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Partial Replacement of Vitamin E with Polyphenol in Nursery Pig Diets[1]

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Summary

A total of 300 pigs (241 × 600 DNA; initially 13.1 lb) were used in a 42-d trial to determine the effects of vitamin E levels and partially replacing vitamin E with a polyphenol (Cabanin CSD; R2 Agro, Denmark) on growth performance, complete blood count (CBC), serum thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and cytokine panel. Sixty pens of pigs were weighed and allotted to 1 of 5 dietary treatments in a completely randomized design with 12 pens per treatment. A control treatment was formulated to provide 15 IU/kg of vitamin E equivalence from vitamin E. This control treatment was then used as a base for 3 replacement strategy diets to determine the effects of replacing an additional 60 IU/kg of vitamin E with Cabanin CSD in diets containing a basal level of vitamin E requirement estimate (15 IU/kg). First, an additional 60 IU/kg of vitamin E was added for a total of 75 IU/kg of vitamin E equivalence. Second, 50% of the additional vitamin E (30 IU/kg) was replaced with the equivalency of Cabanin CSD. Third, all 60 IU/kg of the additional vitamin E was replaced with the equivalency of Cabanin CSD. To evaluate whether there are negative effects of feeding nursery pigs a high level of Cabanin CSD, a fifth treatment was formulated to provide 575 IU/kg of vitamin E equivalence with 75 IU/kg from vitamin E and 500 IU/kg from Cabanin CSD. Whole blood and serum samples were collected on d 10 and 42. For growth performance, increasing vitamin E equivalence tended to improve (quadratic, $P < 0.10$) F/G from d 10 to 21, and tended to improve (linear, $P < 0.10$) F/G from d 21 to 42 and 0 to 42. For antioxidant status, increasing vitamin E equivalence improved (linear, $P < 0.05$) d 42 SOD. For cytokine, there was no evidence of differences ($P > 0.10$) between treatments and vitamin E equivalence. Moreover, there was no evidence of differences ($P > 0.10$) in all response variables between the 3 replacement strategies throughout the entire period. In summary, increasing vitamin E equivalence tended to improve F/G, which may be related to the improved SOD activity. Furthermore, Cabanin CSD can effectively

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replace vitamin E provided above the vitamin E requirement to provide similar benefits from increasing vitamin E equivalence.

Introduction

Weaning is a stressful period for piglets due to changes in diet composition, environment, and bacterial challenges, which can result in reduced feed intake and growth rate. During stressful periods, the need for antioxidants increases. Antioxidants neutralize free radicals by electron donation, complex formation between oxidizing elements, or the regeneration of other antioxidants. Besides endogenous enzymatic antioxidants [e. g., superoxide dismutase (SOD), catalase, glutathione peroxidase], natural non-enzymatic antioxidants are also involved (e. g., vitamins E and C, carotenoids, polyphenols) in protecting the cells from free radicals. The natural polyphenol-based product, Cabanin CSD, contains selected extracts from grapes, citrus, blackcurrant, and chestnuts, all with high concentrations of polyphenols that can provide high antioxidative activity. Previous European university trials observed that Cabanin CSD improved growth performance and reduced feed costs when Cabanin CSD partly replaced vitamin E. Moreover, polyphenols have also shown positive effects on the immune system[5] which have the potential to improve the complete blood count (CBC) and cytokine levels of weaned pigs. We hypothesized that this Cabanin CSD could potentially be used as an effective antioxidant replacer above the minimum NRC[6] vitamin E requirement estimate for nursery pigs with no negative effects. Therefore, the objectives of this experiment were to evaluate the effects of vitamin E equivalence levels (15, 75, and 575 IU/kg), and vitamin E replacement strategies of replacing 60 IU/kg of vitamin E with Cabanin CSD in diets above the minimum vitamin E requirement on growth performance, antioxidant status (TBARS and SOD), complete blood count, and cytokine panels 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 conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Two nursery rooms were used in this trial with 30 pens per room. Each pen (5 × 5 ft) was equipped with a 4-hole dry self-feeder, and a nipple waterer to provide *ad libitum* access to feed and water.

