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Non Ruminant Nutrition

Polyphenols as a partial replacement for vitamin E in nursery pig diets

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Abstract

A total of 300 pigs (241 × 600; DNA, Columbus, NE; initially 6.0 ± 0.01 kg) 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 Argo, Denmark) on growth performance, complete blood count, serum thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and cytokine panel. Sixty pens of pigs were weighed and allotted to one of the five 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 three replacement strategy diets to determine the effects of replacing an additional 60 IU/kg of vitamin E with polyphenol 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 polyphenol. Third, all 60 IU/kg of the additional vitamin E was replaced with the equivalency of polyphenol. To evaluate whether there are negative effects of feeding nursery pigs a high level of polyphenol, 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 polyphenol. Whole blood and serum samples were collected on days 10 and 42, and pig weights and feed disappearance were measured on days 10, 21, 31, 38, and 42. For growth performance, increasing vitamin E equivalence tended to improve (quadratic, $P < 0.10$) gain-to-feed ratio (G:F) from days 10 to 21, and tended to improve (linear, $P < 0.10$) G:F from days 21 to 42 and 0 to 42. There was a vitamin E equivalence × day interaction ($P = 0.050$) for serum SOD activity. Increasing vitamin E equivalence increased (linear, $P < 0.05$) serum SOD activity on day 42 but not on days 10 ($P > 0.10$). For serum cytokines, 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 three replacement strategies throughout the entire periods. In summary, increasing vitamin E equivalence tended to improve G:F, which may be related to the improved SOD activity. Furthermore, polyphenol can effectively replace vitamin E provided above the vitamin E requirement to provide similar benefits from increasing vitamin E equivalence.

Lay Summary

Weaning is a stressful period that can cause high oxidative stress, which results in inflammation in the intestine and reduced growth performance for piglets. Supplying antioxidants in nursery pig diets theoretically can mitigate the negative effects of weaning oxidative stress. Vitamin E is an antioxidant that has been added to the weaned pig diets for this purpose. Another type of antioxidant is plant-based polyphenols that can be found in fruits and plants. Therefore, we evaluated the effects of feeding different vitamin E equivalence levels that were achieved by combining different ratios of vitamin E and a plant-based polyphenol product (Cabanin CSD; R2 Argo, Denmark) in nursery pig diets. Our experiment found that increasing vitamin E equivalence improved feed efficiency which may be related to the improved antioxidant status. Providing additional vitamin E equivalence above the basal vitamin E requirement through either vitamin E or polyphenol showed similar benefits. Thus, the polyphenol used in this study can be used as an effective replacement for vitamin E supplemented above the basal requirement.

Key words: antioxidant, growth, nursery pig, polyphenol, vitamin E

Introduction

Weaning is a stressful period for piglets due to changes in diet composition, environment, and bacterial challenges, which results in reduced feed intake and growth rate (Campbell et al., 2013). During stressful periods, the need for antioxidants increases because of the increased oxidative stress (Hao et al., 2021). Oxidative stress is caused by the imbalance of excess formation of oxidants (free radicals, such as reactive oxygen species) and insufficient degradation of these radicals by the animal's antioxidant system (Lü et al., 2010; Gessner et al., 2017; Hao et al., 2021). High reactive oxygen species levels damage the cellular components, such as DNA, protein, and lipid, which cause gene mutation, abnormal signaling pathways, energy metabolism disorder, lipid peroxidation, and protein structure change (Lü et al., 2010; Hao et al., 2021). Moreover, during the weaning period, a local inflammation occurs in the small intestine, which can be

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characterized by increased cytokine levels and adverse effects on intestinal morphology, such as villus height, crypt depth, brush border enzyme activity, absorption capacity, and intestinal barrier integrity (Pié et al., 2004; Zheng et al., 2021). As a result, the combination of high oxidative stress and local inflammation from weaning in the small intestine can negatively affect growth performance.

Antioxidants neutralize free radicals by electron donation, complex formation between oxidizing elements, or the regeneration of other antioxidants (Lü et al., 2010). Besides endogenous enzymatic antioxidants (e.g., superoxide dismutase [SOD], catalase, and glutathione peroxidase), natural non-enzymatic antioxidants can also be involved (e.g., vitamin E and C, carotenoids, and polyphenols) in protecting the cells from free radicals (Lü et al., 2010; Gessner et al., 2017; Hao et al., 2021).

Vitamin E is a fat-soluble vitamin that can be found in feed ingredients (green plants or seeds) as natural vitamin E (RRR-α-tocopheryl acetate); however, natural vitamin E is destroyed rapidly through oxidation under the influence of heat, moisture, rancid fat, or trace minerals (NRC, 2012). Thus, synthetic vitamin E (DL-α-tocopheryl acetate) has also been used to meet the vitamin E requirement estimate of the pigs (NRC, 2012). For nursery pigs, the vitamin E requirement estimate is 16 IU/kg (mg/kg) of the complete diet (NRC, 2012). However, surveys of industry nutritionists reported that their typical commercial US nursery diets contained ~75, 66, and 45 IU/kg of vitamin E for weight ranges of weaning to 7 kg, 7 to 11 kg, and 11 to 23 kg, respectively (Flohr et al., 2016; Faccin et al., 2023). These above-requirement levels of vitamin E suggest a belief that providing extra antioxidant support will help the pigs overcome oxidative stress from the weaning process. Nonetheless, the effect of this higher level of dietary vitamin E compared to the NRC (2012) vitamin E requirement estimate on nursery pigs is not well understood.

