# Kansas Agricultural Experiment Station Research Reports

Volume 9 Issue 7 *Swine Day* 

Article 9

2023

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## **Recommended Citation**

Bromm, Jenna J.; Tokach, Mike D.; Woodworth, Jason C.; Goodband, Robert D.; DeRouchey, Joel M.; Flohr, Josh R.; Schmitt, Raymond A. M.; Gebhardt, Jordan T.; and Zarate, Felipe (2023) "Effects of Increasing Alpha-Linolenic Acid on Growth Performance and Mortality Rate in PRRS-Virus Challenged Nursery Pigs," *Kansas Agricultural Experiment Station Research Reports*: Vol. 9: Iss. 7. https://doi.org/10.4148/2378-5977.8510

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# Effects of Increasing Alpha-Linolenic Acid on Growth Performance and Mortality Rate in PRRS-Virus Challenged Nursery Pigs

# **Funding Source**

The authors thank NBO3 (Manhattan, KS) for providing partial funding and O-3 Trial Feed for this project.

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# Effects of Increasing Alpha-Linolenic Acid on Growth Performance and Mortality Rate in PRRS-Virus Challenged Nursery Pigs

Jenna J. Bromm, Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Joel M. DeRouchey, Josh R. Flohr,<sup>1</sup> Raymond A. M. Schmitt,<sup>1</sup> Jordan T. Gebhardt,<sup>2</sup> and Felipe Zarate<sup>1</sup>

# **Summary**

A total of 91,140 weaned pigs, (DNA 600  $\times$  PIC 1050; initially 11.33  $\pm$  0.62 lb) originating from PRRSV-positive sow farms, were used across 8 nursery sites to evaluate growth performance, total removal and mortality rate, and medication usage of nursery pigs fed diets containing 0 or 3% O3 Trial Feed (NBO3 Technologies LLC, Manhattan, KS), a source of omega-3 fatty acids. Each of the 8 sites contained 5 barns with 2 rooms in each barn. Rooms of pigs were blocked by nursery site and allocated by sow source to 1 of 2 dietary treatments. Thus, there were 40 groups (rooms) per treatment with approximately 1,100 pigs per room. The first treatment was a standard nursery diet specific to the production system. The second treatment was the same standard nursery diet with the addition of 3% O3 Trial Feed. At placement, pigs were fed a pre-starter and then fed experimental diets across 3 phases with all diets fed in pelleted form. Overall, there were no significant differences (P > 0.10) observed in growth performance between pigs fed diets containing 0 or 3% O3 Trial Feed. Pigs fed control diets had reduced (P < 0.001) total removals and mortality percentage compared to pigs fed diets containing 3% O3 Trial Feed. When evaluating medication usage, there were no significant differences (P > 0.10) observed in the total number of injections given per 1,000 pig days. However, pigs fed diets containing 3% O3 Trial Feed had a reduced (P < 0.001) number of total injections per pig placed. In summary, the increase in alpha-linolenic acid in the diet, through the inclusion of 3% O3 Trial Feed, did not impact growth performance during the duration of this trial. There was an increase in total removals and mortality in pigs fed diets containing O3 Trial Feed. However, there was a reduction in total injections given per pig placed in pigs fed diets containing O3 Trial Feed. We hypothesize that because of the high prevalence of PRRS at entry, O3 Trial Feed may not have had sufficient time to impact the immune system before the PRRS challenge.

# Introduction

The inclusion of omega-3 fatty acid products in swine diets have been used to reduce the omega-6:3 fatty acid ratio. This reduction in the omega-6:3 fatty acid ratio has been

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shown to improve growth performance and the immune response in nursery pigs.<sup>3</sup> O3 Trial Feed (NBO3 Technologies LLC, Manhattan, KS) is a flax seed and algae-derived source of omega-3 fatty acids (alpha-linolenic acids) that has been used to increase omega-3 content of pork. The profile of fatty acids makes the product a viable option to reduce the omega-6:3 fatty acid ratio for nursery pigs. Recent field research observed that the inclusion of O3 Trial Feed in nursery diets improved growth performance and reduced mortality rate in PRRSV-challenged pigs.<sup>4</sup> However, more research is needed to evaluate the inclusion of O3 Trial Feed in a different commercial system with different production stressors and disease challenges. Therefore, the objective of this study was to determine the influence of O3 Trial Feed, a source of omega-3 fatty acids (alpha-linolenic acid), on growth performance, total removal and mortality rate, and medication usage under field conditions. Our hypothesis was that the addition of omega-3 fatty acids (O3 Trial Feed) would improve growth performance and decrease mortality when fed to pigs in commercial nursery facilities.

