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Effects of Increasing Dietary Alpha-Linolenic Acids on Nursery Pig Growth Performance and Response to Immune Challenge

Jenna J. Bromm, Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Joel M. DeRouchey, and Jordan T. Gebhardt[1]

Summary

A total of 350 weanling pigs (Line 241 × 600, DNA; initially 12.7 ± 0.1 lb BW) were used in a 41-d study to evaluate growth performance and immune response of nursery pigs fed diets containing increasing alpha-linolenic acids using O3 Trial Feed, a source of omega-3 fatty acids. At weaning, pigs were randomly assigned to 1 of 5 dietary treatments with 5 pigs per pen and 14 replications per treatment. Treatments were arranged in a completely randomized design. Dietary treatments consisted of increasing levels (0, 1, 2, 3, or 4%) of O3 Trial Feed. This resulted in omega-6:3 fatty acid ratios ranging from approximately 25:1 to 4:1. Treatment diets were fed in 3 phases with phase 1 fed from d 0 to 13, phase 2 from d 13 to 22, and phase 3 from d 22 to 41. On d 25, two pigs per pen were injected with 20 μg of *Eschrichia coli* (*E. coli*) lipopolysaccharide (LPS) per kg BW and one pig per pen was injected with 2 mL of saline to serve as a control. Body temperature was recorded from the 3 pigs per pen prior to the injection (hour 0) and 2, 4, 6, and 12 h after injection. On d 25 a blood sample was collected 4 h post injection from pigs injected with the LPS challenge to determine IL-1β levels in the serum. For overall growth performance, there were no differences observed in ADG, ADFI, or F/G. Temperature increased at 2 h post LPS injection, then decreased as time from the LPS injection increased, but dietary treatment did not influence change in body temperature or IL-1β. These results indicate dietary alpha-linolenic acid levels did not influence growth performance or immune response to a LPS challenge.

Introduction

Omega-3 fatty acids (alpha linolenic acid) have been found to improve immune function and lessen the febrile response to immune system activation. The mode of action is thought to be through a decrease in the omega-6:3 fatty acid ratio. Research has demonstrated that lowering the ratio of omega-6:3 to a range of 3:1 or 5:1 instead of the normal 10:1 or 20:1 observed in typical swine diets, increases incorporation of omega-3 fatty acids into cell membranes to make it available to be utilized during an immune challenge.[2]

[1] Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.

[2] Huber, L., Hooda, S., Fisher-Heffernan, R. E., Karrow, N. A, De Lange, C. F. (2018). Effect of reducing the ratio of omega-6-to-omega-3 fatty acids in diets of low protein quality on nursery pig growth performance and immune response. J. Anim. Sci. 96(10):4348-4359. doi:10.1093/jas/sky296.

O3 Trial Feed is an algae-derived source of omega-3 fatty acids that has been used to increase omega-3 content of pork. The fatty acid profile makes it a viable option to improve the omega-6:3 fatty acid ratio for nursery pigs and improve immune status. Additions of approximately 3% of O3 Trial Feed would result in a final diet containing 4:1 to 6:1 omega-6:3 fatty acid ratio depending on the composition of the basal diet. Our hypothesis was that by changing the omega-6:3 ratio using O3 Trial Feed, we would observe an improved responsiveness to an immune challenge. Therefore, the objective of this study was to determine the effectiveness of O3 Trial Feed, a source of omega-3 fatty acids, on nursery pig performance and response to an LPS immune challenge.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility is completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (4 × 4 ft) had metal tri-bar floors and allowed approximately 3.2 ft^2/pig.

Animal treatment and structure

A total of 350 weanling pigs (Line 241 × 600, DNA) with initial BW of 12.7 ± 0.1 lb were used in a 41-d trial. There were 5 pigs per pen and 14 replications per treatment. Pens of pigs were randomly assigned to 1 of 5 dietary treatments in a completely randomized design. The dietary treatments included increasing O3 Trial Feed (0, 1, 2, 3, and 4%; Table 1). The addition of O3 Trial Feed decreased the omega-6:3 ratios for the 5 treatment diets within each phase (Table 2). Individual pigs were weighed, and feed disappearance was recorded on d 0, 7, 13, 20, 22, 32, and 41 to determine ADG, ADFI, and F/G.

Diet preparation

Pigs were fed experimental diets from d 0 to 41. Diets were formulated to 1.40% SID Lys for phase 1, 1.35% SID Lys for phase 2, and 1.30% SID Lys for phase 3. All other nutrients were formulated to meet or exceed NRC (2012)[3] requirement estimates. For phase 1 and 2, a single base diet was manufactured at Hubbard Feeds (Beloit, KS) with O3 Trial Feed, corn, and soybean meal additions mixed at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) to make the final diets. Phase 3 diets were manufactured at Hubbard Feeds (Beloit, KS). Phase 1 was fed in pellet form and phases 2 and 3 were fed in meal form.

