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Evaluation of a Polyphenol as a Partial Replacement for Vitamin E in Nursery Pig Diets

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Summary

A total of 300 barrows (DNA 200 × 400; initially 14.1 ± 0.09 lb) were used in a 42-d growth trial to evaluate the effects of vitamin E levels and partially replacing vitamin E with a polyphenol (Boreox BX050; Probiotech International, Saint-Hyacinthe, QC, Canada) on growth performance, serum vitamin E, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX). Sixty pens of pigs were weighed and allotted to one of four dietary treatments in a completely randomized design with 15 pens per treatment. A control diet was formulated to provide 15 IU/kg of vitamin E. The second diet provided an additional 60 IU/kg of vitamin E for a total of 75 IU/kg of vitamin E. The second diet was then used as a base for two replacement strategy diets to determine the effects of replacing the additional 60 IU/kg of vitamin E with a polyphenol. For the first replacement strategy diet, 50% of the additional vitamin E (30 IU/kg) was replaced with the polyphenol to achieve the same vitamin E equivalence. For the second replacement strategy diet, all 60 IU/kg of the additional vitamin E was replaced with the polyphenol to achieve the same vitamin E equivalence. The vitamin E form used in this trial was DL-α-tocopherol acetate and Boreox was estimated to provide 50% vitamin E equivalency. Experimental diets were fed in three phases: phase 1 from d 0 to 10, phase 2 from d 10 to 22, and phase 3 from d 22 to 42. Blood samples were collected on d 10 and 42 to determine serum vitamin E, TBARS, SOD, and GSH-PX. For growth performance, there were no differences from d 0 to 10 in ADG, ADFI, or feed efficiency. From d 10 to 22, there was a tendency ($P = 0.063$) for a treatment effect on ADG, but the means did not separate. From d 22 to 42 and overall, there was no evidence of differences for ADG, ADFI, or F/G between treatments. There was a treatment × day interaction ($P < 0.001$) observed for serum vitamin E concentration. There were no differences in serum vitamin E on d 10; however, on d 42, pigs fed either diet containing 15 mg/kg vitamin E had the lowest ($P < 0.05$) serum vitamin E concentration, followed by pigs fed 45 mg/kg, whereas pigs fed 75 mg/kg had the greatest serum vitamin E concentration. Additionally, serum vitamin E concentrations were greater ($P < 0.001$) on d 42 compared with d 10, indicating that treatment differences in vitamin E status became apparent only after

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prolonged dietary exposure. However, there was a tendency ($P = 0.100$) for a treatment effect on serum TBARS on d 10, but the means did not separate. Despite the difference observed for serum vitamin E concentration, there was no evidence of treatment × day interactions or treatment effects for all serum antioxidant measurements. There was a main effect of day ($P < 0.05$) for TBARS and SOD and there tended to be a main effect of day ($P = 0.097$) for GSH-PX, with all serum measurements being greater on d 42 than on d 10. In conclusion, pigs provided additional vitamin E or a combination of vitamin E and polyphenol did not exhibit a growth or antioxidant status response above the 15 IU/kg vitamin E, which is estimated to be the requirement. However, a tendency for a treatment effect on ADG in phase 2 was found, and overall pigs fed 75 IU/kg vitamin E or the polyphenol replacing 100% of the additional 60 IU/kg of vitamin E were 0.9 lb heavier at the end of the study compared with those fed the diet containing vitamin E at the estimated requirement.

Introduction

Weaning is a stressful period for nursery pigs where pigs experience increased oxidative stress due to the imbalance observed between increased production of oxidants and their poor degradation by the pig's antioxidant system. Antioxidants play an important role in mitigating oxidative damage by neutralizing free radicals through electron donation, complex formation with oxidizing compounds, or regeneration of other antioxidants. In addition to endogenous enzymatic antioxidants, dietary antioxidants such as vitamin E are commonly used in nursery pig diets to support health and performance. Vitamin E is widely supplemented above requirement estimates in commercial diets; however, the benefits of feeding high levels and the potential for alternative sources of antioxidants remain areas of interest for research.

