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Effects of Butyrate-Based Feed Additives on Nursery Pig Performance, Fecal Consistency, Blood Criteria, and Short Chain Fatty Acid Production[1]

Ethan B. Stas, Ying Chen,[1] Ross Wolfenden,[1] Jamil E. G. Faccin, Jason C. Woodworth, Mike D. Tokach, Joel M. DeRouchey, Robert D. Goodband, and Jordan T. Gebhardt[2]

Summary

A total of 300 pigs (241 × 600, DNA, initially 12.2 lb) were used to evaluate the effects of butyrate-based feed additives on nursery pig performance, fecal consistency, blood criteria, and short chain fatty acid (SCFA) concentration in the duodenum, jejunum, ileum, and cecum. At weaning, pigs were allotted to one of six dietary treatments based on initial body weight. There were five pigs per pen and 10 replications per treatment. Dietary treatments were arranged in a one-way treatment structure consisting of a negative control, a positive control, and four additional diets containing various butyrate-based feed additives. The negative control was a standard corn-soybean meal-based diet with no antibiotics or pharmacological levels of Zn or Cu. The positive control contained 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2 respectively, and 250 ppm of Cu from $CuSO_4$ in phase 3. The positive control also contained 55 ppm of carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) across all three phases. The four butyrate-based feed additives were added at 0.1% to the negative control diet and consisted of encapsulated butyrate, monobutyrin, mono + tributyrin, and tributyrin (Eastman Chemical, Kingsport, TN). The mono + tributyrin additive contained 71.5% monobutyrin and 28.5% tributyrin. Pigs were fed phase 1 diets for 10 d (d 0 to 10), phase 2 diets for 14 days (d 10 to 24), and phase 3 diets for 14 d (d 24 to 38). Overall (d 0 to 38), pigs fed the positive control had increased ($P < 0.05$) ADG and ADFI compared to the other treatments. In addition, pigs fed mono + tributyrin had improved ($P < 0.05$) F/G compared to pigs fed monobutyrin with other treatments intermediate. There was a treatment × day interaction ($P = 0.042$) for fecal DM where on d 5, there were no differences in fecal DM between treatments ($P > 0.10$); however, on d 10, pigs fed the positive control had increased ($P < 0.05$) fecal DM compared to all other treatments. On d 38, pigs fed encapsulated butyrate had increased ($P < 0.05$) haptoglobin concentration compared to pigs fed the positive control with the other treatments intermediate. There was a treatment × day interaction observed ($P < 0.001$)

[1] Appreciation is expressed to Eastman Chemical (Kingsport, TN) for partial financial support of this trial.

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for vitamin E where pigs fed tributyrin had higher ($P < 0.05$) vitamin E concentration on d 10 compared to pigs fed the negative control, the positive control, and monobutyrin with the other treatments intermediate, and no differences observed on d 38. Pigs fed mono + tributyrin had higher ($P < 0.05$) butyrate concentrations in the cecum compared to pigs fed the negative control with all other treatments intermediate. In summary, nursery pigs fed pharmacological levels of Zn and Cu along with carbadox improved growth performance as expected. Various butyrate-based feed additives had similar effects on nursery pig performance and SCFA in the small intestine. However, mono + tributyrin was associated with higher butyrate levels in cecum, potentially benefiting the epithelial cells.

Introduction

In the swine industry much attention has been directed toward gastrointestinal health of pigs to reduce use of antibiotics and minimize bacterial resistance. Pathogenic bacteria such as *Salmonella* and *Escherichia coli* are causing economic losses for producers throughout the industry. In addition, there are growing concerns with the use of pharmacological levels of Zn. In the European Union, use of pharmacological levels of Zn has been banned in swine diets due to environmental heavy metal accumulation, and other countries are considering similar regulations.

