

## Investigating Potential Additive Effects of Formic Acid and Glycerol Monolaurate in Nursery Pig Diets<sup>1</sup>

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### Summary

A total of 350 weanling pigs (200 × 400, DNA; initially, 12.5 ± 0.3 lb BW) were used in a 42-d study with 5 pigs per pen and 14 replicate pens per treatment. At weaning, pigs were allotted to pens in a completely randomized design and pens of pigs were randomly assigned to one of five dietary treatments: 1) negative control (standard nursery diet with no additives); 2) control diet with 3,000 ppm ZnO included in phase 1 and 2,000 ppm ZnO included in phase 2; 3) control diet with 0.7% formic acid (Amasil NA, BASF, Florham, NJ); 4) control diet with 0.18% glycerol monolaurate (Natural Biologics GML, Natural Biologics, Newfield, NY); and 5) control diet with a 1.0% blend of formic acid, sodium diformate, and glycerol monolaurate (FORMI 3G, ADDCON GmbH, Bitterfeld-Wolfen, Germany). Pigs were fed treatment diets from d 0 to d 28 and were then fed a common diet from d 28 to d 42. This allowed diets to be fed as part of a standard 3-phase nursery program. From d 0 to d 7, pigs fed a diet containing ZnO or the 1.0% blend of formic acid, sodium diformate, and glycerol monolaurate had significantly increased ( $P = 0.03$ ) ADG compared to pigs fed the control. Feed intake did not differ ( $P > 0.05$ ) during this period. Overall, pigs fed GML had reduced ADG compared to their counterparts fed the negative control, ZnO, or FORMI diets. Feed intake was also not impacted ( $P = 0.233$ ) by dietary treatments. Fecal DM was evaluated from d 7 to d 28 and there was a significant treatment × day interaction ( $P = 0.035$ ). Pigs fed GML had significantly lower fecal DM % on d 7, but a higher fecal DM % on d 14 and 21. There was no evidence of difference between treatments for fecal DM by d 28. In summary, there is potential for a blend of formic acid and GML to improve growth performance immediately post-weaning without negatively impacting fecal consistency. Further research is warranted to determine the mode of action of these acids and elucidate their efficacy as alternative feed ingredients to combat post-weaning challenges in swine production.

### Introduction

The period immediately post-weaning is a time of increased stress and health challenge for pigs. The changes in diet composition, environment, and potential exposure to pathogens can drastically alter the pig's intestinal morphology, and gastric function can be negatively impacted. Dietary additives such as antibiotics, or compounds such as

<sup>1</sup> Appreciation is expressed to BASF and ADDCON-GmbH for their financial contributions to this research.

zinc oxide (ZnO) are typically used in this setting to combat these challenges. However, such additives are accompanied by scrutiny from consumers and regulatory officials for the potential of antimicrobial resistance in humans, or the excretion of heavy metals in manure.

Thus, biological alternatives such acidifiers have been investigated as potential antibiotic alternatives. Organic acids work primarily to reduce or stabilize gastric pH, ultimately increasing nutrient digestibility and limiting the growth of pathogenic bacteria (Jacela et al., 2009).<sup>2</sup> The addition of MCFA to nursery diets has been shown to reduce the risks of viruses in swine feed and replace antibiotics such as chlortetracycline (CTC) (Cochrane et al., 2018).<sup>3</sup> However, data surrounding the efficacy of these acids are variable, given many additional factors can impact their success including diet composition, type and dose of acid added, and the existing health of the pigs. While these acids can be fed freely, blends of acids are more typical in production due to the synergistic effects. There is little published research surrounding the use of formic acid and MCFA together, yet commercial feed additives are available with this specific blend. Knowledge of how these acids work together and their impacts on piglet health and performance is scarce. Thus, the objective of this study is to evaluate the impacts of formic acid and the MCFA, glycerol monolaurate (GML), on nursery pig growth performance fecal dry matter when fed alone or in combination.