Chemical analysis

Phase 1 and 2 diet samples were collected at manufacturing, and phase 3 diet samples were collected from every fifth 50-lb bag using a feed probe to obtain a representative sample for each respective diet and phase. Complete diet samples were stored at -4°F until they were homogenized, subsampled, and submitted for analysis. Samples for each

[3] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. doi:10.17226/13298.

dietary treatment in each phase were analyzed (NBO3 Technologies LLC, Manhattan, KS) for fatty acid profiles.

Immune challenge

On d 25, two pigs per pen (those closest to the average weight of the pen) were injected intramuscularly in the neck with 20 micrograms *Escherichia coli* (*E. coli*) LPS per kg BW. An additional pig in each pen was injected with 2 mL of saline to serve as a control. Body temperature was taken from all 3 pigs prior to the injection (hour 0) and at 2, 4, 6, and 12 h after injection. A blood sample was taken from pigs injected with the *E. coli* LPS challenge on d 24 (day prior to challenge) and 4 hours after the *E. coli* LPS injection to determine immune response. Blood samples were centrifuged at 39.2°F (4°C) at 1800 × g for 30 min. Serum was frozen in separate aliquots for later analysis for IL-1β.

Cytokine analysis

For IL-1 β, samples were analyzed in triplicate within a single assay. Serum concentrations of IL-1 β were determined using an IL-1 β ELISA kit per the instructions of the manufacturer (R & D Systems, Minneapolis, MN).

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 a completely randomized design with pen as the experimental unit. Linear and quadratic contrasts in response to increasing O3 Trial Feed were measured among treatments. Differences between treatments 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 13), there was a tendency (linear, $P = 0.065$) for increased ADG with increasing alpha-linolenic acids supplied by O3 Trial Feed (Table 3). There was a significant (quadratic, $P = 0.046$) effect on ADFI, with feed intake decreasing from 0 to 1% inclusion of O3 Trial Feed and then increasing as O3 Trial Feed increased up to 4% in the diet. There was no difference observed for F/G. On d 13, increasing O3 Trial Feed increased BW (linear, $P = 0.042$).

In Phase 2 (d 13 to 22), there were no differences observed in ADG or F/G. However, increasing O3 Trial Feed resulted in a quadratic response ($P = 0.013$) for ADFI, with pigs fed 2% O3 Trial Feed having the highest ADFI. There were no differences observed for BW during this period.

In Phase 3 (d 22 to 41), no differences were observed in ADG, ADFI, or BW. However, during this period, feeding increasing levels of O3 Trial Feed improved (linear, $P = 0.046$) F/G. For overall growth performance, there were no differences observed in ADG, ADFI, or F/G.

There was no interaction between change in body temperature and inclusion of O3 Trial Feed. However, there was a main effect of time. Pigs responded as expected with an increase in body temperature at 2 h post LPS challenge. Then, body tempera-

ture decreased as time post-challenge increased (Figure 1). There were no differences observed in IL-1β concentrations from baseline to 4 h post LPS challenge (Table 3).

These results indicate that dietary alpha linoleic acid level did not influence growth performance or immune response to LPS challenge. These results are in contrast to the benefit of dietary alpha linoleic acid from O3 Trial Feed found in a companion experiment[4] conducted with PRRSV-positive pigs.

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.

[4] Bromm, J.J; Tokach, M.D.; Woodworth, J.C.; Goodband, R.D.; DeRouchey, J.M.; Hastad, C.W.; Post, Z.B.; and Gebhardt, J.T. 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.

Table 1. Diet composition[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	40.32	56.37	66.12
Soybean meal (46.5% CP)	18.98	24.31	29.00
Dried whey	25.00	---	---
Whey permeate, 80% lactose	---	9.00	---
Corn DDGS, 7.5% oil	5.00	---	---
Enzymatically-treated soybean meal[2]	5.00	5.00	---
Corn oil	2.00	1.00	1.00
Calcium carbonate	0.50	0.75	0.75
Monocalcium phosphate (21% P)	0.80	1.10	0.95
Sodium chloride	0.30	0.55	0.60
L-Lys-HCl	0.55	0.55	0.53
DL-Met	0.25	0.25	0.22
L-Thr	0.22	0.25	0.23
L-Trp	0.05	0.04	0.04
L-Val	0.17	0.17	0.16
Vitamin premix with phytase	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15
Choline chloride	0.04	---	---
Zinc oxide	0.41	0.25	---
Phytase[3]	0.02	0.02	0.02
O3 Trial Feed[4]	+/-	+/-	+/-

continued

Table 1. Diet composition[1]