Short chain fatty acids (SCFA) in diets of nursery pigs could serve as a method to improve gastrointestinal health and performance. Epithelial cells line the digestive system and are important for nutrient absorption. Butyrate is the main source of energy for epithelial cells and as a result plays a key role in nutrient utilization.[3] Butyrate has been shown to improve intestinal epithelial permeability and modulation of pro-inflammatory cytokines.[4] It has also shown an enhancement of epithelial morphology, homeostasis, and microbiota balance.[5] Other studies have shown butyrate can reduce the transmission of pathogenic bacteria in pigs.[6] However, additional research is warranted to evaluate the effects of butyrate, supplied from different sources, on nursery pig performance and if performance will be similar to that from feeding pharmacological levels of Zn, Cu and/or antibiotics. Therefore, the objective of this study was to evaluate the effects of butyrate-based feed additives on nursery pig performance, fecal consistency, blood criteria, and SCFA in the duodenum, jejunum, ileum, and cecum.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Each pen was

[3] Chapman, M. A., M. F. Grahn, M. Hutton, and N. S. Williams. 1995. Butyrate metabolism in the terminal ileal mucosa of patients with ulcerative colitis. Br. J. Surg. 82:36–38. doi:10.1002/bjs.1800820115

[4] Guilloteau, P., L. Martin, V. Eeckhaut, R. Ducatelle, R. Zabielski, and F. Van Immerseel. 2010. From the gut to the peripheral tissue: the multiple effects of butyrate. Nutr. Res. Rev. doi:10.1017/S0954422410000247.

[5] Fang, C. L., H. Sun, J. Wu, H. H. Niu, and J. Feng. 2014. Effects of sodium butyrate on growth performance, haematological and immunological characteristics of weanling piglets. J. Anim. Physiol. Anim. Nutr. (Berl.). 98:680–685. doi:10.1111/jpn.12122

[6] De Ridder, L., D. Maes, J. Dewulf, F. Pasmans, F. Boyen, F. Haesebrouck, E. Méroc, P. Butaye, and Y. Van der Stede. 2013. Evaluation of three intervention strategies to reduce the transmission of Salmonella Typhimurium in pigs. Vet. J. 197:613–618. doi:10.1016/j.tvjl.2013.03.026

equipped with a 4-hole, dry self-feeder and nipple waterer to provide ad libitum access to feed and water.

Animals and diets

A total of 300 pigs (241 × 600, DNA, initially 12.2 lb) were used in 38-d nursery trial. Pigs were weaned at approximately 19 d of age and placed in pens of five pigs each based on initial weight and gender. At weaning, pigs were randomly allotted to one of six dietary treatments with 10 replications per treatment. Dietary treatments were fed in three phases and arranged in a one-way treatment structure consisting of a negative control, a positive control, and four additional diets containing various butyrate-based feed additives (Table 1). The negative control was a standard corn-soybean meal-based diet with no antibiotics or pharmacological levels of Zn or Cu. The positive control contained 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2 respectively, and 250 ppm of Cu from $CuSO_4$ in phase 3. The positive control also contained 55 ppm of carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) across all three phases. The four butyrate-based feed additives were added at 0.1% to the negative control diet and consisted of encapsulated butyrate, monobutyrin, mono + tributyrin (71.5% monobutyrin and 28.5% tributyrin), and tributyrin (Eastman Chemical, Kingsport, TN). Diets were formulated to contain 1.35% (phase 1 and 2) and 1.30% (phase 3) SID Lys and met or exceeded other nutrient requirement estimates established by the NRC (2012).[7] Dietary treatments were fed for 10 d in phase 1 (d 0 to 10), 14 d in phase 2 (d 10 to 24) and 14 d in phase 3 (d 24 to 38). The basal diets for phases 1 and 2 were manufactured at Hubbard Feeds in Beloit, KS. The basal diets were divided into six batches and feed additives were added and mixed at the Kansas State University O.H. Kruse Feed and Technology Center in Manhattan, KS to form six experimental diets. Phase 3 diets were manufactured at Hubbard Feeds in Beloit, KS. Individual pig weights and feed disappearance were measured on d 10 and weekly thereafter to determine ADG, ADFI, and F/G.

Fecal samples were collected on d 5, 10, 17, and 24 to determine fecal dry matter (DM) percentage from the same three medium weight pigs from each pen. After collection, fecal samples were dried at 55°C (131°F) in a forced-air oven and the ratio of dried to wet fecal weight determined the fecal percentage DM.

Fecal scores were evaluated from fecal samples collected on d 5, 10, 17, and 24. Each fecal sample was given a fecal score from 0 to 4, with 0 being a firm stool and 4 severe diarrhea. Fecal scores were evaluated by the same person on all four collection days.

Blood was collected from the same medium weight pig per pen on d 10 and 38 into one serum blood tube and K2 EDTA anti-coagulant blood collection tube. Serum blood tubes were spun down using a centrifuge and serum was submitted for haptoglobin and vitamin E analysis. Whole blood tubes were submitted for a complete blood count (CBC) analysis. Both serum and whole blood were submitted to the Kansas State Veterinary Diagnostic Laboratory in Manhattan, KS.

