Kansas Agricultural Experiment Station Research Reports

Volume 10
Issue 6 *Swine Day*

Article 11

2024

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Recommended Citation

Stas, Ethan B.; Chen, Ying; Wolfenden, Ross; Woodworth, Jason C.; Tokach, Mike D.; DeRouchey, Joel M.; Goodband, Robert D.; and Gebhardt, Jordan T. (2024) "Effects of Butyrate Glyceride-based Ingredient Supplementation on Nursery Pig Performance, Fecal Dry Matter, Serum Chemistry, and Blood Characteristics," *Kansas Agricultural Experiment Station Research Reports*: Vol. 10: Iss. 6. https://doi.org/10.4148/2378-5977.8626

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Funding Source

Appreciation is expressed to Eastman Chemical (Kingsport, TN) for partial financial support of this trial and to New Horizon Farms (Pipestone, MN) for technical support and expertise in conducting this trial.

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K-STATE Research and Extension

SWINE DAY 2024

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2024

Effects of Butyrate Glyceride-based Ingredient Supplementation on Nursery Pig Performance, Fecal Dry Matter, Serum Chemistry, and Blood Characteristics[1]

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

Summary

A total of 2,238 pigs (Line 337 × 1050, PIC, Hendersonville, TN; initially 11.3 lb) were used to determine the effects of butyrate glyceride-containing feed additive supplementation on nursery pig growth performance, fecal consistency, blood criteria, and inflammatory markers. Butyrate is the main source of energy for epithelial cells and plays a key role in nutrient utilization. At weaning, pigs were allotted to one of six dietary treatments consisting of a control diet and four diets containing various butyrate glyceride-based feed additives (MTB C, C4 C Mix, MTB 40C, MTB 400C, Eastman Chemical, Kingsport, TN). The final diet contained a commercial feed additive (AviPlus S, Vetagro Inc., Chicago, IL) that is a combination of micro-encapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals. Dietary treatments were fed over three phases. The control diet contained 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2, respectively. The control diet also contained 55 ppm of carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phase 1 and 2. Each feed additive was added at 0.3% to the control diet in phases 1 and 2, and 0.1% to the control diet in phase 3. Phase 1 and 2 diets were fed according to a feed budget of 5 and 12 lb/pig, respectively. Following phase 2 diets, pigs were fed phase 3 diets until the completion of the study. There were no significant differences between treatments for growth performance or serum haptoglobin for the duration of the study ($P > 0.10$). On d 10 of the study, pigs fed MTB 40C diets had increased ($P < 0.05$) fecal DM compared to pigs fed C4 C Mix and AviPlus S diets with other treatments intermediate. There was a treatment × day interaction ($P = 0.041$) for fibrinogen where pigs fed C4 C Mix diets had increased ($P < 0.05$) fibrinogen compared to pigs fed AviPlus S diets on d 10 of the study, but no differences between treatments on d 24 and 42. For IFNγ on d 10, pigs fed C4 C Mix had increased ($P < 0.05$) IFNγ concentration compared to MTB C and MTB 40C with other treatments intermediate. Minor treatment or day effects

[1] Appreciation is expressed to Eastman Chemical (Kingsport, TN) for partial financial support of this trial and to New Horizon Farms (Pipestone, MN) for technical support and expertise in conducting this trial.

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were also observed for other complete blood count measurements and cytokines. In conclusion, although no differences in growth performance were observed, pigs fed MTB 40C had improved d 10 fecal dry matter and exhibited lower concentrations of the pro-inflammatory cytokine IFNγ compared to C4 C Mix.

Introduction

Short-chain fatty acids (SCFA) are organic fatty acids with one to six carbons produced within the intestinal lumen via bacterial fermentation. The most abundant SCFA in the gastrointestinal tract (GIT) are acetate, propionate, and butyrate. Despite being the least abundant of the three primary SCFA, butyrate is important as it is a major metabolite for the colonic epithelial cells: as much as 90% of butyrate is metabolized by colonocytes. The SCFA, including butyrate, possess microbiota and immunity modulation activity and have been widely used as feed additives in an effort to control pathogenic bacteria and improve gut health. Butyrate glycerides, including mono-, di-, and tri-butyrin, consist of a varied number of butyric acid molecules attached to a glycerol backbone. In the small intestine, the butyrate is liberated from the glycerol through the action of lipase. In this form, the butyrate is protected from absorption in the upper GIT.