Ingredient, %	Phase 1	Phase 2	Phase 3
Calculated analysis			
SID Amino acids, %			
Lys	1.40	1.35	1.30
Ile:Lys	55	54	54
Leu:Lys	110	108	114
Met and Cys:Lys	58	58	58
Thr:Lys	64	63	64
Trp:Lys	19.5	19.1	18.9
Val:Lys	70	70	71
His:Lys	32	34	36
Total Lys, %	1.53	1.48	1.44
NE, kcal/lb	1,174	1,144	1,129
SID Lys:NE, g/Mcal	5.41	5.35	5.22
CP, %	20.5	20.3	20.2
Ca, %	0.65	0.69	0.64
P, %	0.66	0.63	0.58
STTD P, %	0.58	0.51	0.46
Ca:P	0.99	1.10	1.10

[1]Phase 1 diets were fed from d 0 to 13 (approximately 12.7 to 16.0 lb BW). Phase 2 diets were fed from d 13 to 22 (approximately 16.0 to 25.4 lb BW). Phase 3 was fed from d 22 to 41 (approximately 24.5 to 50.2 lb BW).
[2]Hamlet Protein, Findlay, OH.
[3]Quantum Blue 5G (AB Vista, Marlborough, UK) provided a release of 0.13% STTD P with 907 FTU/lb.
[4]O3 Trial Feed was added at 0, 1, 2, 3, and 4% at the expense of corn from the experimental diets (NBO3 Technologies LLC, Manhattan, KS).

Table 2. Analyzed fatty acid composition of experimental diets[1]

	O3 Trial Feed,[2] %				
Fatty Acid, %	**0**	**1**	**2**	**3**	**4**
Phase 1 (d 0 to 13)					
Total fatty acids	4.21	4.34	4.72	4.96	4.89
Total fat	4.68	4.82	5.25	5.51	5.44
Omega-6:3	18.57	9.56	6.44	4.94	4.07
C16:0	0.61	0.61	0.64	0.66	0.64
C18:1n9c	1.01	1.01	1.10	1.15	1.12
C18:2n6c[3]	2.26	2.25	2.36	2.11	2.32
C18:3n3[4]	0.12	0.24	0.37	0.49	0.57
Phase 2 (d 13 to 22)					
Total fatty acids	3.95	9.97	4.64	4.53	4.56
Total fat	4.39	4.41	5.15	5.04	5.06
Omega-6:3	15.03	9.60	5.38	4.29	3.78
C16:0	0.57	0.56	0.61	0.59	0.58
C18:1n9c	1.01	0.01	1.10	1.15	1.12
C18:2n6c	2.26	2.25	2.36	2.41	2.32
C18:3n3	0.12	0.24	0.37	0.49	0.57
Phase 3 (d 22 to 41)					
Total fatty acids	4.47	4.77	5.01	4.49	4.71
Total fat	4.96	5.30	5.61	4.99	5.23
Omega-6:3	20.69	41.44	6.71	4.86	3.80
C16:0	0.66	0.67	0.68	0.61	0.61
C18:1n9c	1.06	1.11	1.18	0.99	1.03
C18:2n6c	2.36	2.46	2.53	2.17	2.20
C18:3n3	0.11	0.24	0.38	0.45	0.58

[1]Complete diets contained trace levels of C12:0, C14:0, C15:0, C16:1n7, C17:0, C18:0, C18:1n9t, C18:1n7c, C18:3n6, CLA 9c, 11t (n7), C20:0, C20:2n6, C22:0, C23:0, and C24:0 of < 0.10%. Other fatty acids levels were too low to be detected in the analysis.

[2]O3 Trial Feed was analyzed to contain 23.29% total FA, 25.87% total fat, 0.36% omega-6:3, 1.43% C16:0, 4.17% C18:1n9c, 4.28%, C18:2n6c, and 12.09% C18:3n3.

[3]Major omega-6 fatty acid.

[4]Major omega-3 fatty acid.

Table 3. Influence of O3 Trial Feed on nursery pig performance[1]