## Materials and Methods

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

### *Animals and diets*

A total of 350 pigs (200 × 400, DNA; initially, 12.5 ± 0.3 lb BW) were weaned at an average of 21 d of age and used in a 42-d experiment. Weaning was considered d 0 of the trial and at this point pigs were individually weighed and allotted to pens in a completely randomized design. There were 5 pigs per pen and 14 replicate pens per treatment. Each pen (5 × 5 ft) was equipped with a 4-hole dry self-feeder and nipple waterer to supply *ad libitum* access to feed and water. Pens of pigs were randomly allotted to one of five dietary treatments: 1) negative control (standard nursery diet with no additives); 2) control diet with 3,000 ppm ZnO included in phase 1 and 2,000 ppm ZnO included in phase 2; 3) control diet with 0.7% formic acid (Amasil NA, BASF, Florham, NJ); 4) control diet with 0.18% glycerol monolaurate (Natural Biologics GML, Natural Biologics, Newfield, NY); and 5) control diet with a 1.0% blend of formic acid, sodium diformate, and glycerol monolaurate (FORMI 3G, ADDCON GmbH, Bitterfeld-Wolfen, Germany). All feed additives were included according to manufacturer recommendations. Pigs were fed treatment diets from d 0 to d 28 and were then fed a common diet from d 28 to d 42. This allowed for diets to

<sup>2</sup> Jacela, J.Y., J.M. DeRouchey, M.D. Tokach, R.D. Goodband, J.L. Nelssen, D.G. Renter, and S.S. Dritz. 2009. Feed additives for swine: Fact sheets – acidifiers and antibiotics. *J. Swine Health Prod.* 17(5):270-275. doi: 0.4148/2378-5977.7071.

<sup>3</sup> Cochrane, R.A., J.R. Pluske, J.P. Mansfield, S.S. Dritz, J.C. Woodworth, M.D. Tokach, M.C. Niederwerder, C.B. Paulk, and C.K. Jones. 2018. Evaluating medium chain fatty acids as an alternative to chlortetracycline in nursery pig diets. *Kansas Agric. Exp. Stat. Res. Rep.* 4(9):1-10. doi:10.4148/2378-4977.7659.

be fed as part of a standard 3-phase nursery program. Diets were pelleted in phase 1 and meal in phases 2 and 3.

### *Data collection*

All pigs were weighed individually on d 0, 7, 14, 21, and 28, while pens of pigs were weighed using a floor scale on d 35 and 42 to determine ADG. Feeders were individually weighed on each of these days to calculate ADFI on a weekly basis. Additionally, on d 7, 13, 21, and 28 fecal samples were collected from the same 3 pigs from every pen to be analyzed for fecal DM. To determine the DM percentage, samples were dried in a 105°F oven for 48 h.

### *Statistical analysis*

Data were analyzed as a completely randomized design using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Cary, NC) with pen as the experimental unit. All comparisons incorporated Tukey-Kramer multiple comparison adjustments. Preplanned pairwise contrasts were run to compare the negative control diet and those with feed additives, and the diet containing ZnO compared to those with an acidifier included. For fecal DM, data were analyzed with repeated measures. Results were considered significant if  $P < 0.05$  and marginally significant if  $0.05 < P < 0.10$ .