Animals and diets

A total of 300 pigs (241 × 600 DNA; initially 13.1 lb) were weaned at approximately 21 d of age and placed in pens of 5 pigs each based on initial BW and gender. The gender was balanced between dietary treatments. Pens of pigs were then randomly allotted to the 5 treatments in a completely randomized design with 12 replicate pens per treatment. Treatment diets were fed in 3 phases (phase 1: d 0 to 10; phase 2: d 10 to 21; and phase 3: d 21 to 42) in meal form. The vitamin E form (20,000 IU/lb, DSM, Parsippany, NJ) used in this trial was DL-α-tocopherol acetate with 1 mg providing 1 IU of vitamin E equivalence. The natural polyphenol-based product (Cabanin CSD, R2 Agro, Denmark; Lot number: 220120) contained selected extracts from grapes,

[5] Gessner, D. K., R. Ringseis, and K. Eder. 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J. Anim. Physiol. Anim. Nutr. (Berl.) 101(4):605-628. doi: https://doi.org/10.1111/jpn.12579

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

citrus, blackcurrant, and chestnuts. These ingredients contained high concentrations of polyphenols in the form of phenolic acids, flavonoids, and tannins, which have shown great antioxidative activity. The Cabanin CSD was assumed to have a 50% equivalency to vitamin E (DL-α-tocopherol acetate) based on a previous university trial conducted at Freie Universität Berlin (Germany) for weaned pigs. One mg of Cabanin CSD provided 0.5 IU of vitamin E equivalence. The total polyphenol content was 9.2% for this specific lot of Cabanin CSD product used in this trial. A control treatment was formulated to provide 15 IU/kg of vitamin E equivalence from vitamin E to meet the requirement estimate for vitamin E. This control diet with 15 IU/kg of vitamin E was then used as the basal diet for three replacement strategy diets (Table 1). First, an additional 60 IU/kg of vitamin E was added for a total of 75 IU/kg of vitamin E equivalence. Second, 50% of the additional vitamin E (30 IU/kg) was replaced with the vitamin E equivalence from Cabanin CSD. Third, all 60 IU/kg of supplemental vitamin E was replaced with the equivalency of Cabanin CSD. These three replacement strategies allowed us to determine the effects of replacing vitamin E with Cabanin CSD at different ratios for the additional 60 IU/kg of vitamin E equivalence added to diets containing a minimum vitamin E requirement estimate (15 IU/kg). The fifth treatment was formulated to provide a total of 575 IU/kg of vitamin E equivalence with 75 IU/kg from vitamin E and 500 IU/kg from Cabanin CSD to evaluate whether there are negative effects of feeding nursery pigs a high level of Cabanin CSD.

Basal diets for all 3 phases (Table 2) were manufactured at Hubbard Feeds, Beloit, KS. The basal diets were mixed with remaining ingredients (e.g., vitamin E-free vitamin premix, vitamin E, Cabanin CSD) at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) to make the 5 treatment diets. The remaining ingredients were mixed thoroughly for each dietary treatment before mixing with the basal diet. All diets met or exceeded the NRC[6] nutrient requirement estimates, except for the low vitamin E treatment diet (15 IU/kg of vitamin E) and the phase 1 Lys level, which was formulated at 1.35% SID Lys for all treatments. Diet samples were collected and thoroughly mixed within treatment before analysis for vitamin E concentration with HPLC at the Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, CO).

Data and sample collection

Pen weights and feed disappearance were measured on d 0, 10, 21, 31, 38, and 42 to determine ADG, ADFI, and F/G. The pigs were healthy as there were few medical treatments and no mortality throughout the 42-d trial. Whole blood and serum samples were collected from one median-weight pig of each pen on d 10 and 42 of the experiment for CBC, serum cytokine panel, serum SOD, and serum thiobarbituric acid reactive substances (TBARS). The same pig per experimental unit was used in all subsequent whole blood and serum collections. The gender of the selected pigs was balanced between treatments. Whole blood samples were collected using EDTA blood collection tubes and analyzed for CBC at the Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS) using an Advia 2120 hematology analyzer (Siemens Healthineers, Malvern, PA). For serum samples, whole blood was collected with blood collection tubes that contained no anticoagulant or preservative. The blood was allowed to clot for at least 30 min, centrifuged at 1,500 × g for 30 min, and the resulting serum supernatants were divided into 4 polypropylene tubes as aliquots, and transferred and stored at -112°F. Serum cytokine panel (GM-CSF, IFNγ, IL-1α, IL-1ra,

IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNFα) was evaluated at Eve Technologies (Calgary, Canada). Serum TBARS and SOD were evaluated at the Kansas State University Swine Nutrition Laboratory (Manhattan, KS). For serum SOD, assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI) and samples were run in triplicate in 96-well microplates with intra-assay coefficient of variation (CV) of ≤ 5.0%. For serum TBARS, the assay used in the experiment was described in Rao et al.[7]

Statistical analysis

Data were analyzed as a complete randomized design for one-way ANOVA using the lmer function from the lme4 package for growth performance and blood parameters (CBC, cytokine panel, SOD, and TBARS) in R program.[8] Pen was considered as the experimental unit. Treatment was used as the fixed effect. Nursery room was included in the model as a random intercept. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing vitamin E equivalence levels (15, 75, and 575 IU/kg) for all response criteria. Contrast coefficients were adjusted for unequally spaced treatments. Interactive effects of vitamin E equivalence levels × day (d 10 and 42) interaction and dietary treatment × day (d 10 and 42) interaction were tested for blood parameters. For serum cytokine, results were analyzed with the raw fluorescence intensity value[9] with a log10 transformation. For serum TBARS and SOD assay, microtiter plate was included in the model as a random intercept. Tukey adjustment was used for multiple comparisons. All results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Growth performance

There was no evidence of differences ($P > 0.10$) in ADG and ADFI as vitamin E equivalence increased or between replacement strategies throughout the entire 42-d experimental period (Table 3). From d 10 to 21, increasing vitamin E equivalence improved (quadratic, $P = 0.086$) F/G from 15 to 75 IU/kg of vitamin E equivalence with no further improvement at 575 IU/kg. From d 21 to 42, there was a tendency of improvement (linear, $P = 0.063$) in F/G as the vitamin E equivalence increased. This tendency of improvement in F/G was also observed in overall (d 0 to 42) F/G (linear, $P = 0.075$).

Antioxidant status (TBARS and SOD)

For serum TBARS, there was no evidence of vitamin E equivalence × day interaction, treatment × day interaction, vitamin E equivalence effect, treatment effect, or day effect ($P > 0.10$). However, there was a vitamin E equivalence × day interaction ($P = 0.050$) on serum SOD activity (Figure 1). Increasing vitamin E equivalence increased (linear,

[7] Rao, Zhong-Xing; Tokach, Mike D.; Woodworth, Jason C.; DeRouchey, Joel M.; Goodband, Robert D.; De Marco, Michele; Moreland, Steven; and Gebhardt, Jordan T. (2021) "Evaluation of Selenium Source on Nursery Pig Growth Performance, Serum and Tissue Selenium Concentrations, and Serum Antioxidant Status," Kansas Agricultural Experiment Station Research Reports: Vol. 7: Iss. 11. Doi: 10.4148/2378-5977.8188

[8] R Core Team. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

[9] Breen, E. J., V. Polaskova, and A. Khan. 2015. Bead-based multiplex immuno-assays for cytokines, chemokines, growth factors and other analytes: Median fluorescence intensities versus their derived absolute concentration values for statistical analysis. Cytokine 71(2):188-198. doi: 10.1016/j.cyto.2014.10.030

$P = 0.036$) serum SOD activity on d 42 but not on d 10 (linear, $P = 0.616$). Moreover, there was no treatment effect, day effect, or treatment × day interaction ($P > 0.10$) in serum SOD activity between the five dietary treatments on d 10 and 42.

Complete blood count

All CBC variables were approximate to or within the reference intervals for these ages of pigs according to the Iowa State University Clinical Pathology Laboratory reference intervals for swine (ISU, 2011).[10] There was no evidence ($P > 0.10$) of vitamin E equivalence × day interaction and treatment × day interaction for all CBC criteria (Table 4). Increasing vitamin E equivalence tended to increase (quadratic, $P = 0.070$) leukocyte concentration and increased (quadratic, $P = 0.045$) eosinophil concentration from 15 to 75 IU/kg of vitamin E equivalence then reduced at 575 IU/kg. Additionally, there was a tendency (treatment, $P = 0.089$) of treatment difference in segmented neutrophil concentration; however, no pairwise mean separation was observed. Lymphocyte and monocyte concentration were increased (day, $P < 0.05$); platelets and segmented neutrophil concentration showed a tendency to increase (day, $P < 0.10$), while RBC distribution width was decreased (day, $P < 0.001$) from d 10 to 42. These differences between days can be expected as pigs aged.[8]

