Kansas Agricultural Experiment Station Research Reports

Volume 9
Issue 7 *Swine Day*

Article 11

2023

Effects of Folic Acid on Nursery Pig Growth Performance and Serum Homocysteine

Larissa L. Becker
Kansas State University, Manhattan, lbecker9@ksu.edu

Jordan T. Gebhardt
Kansas State University, Manhattan, jgebhardt@k-state.edu

Mike D. Tokach
Kansas State University, Manhattan, mtokach@k-state.edu

See next page for additional authors

Recommended Citation

Becker, Larissa L.; Gebhardt, Jordan T.; Tokach, Mike D.; Goodband, Robert D.; DeRouchey, Joel M.; and Woodworth, Jason C. (2023) "Effects of Folic Acid on Nursery Pig Growth Performance and Serum Homocysteine," *Kansas Agricultural Experiment Station Research Reports*: Vol. 9: Iss. 7. https://doi.org/10.4148/2378-5977.8512

Effects of Folic Acid on Nursery Pig Growth Performance and Serum Homocysteine

Authors

Larissa L. Becker, Jordan T. Gebhardt, Mike D. Tokach, Robert D. Goodband, Joel M. DeRouchey, and Jason C. Woodworth

K-STATE Research and Extension

SWINE DAY 2023

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2023

Effects of Folic Acid on Nursery Pig Growth Performance and Serum Homocysteine

Larissa L. Becker, Jordan T. Gebhardt,[1] Mike D. Tokach, Robert D. Goodband, Joel M. DeRouchey, and Jason C. Woodworth

Summary

A total of 350 barrows (DNA 200 × 400; initially 13.2 ± 0.12 lb) were used in a 38-d growth study to determine the effects of folic acid on nursery pig growth performance and blood measurements. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments in a completely randomized design. A total of 70 pens were used with 5 pigs per pen and 14 replications per treatment. Dietary treatments were corn-soybean meal-based and consisted of increasing folic acid: 0, 5, 10, 20, or 40 ppm. Treatment diets were fed in three phases from d 0 to 10 (phase 1), d 10 to 23 (phase 2), and d 23 to 38 (phase 3) after weaning. For phase 1 (d 0 to 10), there were no differences ($P > 0.10$) in BW, ADG, or ADFI across treatments. However, increasing folic acid resulted in poorer F/G (linear, $P = 0.032$). For phase 2 (d 10 to 23), there was a marginally significant response in BW, ADG, and ADFI where performance was reduced as folic acid increased with the poorest performance observed when pigs were fed 20 ppm (quadratic, $P \leq 0.079$). No treatment differences ($P > 0.10$) were observed for F/G. For phase 3 (d 23 to 38) and overall (d 0 to 38), there was a significant response in final BW, ADG, ADFI, and F/G where performance was reduced with increasing folic acid with the poorest performance observed when pigs were fed 20 ppm (quadratic, $P \leq 0.049$). On days 10 and 23, 70 pigs were bled to determine serum homocysteine concentration, and a marginally significant treatment × day interaction was observed (linear folic acid, $P = 0.069$). An increase (linear, $P = 0.037$) in homocysteine concentrations was observed as folic acid increased from 0 to 40 ppm in the diet on d 10; however, no differences were observed across increasing folic acid treatments on d 23 ($P = 0.450$). Pigs had increased ($P < 0.001$) homocysteine concentrations on d 10 compared to d 23. In summary, the addition of folic acid resulted in reduced growth performance with the greatest impact being observed when pigs were fed 20 ppm.

Introduction

Folic acid, vitamin B_9, is routinely added to gestation and lactation diets to increase litter size, but its addition to growing pig diets is not as common. Folic acid serves as a co-factor in the methionine cycle specifically in the process of methylating homocysteine into methionine or transforming into cysteine. Homocysteine is an amino acid

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

that can lead to cardiovascular disease and serves as an intermediate product of the synthesis of methionine and cysteine.