## **Results and Discussion**

In the first week post-weaning, pigs fed a diet containing ZnO or the 1.0% blend of formic acid, sodium diformate, and glycerol monolaurate had significantly increased ( $P = 0.03$ ) ADG compared to pigs fed the control. There were no differences among treatment diets for ADFI ( $P > 0.05$ ) during this period. Pigs fed a diet containing 0.18% glycerol monolaurate had improved feed efficiency compared to those fed the negative control or a diet with 0.7% formic acid, while pigs fed the remaining treatments were intermediate. For the entire treatment period (d 0 to d 28), pigs fed ZnO or FORMI had improved ADG ( $P < 0.0001$ ) compared to those fed GML. Dietary treatment did not significantly impact ADFI ( $P = 0.119$ ). During this time, pigs fed GML had poorer ( $P < 0.0001$ ) feed conversion than those fed the negative control, ZnO, and FORMI treatments. There was no evidence of differences for any growth response criteria ( $P \geq 0.254$ ) during the common period. Overall, pigs fed GML had reduced ADG compared to their counterparts fed the negative control, ZnO, or FORMI diets. Feed intake was not impacted ( $P = 0.233$ ) by dietary treatments, but F/G differed ( $P = 0.031$ ), with differences driven by the reduced gain in pigs fed GML.

For fecal dry matter, the treatment  $\times$  sampling day interaction was significant ( $P = 0.035$ ). In the first week post-weaning, pigs fed glycerol monolaurate alone had significantly lower ( $P = 0.043$ ) fecal DM % compared to pigs fed any other treatment. However, by d 14, this response had shifted, and pigs fed GML had significantly higher ( $P = 0.007$ ) fecal DM % compared to pigs fed the remaining treatments, and this continued until d 21 of the experiment. By d 28, fecal DM % standardized across dietary treatments.

In conclusion, these data indicate that in the first week post-weaning, feeding formic acid or glycerol monolaurate alone did not positively impact growth performance; however, their combinational use improved ADG without an effect on ADFI. For the entire treatment period, ZnO was the only examined feed additive to improve growth

performance compared to a negative control diet, but this difference diminished by the end of the trial as a result of compensatory growth during the common period. Overall, the addition of GML alone negatively impacted growth performance, when these pigs were an average of 2.8 lb lighter by the end of the experiment compared to pigs fed the control. Finally, feeding GML reduced the fecal DM percentage in the first week post-weaning, but then improved the fecal consistency for the remainder of the trial. Further research should look deeper into the mechanism of action behind these acidifiers.

*Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.*

**Table 1. Phase 1 diet composition (as-fed basis)<sup>1</sup>**

<b>Ingredient, %</b>	<b>Control</b>	<b>Zinc oxide<sup>2</sup></b>	<b>Formic acid<sup>3</sup></b>	<b>Glycerol</b>	<b>Formic acid +</b>
				<b>monolaurate (GML)<sup>4</sup></b>	<b>GML blend<sup>5</sup></b>
Corn	40.08	39.69	40.07	39.90	40.07
Spray dried whey	25.00	25.00	25.00	25.00	25.00
Soybean meal, 47.5% CP	17.30	17.30	17.30	17.30	17.30
Corn DDGS, 7.5% oil	5.00	5.00	5.00	5.00	5.00
Spray dried bovine plasma	4.00	4.00	4.00	4.00	4.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Choice white grease	3.00	3.00	3.00	3.00	3.00
Calcium carbonate, 38.5% Ca	0.65	0.65	0.65	0.65	0.65
Monocalcium phosphate, 21.5% P	0.55	0.55	0.55	0.55	0.55
NaCl	0.30	0.30	0.30	0.30	0.30
L-Lys HCL	0.35	0.35	0.35	0.35	0.35
DL-Met	0.15	0.15	0.15	0.15	0.15
L-Thr	0.13	0.13	0.13	0.13	0.13
L-Trp	0.03	0.03	0.03	0.03	0.03
L-Val	0.07	0.07	0.07	0.07	0.07
Vitamin premix w/phytase	0.25	0.25	0.25	0.25	0.25
Trace mineral premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15
Additive	---	0.39	0.70	0.18	1.00
Total	100.00	100.00	100.00	100.00	100.00