# Procedures

## General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at Seaboard Foods in northwest Oklahoma and southwest Kansas. At weaning, pigs were moved and housed in temperature-controlled nursery facilities. Each barn contained 2 rooms and each room contained 40 pens with 27 to 28 pigs per pen (approximately 1,100 pigs per room). Each pen (9.75 × 6.46 ft) contained one nipple waterer and one 6-hole stainless steel self-feeder. Feed was provided to each room through a single auger feedline system. Access to feed and water was provided *ad libitum*. Pigs were allowed approximately 2.25 ft<sup>2</sup>/pig.

## Animal and treatment structure

A total of 91,140 weaned pigs, (DNA 600 × PIC 1050; initially  $11.33 \pm 0.62$  lb) originating from a PRRSV-positive sow source were used across 8 nursery sites. Each site contained 5 barns with 2 rooms in each barn and approximately 1,100 pigs per room. Rooms of pigs were blocked by nursery site and allocated, by sow source, to 1 of 2 dietary treatments with 40 groups (rooms) per treatment. The first treatment was a standard nursery diet specific to the production system and did not contain O3 Trial Feed. The second treatment was the same standard nursery diet with the supplementation of 3% O3 Trial Feed. An initial BW was estimated for all pigs at placement. At the end of each nursery turn, pigs were loaded onto a semi-truck which was weighed to determine close-out weights. Feed intake was determined by the difference between the amount of feed delivered and the feed remaining upon completion of the nursery group. The data were used to determine ADG, ADFI, and F/G. Adjusted ADG was calculated by adding total removal and mortality weight to the total gain to create an adjusted total gain, which then was divided by pig days. Adjusted gain was then used to

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<sup>&</sup>lt;sup>3</sup> Huber L. A., S. Hooda, R. E. Fisher-Heffernan, N. A. Karrow, and C. F. M. de Lange. 2018. Effect of reducing the ratio of omega-6-to-3 fatty acids in diets of low protein quality on nursery pig growth performance and immune response. J. Anim. Sci. 96:4348-4359. doi:10.1093/jas/sky296.

<sup>&</sup>lt;sup>4</sup> Bromm J. B., M. T. Tokach, J. W. Woodworth, R. D. Goodband, J. M. DeRouchey, C. W. Hastad, Z. B. Post, and J. T. Gebhardt. 2021. Use of O3 trial feed to reduce omega-6:3 ratio in PRRS-Virus challenged nursery pigs. Kansas Agricultural Experiment Station Research Reports: Vol. 7 Issue 11. doi:10.4148/2378-5977.8181.

calculate an adjusted F/G. After the shipping of pigs in each nursery room, all water and injectable treatment records were collected to evaluate medication usage.

# **Diet preparation**

At placement, all pigs received 1 lb/pig of a common pre-starter diet containing no O3 Trial Feed. Pigs were then fed experimental diets across 3 phases (Table 1). Pigs were fed on a feed budget, receiving 6 lb/pig of phase 1 and 15 lb of phase 2 before being fed phase 3 for the remainder of the study. The SID Lys concentration was formulated to 1.35% for phase 1, 1.30% for phase 2, and 1.28% for phase 3. All other nutrients were formulated to meet or exceed NRC<sup>5</sup> requirement estimates. O3 Trial Feed was added at the expense of corn while adjusting feed grade amino acids and enzymatically treated soybean meal to maintain similar soybean meal levels and amino acid profiles. Diets for phase 1 and phase 2 were manufactured at Seaboard Feed Mill (Leoti, KS) and phase 3 was manufactured at Seaboard Feed Mill (Hugoton, KS). All diets were fed in pelleted form.

# Chemical analysis

Diet samples for each treatment were collected weekly from feeders in each room throughout the study. Complete diet samples were sent to the Kansas State University Swine Laboratory and stored at -4°F. Samples were then subsampled to create a composite sample for each treatment and submitted for analysis. Samples of each dietary treatment were analyzed (NBO3 Technologies LLC; Manhattan, KS) for fatty acid profiles (Table 2).