After the 38-d study, six pigs per treatment group were humanely euthanized via penetrating captive bolt. Approximately 4 mL of digesta was taken from the duodenum, proximal jejunum, ileum, and cecum for analysis of short chain fatty acids (SCFA). The

[7] NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

pigs euthanized for digesta collection were the same pigs bled on d 10 and 38. Digesta was analyzed using gas chromatography and measured concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaporate, caporate, and heptanoate. Any sample with non-detectable levels of SCFA was assumed to have a concentration of 0.50 μmol (lowest detection limit).

Statistical analysis

Data were analyzed as a completely randomized design using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) using R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen as the experimental unit. Differences between treatments were analyzed as a pairwise comparison. Fecal DM, haptoglobin, vitamin E, and CBC were analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. Fecal score data were analyzed as categorical outcomes using a generalized linear mixed model using a multinomial response distribution using a cumulative logit link function. Treatment, day, and the associated interactions were considered fixed effects within the statistical model. Data were analyzed as repeated measures and pen was included in the model as a random intercept to account for subsampling attributed to the multiple evaluators on each day. Fecal score data were fit using the GLIMMIX procedure of SAS (version 9.4, Cary, NC) and data were summarized using the FREQ procedure and reported as percentage of observations within each fecal score category by treatment and day. Differences between treatments and day (where appropriate), as well as their interaction, were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

In phase 1 (d 0 to 10), pigs fed the positive control diet had increased ($P < 0.05$) BW and ADG compared to pigs fed encapsulated butyrate and mono + tributyrin with the other treatments intermediate (Table 2). Pigs fed the positive control diet had improved ($P < 0.05$) F/G compared to pigs fed encapsulated butyrate, mono + tributyrin, and tributyrin with the other treatments intermediate. In phase 2 (d 10 to 24), pigs fed the positive control diet had increased ($P < 0.05$) BW, ADG, and ADFI compared to all other treatments with no differences observed for F/G. In phase 3 (d 24 to 38), there was no treatment difference for ADG, but pigs fed the positive control diet had increased ($P < 0.05$) ADFI compared to mono + tributyrin and tributyrin with the other treatments intermediate. Pigs fed mono + tributyrin had improved ($P < 0.05$) F/G compared to pigs fed monobutyrin with the other treatments intermediate. Overall (d 0 to 38), pigs fed the positive control diet had increased ($P < 0.05$) BW and ADG compared to all other treatments. Pigs fed the positive control diet had increased ($P < 0.05$) ADFI compared the other treatments except pigs fed monobutyrin, which was intermediate. Pigs fed mono + tributyrin had improved ($P < 0.05$) F/G compared to pigs fed monobutyrin with the other treatments intermediate.

There was a treatment × day interaction observed ($P = 0.035$) for fecal DM where on d 5, there were no differences in fecal DM between treatments, but on d 10, pigs fed the positive control diet had increased ($P < 0.05$) fecal DM compared to all other treatments. On d 17, there were no differences in fecal DM between treatments. On d 24, while a treatment effect was present ($P = 0.003$), no treatment means were significantly different; however, pigs fed the positive control diet and mono + tributyrin had

the highest fecal DM. For day effect of fecal DM across all treatments, DM decreased ($P < 0.05$) from d 5 to 10 and then increased ($P < 0.05$) from d 10 to 17 with no differences ($P > 0.05$) from d 17 to 24. There was a treatment × day interaction observed ($P = 0.035$; data not shown) for fecal scores driven by the change in fecal consistency except for pigs fed the positive control diet. From d 5 to 10, pigs fed the negative control diet and butyrate-based feed additives shifted toward a looser consistency with the positive control remaining relatively unchanged. In most instances, fecal scores are reflective of fecal DM percentage.

There was no treatment × day interaction observed for haptoglobin (Table 2). On d 38, pigs fed encapsulated butyrate had increased ($P < 0.05$) haptoglobin concentration compared to pigs fed the positive control diet with the other treatments intermediate. Haptoglobin concentration increased ($P < 0.05$) from d 10 to 38 across all treatments. There was a treatment × day interaction observed ($P < 0.001$) for vitamin E where pigs fed tributyrin had higher ($P < 0.05$) vitamin E concentration on d 10 compared to pigs fed all other treatments (Table 2). However, there was no difference in vitamin E concentration between treatments on d 38 ($P > 0.05$). Vitamin E concentration decreased ($P < 0.001$) from d 10 to 38.