*continued*

**Table 1. Phase 1 diet composition (as-fed basis)<sup>1</sup>**

Ingredient, %	Control	Zinc oxide <sup>2</sup>	Formic acid <sup>3</sup>	Glycerol	Formic acid + GML blend <sup>5</sup>
				monolaurate (GML) <sup>4</sup>	
Calculated analysis					
Standardized ileal digestible (SID) amino acids					
Lysine	1.40	1.40		1.40	1.40
Ile:Lys	55	55		55	55
Leu:Lys	118	118		118	118
Met:Lys	32	32		32	32
Met and Cys:Lys	56	56		56	56
Thr:Lys	63	63		63	63
Trp:Lys	19.3	19.3		19.3	19.3
Val:Lys	69	69		69	69
ME, kcal/lb	1,577	1,577		1,577	1,577
NE kcal/lb	1,192	1,192		1,192	1,192
SID lysine:NE, g/mcal	5.33	5.33		5.33	5.33
CP, %	21.4	21.4		21.4	21.4
Ca, %	0.78	0.78		0.78	0.78
STTD P, %	0.63	0.63		0.63	0.63

<sup>1</sup>Treatment diets were fed to 350 pigs (200 × 400, DNA; initially 12.5 ± 0.3 lb BW) from d 0 to d 7 of the study, which was considered dietary Phase 1.

<sup>2</sup>ZnO was included to provide 3,000 ppm Zn.

<sup>3</sup>Formic acid (Amasil-NA; BASF, Florham, NJ) was included at 0.70% at the expense of ground corn.

<sup>4</sup>Glycerol monolaurate (GML; Natural Biologics, Newfield, NY) was guaranteed 90% purity and included in the diet at 0.18% at the expense of ground corn.

<sup>5</sup>A formic acid and glycerol monolaurate blend (FORMI-3G, ADDCON GmbH, Bitterfeld-Wolfen, Germany) was included at 1.0% of the diet at the expense of ground corn.

<sup>6</sup>Premix provided per kg of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 26.4 g Mn from manganese oxide; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

**Table 2. Phase 2 diet composition (as-fed basis)<sup>1</sup>**

<b>Ingredient, %</b>	<b>Control</b>	<b>Zinc oxide<sup>2</sup></b>	<b>Formic acid<sup>3</sup></b>	<b>Glycerol monolaurate<sup>4</sup></b>	<b>Formic acid + GML blend<sup>5</sup></b>
Corn	45.98	45.73	45.28	45.80	44.98
Soybean meal, 47.5% CP	22.75	22.75	22.75	22.75	22.75
Spray dried whey	10.00	10.00	10.00	10.00	10.00
Corn DDGS, 7.5% oil	10.00	10.00	10.00	10.00	10.00
Enzyme-treated soybean meal <sup>6</sup>	4.50	4.50	4.50	4.50	4.50
Corn oil	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate, 21% P	1.00	1.00	1.00	1.00	1.00
NaCl	0.55	0.55	0.55	0.55	0.55
L-Lysine HCL	0.50	0.50	0.50	0.50	0.50
Vitamin premix w/phytase	0.25	0.25	0.25	0.25	0.25
L-Threonine	0.18	0.18	0.18	0.18	0.18
DL-Methionine	0.17	0.17	0.17	0.17	0.17
Trace mineral premix <sup>6</sup>	0.15	0.15	0.15	0.15	0.15
L-Valine	0.08	0.08	0.08	0.08	0.08
L- Tryptophan	0.04	0.04	0.04	0.04	0.04
Additive	-	0.25	0.70	0.18	1.00
Total	100.00	100.00	100.00	100.00	100.00

## Calculated analysis

## Standardized ileal digestible (SID) amino acids

Lysine	1.49	1.49	1.49	1.49	1.49
Ile:Lys	57	57	57	57	57
Leu:Lys	119	119	119	119	119
Met:Lys	34.9	34.9	34.9	34.9	34.9
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	64	64	64	64	64
Trp:Lys	18.7	18.7	18.7	18.7	18.7
Val:Lys	70	70	70	70	70
ME, kcal/lb	1,521	1,517	1,517	1,518	1,506
NE kcal/lb	1,176	1,173	1,173	1,174	1,164
SID lysine:NE, g/mcal	5.28	5.28	5.28	5.28	5.28
CP, %	21.2	21.2	21.2	21.2	21.2
Ca, %	0.75	0.75	0.75	0.75	0.75
STTD P, %	0.68	0.68	0.68	0.68	0.68