## Collection and analysis of oral fluid samples

Cotton ropes were placed in each room every other week to determine if pigs were positive for PRRSV North American and European strains. New ropes were placed in each room for 30 minutes on each sample day, and then the oral fluid from each rope were collected to create 2 duplicate samples for each room. The oral fluid samples were then frozen and sent to the Kansas State University Swine Laboratory and stored at -4°F. Samples were processed at the Kansas State University Veterinary Diagnostics Laboratory using the Tetracore PRRS Multiplex real time PCR procedures.

# Statistical analysis

Growth performance data were analyzed using the nlme package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as randomized complete block design with room serving as the experimental unit. Mortality and medication data were analyzed using the GLIMMIX procedure of SAS (version 9.4, Cary, NC). Total removals and mortality data were analyzed assuming a binomial distribution with a logit link function. Medication data were analyzed using a Poisson distribution with an offset function using the log transformed number of days at risk for each experimental unit, or count of pigs placed, and data were reported as count of injections per 1,000 pig days and count of injections per pig placed, respectively. Differences between treatments were considered significant at  $P \le 0.05$  and marginally significant at  $0.05 < P \le 0.10$ .

<sup>&</sup>lt;sup>5</sup> National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. doi:10.17226/13298.

# **Results and Discussion**

Of the oral fluids taken from the ropes placed in each room, 61 of the 80 rooms tested positive for PRRSV North American one-week post-placement into the nursery. Oral fluid samples from 78 of the 80 rooms tested positive for PRRSV North American 3 weeks post-placement into the nursery, and all oral fluid samples from each room tested positive for PRRSV North American for the remainder of the nursery turn. All samples tested negative for PRRSV European on each collection day.

Overall, there were no significant differences observed in ADG, ADFI, or F/G between pigs fed the control diets or those containing 3% O3 Trial Feed (P > 0.10; Table 3). Similarly, there were no significant differences observed for adjusted ADG or F/G (P > 0.10). There were also no main effects of sow flow between pigs fed the control diets or those fed diets containing 3% O3 Trial Feed (P > 0.10). Pigs fed control diets had reduced (P < 0.001) total removals and mortalities compared to pigs fed diets containing 3% O3 Trial Feed.

For medications given per 1,000 pig days, pigs fed diets containing 3% O3 Trial Feed tended to have reduced (P = 0.097) number of injections of Enrofloxacin compared to pigs fed control diets (Table 3). However, there was an increase ( $P \le 0.036$ ) in the number of injections of Dexamethasone and Ceftiofur hydrochloride given to pigs fed diets containing 3% O3 Trial Feed. There were no significant differences observed in the total number of injections given per 1,000 pig days (P > 0.10).

For injections given per pig placed, pigs fed diets containing 3% O3 Trial Feed tended to have increased ( $P \le 0.065$ ) number of injections of Dexamethasone and Ceftiofur hydrochloride compared to pigs fed control diets without O3 Trial Feed (Table 3). However, pigs fed diets containing 3% O3 Trial Feed had reduced (P < 0.001) number of injections of Enrofloxacin and total injections per pigs placed compared to pigs fed diets without O3 Trial Feed.

In summary, the increase in alpha-linolenic acid in the diet, through the inclusion of 3% O3 Trial Feed, did not impact growth performance during the duration of this trial. There was an increase in total removals and mortality in pigs fed diets containing O3 Trial Feed. However, there was a reduction in total injections given per pig placed in pigs fed diets containing O3 Trial Feed. We hypothesize that because of the high prevalence of PRRS at entry, O3 Trial Feed may not have had sufficient time to impact the immune system before the PRRS challenge.

# Acknowledgments

The authors thank NBO3 (Manhattan, KS) for providing partial funding and O-3 Trial Feed for this project.

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.