There are 2 pathways for converting homocysteine to methionine. In the liver, homocysteine is remethylated via betaine-homocysteine methyltransferase using betaine as a methyl donor. The other pathway occurs in tissues in the body where homocysteine is remethylated to methionine via methionine synthase (MS) using folic acid as a methyl donor. Therefore, the addition of folic acid decreases homocysteine concentrations in nursery pigs because of the remethylation of homocysteine via MS.[2] However, it is speculated that over-fortifying folic acid in the diet may interfere with the metabolism of the folic acid by downregulating the enzyme methylenetetrahydrofolate reductase (MTHFR). Methylenetetrahydrofolate reductase converts folic acid to a more active form. When MTHFR is downregulated, the conversion process is slowed down causing reduced levels of the active form of folic acid.[3] The active form of folic acid functions as a methyl donor to help convert homocysteine into methionine. When the conversion of homocysteine to methionine is reduced due to low levels of the active form of folic acid, homocysteine levels in the blood can increase. High levels of homocysteine can increase the risk of vascular disease and affect protein metabolism by accelerating protein breakdown.

The folic acid requirement estimate for nursery pigs is 0.30 ppm.[4] Recent research showed that supplementing pigs with 20 ppm of added folic acid resulted in negative growth performance compared to 0 ppm.[5] Researchers also observed numerical differences in mortality with pigs fed 20 or 40 ppm of folic acid having 7.5 or 10% mortality, respectively, compared to 0% mortality in pigs fed no added folic acid.

Our objective of this study is to determine the effects of including folic acid in nursery pig diets on growth performance and serum homocysteine. We hypothesize that excess folic acid might result in poorer growth performance because of the increased homocysteine and its effects on protein synthesis.

Procedures

Animals and diets

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 Segregated Early Weaning Research Facility in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water.

---

[2] Bing, Y. U., G. Yang, J. Liu, and D. Chen. 2010. Effects of folic acid supplementation on growth performance and hepatic folate metabolism-related gene expressions in weaned piglets. Front. Agric. China. 4(4): 494-500. doi:10.10007/s11703-010-1047-1.

[3] Stern, F., Y.N. Berner, Z. Polyak, M. Komarnitsky, A. B. Sela, M. Hopp, and Y. Dror. 2004. Homocysteine effect on protein degradation rates. Clin. Biochem. 37:1002-1009. doi:10.1016/j.clinbiochem.2004.07.011

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

[5] Becker, L. L., M. D. Tokach, J. C. Woodworth, R. D. Goodband, J. M. DeRouchey, and J. T. Gebhardt. 2022. Effects of Folic Acid and Zinc Oxide on Nursery Pig Growth Performance. Kansas Agricultural Experiment Station Research Reports (8): 10. doi:10.4148/2378-5977.8367

A total of 350 barrows (DNA 200 × 400; initially 13.2 ± 0.12 lb) were used in a 38-d growth trial. Pigs were weaned at approximately 21 d of age and randomly allotted to 1 of 5 dietary treatments in a completely randomized design. A total of 70 pens were used with 5 pigs per pen and 14 replications per treatment. Dietary treatments were corn-soybean meal-based and consisted of increasing added folic acid: 0, 5, 10, 20, or 40 ppm (Rovimix Folic Acid, DSM, Parsippany, NJ; Table 1). The vitamin premix utilized in these diets did not contain folic acid. Treatment diets were fed in three phases from d 0 to 10 (phase 1), d 10 to 23 (phase 2), and d 23 to 38 (phase 3) after weaning. Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Complete diet samples were taken using a grain probe during bagging from every fourth bag and pooled into one homogenized sample per dietary treatment, then stored at -4°F (-20°C). Pigs were weighed on d 0, 10, 17, 23, 31, and 38 to determine ADG, ADFI, and feed efficiency.

Blood sampling

On d 10 and 23, one average weight barrow from each pen (14 per treatment) was bled for analysis of serum homocysteine concentration. Blood was collected in tubes without anticoagulant to obtain serum. The blood was allowed to clot before centrifuging for 15 min at 1,500 × *g* to collect serum, and samples were stored at 0°F (-18°C) until analyzed. Samples were analyzed in duplicate within a single assay. Serum concentrations were determined using ELISA kits per the instructions of the manufacturer (Aspira Chemical, Oakland, CA).