Plant-derived polyphenols are a class of natural compounds with strong antioxidative properties and have been evaluated as potential alternatives to vitamin E. These compounds can act as free radical scavengers and may enhance endogenous antioxidant systems. Previous research has demonstrated that polyphenol-based products can partially replace supplemental vitamin E above the requirement without negatively affecting growth performance or antioxidant status in nursery pigs.[4] Boreox BX050 (Probiotech International, Saint-Hyacinthe, QC, Canada) is a polyphenol-based product derived from conifer bark extracts and contains a diverse mixture of bioactive compounds with proposed antioxidant activity. Based on prior estimates, Boreox is assumed to provide approximately 50% of the antioxidant equivalence of DL-α-tocopherol acetate, allowing it to potentially replace a portion of supplemental vitamin E in nursery pig diets. Therefore, the objective of this experiment was to evaluate the effects of partially replacing vitamin E with a polyphenol on growth performance and antioxidant response of nursery pigs from weaning to 42 d post-weaning.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. This study was conducted at the Kansas State University Segregated Early Weaning facility in Manhattan, KS. The facility has two

[4] Rao, Z.-X., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, A. S. Shah, B. H. Foley, K. C. Kjeldsen, G. Brunsgaard, and J. T. Gebhardt. 2023. Polyphenols as a partial replacement for vitamin E in nursery pig diets. Transl. Anim. Sci. 7:txad116. doi:10.1093/tas/txad116.

identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a cup waterer for *ad libitum* access to feed and water. Pens (4 × 4 ft) had metal tri-bar floors and provided approximately 2.7 ft^2/pig.

Animals and diets

A total of 300 barrows (DNA 200 × 400; initially 14.1 ± 0.09 lb) were used across two barns in a 42-d growth trial. Pigs were weaned at approximately 21 d of age, randomly assigned to pens based on initial BW, and then allotted to one of four dietary treatments in a completely randomized design. There were five pigs per pen and 15 replicates per treatment.

The vitamin E form used in this trial was DL-α-tocopherol acetate (20,000 IU/lb, Nutra Blend, Neosho, MO) with 1 mg providing 1 IU of vitamin E equivalence. Boreox BX050 (Boreox) contains a blend of structurally diverse polyphenols with proposed antioxidant activity through free-radical scavenging and enhancement of endogenous antioxidant systems. Boreox was estimated to provide 50% equivalency to DL-α-tocopherol acetate, meaning 1 mg of Boreox provided an estimated 0.5 IU of vitamin E equivalence. This equivalency ratio was used to calculate the required addition of Boreox needed to replace a proportion of supplemental vitamin E in nursery diets (Table 1).

A basal diet was formulated using a vitamin E-free premix (dsm-firmenich, Plainsboro, NJ). A control diet was formulated to provide 15 IU/kg of vitamin E (Table 1). The second diet provided an additional 60 IU/kg of vitamin E for a total of 75 IU/kg of vitamin E. The second diet was then used as a comparison for two replacement strategy diets to determine the effects of replacing the additional 60 IU/kg of vitamin E with polyphenol in diets already containing a basal level of vitamin E at the requirement estimate. For the first replacement strategy diet, 50% of the additional vitamin E (30 IU/kg) was replaced with the equivalency provided by polyphenol. The first replacement treatment supplied 45 IU/kg from vitamin E and 30 IU/kg of vitamin E equivalence from Boreox (60 mg/kg of Boreox). For the second replacement strategy diet, all 60 IU/kg of the additional vitamin E was replaced with the equivalency provided by polyphenol. The second replacement treatment supplied 15 IU/kg from vitamin E and 60 IU/kg of vitamin E equivalence from Boreox (120 mg/kg of Boreox). These inclusion rates allowed evaluation of a polyphenol replacing either 50 or 100% of the supplemental vitamin E above the minimum requirement.

Pigs were fed treatment diets in meal form for all three nursery phases: phase 1 from d 0 to 10, phase 2 from d 10 to 22, and phase 3 from d 22 to 42 (Table 2). Basal and treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Pig weights and feed disappearance were measured on d 0, 10, 22, 30, and 42 to determine ADG, ADFI, and feed efficiency (F/G). For serum samples, blood was collected from the same median weight pig per pen on d 10 and 42 and allowed to clot for at least 30 min, centrifuged at 1,500 × g for 30 min at 39°F, and resulting serum supernatants were divided into four polypropylene tubes as aliquots stored at -112°F. Serum was analyzed for vitamin E, thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX).

Statistical analysis

Data were analyzed as a completely randomized design for a one-way ANOVA using the lmer function from the lme4 package for growth performance and blood serum parameters in R Studio (Version 3.5.2, R Core Team, Vienna, Austria). Pen served as the experimental unit, dietary treatment was included as a fixed effect, and barn was included as a random effect. The main effects of treatment, day, and interaction between treatment and day were tested for blood serum parameters with barn, pen, and microtiter plate included as random effects. All results were considered significant with $P < 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Growth performance

From d 0 to 10, there was no evidence of differences in ADG, ADFI, or F/G between dietary treatments (Table 3). From d 10 to 22, there was a tendency ($P = 0.063$) for a treatment effect for ADG; however, the means did not separate. This effect was driven by the highest numerical ADG for pigs fed 75 IU/kg vitamin E, with the pigs fed the 50% equivalence from polyphenol having the lowest. From d 22 to 42 and overall, there were no differences observed for ADG, ADFI, or F/G between treatments.