Because of their microbiota modulation activity and effect on host immune response, butyrate and its derivatives are considered a potential option to improve health and performance of post-weaning pigs. However, limited research is available that compares different butyrate glyceride-based ingredients to each other or to other commercially available products that are reported to elicit similar benefits. Therefore, the objective of this study was to evaluate butyrate glyceride feed additive supplementation on nursery pig growth performance and gut health under post-weaning stress conditions in a commercial environment.

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 a commercial research site owned and operated by New Horizon Farms in Pipestone, MN. Each pen was equipped with a 6-hole, dry self-feeder and pan waterer to provide ad libitum access to feed and water.

Animals and diets

A total of 2,238 pigs (Line 337 × 1050, PIC, Hendersonville, TN; initially 11.3 lb) were used in a 42-d study with either 26 or 27 pigs per pen and 14 replications per treatment. Pigs were weaned at approximately 21 d of age, and pens were randomly assigned to treatment based on initial body weight and weaning date. At weaning, pigs were randomly allotted to one of six dietary treatments fed in three phases (Table 1). The control diet was a standard corn-soybean meal-based diet containing 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2, respectively, and 55 ppm of carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phases 1 and 2. The next four treatments consisted of one of four butyrate glyceride-containing feed additives added to the control diet that consisted of MTB C, C4 C Mix, MTB 40C, or MTB 400C (Eastman Chemical, Kingsport, TN). The final diet utilized a commercially available feed additive (AviPlus S, Vetagro Inc., Chicago, IL) in the control diet. AviPlus S is a

combination of micro-encapsulated sorbic and citric acids and synthetic thymol and vanillin botanicals. The butyrate glyceride-containing feed additives and AviPlus S were added to the control diet at 0.3% in phase 1 and 2, and 0.1% in phase 3. 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).[3] Phase 1 and 2 diets were fed according to a feed budget of 5 and 12 lb/pig, respectively. Following phase 2 diets, pigs were placed on phase 3 diets until the completion of the study. Phase 1 diets were fed for approximately the first 10 d (d 0 to 10), phase 2 diets were fed for approximately 14 d (d 10 to 24), and phase 3 diets were fed for approximately 18 d (d 24 to 42). Phase 1 diets were manufactured at Hubbard Feeds in Mankato, MN, and phase 2 and 3 diets were manufactured at the New Horizon feed mill located in Pipestone, MN. Pens of pigs were weighed, and feed disappearance was calculated weekly from d 7 to 42 to determine ADG, ADFI, and F/G.

Fecal samples were collected on approximately d 10, 24, and 42 to determine fecal dry matter (DM) percentage from the same three medium-weight pigs from each pen on each collection day. All fecal samples were collected before the transition to the next dietary phase. 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 DM percentage.

Blood was collected from the same medium-weight pig per pen on approximately d 10, 24, and 42 into one serum blood tube and a K2 EDTA anti-coagulant blood collection tube. The serum blood tubes were spun down using a centrifuge, and serum was submitted for haptoglobin and cytokine profile. The whole blood tube was submitted to South Dakota State University Animal Research and Diagnostic Laboratory in Brookings, SD for a complete blood count (CBC) analysis. Serum haptoglobin was submitted to the Kansas State University Diagnostic Laboratory in Manhattan, KS. Serum cytokines were submitted to Eve Technologies in Calgary, AB Canada.