Serum cytokine

There was no evidence of vitamin E equivalence × day interaction, vitamin E equivalence effect, treatment × day interaction, or treatment effect ($P > 0.10$) for any measured cytokine (Table 5). Even though there were no statistical differences, several proinflammatory cytokines showed numeric reduction in pigs fed diets formulated with 75 or 575 IU/kg of vitamin E equivalence compared to the control diet (15 IU/kg). Moreover, cytokine IL-1α, IL-2, IL-4, and IL-6 were increased (day, $P < 0.05$); IL-1β, IL-10, and IL-12 showed a tendency to increase (day, $P < 0.10$), and GM-CSF showed a tendency to decrease (day, $P = 0.069$) from d 10 to 42. There is no reference interval for cytokine level based on pig's age; however, there is some evidence that cytokine levels tend to increase as weaned pigs age.[11]

In summary, increasing vitamin E equivalence by the addition of vitamin E or Cabanin CSD improves feed efficiency, which may be related to the improved serum SOD activity. Moreover, we found no evidence of difference between the three vitamin E replacement strategies in all response criteria. Thus, this suggests that Cabanin CSD can be used as an effective replacement for the 60 IU/kg of additional vitamin E added to diets that met the basal vitamin E requirement (15 IU/kg) for nursery pigs.

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[10] Iowa State University Clinical Pathology Laboratory. Reference Intervals. 2011. Retrieved from: https://vetmed.iastate.edu/vpath/services/diagnostic-services/clinical-pathology/testing-and-fees/reference-intervals?field_p_type_tid=287 (Accessed 10 March 2023)

[11] de Groot, J., Kruijt, L., Scholten, J. W., Boersma, W. J., Buist, W. G., Engel, B., and van Reenen, C. G. 2005. Age, gender and litter-related variation in T-lymphocyte cytokine production in young pigs. Immunology, 115(4), 495–505. doi:10.1111/j.1365-2567.2005.02184.x

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

Vitamin E[1], mg/kg:	**15**	**75**	**45**	**15**	**75**
Cabanin CSD[2], mg/kg:	**0**	**0**	**60**	**120**	**1,000**
Vitamin E equivalence, IU/kg					
Vitamin E requirement	15	15	15	15	15
Additional vitamin E equivalence					
Vitamin E	0	60	30	0	60
Cabanin CSD	0	0	30	60	500
Total vitamin E equivalence[3]	15	75	75	75	575
Analyzed vitamin E, mg/kg[4]					
Phase 1	17.0	63.5	55.0	16.0	76.0
Phase 2	16.2	65.0	51.0	11.0	38.5
Phase 3	23.0	85.0	69.0	13.0	98.0
Weighted average[5]	21.0	79.1	64.1	12.7	83.0

[1] Vitamin E (20,000 IU/lb, DSM, Parsippany, NJ). The vitamin E form was DL-α-tocopherol acetate. One mg of DL-α-tocopherol acetate provides 1 IU of vitamin E equivalence.
[2] Cabanin CSD (R2 Agro, Denmark) was assumed to have a 50% equivalency to vitamin E. One mg of Cabanin CSD 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 Cabanin CSD.
[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 3 phases ÷ total feed intake.

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

Item	Phase 1	Phase 2	Phase 3
Ingredients, %			
Corn	43.4	44.7	52.4
Soybean meal, 46.5% CP[2]	20.6	26.4	29.1
Corn DDGS	5.0	10.0	15.0
Fish meal	2.5	--	--
Dried whey	10.0	--	--
Dried whey permeate, 80% lactose	10.0	--	--
Fermented soybean meal[3]	4.0	4.0	--
Choice white grease	1.0	1.0	--
Calcium carbonate	0.50	0.83	0.90
Monocalcium phosphate	0.80	0.90	0.70
Sodium chloride	0.30	0.50	0.60
L-Lys-HCl	0.45	0.45	0.45
DL-Met	0.22	0.19	0.11
L-Thr	0.18	0.17	0.15
L-Trp	0.03	0.02	0.03
L-Val	0.09	0.04	0.02
Trace mineral premix[4]	0.15	0.15	0.15
Zinc oxide	0.40	0.26	--
Phytase[5]	0.01	0.01	0.01
Vitamin premix[6]	0.11	0.11	0.11
Treatment premix[7]	0.29	0.29	0.29
Total	100.00	100.00	100.00

continued

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

Item	Phase 1	Phase 2	Phase 3
Calculated analysis			
SID AA, %			
Lys	1.35	1.35	1.30
Ile:Lys	58	61	61
Leu:Lys	117	127	137
Met:Lys	38	37	33
Met and Cys:Lys	56	56	56
Thr:Lys	63	63	63
Trp:Lys	19.0	19.1	18.9
Val:Lys	69	69	69
His:Lys	34	38	40
Net energy, kcal/lb	1,147	1,120	1,085
CP, %	21.4	22.9	23.0
Ca, %	0.67	0.67	0.64
STTD P, %	0.60	0.53	0.49