Another type of antioxidant that has been used in swine diets is plant-based polyphenols derived from the fruit and plant byproduct industry. These polyphenols are secondary plant metabolites that consist of a diverse group of compounds, including phenolic acids, flavonoids, tannins, and other phenolics (Naczk and Shahidi, 2006). They have shown some antioxidative and anti-inflammatory properties in several in vitro and in vivo studies and have the potential to partially replace vitamin E in swine, poultry, or dairy cow diets (Gessner et al., 2017; Lipiński et al., 2017). However, the effects of dietary polyphenols on nursery pigs' growth performance, antioxidant status (TBARS and SOD), CBC, and cytokine panels have not been well investigated. Cabanin CSD (R2 Argo, Denmark) is a natural plant-based polyphenol product that contains selected extracts from grapes, citrus, blackcurrant, and chestnuts. These ingredients contain high concentrations of polyphenols; therefore, we hypothesized that this polyphenol product could potentially be used as an effective antioxidant replacer above the minimum NRC (2012) vitamin E requirement estimate for nursery pigs with no negative effects.

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 polyphenols in diets above the minimum vitamin E requirement on growth performance, antioxidant status (TBARS and SOD), CBC, and cytokine panels of nursery pigs from weaning to 42 d post-weaning.

Material and Methods

General

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. Pigs were housed in pens with each pen (1.5 × 1.5 m) equipped with a four-hole dry self-feeder, and a nipple waterer to provide ad libitum access to feed and water. A total of 300 pigs (241 × 600, DNA, Columbus, NE; initially 6.0 ± 0.01 kg) were weaned at ~21 d of age and placed in pens of five pigs each based on initial body weight and sex. Pens of pigs were then randomly allotted to the five treatments in a completely randomized design with 12 replicate pens per treatment. The sex was balanced between dietary treatments.

Diets

The vitamin E form (44 092 IU/kg, 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 Argo, Denmark; Lot number: 220120) contained selected extracts from grapes, 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 in in vitro studies (Naczk and Shahidi, 2006). The polyphenol product 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 (data not published). One mg of this polyphenol product was estimated to provide 0.5 IU of vitamin E equivalence based on this previous research. The total polyphenol content was 9.2% for this specific lot of polyphenol 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 polyphenol. Third, all 60 IU/kg of supplemental vitamin E was replaced with the equivalency of polyphenol. These three replacement strategies allowed us to determine the effects of replacing vitamin E with polyphenol 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 polyphenol to evaluate whether there are negative effects of feeding nursery pigs a high level of polyphenol. Treatment diets were fed in three phases based on body weight (phase 1: 6 to 7 kg; phase 2: 7 to 12 kg; and phase 3: 12 to 25 kg) in meal form.

Basal diets for all three 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, and/or polyphenol) at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) to make the five treatment diets. The

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

Vitamin E[1], mg/kg:	**15**	**75**	**45**	**15**	**75**
Polyphenol[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
Polyphenol	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 (44,092 IU/kg, 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 Argo, Denmark) was used as the polyphenol source in this trial and 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 polyphenol.
[4]Vitamin E concentration was analyzed at Colorado State University Veterinary Diagnostic Laboratory (Fort Collins, Colorado).
[5]Weighted averages = Sum of the calculated vitamin E intake of the three phases/ total feed intake.

remaining ingredients were mixed thoroughly for each dietary treatment before mixing with the basal diet. All diets met or exceeded the NRC (2012) 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, Colorado).

Data and Sample Collection

Pen weights and feed disappearance were measured on days 0, 10, 21, 31, 38, and 42 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). 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 days 10 and 42 of the experiment for complete blood count (CBC), serum SOD activity, serum thiobarbituric acid reactive substances (TBARS), and serum cytokine panel. The same pig per experimental unit was used in all subsequent whole blood and serum collections. The sex of the selected pigs was balanced between treatments. Whole blood samples were collected with EDTA-containing blood tubes (VACUETTE tube 6 mL K3E K3EDTA separator 13 × 100, non-ridged; Greiner Bio-One North America Inc., Monroe, NC) 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 (Covidien Monoject blood collection tubes, silicone-coated tubes with red stoppers, no additive, 7 mL draw; Medtronic, Minneapolis, MN) that contained no anticoagulant or preservative, and 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 (PR1MA microcentrifuge tubes, natural boil-proof; Midwest Scientific, St. Louis, MO) as aliquots, and stored at −80°C. 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, and TNFα) was evaluated at Eve Technologies (Calgary, AB, Canada). Serum TBARS and SOD were evaluated at the Kansas State University Swine Nutrition Laboratory (Manhattan, KS). For serum TBARS, the assay used in the experiment was described in Rao et al. (2023) and the samples were run in triplicate in 96-well microplates with intra-assay CV of ≤ 5.0%. For serum SOD, assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI; # 703102) and samples were run in triplicate in 96-well microplates with intra-assay CV of ≤ 5.0%.