<sup>1</sup>Treatment diets were fed to 350 pigs (200 × 400, DNA; initially 12.5 ± 0.3 lb BW) from d 7 to d 28 of the study, which was considered dietary Phase 2.

<sup>2</sup>ZnO was included to provide 3,000 ppm Zn.

<sup>3</sup>Formic acid (Amasil-NA; BASF, Florham, NJ) was included at 0.70% at the expense of ground corn.

<sup>4</sup>Glycerol monolaurate (GML; Natural Biologics, Newfield, NY) was guaranteed 90% purity and included in the diet at 0.18% at the expense of ground corn.

<sup>5</sup>A formic acid and glycerol monolaurate blend (FORMI-3G, ADDCON GmbH, Bitterfeld-Wolfen, Germany) was included at 1.0% of the diet at the expense of ground corn.

<sup>6</sup>Premix provided per kg of premix: 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 26.4 g Mn from manganese oxide; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

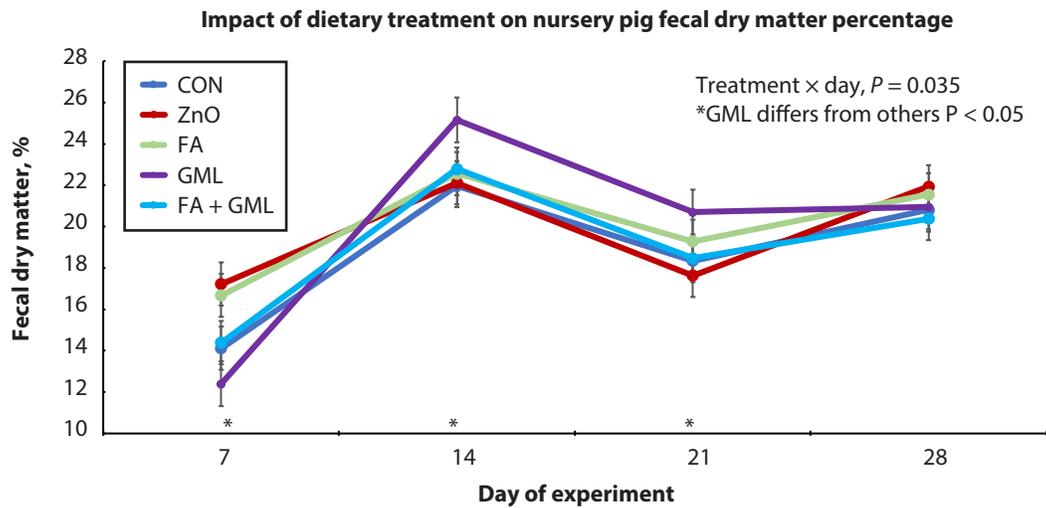
**Table 3. Impact of dietary treatment on nursery pig growth performance<sup>1</sup>**