	Phase 1		Phase 2		Phase 3	
-	O3 Tr		O3 Tria	al Feed, %		
Ingredient, %	0	3	0	3	0	3
Corn	43.97	40.58	59.30	56.13	56.72	53.30
Soybean meal (47%)	20.00	20.00	32.50	32.50	37.25	37.83
Base mix <sup>2</sup>	21.13	21.13				
Enzymatically treated soybean meal <sup>3</sup>	6.09	6.42	0.78	1.09		
Lucrafit TM 50 <sup>4</sup>	2.15	2.15	1.25	1.25		
Monocalcium phosphate <sup>5</sup>	1.52	1.58	1.15	1.17	0.84	0.86
Beef tallow	0.86	0.86	1.64	1.54	2.48	2.39
Limestone	0.41	0.40	0.54	0.54	0.66	0.66
L-Lys	0.68	0.64	0.65	0.61	0.46	0.41
L-Trp	0.10	0.10	0.10	0.09	0.08	0.07
L-Val	0.06	0.10	0.09	0.10		
L-Thr	0.20	0.20	0.22	0.20	0.17	0.15
Vitamin premix-nursery <sup>6</sup>	0.05	0.05	0.05	0.05		
Vitamin premix-GF <sup>7</sup>					0.08	0.08
Trace mineral premix <sup>8</sup>	0.10	0.10	0.08	0.08	0.08	0.08
Salt	0.73	0.73	0.60	0.60	0.40	0.40
Phytase <sup>9</sup>	0.06	0.06	0.06	0.06	0.06	0.06
Copper chloride	0.03	0.03	0.03	0.03	0.03	0.03
Choline chloride	0.03	0.03				
Zinc oxide (72%)	0.32	0.32	0.32	0.32		
FXP <sup>10</sup>	0.40	0.40	0.20	0.20		
Liquid methionine <sup>11</sup>	0.30	0.31	0.21	0.21	0.17	0.15
N-hance <sup>10</sup>	0.30	0.30				
CTC 100 g	0.25	0.25			0.25	0.25
Oxytetracycline 200 g			0.13	0.13		
Denagard 10 g	0.18	0.18			0.18	0.18
Synthetic red dye		0.01		0.01		0.01
Synthetic blue dye	0.01		0.01		0.01	
O3 Trial Feed <sup>12</sup>		3.00		3.00		3.00

### Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>

continued

•	Pha	Phase 1		Phase 2		Phase 3	
		O3 Trial Feed, %					
Ingredient, %	0	3	0	3	0	3	
Calculated analysis							
SID Amino acids, %							
Lys	1.35	1.35	1.30	1.30	1.28	1.28	
Ile:Lys	58	58	58	58	64	64	
Met and Cys:Lys	58	58	58	58	58	58	
Thr:Lys	64	64	64	64	64	64	
Trp:Lys	24	24	24	24	24	24	
Val:Lys	72	72	72	72	70	70	
Total Lys, %	1.46	1.46	1.43	1.43	1.42	1.43	
NE NRC, kcal/lb	1,190	1,180	1,220	1,220	1,260	1,260	
SID Lys:NE, g/Mcal	5.15	5.19	4.83	4.83	4.61	4.61	
CP, % <sup>13</sup>	21.79	22.68	21.51	22.00	22.58	23.14	
Crude fat, %	4.10	4.42	4.10	4.52	4.80	5.22	
Ca, %	0.68	0.68	0.67	0.67	0.70	0.70	
P, %	0.67	0.68	0.59	0.59	0.54	0.54	
STTD P, %	0.50	0.50	0.47	0.47	0.43	0.43	

#### Table 1. Experimental diet composition (as-fed basis)<sup>1</sup>

<sup>1</sup>Pigs were fed experimental diets on a feed budget with Phase 1 and 2 provided at 6 and 15 lb per pig. Phase 3 was provided for the remainder of the study.

<sup>2</sup>Quincy Farms, Quincy, IL.

<sup>3</sup>HP300; Hamlet Protein, Findley, OH.

<sup>4</sup>Purina Animal Nutrition, Arden Hills, MN.

5NexFos; The Mosaic Company, Plymouth, MN.

<sup>6</sup>Provided per lb of premix: 11,000,000 IU vitamin A; 1,700,000 IU vitamin  $D_3$ ; 100,000 IU vitamin E; 3,000 mg menadione; 9,000 mg riboflavin; 44,000 mg niacin; 36,000 mg pantothenic acid; 42 mg vitamin  $B_{12}$ ; 100 mg biotin; 1,600 mg folic acid; 3,000 mg pyridoxine; 272 mg selenium; and 14,528,000 BXU xylanase.

<sup>7</sup>Provided per lb of premix: 4,000,000 IU vitamin A; 700,000 IU vitamin D<sub>3</sub>; 20,000 IU vitamin E; 1,500 mg menadione; 3,000 mg riboflavin; 12,500 mg niacin; 12,000 mg pantothenic acid; 12 mg vitamin  $B_{12}$ ; 170 mg selenium; and 11,622,400 BXU xylanase.