Statistical Analysis

Data were analyzed as a completely randomized design for one-way ANOVA using the Elmer function from the lme4 package for growth performance and blood parameters (CBC, cytokine panel, SOD, and TBARS) in R program (R Core Team, 2022). Pen was considered 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 (days 10 and 42) interaction and dietary treatment × day (days 10 and 42) interaction were tested for blood parameters. For serum cytokine data, the data were analyzed with the raw fluorescence intensity value based on Breen et al. (2015) with a log10 transformation for statistical analysis. All serum samples were analyzed at the same time with a single standard curve for each cytokine criterion. For serum TBARS and SOD assay, microtiter plate was used in the model as a random intercept. A Tukey multiple comparison adjustment was used, and all results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

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)	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[2]	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[3]	0.15	0.15	0.15
Zinc oxide	0.40	0.26	--
Phytase[4]	0.01	0.01	0.01
Vitamin premix[5]	0.11	0.11	0.11
Treatment premix[6]	0.29	0.29	0.29
Total	100.00	100.00	100.00
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/kg	2,529	2,469	2,392
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 body weight (phase 1: 6 to 7 kg; phase 2: 7 to 12 kg; and phase 3: 12 to 25 kg) in meal form.
[2]MEpro (Prairie Aquatech, Brookings, SD).
[3]Trace mineral premix provided per kg of diet: 110 mg Zn, 110 mg Fe, 33 mg Mn, 16.5 mg Cu, 0.29 mg I, and 0.29 mg Se.
[4]Quantum Blue 5G (AB Vista, Plantation, FL) provided 626 FTU per kg of diet with an expected STTD P release of 0.15%.
[5]Vitamin premix without vitamin E provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 3.3 mg vitamin K; 0.033 mg vitamin B_{12}; 49.6 mg niacin; 27.6 mg pantothenic acid; and 8.27 mg riboflavin.
[6]For the 5 treatments, treatment premix provided per ton of diet: 0.35, 1.7, 1.05, 0.35, and 1.7 kg of vitamin E (44,092 IU/kg, DSM, Parsippany, NJ), respectively; 2.35, 1.0, 1.6, 2.25, and 0.0 kg of ground corn, respectively; and 0.0, 0.0, 0.06, 0.12, and 1.0 kg of polyphenol (Cabanin CSD, R2 Argo, Denmark), respectively.

Results

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 days 10 to 21, increasing vitamin E equivalence increased (quadratic, $P = 0.086$) G:F from 15 to 75 IU/kg of vitamin E equivalence with no further increase at 575 IU/kg. From d 21 to 42, there was a tendency for improvement (linear, $P = 0.063$) in G:F as the vitamin E equivalence increased. This tendency of increasing improvement in G:F was also observed in overall (d 0 to 42) G:F (linear, $P = 0.075$).

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			
Polyphenol, 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]
Days 0 to 10 (phase 1)								
day 0 BW, kg	6.0	6.0	6.0	6.0	6.0	0.01	0.998	0.803
day 10 BW, kg	7.4	7.2	7.2	7.4	7.3	0.11	0.945	0.451
ADG, g	140	128	125	139	137	11.3	0.943	0.429
ADFI, g	168	165	154	161	162	8.0	0.776	0.394
G:F, g/kg	824	771	807	858	835	42.3	0.678	0.693
Days 10 to 21 (phase 2)								
day 21 BW, kg	12.4	12.4	12.4	12.5	12.3	0.21	0.588	0.931
ADG, g	460	465	475	465	451	19.7	0.405	0.485
ADFI, g	591	584	585	576	567	20.1	0.244	0.670
G:F, g/kg	779	797	810	807	798	13.8	0.574	0.086
Days 21 to 42 (phase 3)								
day 42 BW, kg	25.1	25.3	25.5	25.8	25.2	0.38	0.867	0.319
ADG, g	604	617	620	633	614	12.2	0.878	0.159
ADFI, g	929	936	947	959	917	19.2	0.400	0.348
G:F, g/kg	649	660	657	660	670	7.3	0.063	0.337
Days 0 to 42 (overall)								
ADG, g	455	461	464	471	458	9.0	0.867	0.321
ADFI, g	659	660	663	669	646	13.0	0.317	0.655
G:F, g/kg	691	698	701	705	709	6.5	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 polyphenol (Cabanin CSD, R2 Argo, Denmark).
[2]BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = 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).

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 ($P > 0.10$; Figure 1). However, there was a vitamin E equivalence × day interaction ($P = 0.050$) on serum SOD activity (Figure 2). Increasing vitamin E equivalence increased (linear, $P = 0.036$) serum SOD activity on day 42 but not on day 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 days 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). 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 days 10 to 42.

Serum Cytokines

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 days 10 to 42.

