 4. Interactive means of treatments × day on Enterotoxigenic *E. coli* fecal shedding¹

Item	Control	CTC	1:1:1 MCFA			SEM	P =
			blend ²	Product A ³	Product B ⁴		
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}		

¹Fecal 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.

²1:1:1 ratio of C6:0, C8:0, and C10:0. Each fatty acid was supplied from Nuscience Group, Ghent (Drogen), Belgium.

³Nuscience Group, Ghent (Drogen), Belgium.

⁴Kemin Industries, Des Moines, IA, USA.

⁵Baseline levels were taken for each pig on the day of arrival.

⁶Baseline 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.