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, cytokines, and CBC criteria were analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. 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

For the duration of the trial there were no significant differences between treatments for growth performance, removals, and mortalities (Table 2). For fecal DM, there was no treatment × day interaction observed; however, pigs fed MTB 40C had increased ($P < 0.05$) fecal DM compared to pigs fed C4 C Mix and AviPlus S on d 10 of the study with pigs fed other treatments intermediate. Fecal DM increased ($P < 0.001$) from d 10 to 42 across all treatments. For haptoglobin concentration, there was no treatment ×

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

day interaction or treatment effects observed ($P > 0.10$). Haptoglobin concentration increased ($P < 0.001$) from d 10 to 42 across all treatments.

For CBC analysis, there was treatment × day interaction ($P = 0.041$) for fibrinogen where pigs fed C4 C Mix diets had increased ($P < 0.05$) fibrinogen compared to pigs fed AviPlus S diets on d 10 of the study, but no differences between treatments were observed on d 24 or 42 (Table 3). Red blood cell count on d 42 was increased ($P < 0.05$) for pigs fed MTB 400C diets compared to pigs fed MTB 40C diets with other treatments intermediate. On d 10, pigs fed the control diet had increased ($P < 0.05$) mean corpuscular volume compared to pigs fed MTB 400C with other treatments intermediate. Mean corpuscular hemoglobin concentration on d 42 was increased ($P < 0.05$) for pigs fed the control diet compared to the pigs fed the MTB C, MTB 40C, and AviPlus S diets with other treatments intermediate. For platelets on d 24, there was a tendency for a treatment effect ($P = 0.058$); however, the means did not separate, with pigs fed AviPlus S diets having the highest numerical platelet concentration and pigs fed MTB 40C diets having the lowest. For basophils on d 42, there was a tendency for a treatment effect ($P = 0.055$) with no treatment means separation. There was a significant main effect of day ($P < 0.05$) observed for white blood cell count, red blood cell count, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, platelets, segmental neutrophils, monocytes, protein, and fibrinogen.

For cytokine profile, both fluorescence intensity and concentration are reported (Table 4). Differences between treatments will only be discussed for concentration, as fluorescence intensity differences matched reasonably well. There were no significant treatment × day interactions for any cytokines measured. For granulocyte-macrophage colony stimulating factor (GM-CSF) on d 10, pigs fed C4 C Mix and Aviplus S had increased ($P < 0.05$) GM-CSF concentration compared to pigs fed MTB C, with other treatments intermediate. There were no differences between treatments for GM-CSF on d 24 and 42 ($P > 0.10$). For interferon gamma (IFNγ) on d 10, pigs fed C4 C Mix had increased ($P < 0.05$) IFNγ concentration compared to pigs fed MTB C and MTB 40C with other treatments intermediate. There were no differences between treatments for IFNγ on d 24 and 42. For interleukin 12 (IL-12) on d 42, there was a significant treatment effect ($P = 0.036$) without mean separation. All other cytokines were not influenced by treatment ($P > 0.10$). There was a significant main effect of day ($P < 0.05$) observed for GM-CSF, IFNγ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-10, IL-12, IL-18, and TNFα.

In conclusion, there were no differences in growth performance or serum haptoglobin for the duration of the study. However, pigs fed MTB 40C had improved fecal DM on d 10 of the study and lower concentrations of the proinflammatory cytokine IFNγ compared to pigs fed C4 C Mix. In addition, on d 42, pigs fed MTB 40C had decreased red blood cell count which may be an indication of better hydration. Therefore, the results of this study suggest MTB 40C may provide the most benefit at improving gut health in nursery pigs.