Discussion

Although vitamin E and polyphenols are both added to swine diets for their antioxidative properties, their mechanism of action are different. Vitamin E can be absorbed in the intestine and enter the systemic circulation, as supplementing vitamin E in pig diets has shown increased serum and tissue (loin muscle, liver, and fat) vitamin E concentrations throughout the literature (Lauridsen, 2010; Song et al., 2014; Rey et al., 2017). The absorbed vitamin E can be used directly as

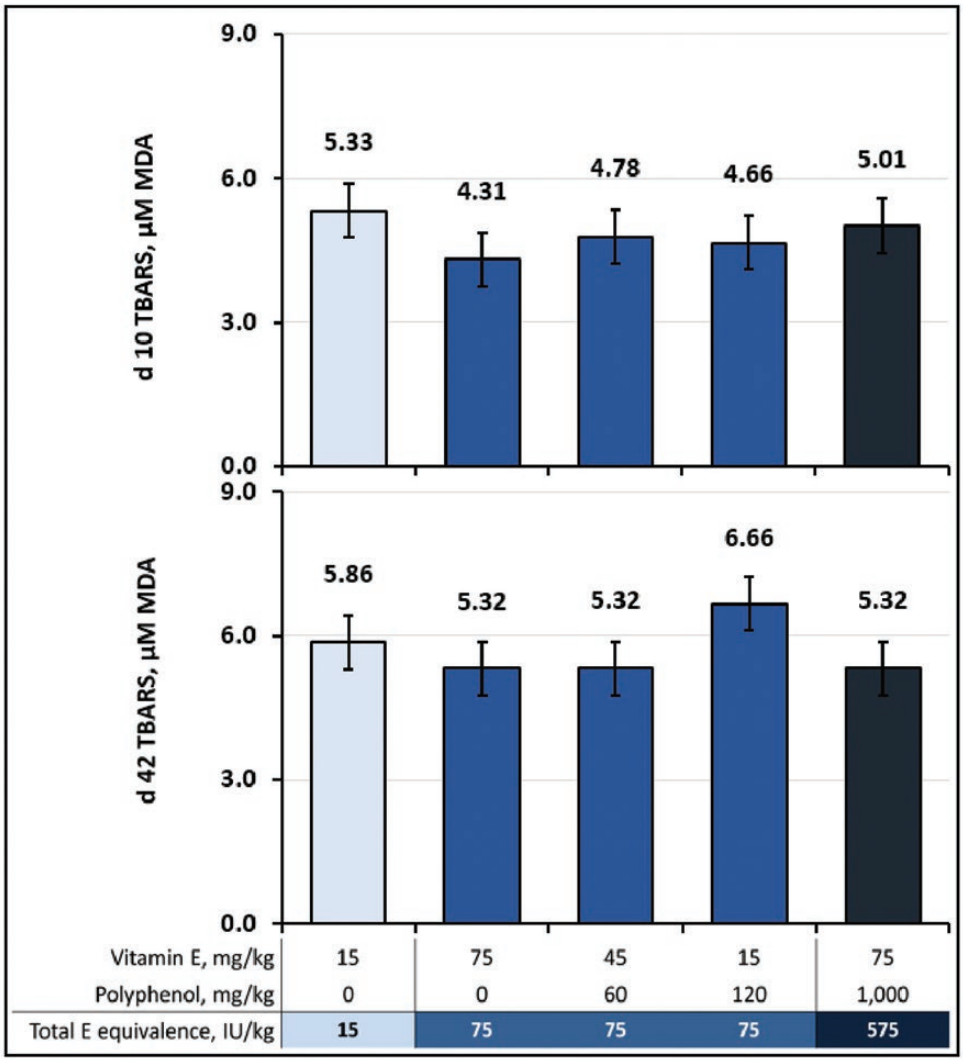

Figure 1. Serum TBARS concentration on days 10 and 42. Error bar equals to 1 SEM. A total of 300 pigs (initially 6.0 kg) were used with 60 pigs per replicate and 12 replicates per treatment. Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and polyphenol (Cabanin CSD, R2 Argo, Denmark). There was no evidence of total vitamin E equivalence × day interaction, vitamin E equivalence, treatment × day interaction, treatment, or day effect ($P > 0.10$).