For CBC analysis, there was a treatment × day interaction ($P = 0.023$) for band neutrophil concentration where there were no differences between treatments on d 10, but pigs fed mono + tributyrin had increased ($P < 0.05$) band neutrophil concentration on d 38 compared to pigs fed all other treatments (Table 3). For eosinophil concentration on d 38, while a treatment effect was present ($P = 0.029$) no treatment means were significantly different, however pigs fed mono + tributyrin and tributyrin had the highest eosinophil concentration. For basophil concentration on d 38, pigs fed the negative control diet had increased ($P < 0.05$) basophil concentration compared to pigs fed the positive control diet with all other treatments intermediate. For hemoglobin and hematocrit concentration on d 10, while a treatment effect was present ($P \leq 0.046$) no treatment means were significantly different; however, pigs fed mono + tributyrin and tributyrin had the highest hemoglobin concentration. All other CBC criteria were not different between treatments. There was a significant main effect of day ($P < 0.05$) observed for white blood cell count, segmental neutrophil concentration, lymphocyte concentration, monocyte concentration, eosinophil concentration, red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean cellular hemoglobin concentration, red cell distribution width, packed cell volume, protein, and fibrinogen.

For SCFA analysis, there were only five pigs total that had detectable levels of acetate present in the duodenum with no differences between treatments (Table 4). Pigs fed mono + tributyrin and tributyrin did not have any detectable levels of acetate in the duodenum. In the jejunum, there were no differences between treatments for acetate concentration, although pigs fed the positive control diet were the only ones that did not have any detectable levels. In the ileum, there were no differences between treatments for acetate or butyrate concentration. For propionate, one pig fed encapsulated butyrate that had detectable levels in the ileum. In the cecum, there were no differences between treatments for acetate or propionate concentration. Pigs fed mono + tributyrin had higher ($P < 0.05$) butyrate concentration compared to pigs fed the negative control with all other treatments intermediate. Pigs fed monobutyrin had higher

($P < 0.05$) valerate concentration compared to pigs fed the positive control with all other treatments intermediate.

In conclusion, butyrate-based feed additives were not able to improve growth performance or fecal consistency to the level of the positive control. The various dietary treatments had minimal effects on blood criteria. However, pigs fed mono + tributyrin had greater butyrate in the cecum compared to the other treatments, potentially supplying more energy for intestinal epithelial cells.

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

	Phase 1		Phase 2		Phase 3	
Item	Negative Control[2]	Positive Control	Negative Control[2]	Positive Control	Negative Control[2]	Positive Control
Ingredients, %						
Corn	43.90	43.90	46.05	46.05	65.10	64.35
Soybean meal (46.5% CP)	21.95	21.95	30.75	30.75	31.30	31.30
Corn DDGS	5.00	5.00	7.50	7.50	---	---
Whey powder	10.00	10.00	---	---	---	---
Whey permeate	10.00	10.00	10.00	10.00	---	---
Microbial enhanced soybean meal[3]	3.50	3.50	---	---	---	---
Choice white grease	1.00	1.00	1.00	1.00	---	---
Limestone	0.60	0.20	0.80	0.45	0.70	0.35
Monocalcium phosphate (21% P)	1.15	1.15	1.00	1.00	0.95	0.95
Salt	0.30	0.30	0.50	0.50	0.60	0.60
L-Lys HCl	0.55	0.55	0.50	0.50	0.45	0.45
DL-Met	0.23	0.23	0.21	0.21	0.17	0.17
L-Thr	0.22	0.22	0.22	0.22	0.20	0.20
L-Trp	0.04	0.04	0.03	0.03	0.03	0.03
L-Val	0.15	0.15	0.12	0.12	0.10	0.10
Vitamin premix with phytase[4]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Zinc oxide	---	0.41	---	0.27	---	---
Copper sulfate	---	---	---	---	---	0.10
Mecadox[5]	---	1.00	---	1.00	---	1.00
Sand	1.01	---	0.92	---	---	---
Total	100	100	100	100	100	100