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

	Phase 1: Control	Phase 2: Control	Phase 3: Control
Ingredients, %			
Corn	38.51	56.35	56.21
Soybean meal (46.5% CP)	22.00	20.28	24.77
Whey powder	25.00	10.00	---
Dried distillers grains with solubles	---	---	15.00
Enzymatically treated soybean meal[2]	7.00	7.00	---
Soybean oil	3.00	---	---
Corn oil	---	1.00	---
Limestone	0.60	0.70	1.30
Monocalcium phosphate (21% P)	1.15	1.15	0.45
Salt	0.35	0.55	0.60
L-Lys HCl	0.35	---	---
Liquid Lys (55%)	---	0.75	0.85
DL-Met	0.22	0.24	0.16
L-Thr	0.15	---	---
Thr-Pro[3]	---	0.30	0.28
L-Trp	0.02	0.05	0.05
L-Val	0.08	0.14	0.09
Vitamin premix	0.05	---	---
Trace mineral premix	0.08	---	---
Vitamin-trace mineral premix	---	0.20	0.20
Selenium premix	0.05	---	---
Phytase[4]	0.02	0.05	0.05
Zinc oxide	0.40	0.27	---
Mecadox[5]	1.00	1.00	---
Total	100	100	100

continued

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

	Phase 1: Control	Phase 2: Control	Phase 3: Control
SID amino acids, %			
Lys	1.35	1.35	1.30
Ile:Lys	62	55	56
Leu:Lys	116	111	128
Met:Lys	36	38	35
Met and Cys:Lys	58	58	58
Thr:Lys	64	65	65
Trp:Lys	19.4	19.3	19.1
Val:Lys	70	69	69
His:Lys	35	34	37
Total Lys, %	1.48	1.49	1.46
NE NRC, kcal/lb	1,170	1,124	1,097
CP, %	21.1	20.4	21.6
Ca, %	0.85	0.79	0.68
P, %	0.73	0.66	0.55
STTD P, %	0.62	0.55	0.42

[1] Phase 1 and 2 diets were provided with a feed budget of 5 and 12 lb/pig, respectively. Feed additives were added at 0.3% of the control diet for phases 1 and 2, and 0.1% of the control diet in phase 3. Feed additives included: MTB C, C4 C Mix, MTB 40C, or MTB 400C (Eastman Chemical, Kingsport, TN), and AviPlus S (Vetagro Inc., Chicago, IL).

[2] HP 300, Hamlet Protein, Findlay, OH.

[3] CJ America, Los Angeles, CA.

[4] Phase 1: Quantum Blue 5G (ABVista, Plantation, FL), which provided an estimated release of 0.12% STTD P with 341 FTU/lb. Phase 2 and 3: Optiphos Plus 2500 G (Huvepharma Inc.; Peachtree City, Georgia), which provided an estimated release of 0.13% STTD P with 567 FTU/lb.

[5] Mecadox (Phibro Animal Health Corp., Teaneck, NJ) provided 55 ppm of carbadox in the diet.

Table 2. Effects of butyrate glyceride-containing feed additives on nursery pig growth performance, removals and mortality, fecal DM, and serum haptoglobin[1]