Statistical analysis

Growth performance data were analyzed as a completely randomized design. Pen was considered the experimental unit. Treatment was used as the fixed effect and barn was included in the model as a random effect. Linear and quadratic contrasts were evaluated within increasing levels of folic acid. Homocysteine concentrations were analyzed as repeated measures representing multiple observations from each pen over time with pen included in the model as a random intercept to account for each sample being analyzed in duplicate, and microplate was used as a random effect. Treatment, day, and the associated interaction were considered fixed effects. All models were fit using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

For phase 1 (d 0 to 10), there were no differences ($P > 0.10$) in d 10 BW, ADG, or ADFI across treatments (Table 2). However, a linear response was observed for F/G, with poorer feed efficiency observed as folic acid increased in the diet from 0 ppm to 40 ppm (linear, $P = 0.032$).

For phase 2 (d 10 to 23), there was a marginally significant response observed for d 23 BW, ADG, and ADFI where performance decreased as folic acid increased in the diet, with pigs fed 20 ppm having the poorest performance (quadratic, $P \leq 0.079$). No treatment differences ($P > 0.10$) were observed for F/G.

For phase 3 (d 23 to 38) and overall (d 0 to 38), there was a significant response observed for final BW, ADG, ADFI, and F/G where performance decreased as folic acid increased in the diet, with pigs fed 20 ppm having the poorest performance (quadratic, $P \leq 0.047$).

Blood analysis

For homocysteine concentration, a marginally significant linear treatment × day interaction was observed ($P = 0.069$; Table 3) as folic acid increased across treatments. An increase (linear, $P = 0.037$) in homocysteine concentration was observed as folic acid increased from 0 to 40 ppm in the diet on d 10. However, no differences were observed across increasing levels of folic acid on d 23 ($P = 0.450$). Pigs had increased ($P < 0.001$) homocysteine concentrations on d 10 compared to d 23. An increase in serum homocysteine concentrations indicates that the treatment diets with added folic acid downregulated MTHFR. This enzyme is responsible for converting folic acid into the active form in order to serve as a methyl donor needed for the conversion of homocysteine to methionine.

In conclusion, the addition of folic acid resulted in reduced growth performance with the greatest impact observed when pigs were fed 20 ppm. These data suggests that the basal diets contained adequate folic acid and that added folic acid is not necessary in nursery diets.

Table 1. Diet composition (as-fed basis)

Ingredient, %	Phase 1[1]	Phase 2[2]	Phase 3[3]
Corn	43.08	53.55	65.73
Soybean meal, dehulled	17.25	23.36	30.52
Bovine blood plasma	2.00	---	---
DDGS	5.00	7.50	---
Fish meal	2.50	---	---
Whey powder	10.00	---	---
Whey permeate	10.00	5.50	---
Microbial-enhanced soy protein[4]	5.00	5.00	---
Soybean oil	2.00	1.00	---
Calcium carbonate	0.40	0.70	0.75
Monocalcium P	0.75	1.00	0.90
Salt	0.30	0.50	0.60
L-Lys-HCl	0.40	0.55	0.48
DL-Met	0.18	0.21	0.19
L-Thr	0.17	0.23	0.21
L-Trp	0.03	0.04	0.04
L-Val	0.08	0.12	0.11
Vitamin and trace mineral premixes[5]	0.40	0.40	0.40
Zinc oxide	0.40	0.26	---
Folic acid[6]	+/-	+/-	+/-
Total	100	100	100

continued

Table 1. Diet composition (as-fed basis)

Ingredient, %	Phase 1[1]	Phase 2[2]	Phase 3[3]
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.30
Ile:Lys	56	56	56
Leu:Lys	118	119	117
Met:Lys	35	37	36
Met and Cys:Lys	57	57	57
Thr:Lys	64	64	64
Trp:Lys	19.3	19.0	19.2
Val:Lys	69	70	69
Total Lys, %	1.51	1.51	1.44
NE, kcal/lb	1,177	1,127	1,102
SID Lys:NE, g/Mcal	5.20	5.43	5.35
CP, %[7]	21.2	21.5	20.8
Ca, %	0.66	0.66	0.65
STTD P, %	0.58	0.51	0.46