Serum vitamin E

There was an interaction ($P < 0.001$) between treatment and day for serum vitamin E (Table 4). There were no differences in serum vitamin E on d 10. However, on d 42, pigs fed either diet containing 15 mg/kg vitamin E had the lowest ($P < 0.05$) serum vitamin E concentration, followed by pigs fed 45 mg/kg, and pigs fed diets containing 75 mg/kg had the greatest serum vitamin E concentration. The observed treatment × day interaction indicates that differences in vitamin E status required prolonged dietary exposure before becoming detectable. Furthermore, serum vitamin E concentration increased in a dose-dependent manner with increasing dietary vitamin E equivalence, confirming that circulating vitamin E concentrations were responsive to dietary vitamin E intake, which demonstrated that the polyphenol replacement strategy did not increase circulating vitamin E concentrations beyond those from the amount of supplemental vitamin E provided. Additionally, there was a main effect of day ($P < 0.001$), as serum measurements were greater on d 42 than on d 10.

Antioxidant status

For all serum antioxidant measurements, there was no evidence of treatment × day interactions or treatment effects (Table 4). There was a main effect of day ($P < 0.05$) for TBARS and SOD and tended to be a main effect of day ($P = 0.097$) for GSH-PX, with all serum measurements being greater on d 42 than on d 10. However, there was a tendency ($P = 0.100$) for a treatment effect on serum TBARS on d 10, but the means did not separate. This was the result of the lowest TBARS concentration observed in pigs fed the diet in which the polyphenol replaced 100% of the additional 60 IU/kg vitamin E, with pigs fed 75 IU/kg vitamin E having the highest. Despite the differences observed in serum vitamin E concentration, increasing dietary vitamin E equivalence did not influence the measured antioxidant biomarkers, suggesting that the basal dietary concentration of 15 IU/kg vitamin E equivalence was sufficient to maintain antioxidant status under the conditions present in this study.

In conclusion, pigs in this study provided additional vitamin E or a combination of vitamin E and polyphenol did not elicit a growth or antioxidant status response above the 15 IU/kg estimated requirement. However, a tendency for a treatment effect on ADG was observed in phase 2, and overall pigs fed 75 IU/kg vitamin E or the polyphenol replacing 100% of the additional 60 IU/kg of vitamin E were 0.9 lb heavier at the end of the study compared with those fed the diet formulated to meet the estimated requirement of vitamin E, although this difference was not statistically significant.

Acknowledgments

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Table 1. Treatment dietary vitamin E equivalence provided by vitamin E sources

Vitamin E[1], mg/kg:	15	75	45	15
Boreox[2], mg/kg:	0	0	60	120
Vitamin E equivalence, IU/kg				
Vitamin E requirement	15	15	15	15
Additional vitamin E equivalence				
Vitamin E	0	60	30	0
Boreox	0	0	30	60
Total vitamin E equivalence[3]	15	75	75	75
Analyzed vitamin E, mg/kg[4]				
Phase 1	27	70	41	15
Phase 2	22	88	35	20
Phase 3	21	74	44	21
Weighted average[5]	21.6	77.2	41.6	20.4

[1] Vitamin E (20,000 IU/lb, Nutra Blend, Neosho, MO). The vitamin E form was DL-α-tocopherol acetate. One mg of DL-α-tocopherol acetate provides 1 IU of vitamin E equivalence.

[2] Boreox BX050 (Probiotech International, Saint-Hyacinthe, QC, Canada) assumed to have a 50% equivalency to vitamin E. One mg of Boreox provides 0.5 IU of vitamin E equivalence.

[3] Total vitamin E equivalence is the combination of vitamin E equivalence provided by vitamin E and Boreox.

[4] Vitamin E concentration was analyzed at the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, CO).

[5] Weighted averages = Sum of the calculated vitamin E intake of the three phases ÷ total feed intake.