antioxidant in the animals at cell membrane level, and also has a structural role in the cell membranes (NRC, 2012). On the other hand, there are few in vivo swine studies on the digestibility and bioavailability of polyphenols. Several human research studies suggest that only low percentages of the dietary polyphenols may be absorbed in the small intestine and have low bioavailability because of their molecular structures (Han et al., 2007; Landete, 2013; Faria et al., 2014). The polyphenols are expected to have direct antioxidant effects in vivo in the intestinal lumen because of the higher concentration of polyphenols in the lumen compared to the systemic concentrations (Gessner et al., 2017). Moreover, the low amount of absorbed polyphenols are then extensively bio-transformed in the liver and rapidly excreted in urine and bile (Hackman et al., 2008). Next, the bile-excreted polyphenol metabolites and the unabsorbed polyphenols are bio-transformed by the colon microbiota's enzymatic activities to various metabolites (Hein et al., 2008; Gessner et al., 2017). These bio-transformed polyphenol metabolites have shown prebiotic effects as growth-promoting substrates or antimicrobial substances for bacteria of the colon microbiota in human and mice research (Gessner et al., 2017). As these experiments were conducted on other monogastric species, more swine research is needed to determine whether this physiological process occurs similarly in pigs. Although there was no analytical data on the bio-transformed polyphenol metabolites, several swine research found feeding polyphenols modulated the nursery pigs' microbiota composition (Ao et al., 2022; Wei et al., 2022; Xu et al., 2022), lowered the diarrhea incidence rate (Liu et al., 2021; Xu et al., 2022), and improved intestine barrier integrity (Hu et al., 2020; Chen et al., 2022; Guo et al., 2022). These improvements in gastrointestinal health from feeding polyphenols can potentially be a key mechanism of action in improving the overall health status, such as growth performance, systemic antioxidant status, and immune system of the nursery pigs.

Because the cell structural need for the lipophilic vitamin E and pigs cannot synthesize vitamin E endogenously (NRC, 2012), polyphenols, which are relatively hydrophilic (Tsao, 2010), theoretically cannot completely replace vitamin E in

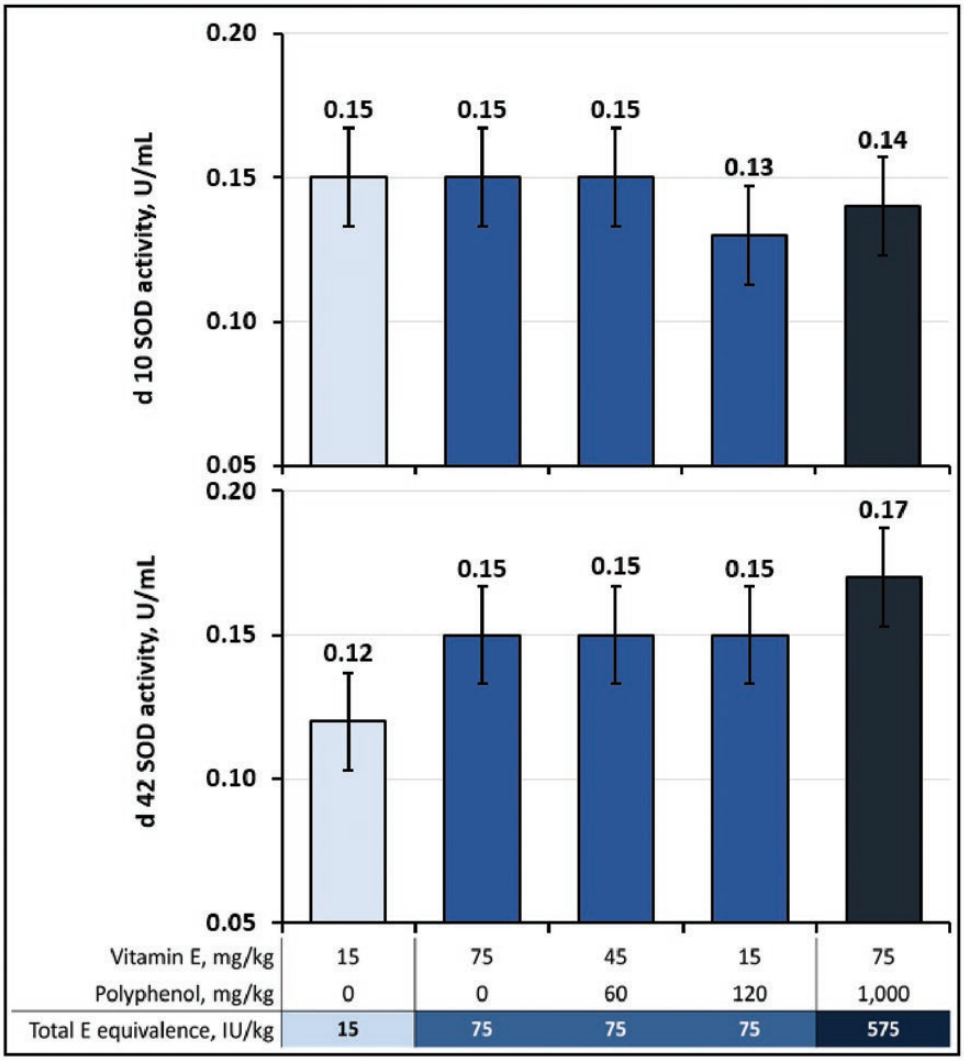

Figure 2. Serum SOD activity on days 10 and 42. Error bar equals to 1 SEM. A total of 300 pigs (initially 6.0 kg) were used with 60 pigs per replicate and 12 replicates per treatment. Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and polyphenol (Cabanin CSD, R2 Argo, 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, $P = 0.616$).