<sup>8</sup>Provided per lb of premix: 85,049 mg Zn from zinc oxide and zinc sulfate, 43,091 mg Fe from ferrous sulfate, 14,175 mg Mn from manganese sulfate and manganese oxide, 8,505 mg Cu from copper sulfate, and 340 mg I from calcium iodate.

<sup>9</sup>Axtra PHY Gold (Danisco Animal Nutrition, Cedar Rapids, IA) was included to provide approximately 758 FTU/lb in phase 1, 850 FTU/lb in phase 2, and 870 FTU/lb in phase 3 providing an estimated release of 0.08, 0.11, and 0.12% STTD P, for phase 1, 2, and 3, respectively.

<sup>10</sup>Ani-Tek, Social Circle, GA.

<sup>11</sup>Aliment; Novus International Inc., Saint Charles, MS.

<sup>12</sup>O3 Trial Feed (NBO3 Technologies LLC, Manhattan, KS) was added at 3% at the expense of corn while adjusting feed grade amino acids and enzymatically treated soybean meal to maintain similar soybean meal levels and amino acid profiles. <sup>13</sup>CP = crude protein.

	O3 Trial Feed, <sup>2</sup> %			
Fatty acid, %	0	3		
Total fatty acid	5.14	5.44		
Total fat	5.72	6.05		
Omega-6:3	14.74	4.57		
C16:0	0.99	0.95		
C18:1n9c	1.41	1.44		
C18:2n6c <sup>3</sup>	1.67	1.79		
C18:3n3 <sup>4</sup>	0.11	0.39		

Table 2. Analy	vzed fattv	acid com	position of	experimental	diets <sup>1</sup>
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<sup>1</sup>Composites of complete diets contained trace levels of C6:0, C8:0, C10:0, C12:0, C14:0, C14:1n5, C15:0, C16:1n7, C17:0, C18:0, C18:1n9t, C18:1n7t, C18:1n7c, C18:3n6, CLA 9c, 11t (n7), C20:0, C201n9, C20:2n6, C22:0, C23:0, C24:0, and C24:1n9 of < 0.10%. Other fatty acids levels were too low to be detected in the analysis. <sup>2</sup>O3 Trial Feed was analyzed to contain 20.13% total FA, 22.37% total fat, 0.46% omega-6:3, 1.38% C16:0, 4.67% C18:1n9c, 4.03% C18:2n6c, and 8.73% C18:3n3.

<sup>3</sup>Major omega-6 fatty acid.

<sup>4</sup>Major omega-3 fatty acid.

	O3 Trial Feed, <sup>2</sup> %			
Item	0	3	SEM	P =
BW, lb				
d 0	11.4	11.3	0.11	0.316
d 43	39.0	38.8	0.67	0.707
Overall (d 0 to 43)				
No adjustment				
ADG, lb	0.63	0.62	0.015	0.555
ADFI, lb	0.96	0.94	0.028	0.313
F/G	1.56	1.53	0.058	0.559
Mortality/removal adjustment				
ADG, lb	0.67	0.67	0.012	0.912
F/G	1.43	1.40	0.056	0.268
Total removals and mortality, %	7.7	8.9	1.13	< 0.001
Injections per 1,000 pig days, n				
Total injections	18.26	18.03	1.170	0.226
Enrofloxacin	17.57	17.30	1.169	0.097
Dexamethasone	0.31	0.35	0.046	0.036
Ceftiofur hydrochloride	0.30	0.34	0.040	0.032
Injections per pig placed, n				
Total injections	0.70	0.68	0.042	< 0.001
Enrofloxacin	0.68	0.65	0.042	< 0.001
Dexamethasone	0.136	0.135	0.0018	0.065
Ceftiofur hydrochloride	0.012	0.013	0.0016	0.059

Table 3. Effects of O3 Trial Feed on growth performance, total removals and mortality, and medication usage in nursery pigs<sup>1</sup>

 $^{1}$ A total of 91,140 (initially 11.33 ±0.62 lb) were used with 40 rooms per treatment and approximately 1,100 pigs per room.

<sup>2</sup>O3 Trial feed is a flax seed and algae-derived source of omega-3 fatty acids (alpha-linolenic acids).