	O3 Trial Feed,[2] %						*P* =	
Item	**0**	**1**	**2**	**3**	**4**	**SEM**	**Linear**	**Quadratic**
BW, lb								
d 0	12.7	12.6	12.8	12.7	12.8	0.07	0.366	0.927
d 13	15.8	15.4	16.4	16.0	16.4	0.25	0.042	0.831
d 22	25.5	24.5	26.0	26.1	25.2	0.39	0.361	0.441
d 41	50.7	48.1	51.4	50.6	50.1	0.69	0.534	0.975
Phase 1 (d 0 to 13)								
ADG, lb	0.24	0.21	0.28	0.25	0.28	0.017	0.065	0.732
ADFI, lb	0.43	0.38	0.44	0.45	0.52	0.026	0.003	0.046
F/G	1.91	1.87	1.62	1.94	1.92	0.129	0.993	0.245
Phase 2 (d 13 to 22)								
ADG, lb	1.06	0.99	1.06	1.08	0.98	0.027	0.502	0.281
ADFI, lb	1.32	1.34	1.40	1.37	1.27	0.034	0.487	0.013
F/G	1.25	1.37	1.32	1.28	1.29	0.033	0.947	0.116
Phase 3 (d 22 to 41)[3]								
ADG, lb	1.33	1.25	1.34	1.29	1.31	0.028	0.914	0.544
ADFI, lb	2.10	2.10	2.07	2.02	2.04	0.048	0.188	0.869
F/G	1.59	1.68	1.56	1.57	1.56	0.028	0.046	0.646
Overall (d 0 to 41)								
ADG, lb	0.92	0.85	0.94	0.90	0.91	0.017	0.497	0.695
ADFI, lb	1.39	1.36	1.40	1.36	1.38	0.025	0.889	0.726
F/G	1.52	1.61	1.50	1.51	1.53	0.024	0.264	0.906
IL-1β change, pg/mL[4]	506.1	615.5	777.8	430.6	543.2	147.8	0.796	0.308

[1]A total of 350 pigs (Line 241 × 600, DNA; initial BW of 12.7 lb) were used with 5 pigs per pen and 14 replications per treatment and were fed trial diets for a 41-day period.

[2]Omega-6:3 ratios for the five treatments within each phase were: Phase 1 (27.3:1, 11.6:1, 7.4:1, 5.4:1, 4.3:1); Phase 2 (23.0:1, 9.6:1, 6.1:1, 4.5:1, 3.6:1); and Phase 3 (24.4:1, 10.2:1, 6.5:1, 4.8:1, 3.8:1), respectively.

[3]Two pigs per pen were injected intramuscularly with 20 micrograms *Escherichia coli* (*E. coli*) LPS per kg BW on d 25 to measure immune responses.

[4]Change in IL-1β from baseline (0 h) to 4 h after intramuscular injection with 20 µg *Escherichia coli* LPS per kg BW on d 25. The average IL-1β baseline was 4.1 ± 1.68 pg/mL across all treatments and with all baseline values below 21.5 pg/mL.

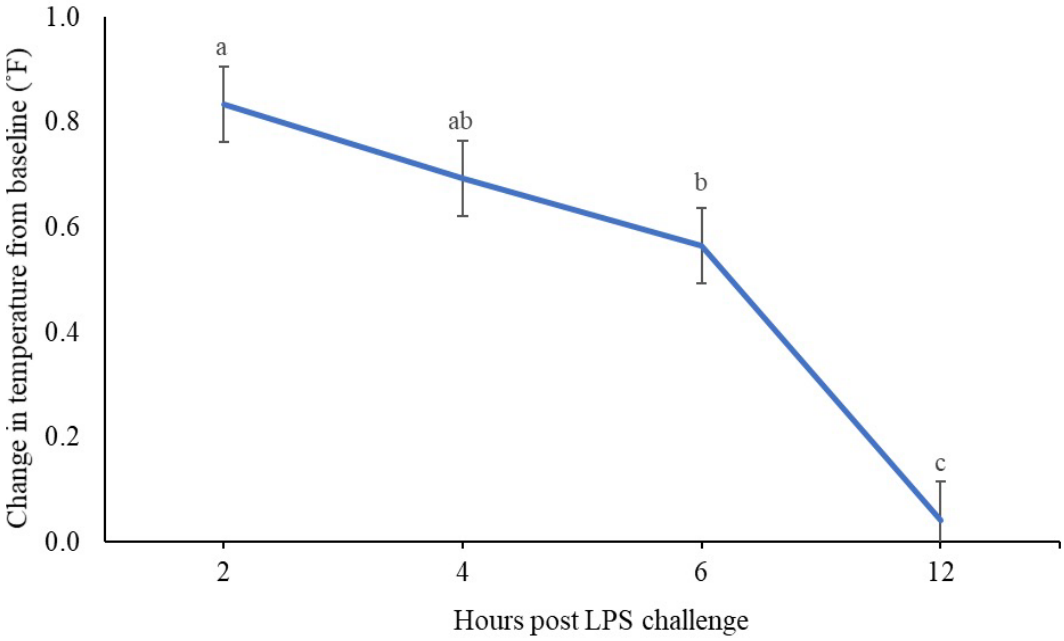

Figure 1. Change in temperature showing main effects of time post LPS challenge. Temperature was measured in change from baseline temperature. Effect of treatment × time and effect of treatment, $P > 0.10$; main effect of time, $P < 0.0001$. Data labels represent differing means among hours post LPS challenge with different superscripts.
[abc] Means with different superscripts differ, $P < 0.05$.