the diets (Gessner et al., 2017). Thus, our treatment diets all contained a minimum basal level of vitamin E equivalence (15 IU/kg) from vitamin E to meet the baseline requirement of vitamin E. This design allowed us to determine the effect of replacing vitamin E with polyphenols for the additional vitamin E equivalence above vitamin E requirement. In our study, we found no evidence of difference in ADG and ADFI throughout the 42-d experimental period; however, G:F was improved as vitamin E equivalence increased in our trial. Moreover, the three vitamin E replacement strategies showed similar results in all response variables, which suggests that polyphenols can effectively replace vitamin E for the additional 60 IU/kg of vitamin E equivalence in nursery pig diets that contained a baseline requirement (15 IU/kg) of vitamin E. Similar to our results, some studies found improved G:F with no evidence of difference in ADG and ADFI when additional vitamin E (Wilburn et al., 2008) or polyphenols (Fiesel et al., 2014; Silva-Guillen et al., 2020) were added to the nursery pig diets contained above-requirement level of vitamin E. Differently, some studies found polyphenols improved nursery pigs' ADG or ADFI but not G:F (Dell'Anno et al., 2020; Liu et al., 2021; Ao et al., 2022). The improvement in growth performance in our trial and other experiments may be related to the improvement in antioxidant and immune function. We found increasing vitamin E equivalence increased serum SOD activity. Similar to our results, several studies also found improved ($P \leq 0.05$) antioxidant status indicated by improved SOD, total antioxidant capacity (TAOC), and TBARS when nursery pigs were supplemented with vitamin E (Rey et al., 2017; Silva-Guillen et al., 2020) or polyphenols (Ao et al., 2022; Guo et al., 2022; Wei et al., 2022). For the immune function, even though we only found some numerical reduction, many studies found significant reductions in proinflammatory cytokines, such as TNF-α, NF-kB, IL-1β, IL-1ra, IL-2, IL-4, IL-6, or IL-8, when vitamin E (Silva-Guillen et al., 2020) or polyphenols were fed to the nursery pigs (Fiesel et al., 2014; Pistol et al., 2019; Guo et al., 2022). A reduction in proinflammatory cytokine levels in healthy pigs indicates an improvement in immune status, which suggests that these pigs may be able to spend less energy and amino acid (AAs)

Table 4. Evaluation of vitamin E levels and vitamin E replacement strategies on nursery pig complete blood count[1]

Vitamin E, mg/kg		15	75	45	15	75					
Polyphenol, mg/kg		0	0	60	120	1,000		Probability, *P* =			
Total E equivalence, IU/kg		15	75	75	75	575	SEM	Treatment[2]	Day[2]	Linear[3]	Quadratic[3]
Erythrocyte, M/uL	day 10	6.33	6.38	6.20	6.34	6.29	0.118	0.818	0.456	0.770	0.492
	day 42	6.43	6.31	6.32	6.31	6.35					
Hemoglobin, g/dL	day 10	11.3	11.5	11.5	11.7	11.2	0.21	0.702	0.540	0.915	0.613
	day 42	11.5	11.3	11.7	11.4	11.7					
Mean cell volume, fL	day 10	60.3	60.9	62.5	62.1	60.4	1.06	0.244	0.530	0.828	0.163
	day 42	60.9	60.8	62.5	61.7	62.1					
Mean cell hemoglobin, pg	day 10	18.0	18.0	18.5	18.4	17.9	0.35	0.376	0.757	0.872	0.225
	day 42	17.9	17.9	18.6	18.2	18.3					
Mean cell hemoglobin, g/dL	day 10	29.8	29.5	29.6	29.7	29.6	0.25	0.970	0.230	0.977	0.938
	day 42	29.4	29.5	29.7	29.4	29.6					
Hematocrit, %	day 10	35.5	35.9	36.0	36.6	35.3	0.67	0.666	0.389	0.702	0.745
	day 42	36.3	35.3	36.5	35.8	36.2					
RBC distribution width, %	day 10	23.5	23.3	23.1	23.7	22.6	0.75	0.872	< 0.001	0.324	0.871
	day 42	18.9	18.4	18.2	19.2	18.2					
Leukocyte, K/uL	day 10	13.0	17.3	14.3	13.9	14.1	1.50	0.149	0.003	0.843	0.070
	day 42	18.1	19.7	19.3	20.0	19.0					
Segmented neutrophil, K/uL	day 10	5.47	8.53	6.5	5.7	5.84	0.919	0.089	0.081	0.812	0.329
	day 42	7.43	7.39	7.66	7.22	7.28					
Band neutrophil, K/uL	day 10	0.008	0.100	0.025	0.027	0.033	0.0402	0.527	0.456	0.716	0.354
	day 42	0.050	0.030	0.080	0.090	0.080					
Lymphocyte, K/uL	day 10	6.51	7.17	6.72	7.07	7.11	0.852	0.967	0.024	0.462	0.144
	day 42	8.8	10.3	10.0	10.6	10.0					
Monocyte, K/uL	day 10	0.67	0.95	0.75	0.67	0.70	0.15	0.667	< 0.001	0.345	0.748
	day 42	1.48	1.37	1.23	1.61	1.22					
Eosinophil, K/uL	day 10	0.24	0.52	0.29	0.41	0.34	0.084	0.163	0.833	0.885	0.045
	day 42	0.27	0.54	0.29	0.28	0.26					
Basophil, K/uL	day 10	0.06	0.03	0.08	0.02	0.05	0.038	0.753	0.426	0.934	0.587
	day 42	0.10	0.06	0.03	0.16	0.10					
Platelets, K/uL	day 10	342.2	430.2	378.5	395.9	310.7	53.03	0.315	0.068	0.117	0.207
	day 42	437.4	407.3	528.3	438.0	382.0					