continued

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

	Phase 1		Phase 2		Phase 3	
Item	Negative Control[2]	Positive Control	Negative Control[2]	Positive Control	Negative Control[2]	Positive Control
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.30	1.30
Ile:Lys	55	55	57	57	57	57
Leu:Lys	112	112	118	118	119	119
Met:Lys	37	37	37	37	35	35
Met and Cys:Lys	55	55	57	57	57	57
Thr:Lys	63	63	64	64	64	64
Trp:Lys	19.1	19.1	19.2	19.2	19.1	19.1
Val:Lys	70	70	70	70	70	70
His:Lys	33	33	36	36	38	38
Total Lys, %	1.49	1.49	1.51	1.51	1.44	1.44
NE NRC, kcal/lb	1,135	1,135	1,111	1,111	1,101	1,101
SID Lys:NE, g/Mcal	5.39	5.39	5.51	5.51	5.36	5.36
CP, %	20.3	20.3	21.7	21.7	21.1	21.1
Ca, %	0.69	0.69	0.71	0.71	0.63	0.63
P, %	0.66	0.66	0.63	0.63	0.60	0.60
STTD P, %	0.58	0.58	0.51	0.51	0.46	0.46

[1] Phase 1 diets were fed from approximately 12 to 14 lbs. Phase 2 diets were fed from approximately 14 to 24 lbs. Phase 3 diets were fed from approximately 24 to 40 lbs.
[2] Encapsulated butyrate, monobutyrin, tributyrin, and mono + tributyrin (Eastman Chemical, Kingsport, TN) were all added at 0.1% of the negative control diet. Mono + tributyrin was approximately 71.5% monobutyrin and 28.5% tributyrin.
[3] ME-PRO, Prairie Aquatech, Brookings, SD.
[4] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 568 FTU/lb for all three phases.
[5] Mecadox (Phibro Animal Health Corp., Teaneck, NJ) provided 55 ppm of carbadox in the diet.

Table 2. Effects of Butyrate-based feed additives on nursery pig growth performance and fecal dry matter[1,2]

Protein source:	Negative Control[3]	Positive Control[4]	Encapsulated Butyrate[5]	Monobutyrin[5]	Mono + Tributyrin[5,6]	Tributyrin[5]	SEM	*P* =
BW, lb								
d 0	12.2	12.2	12.2	12.3	12.2	12.2	0.05	0.922
d 10	13.6^{ab}	14.2^{a}	13.4^{b}	13.7^{ab}	13.4^{b}	13.6^{ab}	0.29	0.013
d 24	23.6^{b}	27.6^{a}	23.3^{b}	25.0^{b}	23.8^{b}	23.6^{b}	0.57	< 0.001
d 38	39.5^{b}	43.8^{a}	38.8^{b}	39.6^{b}	38.5^{b}	38.6^{b}	0.91	< 0.001
Day 0 to 10 (Phase 1)								
ADG, lb	0.13^{ab}	0.20^{a}	0.12^{b}	0.14^{ab}	0.12^{b}	0.14^{ab}	0.028	0.006
ADFI, lb	0.26	0.30	0.29	0.29	0.26	0.28	0.024	0.283
G:F	0.49^{ab}	0.67^{a}	0.41^{b}	0.47^{ab}	0.43^{b}	0.44^{b}	0.052	0.007
F/G[7]	2.04^{ab}	1.50^{a}	2.42^{b}	2.11^{ab}	2.32^{b}	2.28^{b}	---	0.007
Day 10 to 24 (Phase 2)								
ADG, lb	0.72^{b}	0.96^{a}	0.71^{b}	0.80^{b}	0.74^{b}	0.72^{b}	0.031	< 0.001
ADFI, lb	0.85^{b}	1.13^{a}	0.88^{b}	0.96^{b}	0.87^{b}	0.85^{b}	0.043	< 0.001
G:F	0.84	0.85	0.80	0.84	0.85	0.85	0.027	0.439
F/G[7]	1.19	1.18	1.25	1.19	1.17	1.18	---	0.439
Day 24 to 38 (Phase 3)								
ADG, lb	1.13	1.16	1.11	1.04	1.05	1.08	0.042	0.289
ADFI, lb	1.65^{ab}	1.78^{a}	1.62^{ab}	1.66^{ab}	1.51^{b}	1.56^{b}	0.047	0.003
G:F	0.69^{ab}	0.65^{ab}	0.69^{ab}	0.63^{b}	0.70^{a}	0.69^{ab}	0.014	0.008
F/G[7]	1.46^{ab}	1.53^{ab}	1.46^{ab}	1.59^{b}	1.44^{a}	1.46^{ab}	---	0.008
Day 0 to 38 (Overall)								
ADG, lb	0.71^{b}	0.83^{a}	0.70^{b}	0.71^{b}	0.69^{b}	0.70^{b}	0.023	< 0.001
ADFI, lb	0.98^{b}	1.15^{a}	1.00^{b}	1.04^{ab}	0.95^{b}	0.96^{b}	0.032	< 0.001
G:F	0.72^{ab}	0.73^{ab}	0.70^{ab}	0.69^{b}	0.73^{a}	0.72^{ab}	0.009	0.012
F/G[7]	1.38^{ab}	1.38^{ab}	1.42^{ab}	1.46^{b}	1.37^{a}	1.38^{ab}	---	0.012