Item	Control[2]	MTB C[3]	C4 C Mix[3]	MTB 40C[3]	MTB 400C[3]	Aviplus S[4]	SEM	*P* =
BW, lb								
d 0	11.3	11.3	11.3	11.3	11.3	11.3	0.28	0.999
d 7	12.5	12.4	12.5	12.5	12.4	12.4	0.29	0.988
d 21	21.8	21.8	22.2	22.2	22.4	22.0	0.45	0.593
d 42	39.7	39.5	40.2	40.9	40.5	40.0	0.77	0.352
d 42 per pig placed	34.4	32.4	33.8	34.9	33.7	34.1	1.12	0.320
Day 0 to 7[5]								
ADG, lb	0.17	0.15	0.16	0.17	0.15	0.16	0.015	0.804
ADFI, lb	0.37	0.35	0.37	0.37	0.36	0.37	0.012	0.631
G:F	0.45	0.42	0.44	0.46	0.42	0.43	0.037	0.890
F/G[6]	2.21	2.41	2.27	2.16	2.39	2.34	---	0.890
Day 7 to 21[7]								
ADG, lb	0.64	0.64	0.66	0.66	0.67	0.66	0.018	0.463
ADFI, lb	0.83	0.85	0.86	0.85	0.88	0.87	0.020	0.481
G:F	0.77	0.75	0.77	0.78	0.76	0.76	0.011	0.379
F/G[6]	1.31	1.34	1.29	1.29	1.31	1.32	---	0.379
Day 21 to 42[8]								
ADG, lb	0.83	0.82	0.83	0.87	0.84	0.84	0.022	0.351
ADFI, lb	1.24	1.23	1.24	1.30	1.28	1.24	0.027	0.223
G:F	0.67	0.67	0.68	0.67	0.66	0.67	0.009	0.338
F/G[6]	1.50	1.50	1.48	1.49	1.53	1.48	---	0.338
Day 0 to 42 (Overall)								
ADG, lb	0.64	0.63	0.65	0.67	0.65	0.65	0.015	0.234
ADFI, lb	0.95	0.94	0.95	0.98	0.97	0.96	0.018	0.365
G:F	0.68	0.67	0.69	0.69	0.67	0.68	0.006	0.182
F/G[6]	1.47	1.49	1.46	1.46	1.49	1.47	---	0.182
Day 0 to 42 (Overall) per pig placed								
ADG, lb[9]	0.58	0.54	0.57	0.60	0.57	0.58	0.030	0.271
ADFI, lb[10]	0.86	0.82	0.85	0.87	0.86	0.85	0.023	0.596
G:F	0.68	0.64	0.67	0.69	0.66	0.68	0.034	0.151
F/G[6]	1.46	1.55	1.49	1.46	1.51	1.48	---	0.151
Day 0 to 42 (Overall)								
Removals, %[11]	8.8	12.6	10.2	9.7	11.3	9.4	1.71	0.630
Mortality, %[12]	2.9	3.8	2.7	2.7	3.2	2.1	0.96	0.887
Total mortality[13]	4.3	7.0	5.4	4.8	6.7	5.1	1.28	0.600
Total rem and mort, %	11.8	16.4	12.9	12.3	14.5	11.5	1.88	0.408

continued

Table 2. Effects of butyrate glyceride-containing feed additives on nursery pig growth performance, removals and mortality, fecal DM, and serum haptoglobin[1]

Item	Control[2]	MTB C[3]	C4 C Mix[3]	MTB 40C[3]	MTB 400C[3]	Aviplus S[4]	SEM	*P* =
Fecal DM (%)[14]								
d 10	19.1[ab]	20.5[ab]	17.8[b]	21.4[a]	18.2[ab]	17.8[b]	0.86	0.008
d 24	21.0	21.7	21.7	22.9	22.5	22.5		0.604
d 42	24.9	25.8	24.6	25.3	26.3	25.8		0.718
Haptoglobin (mg/dL)[15]								
d 10	57.9	55.6	68.6	71.3	55.4	51.9	27.33	0.975
d 24	193.3	194.4	187.8	170.1	201.6	182.6		0.898
d 42	230.9	218.9	239.0	242.9	213.4	216.4		0.837

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

[1] A total of 2,238 pigs (initial BW of 11.3 ± 0.28 lb) were used in a 42-d nursery trial with 26 or 27 pigs per pen and 14 pens per treatment spread across two rooms. Pigs were weaned at approximately 21 d of age and allotted to treatment in a completely randomized design. Experimental diets were fed in three phases. Pigs received a feed budget of 5 and 12 lbs/pig for phases 1 and 2, respectively. Following phase 2, pigs were fed phase 3 diets until the completion of the study.

[2] The control consisted of a standard corn-soybean meal-based diet with 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. The control diet also contained 55 ppm carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phases 1 and 2.

[3] Eastman Chemical, Kingsport, TN. Butyrate glyceride-based and were added at 0.3% of the diet in phase 1 and 2, and 0.1% of the diet in phase 3.

[4] Vetagro, Chicago, IL. Added at 0.3% of the diet in phase 1 and 2, and 0.1% of the diet in phase 3.

[5] Approximate feeding period of phase 1 diets.

[6] F/G was calculated by taking the inverse of G:F. Statistics were not ran on F/G therefore no SEM is reported and *P*-values are consistent with G:F.

[7] Approximate feeding period of phase 2 diets.