[1] Phase 1, 2, and 3 diets were formulated based on pig bodyweight (phase 1: 12 to 15 lb; phase 2: 15 to 25 lb; and phase 3: 25 to 55 lb) in meal form.
[2]CP = crude protein.
[3] MEpro, Prairie Aquatech, Brookings, SD.
[4] Trace mineral premix provided per lb of diet: 49.9 mg Zn, 49.9 mg Fe, 15 mg Mn, 7.5 mg Cu, 0.13 mg I, and 0.13 mg Se.
[5] Quantum Blue 5G (AB Vista, Plantation, FL) was used in phase 1 diet and provided 1,379 FTU per lb of diet with an expected STTD P release of 0.15%.
[6] 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 B_{12}; 22.5 mg niacin; 12.5 mg pantothenic acid; and 3.75 mg riboflavin.
[7] For the 5 treatments, treatment premix provided per ton of diet: 0.7, 3.4, 2.1, 0.7, and 3.4 lb of vitamin E (20,000 IU/lb, DSM, Parsippany, NJ), respectively; 4.7, 2.0, 3.2, 4.5, and 0.0 lb of ground corn, respectively; and 0.0, 0.0, 0.12, 0.24, 2.0 lb of Cabanin CSD (R2 Agro, Denmark), respectively.

Table 3. Evaluation of vitamin E levels and vitamin E replacement strategies on nursery pig growth performance[1,2]

Vitamin E, mg/kg:	15	75	45	15	75			
Cabanin CSD, mg/kg:	0	0	60	120	1,000		Probability, P =[3]	
Total E equivalence, IU/kg:	15	75	75	75	575	SEM	Linear[4]	Quadratic[4]
d 0 to 10 (Phase 1)								
d 0 BW, lb	13.1	13.1	13.1	13.2	13.1	0.02	0.998	0.803
d 10 BW, lb	16.2	16.0	15.9	16.2	16.2	0.25	0.945	0.451
ADG, lb	0.31	0.28	0.28	0.31	0.30	0.025	0.943	0.429
ADFI, lb	0.37	0.36	0.34	0.36	0.36	0.018	0.776	0.394
F/G	1.25	1.32	1.25	1.19	1.22	0.063	0.678	0.693
d 10 to 21 (Phase 2)								
d 21 BW, lb	27.4	27.3	27.4	27.5	27.1	0.45	0.588	0.931
ADG, lb	1.01	1.03	1.05	1.02	1.00	0.043	0.405	0.485
ADFI, lb	1.30	1.29	1.29	1.27	1.25	0.044	0.244	0.670
F/G	1.29	1.26	1.24	1.24	1.26	0.021	0.574	0.086
d 21 to 42 (Phase 3)								
d 42 BW, lb	55.3	55.8	56.1	56.8	55.5	0.84	0.867	0.319
ADG, lb	1.33	1.36	1.37	1.40	1.35	0.027	0.878	0.159
ADFI, lb	2.05	2.06	2.09	2.11	2.02	0.042	0.400	0.348
F/G	1.54	1.52	1.53	1.52	1.49	0.018	0.063	0.337
d 0 to 42 (Overall)								
ADG, lb	1.00	1.02	1.02	1.04	1.01	0.020	0.867	0.321
ADFI, lb	1.45	1.46	1.46	1.47	1.42	0.029	0.317	0.655
F/G	1.45	1.43	1.43	1.42	1.41	0.013	0.075	0.212

[1]A total of 300 pigs were used with 60 pigs per replicate and 12 replicates per treatment. Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and Cabanin CSD (R2 Agro, Denmark).

[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency.

[3]Treatment was not significant, $P > 0.10$.

[4]Polynomial contrasts were utilized to analyze the effects of dietary total E equivalence levels (15, 75, and 575 IU/kg).