continued

Table 2. Effects of Butyrate-based feed additives on nursery pig growth performance and fecal dry matter[1,2]

Protein source:	Negative Control[3]	Positive Control[4]	Encapsulated Butyrate[5]	Monobutyrin[5]	Mono + Tributyrin[5,6]	Tributyrin[5]	SEM	*P* =
Fecal DM, %[8]								
d 5	19.8	23.7	22.4	21.4	20.8	22.2	1.16	0.243
d 10	15.9[b]	23.6[a]	16.3[b]	15.3[b]	16.7[b]	13.7[b]		< 0.001
d 17	18.6	21.8	18.3	18.9	19.1	18.2		0.249
d 24	19.8	22.5	18.2	17.9	22.5	18.0		0.003
Haptoglobin, mg/dL[9]								
d 10	69	35	49	81	67	66	17.1	0.334
d 38	100[ab]	48[b]	130[a]	101[ab]	117[a]	101[ab]		0.007
Vitamin E, ppm[10]								
d 10	0.54[b]	0.45[b]	0.64[b]	0.50[b]	0.68[b]	1.09[a]	0.085	< 0.001
d 38	0.49	0.35	0.40	0.36	0.54	0.37		0.359

[a,b] Means within a row with different superscripts differ ($P < 0.05$).

[1] A total of 300 pigs (initial BW of 12.2 ± 2.18 lb) were used in a 38-d nursery trial with five pigs per pen and 10 pens per treatment spread across two rooms. Pigs were weaned at approximately 19 d of age and allotted to treatment in a completely randomized design. On d 10 and 38 of the trial, blood was collected from one pig per pen (10 pigs per treatment and 60 pigs total) and analyzed for advanced oxidation protein products, haptoglobin, and vitamin E. Haptoglobin and vitamin E were submitted to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS), while advanced oxidation protein products were analyzed in the Kansas State Swine Nutrition Laboratory (Manhattan, KS). Advanced oxidation protein products were not able to detect due to the analytical method limitation. The same pig was bled on d 10 and 38.

[2] Pens of pigs were fed diets in 3 phases. Pigs were fed phase 1 diets from 0 to 10 d after weaning, phase 2 diets from d 10 to 24, and phase 3 diets from d 24 to 38.

[3] The negative control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives.

[4] The positive control contained 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 ppm of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 ppm of carbadox from Mecadox across all three phases.

[5] All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the negative control diet.

[6] Mono + Trtibutyrin product contained 71.5% Monobutyrin and 28.5% Tributyrin.

[7] F/G was calculated by taking the inverse of G:F. Therefore no SEM is reported and *P*-values are consistent with G:F.

[8] Treatment × day, $P = 0.035$; Treatment, $P < 0.001$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day. The SEM is representative of all collection periods.

[9] Treatment × day, $P = 0.272$; Treatment, $P = 0.009$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day. The SEM is representative of both collection periods.

[10] Treatment × day, $P < 0.001$; Treatment, $P = 0.001$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day. The SEM is representative of both collection periods.