[8] Approximate feeding period of phase 3 diets.

[9] ADG per pig placed = (final weight – initial weight) ÷ (Pigs initially placed × days of trial).

[10] ADFI per pig placed = (total feed intake) ÷ (Pigs initially placed × days of trial).

[11] Percentage of pigs that were removed into a hospital pen.

[12] Percentage of pigs that died or were euthanized in original pen.

[13] Percentage of pigs that died or were euthanized in original pen or hospital pen after being removed.

[14] Treatment × day, $P = 0.269$; Treatment, $P = 0.078$; Day, $P < 0.001$. The *P*-values represented in data table show the effect of treatment within day.

[15] Treatment × day, $P = 0.967$; Treatment, $P = 0.957$; Day, $P < 0.001$. The *P*-values represented in data table show the effect of treatment within day.

Table 3. Effects of butyrate glyceride-containing feed additives on nursery pig complete blood count[1,2]

Item	Control[3]	MTB C[4]	C4 C Mix[4]	MTB 40C[4]	MTB 400C[4]	Aviplus S[5]	SEM	*P* =[6]
Red blood cell count, M/uL[6,7]								
d 10	6.14	6.40	6.38	6.21	6.66	6.41	0.258	0.238
d 24	5.60	5.41	5.46	5.39	5.55	5.33		0.817
d 42	5.87^{ab}	5.84^{ab}	5.86^{ab}	5.46^{b}	6.21^{a}	5.77^{ab}		0.064
Mean corpuscular volume, fL[6]								
d 10	63.3^{a}	61.8^{ab}	62.4^{ab}	62.4^{ab}	57.7^{b}	61.4^{ab}	1.95	0.022
d 24	60.4	59.5	59.7	60.1	59.3	60.8		0.940
d 42	60.1	59.7	59.4	59.1	58.4	59.5		0.957
Mean corpuscular hemoglobin concentration, g/dL								
d 10	30.7	30.5	31.5	31.6	31.8	31.7	1.12	0.644
d 24	30.7	30.8	30.9	30.9	30.6	30.5		0.997
d 42	33.6^{a}	30.6^{b}	31.0^{ab}	30.7^{b}	30.8^{ab}	30.4^{b}		0.022
Platelets, K/uL[6]								
d 10	286	276	298	278	286	292	61.6	0.999
d 24	460	393	421	345	391	501		0.058
d 42	471	488	515	504	498	493		0.980
Basophils, %								
d 10	0.00	0.00	0.00	0.00	0.00	0.00	0.071	1.000
d 24	0.00	0.00	0.00	0.00	0.00	0.00		1.000
d 42	0.00	0.15	0.00	0.08	0.00	0.00		0.055
Fibrinogen, mg/dL[6,8]								
d 10	292^{ab}	215^{ab}	409^{a}	226^{ab}	233^{ab}	171^{b}	104.6	0.020
d 24	371	417	308	282	300	400		0.272
d 42	282	331	292	325	355	250		0.796

^{a,b} Means within a row with different superscripts differ ($P < 0.05$).

[1] On d 10, 24, and 42 of the trial, blood was collected from the pig per pen (14 pigs per treatment and 84 pigs total) and analyzed for complete blood count (CBC) to the South Dakota State Animal Disease Research and Diagnostic Laboratory (Brookings, SD).

[2] On d 10 of blood collection, a total of nine blood tubes clotted and could not be analyzed for CBC (1 control, 1 MTB C, 3 C4 C Mix, 2 MTB 40C, and 2 MTB 400C). On d 24 of blood collection, a total of nine blood tubes clotted and could not be analyzed for CBC (2 MTB C, 2 C4 C Mix, 4 MTB 40C, and 1 MTB 400C). On d 42 of blood collection, a total of 17 blood tubes clotted and could not be analyzed for CBC (3 control, 1 MTB C, 2 C4 C Mix, 2 MTB 40C, 3 MTB 400C, and 6 Aviplus S).