[1]A total of 300 pigs (initially 6.0 kg) were used with 60 pigs per replicate and 12 replicates per treatment. Complete blood count was analyzed at Kansas State University Veterinary Diagnostic Laboratory (Manhattan, KS). Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and polyphenol (Cabanin CSD, R2 Argo, Denmark).
[2]F-test *P*-value. All treatment × day interactions were not statistically significant ($P > 0.10$).
[3]Polynomial contrasts were utilized to analyze the effects of dietary total E equivalence levels (15, 75, and 575 IU/kg) of collection. All total E equivalence levels × day interactions were not statistically significant ($P > 0.10$).

on immune overexpression which can potentially lead to an improved energy and AAs utilization (Gessner et al., 2017). This may explain the reduced cytokines and improved feed efficiency found in several experiments cited above. Moreover, we found several cytokines (GM-CSF, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, and IL-12) increased from d 10 to 42 as nursery pig aged. Though there are no reference values for cytokine levels based on pig's age, some evidences suggests that cytokine levels tend to increase as weaned pigs age (de Groot et al., 2005). For the results of CBC, we observed some differences as the vitamin E equivalence increased (leukocyte and eosinophil) or between dietary treatments (segmented neutrophil); however, whether these differences affected growth performance are not clear as all CBC variables were within the reference intervals for these ages of pigs (ISU, 2011). There is some evidence suggesting that some CBC parameters are associated with growth performance for grow-finish pigs (Lindholm-Perry et al., 2021); nevertheless, more research is needed for nursery pigs. Additionally, we found lymphocyte, monocyte, platelets, and segmented neutrophil increase, while RBC distribution width decreased from days 10 to 42. These differences between days can be expected as pigs age (ISU, 2011).

Though some studies found no evidence of difference ($P > 0.10$) in growth performance (ADG, ADFI, and G:F) following the inclusion of dietary vitamin E (Kim et al., 2016; Rey et al., 2017; Chen et al., 2019) or polyphenols (Zhang et al., 2014; Xu et al., 2019; Hu et al., 2020) in the nursery pig diets, several of these studies found improvement in antioxidant status (TBARS and T-AOC) or gastrointestinal health (tight junction), or reduced cytokines (TNF-α, IL-1β, IL-2, or IL-6). This lack of differences in growth performance may

Table 5. Evaluation of vitamin E levels and vitamin E replacement strategies on nursery pig cytokine profile (fluorescence intensity value)[1]

Vitamin E, mg/kg		15	75	45	15	75					
Polyphenol, mg/kg		0	0	60	120	1,000		Probability, *P* =			
Total E equivalence, IU/kg		15	75	75	75	575	SEM	Treatment[2]	Day[2]	Linear[3]	Quadratic[3]
GM-CSF	day 10	18.0	14.0	11.9	18.8	15.9	1.22	0.476	0.069	0.884	0.856
	day 42	12.2	11.6	11.7	20.0	12.7					
IFNγ	day 10	43.0	46.3	36.8	45.9	42.3	1.31	0.973	0.138	0.889	0.909
	day 42	28.5	30.2	23.7	39.7	32.0					
IL-1α	day 10	47.2	32.7	26.5	71.6	27.0	1.40	0.122	0.004	0.185	0.239
	day 42	162.3	114.9	97.3	79.7	101.8					
IL-1β	day 10	85.4	70.4	56.7	98.3	44.0	1.31	0.220	0.059	0.139	0.288
	day 42	163.5	123.6	103.8	95.9	120.1					
IL-1ra	day 10	359.1	274.0	310.0	464.1	291.9	1.25	0.127	0.631	0.268	0.377
	day 42	314.5	221.1	249.5	235.7	225.3					
IL-2	day 10	51.8	31.6	31.3	74.5	26.8	1.42	0.150	0.008	0.188	0.202
	day 42	166.8	94.4	105.8	81.3	107.1					
IL-4	day 10	71.2	53.0	40.7	98.1	39.6	1.40	0.128	0.020	0.101	0.267
	day 42	174.0	138.0	108.2	89.8	102.9					
IL-6	day 10	63.9	37.4	39.8	82.5	32.3	1.37	0.134	0.041	0.160	0.198
	day 42	147.8	112.0	92.1	76.7	104.0					
IL-8	day 10	680.5	583.7	437.7	674.5	654.6	1.37	0.849	0.166	0.565	0.770
	day 42	406.8	401.2	393.7	417.8	273.1					
IL-10	day 10	58.6	39.9	46.0	80.0	34.5	1.38	0.371	0.067	0.180	0.359
	day 42	125.7	79.5	79.7	72.5	76.1					
IL-12	day 10	612.2	700.3	757.3	774.1	644.3	1.16	0.757	0.064	0.119	0.711
	day 42	900.9	741.8	806.3	777.6	589.5					
IL-18	day 10	91.7	82.8	80.3	128.6	63.4	1.30	0.367	0.135	0.405	0.537
	day 42	148.2	109.9	99.8	107.0	129.8					
TNFα	day 10	37.2	31.4	27.6	32.9	34.1	1.22	0.872	0.132	0.690	0.741
	day 42	26.9	23.6	24.3	44.4	32.9					