Table 3. Effects of Butyrate-based feed additives on nursery pig complete blood count[1]

Protein source:	Negative Control[2]	Positive Control[2]	Encapsulated Butyrate[2]	Monobutyrin[2]	Mono + Tributyrin[2]	Tributyrin[2]	SEM	*P* =[3]
Band neutrophil concentration, K/uL[4]								
d 10	0.03	0.00	0.00	0.00	0.00	0.00	0.011	0.223
d 38	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.05[a]	0.00[b]		0.008
Eosinophil concentration, K/uL[5]								
d 10	0.20	0.20	0.14	0.08	0.11	0.16	0.126	0.975
d 38	0.49	0.32	0.43	0.30	0.70	0.76		0.029
Basophil concentration, K/uL								
d 10	0.01	0.01	0.05	0.00	0.01	0.05	0.027	0.519
d 38	0.11[a]	0.00[b]	0.07[ab]	0.02[ab]	0.03[ab]	0.04[ab]		0.049
Hematocrit, %[5]								
d 10	42.5	42.1	43.7	43.1	46.5	45.3	1.18	0.046
d 38	39.3	38.2	39.8	39.7	39.5	37.3		0.621
Hemoglobin, g/dL[5]								
d 10	12.8	12.7	13.0	12.9	13.7	13.6	0.29	0.044
d 38	11.3	11.2	11.2	11.5	11.4	10.8		0.535

[a,b] Means within a row with different superscripts differ ($P < 0.05$).

[1] On d 10 and 38 of the trial, blood was collected from the same pig per pen (10 pigs per treatment and 60 pigs total) and analyzed for complete blood count to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS). On d 10, a total of six blood tubes clotted and could not be analyzed (1 positive control, 1 encapsulated butyrate, 1 monobutyrin, 2 mono + tributyrin, and 1 tributyrin). On d 38, a total of four blood tubes clotted and could not be analyzed (1 negative control, 2 mono + tributyrin, and 1 tributyrin).

[2] The negative control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives. The positive control contained 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 ppm of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 ppm of carbadox from Mecadox across all three phases. All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the negative control diet. Mono + Trtibutyrin product contained 71.5% Monobutyrin and 28.5% Tributyrin.

[3] There were no significant treatment within day effects for white blood cell count, segmental neutrophil concentration, lymphocyte concentration, monocyte concentration, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, cellular hemoglobin concentration mean, red cell distribution width, platelet count, packed cell volume, protein, fibrinogen, $P > 0.10$.

[4] Treatment × day, $P < 0.05$. The *P*-values represented in the data table show the effect of treatment within day.

[5] Day, $P < 0.05$.

Table 4. Effects of butyrate-based feed additives on intestinal short chain fatty acid composition[1,2]

Item	Negative Control[3]	Positive Control[4]	Encapsulated Butyrate[5]	Monobutyrin[5]	Mono + Tributyrin[5,6]	Tributyrin[5]	SEM	*P* =[7]
Cecum, μmol/L								
Acetate	62.17	62.95	66.55	63.32	66.60	63.20	2.600	0.734
N[8]	6	6	6	6	6	6		
Butyrate	14.19[b]	15.12[ab]	17.02[ab]	18.70[ab]	20.40[a]	16.01[ab]	1.335	0.026
N[8]	6	6	6	6	6	6		
Propionate	28.71	30.15	31.04	29.43	30.05	30.36	0.877	0.533
N[8]	6	6	6	6	6	6		
Valerate	2.64[bc]	1.21[c]	3.26[ab]	4.31[a]	3.47[ab]	2.80[b]	0.350	< 0.001
N[8]	6	4	6	6	6	6		

[a,b,c] Means within a row with different superscripts differ ($P < 0.05$).

[1] On d 38 of the trial, six medium weight pigs from each treatment were humanely euthanized via penetrating captive bolt. Samples of digesta were taken from the duodenum, proximal jejunum, ileum, and cecum for analysis of short chain fatty acids. Pigs euthanized for digesta collection were the same pigs bled on d 10 and 38 of the trial.

[2] Digesta was analyzed using gas chromatography and measured concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaporate, caporate, and heptanoate. Any sample with non-detectable levels of SCFA was assumed to have a concentration of 0.50 μmol (lower detection limit). Short chain fatty acids not reported in the table indicates all samples were below the lower detection limit.

[3] The negative control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives.

[4] The positive control contained 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 ppm of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 ppm of carbadox from Mecadox across all three phases.

[5] All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the negative control diet.

[6] Mono + Trtibutyrin product contained 71.5% Monobutyrin and 28.5% Tributyrin.

[7] There were no significant treatment effects for intestinal samples collected in the duodenum, jejunum, or ileum, $P > 0.10$. Duodenum and Jejunum had detectable levels of acetate. Ileum had detectable levels of acetate, butyrate, and propionate.

[8] Number of samples with detectable short chain fatty acids.