[1]Phase 1 diets were fed from d 0 to 10 (13 to 16 lb).
[2]Phase 2 diets were fed from d 10 to 23 (16 to 28 lb).
[3]Phase 3 diets were fed from d 23 to 38 (28 to 48 lb).
[4]HP 300, Hamlet Protein, Findlay, OH.
[5]Ronozyme HiPhos (DSM, Parsippany, NJ) included at 1,250 FTU/kg provided an estimated release of 0.13% STTD P.
[6]Rovimix Folic Acid (DSM, Parsippany, NJ) included at the expense of corn at 0.0006, 0.0013, 0.0025, or 0.005% of the diet for the respective treatments to provide 5, 10, 20, or 40 ppm folic acid.
[7]CP = crude protein.

Table 2. Effects of folic acid on nursery pig growth performance[1]

	Folic acid, ppm[2]						*P* =	
Item	**0**	**5**	**10**	**20**	**40**	**SEM**	**Linear**	**Quadratic**
BW, lb								
d 0	13.2	13.2	13.1	13.1	13.2	0.12	0.759	0.482
d 10	16.2	16.2	16.2	15.7	15.9	0.44	0.115	0.255
d 23	28.3	27.5	27.9	26.7	27.3	0.47	0.068	0.069
d 38	49.5	48.1	48.3	46.1	47.7	1.20	0.023	0.002
d 0 to 10 (phase 1)								
ADG, lb	0.30	0.31	0.31	0.25	0.28	0.034	0.127	0.315
ADFI, lb	0.31	0.31	0.31	0.29	0.31	0.021	0.484	0.375
F/G	1.06	1.04	1.05	1.22	1.16	0.081	0.032	0.458
d 10 to 23 (phase 2)								
ADG, lb	0.93	0.86	0.89	0.85	0.88	0.022	0.203	0.079
ADFI, lb	1.21	1.13	1.18	1.09	1.12	0.024	0.015	0.055
F/G	1.30	1.32	1.32	1.29	1.28	0.022	0.148	0.780
d 23 to 38 (phase 3)								
ADG, lb	1.42	1.38	1.36	1.29	1.36	0.055	0.059	0.001
ADFI, lb	2.01	1.67	1.96	1.88	1.94	0.084	0.025	0.005
F/G	1.42	1.43	1.45	1.45	1.43	0.014	0.948	0.049
d 0 to 38								
ADG, lb	0.96	0.92	0.92	0.87	0.91	0.028	0.031	0.002
ADFI, lb	1.29	1.25	1.26	1.19	1.23	0.039	0.014	0.008
F/G	1.35	1.36	1.37	1.37	1.35	0.010	0.966	0.047

[1]A total of 350 barrows (initially 13.2 ± 0.12 lb) were used with 5 pigs per pen and 14 replications per treatment.
[2]Rovimix Folic Acid (DSM, Parsippany, NJ).

Table 3. Effects of folic acid on nursery pig serum homocysteine concentrations[1]

	Folic acid, ppm[2]						*P* =	
Homocysteine, umol/L[3]	0	5	10	20	40	SEM	Linear treatment × day[3]	Folic acid, linear
d 10	19.2	20.9	22.2	26.9	29.8	4.70	0.069	0.037
d 23	14.5	17.9	15.2	24.8	17.7			0.450

[1]A total of 350 barrows (initially 13.2 ± 0.12 lb) were used with 5 pigs per pen and 14 replications per treatment. Blood samples were collected from one average weight barrow from each pen on d 10 and 23.
[2]Rovimix Folic Acid (DSM, Parsippany, NJ).
[3]Quadratic folic acid × day interaction also tested, $P = 0.193$. Main effect of day, $P < 0.001$.