Item	Dietary treatment <sup>2</sup>					SEM	P-value Treatment
	Control	ZnO	Formic acid	GML	Formic acid + GML blend		
BW, lb							
d 0	12.4	12.6	12.7	12.5	12.4	0.13	0.343
d 7	13.2	13.7	13.4	13.6	13.7	0.14	0.035
d 14	16.1 <sup>ab</sup>	17.1 <sup>a</sup>	16.1 <sup>b</sup>	15.9 <sup>b</sup>	16.8 <sup>ab</sup>	0.25	0.003
d 28	30.8 <sup>bc</sup>	33.8 <sup>a</sup>	30.8 <sup>bc</sup>	29.1 <sup>c</sup>	32.2 <sup>ab</sup>	0.45	<0.0001
d 42	52.4 <sup>b</sup>	55.4 <sup>a</sup>	51.3 <sup>bc</sup>	49.6 <sup>c</sup>	53.4 <sup>ab</sup>	0.70	<0.0001
d 0 to 7							
ADG, lb/d	0.11 <sup>b</sup>	0.19 <sup>a</sup>	0.13 <sup>ab</sup>	0.16 <sup>ab</sup>	0.19 <sup>a</sup>	0.020	0.003
ADFI, lb/d	0.25	0.29	0.28	0.25	0.30	0.018	0.161
F/G	2.33 <sup>b</sup>	1.52 <sup>ab</sup>	2.15 <sup>b</sup>	1.57 <sup>a</sup>	1.60 <sup>ab</sup>	0.027	0.002
d 7 to 14							
ADG, lb/d	0.43 <sup>ab</sup>	0.50 <sup>a</sup>	0.38 <sup>bc</sup>	0.33 <sup>c</sup>	0.45 <sup>ab</sup>	0.024	<0.0001
ADFI, lb/d	0.49	0.57	0.49	0.52	0.52	0.025	0.154
F/G	1.14 <sup>a</sup>	1.16 <sup>a</sup>	1.29 <sup>a</sup>	1.59 <sup>b</sup>	1.15 <sup>a</sup>	0.039	<0.0001
Overall treatment (d 0 to 28)							
ADG, lb/d	0.65 <sup>bc</sup>	0.73 <sup>a</sup>	0.65 <sup>bc</sup>	0.59 <sup>c</sup>	0.71 <sup>ab</sup>	0.017	<0.0001
ADFI, lb/d	1.02	1.11	1.04	1.01	1.05	0.028	0.119
F/G	1.57 <sup>a</sup>	1.51 <sup>a</sup>	1.58 <sup>ab</sup>	1.70 <sup>b</sup>	1.49 <sup>a</sup>	0.011	<0.0001
Overall common (d 28 to 42)							
ADG, lb/d	1.55	1.54	1.50	1.50	1.52	0.029	0.254
ADFI, lb/d	2.15	2.19	2.17	2.06	2.16	0.056	0.555
F/G	1.39	1.42	1.45	1.40	1.42	0.017	0.732
Overall trial (d 0 to d 42)							
ADG, lb/d	0.95 <sup>ab</sup>	1.00 <sup>a</sup>	0.93 <sup>bc</sup>	0.88 <sup>c</sup>	0.98 <sup>ab</sup>	0.015	<0.0001
ADFI, lb/d	1.39	1.46	1.41	1.36	1.42	0.031	0.233
F/G	1.47	1.46	1.51	1.54	1.45	0.010	0.031

<sup>1</sup>A total of 350 weanling pigs (200 × 400, DNA; initially 12.5 lb BW) were used in a 42-d experiment.

<sup>2</sup>Dietary treatments consisted of: 1) negative control; 2) negative control with 3,000 ppm ZnO in phase 1 and 2,000 ppm ZnO in phase 2; 3) negative control with 0.7% added formic acid; 4) negative control with 0.18% added glycerol monolaurate; and 5) negative control with 1.0% blend of formic acid, sodium diformate, and glycerol monolaurate.

<sup>ab</sup>Means within a row that do not share a common superscript differ  $P < 0.05$ .



**Figure 1. Impact of dietary treatment on nursery pig fecal DM percentage.** A total of 350 pigs (200 × 400, DNA; initially 5.67 kg BW) were fed experimental diets for 28 d. Diets consisted of a negative control (CON); control diet with zinc oxide (ZnO); control diet with formic acid (FA); control diet with glycerol monolaurate (GML); and the control diet with a blend of formic acid and glycerol monolaurate (FA + GML).