[3] The control consisted of a standard corn-soybean meal-based diet with 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2, respectively. The control diet also contained 55 ppm carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phase 1 and 2.

[4] Eastman Chemical, Kingsport, TN. Butyrate glyceride-based and were added at 0.3% of the diet in phase 1 and 2, and 0.1% of the diet in phase 3.

[5] Vetagro, Chicago, IL. Added at 0.3% of the diet in phases 1 and 2, and 0.1% of the diet in phase 3.

[6] There were no significant treatment-within-day effects observed for white blood cell count, hemoglobin, packed cell volume, mean corpuscular hemoglobin, segmental neutrophils, lymphocytes, monocytes, eosinophils, band concentration, and protein.

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

[7] Treatment, $P < 0.05$.

[8] Treatment × Day, $P < 0.05$.

Table 4. Effects of butyrate glyceride-containing feed additives on nursery pig cytokine profile[1]

Item	Control[2]	MTB C[3]	C4 C Mix[3]	MTB 40C[3]	MTB 400C[3]	Aviplus S[4]	SEM	*P*[5] =
GM-CSF								
Fluorescence intensity[6]								
d 10	82.3[abc]	27.0[c]	124.1[a]	40.4[b]	43.0[b]	107.5[ab]	19.64	< 0.001
d 24	19.5	23.4	26.1	19.7	26.1	24.8		1.000
d 42	19.7	22.4	21.3	25.4	22.1	18.9		1.000
Concentration, pg/mL[6]								
d 10	133.9[ab]	42.2[b]	190.9[a]	75.5[ab]	77.4[ab]	182.4[a]	32.08	0.002
d 24	23.5	34.3	40.0	23.8	39.0	37.3		0.998
d 42	23.8	29.4	26.8	38.1	31.3	20.7		0.999
IFNγ								
Fluorescence intensity[6]								
d 10	283.5[ab]	189.2[b]	621.1[a]	205.2[b]	298.9[ab]	289.0[ab]	94.95	0.020
d 24	61.8	48.5	54.8	114.0	160.9	50.0		0.947
d 42	45.7	31.3	45.3	47.7	56.5	29.1		1.000
Concentration, pg/mL[6]								
d 10	6,805[ab]	4,173[b]	20,577[a]	4,506[b]	7,119[ab]	6,789[ab]	3,474.1	0.010
d 24	967	633	787	2,192	3,482	670		0.990
d 42	583	248	576	628	827	206		1.000
IL-12								
Fluorescence intensity[6,7]								
d 10	1,007	1,188	1,128	958	976	974	149.0	0.834
d 24	1,480	1,520	1,081	1,292	1,147	1,278		0.224
d 42	1,956	2,092	1,720	1,514	1,567	1,588		0.030
Concentration, pg/mL[6,7]								
d 10	1,258	1,526	1,409	1,196	1,222	1,215	199.8	0.809
d 24	1,863	1,922	1,351	1,658	1,437	1,607		0.262
d 42	2,498	2,659	2,174	1,906	1,978	2,000		0.036

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

[1] On d 10, 24, and 42 of the trial, blood was collected from the same pig per pen (14 pigs per treatment and 84 pigs total) and analyzed for Porcine Cytokine/Chemkine 13-Plex Discovery Assay at Eve Technologies (Calgary, AB Canada).

[2] The control consisted of a standard corn-soybean meal-based diet with 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. The control diet also contained 55 ppm carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phases 1 and 2.

[3] Eastman Chemical, Kingsport, TN. Butyrate glyceride-based and were added at 0.3% of the diet in phases 1 and 2, and 0.1% of the diet in phase 3.

[4] Vetagro, Chicago, IL. Added at 0.3% of the diet in phases 1 and 2, and 0.1% of the diet in phase 3.

[5] There were no treatment × day interactions, $P > 0.10$. The *P*-values represented in the data table show the effect of treatment within day. There were no significant treatment-within-day effects observed for IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, and TNFα, $P > 0.10$.

[6] Day, $P < 0.05$.

[7] Treatment, $P < 0.05$.