Table 2. Diet composition (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredients, %			
Corn	50.53	58.54	66.15
Soybean meal, 47.7% CP	15.16	22.21	29.41
Whey powder	20.85	10.00	---
Spray-dried blood plasma	2.50	---	---
Microbially enhanced soybean meal[2]	6.25	5.00	---
Soybean oil	1.00	---	---
Calcium carbonate	0.50	0.60	0.90
Monocalcium P, 21% P	0.80	0.87	0.95
Salt	0.30	0.58	0.60
L-Lys-HCl	0.40	0.50	0.51
DL-Met	0.20	0.22	0.21
L-Thr	0.17	0.23	0.23
L-Trp	0.03	0.05	0.06
L-Val	0.09	0.13	0.14
Zinc oxide	0.40	0.26	---
Vitamin premix[3]	0.13	0.13	0.13
Trace mineral premix	0.15	0.15	0.15
Phytase[4]	0.06	0.06	0.06
Treatment premix[5]	0.50	0.50	0.50
Total	100.00	100.00	100.00
Calculated analysis			
SID AA, %			
Lys	1.36	1.35	1.30
Ile:Lys	58	58	55
Leu:Lys	118	115	115
Met:Lys	34	37	37
Met and Cys:Lys	58	58	58
Thr:Lys	65	65	64
Trp:Lys	20.2	20.1	20.2
Val:Lys	70	70	70
His:Lys	35	35	36
NE, kcal/lb	1,157	1,116	1,105
CP, %	20.9	21.0	20.4
Ca, %	0.63	0.63	0.72
STTD P, %	0.56	0.50	0.46

[1] Phase 1 diets were fed from d 0 to 10, phase 2 from d 10 to 22, and phase 3 from d 22 to 42.

[2] MEpro, Prairie Aquatech, Brookings, SD.

[3] Vitamin premix without vitamin E provided per lb of diet: 1,875 IU vitamin A; 750 IU vitamin D; 1.5 mg vitamin K; 0.015 mg vitamin B12; 22.5 mg niacin; 12.5 mg pantothenic acid; and 3.75 mg riboflavin.

[4] HiPhorius 2400 (Novonesis, Kongens Lyngby, Denmark) added at 1,500 FTU/kg feed provided an estimated release of 0.13% STTD P.

[5] For the four treatments, treatment premix provided per ton of diet: 0.7, 3.4, 2.1, and 0.7 lb of vitamin E (20,000 IU/lb, Nutra Blend, Neosho, MO), respectively; 9.3, 6.6, 7.4, and 8.3 lb of ground corn, respectively; and 0.0, 0.0, 0.5, and 1.0 lb of Boreox BX050 premix (Probiotech International, Saint-Hyacinthe, QC, Canada), respectively.

Table 3. Evaluation of dietary vitamin E levels and vitamin E partial replacement from polyphenol strategies on nursery pig growth performance[1]

Vitamin E[2], mg/kg:	15	75	45	15		
Boreox[3], mg/kg:	0	0	60	120		
Total vitamin E equivalence[4], IU/kg:	15	75	75	75	SEM	*P* =
BW, lb						
d 0	14.1	14.1	14.1	14.1	0.09	0.990
d 10	17.1	16.9	17.0	17.2	0.20	0.816
d 22	29.1	29.5	28.8	29.6	0.51	0.398
d 42	57.2	58.1	56.9	58.1	0.86	0.363
Phase 1 (d 0 to 10)						
ADG, lb	0.29	0.28	0.29	0.30	0.019	0.819
ADFI, lb	0.40	0.38	0.39	0.40	0.020	0.728
F/G	1.40	1.37	1.38	1.36	0.042	0.911
Phase 2 (d 10 to 22)						
ADG, lb	0.99	1.05	0.98	1.03	0.035	0.063
ADFI, lb	1.26	1.30	1.24	1.31	0.040	0.312
F/G	1.28	1.24	1.27	1.26	0.014	0.221
Phase 3 (d 22 to 42)						
ADG, lb	1.41	1.43	1.41	1.42	0.021	0.619
ADFI, lb	2.14	2.18	2.14	2.19	0.036	0.617
F/G	1.53	1.52	1.52	1.53	0.014	0.959
Overall (d 0 to 42)						
ADG, lb	1.02	1.05	1.02	1.05	0.017	0.221
ADFI, lb	1.47	1.50	1.47	1.51	0.026	0.447
F/G	1.45	1.43	1.44	1.44	0.009	0.641

[1] A total of 300 barrows (DNA 200 × 400; initially 14.1 ± 0.09 lb) weaned at approximately 21 d of age, were used in a 42-d growth trial with five pigs per pen and 15 replicates per treatment.

[2] Vitamin E (20,000 IU/lb, Nutra Blend, Neosho, MO) in the form of DL-α-tocopherol acetate. One mg of DL-α-tocopherol acetate provided 1 IU of vitamin E equivalence.

[3] Boreox BX050 (Probiotech International, Saint-Hyacinthe, QC, Canada) assumed to have a 50% equivalency to vitamin E. One mg of Boreox provided 0.5 IU of vitamin E equivalence.

[4] Total vitamin E equivalence is the combination of vitamin E equivalence provided by vitamin E and Boreox.