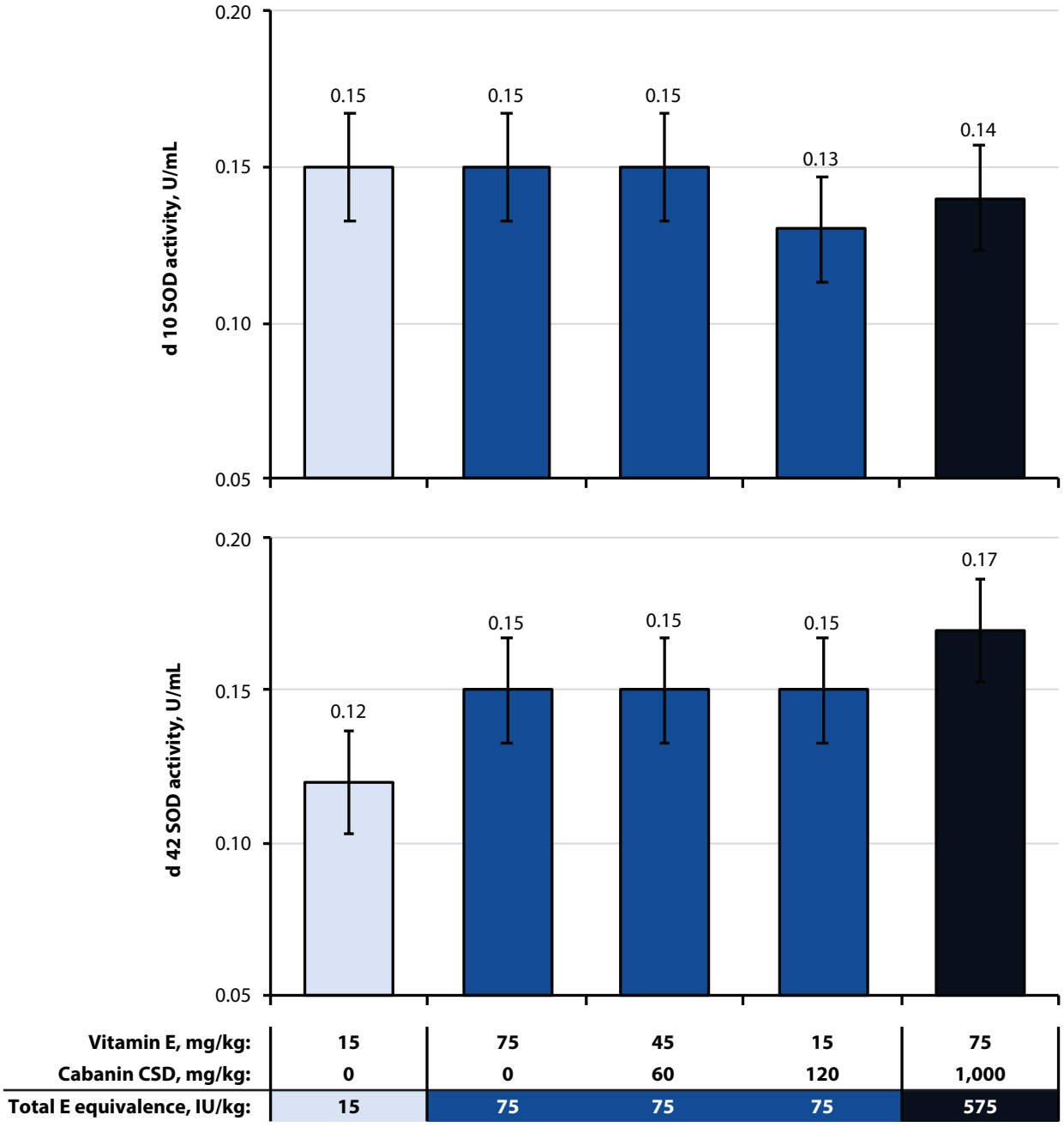

Figure 1. Serum superoxide dismutase (SOD), activity on d 10 and 42. Error bar equals to 1 SEM. A total of 300 pigs (initially 13.1 lb) were used with 60 pigs per replicate and 12 replicates per treatment. Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and Cabanin CSD (R2 Agro, Denmark). There was a total vitamin E equivalence × day interaction (linear interaction, $P = 0.05$), but no evidence of treatment × day interaction, treatment, or day effect ($P > 0.10$). Increasing total vitamin E equivalence increased SOD on d 42 (linear, $P = 0.036$) but not on d 10 (linear, $P = 0.616$).