[1]A total of 300 pigs (initially 6.0 kg) were used with 60 pigs per replicate and 12 replicates per treatment. Serum cytokine panel was evaluated at Eve Technologies (Calgary, AB, Canada). Data were log10 transformed for statistical analysis and transformed back for the cell mean values reported in this table. Total E equivalence is the combination of vitamin E equivalence provided by vitamin E and polyphenol (Cabanin CSD, R2 Argo, Denmark).
[2]F-test *P*-value. All treatment × day interactions were not statistically significant ($P > 0.10$).
[3]Polynomial contrasts were utilized to analyze the effects of dietary total E equivalence levels (15, 75, and 575 IU/kg) of collection. All total E equivalence levels × day interactions were not statistically significant ($P > 0.10$).

be attributed to the lack of sufficiently stressful events that would generate high levels of oxidative stress that allow the improved antioxidant and immune status to demonstrate their beneficial effects on growth. Many of these studies were conducted in high-health-status farms, where the barn environment was highly regulated, and exposure to environmental pathogens was minimized, thereby reducing the challenges that can increase oxidative stress or trigger immune responses in these weaned pigs. Dietary vitamin E or polyphenol levels in these experimental diets might have already provided sufficient antioxidative or immune-promoting effects to optimize the growth performance of these nursery pigs; thus, no evidence of differences in growth was found. To understand the effects of vitamin E and polyphenols fed to pigs under higher oxidative stress, controlled challenged experiments may have the potential to provide us with some insights as they can increase the oxidative stress of pigs (Hao et al., 2021). Jiang et al. (2014) found *E. coli*-challenged nursery pigs fed polyphenols had improved G:F, GSH-Px, and T-AOC compared to the *E. coli*-challenged pig without polyphenols. Chen et al. (2022) found that diquat-challenged nursery pigs fed polyphenols had improved ADG, antioxidant status, and intestinal barrier integrity compared to challenged pigs without polyphenols in the diet. For nursery pigs challenged with LPS, dietary polyphenols reduced TBARS and cytokines, and improved intestine tight junction (Hu et al., 2020, 2022). These results suggest that pigs under high oxidative stress from controlled challenges could benefit from supplementing vitamin E and polyphenols in their diets for growth, antioxidant status, and health. However, more research is needed as only few research has been conducted for pigs under controlled oxidative stress.

Additionally, comparing the results of different polyphenol experiments on nursery pigs poses challenges due to the diversity of polyphenols used as feed additives. These polyphenol additives are derived from various fruits and herbs extracts, and combined in different ratios. The scientific literature lacks a thorough investigation of whether different polyphenols exert distinct or similar effects on nursery pigs' growth, antioxidant status, or immune system. Furthermore, the inclusion

levels of these polyphenol additives vary widely from 0.01% to 10% with different concentrations and compositions of polyphenol content. In our study, when feeding high levels of polyphenolic compounds (1,000 mg/kg polyphenol, providing an estimated 500 IU/kg vitamin E equivalence), we did not see any detrimental effects. Similarly, the effects of vitamin E on nursery pigs may also be variable, since the natural vitamin E concentrations from feed ingredients vary as heat and humidity from environmental conditions could rapidly destroy them (NRC, 2012). Furthermore, vitamin E requirements can also be affected by many dietary factors, including Se, unsaturated fatty acids, and other natural or synthetic antioxidants (NRC, 2012). These factors all contributed to posing challenges in determining the effects of vitamin E and polyphenols on nursery pigs' performance.

In summary, increasing vitamin E equivalence by the addition of vitamin E or polyphenols 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 polyphenol can be used as an effective replacement for the 60 IU/kg of additional vitamin E added to diets that meet the basal vitamin E requirement (15 IU/kg) for nursery pigs. These similar improvements may be driven by different mechanisms of action between vitamin E and polyphenols. Lastly, whether different vitamin E levels or vitamin E replacement strategies can provide similar results in conditions that have higher oxidative stress or pathogen challenge requires further research.

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Author Contributions

ZXR, MDT, ASS, and JTG were responsible for experimental design and conduct, and analyzing the data; JCW, JMD, RDG, and GB assisted in data interpretation; ZXR, MDT, JCW, JMD, RDG, ASS, BHF, KCK, GB, and JTG assisted in writing, editing, and approval of the final manuscript.

Conflict of Interest

The authors declare no conflict of interest. AS, DL, and BF are employees of SAM Nutrition (Bloomington, MN) who distributes the Cabanin CSD. KK and GB are employees of R2Agro (Hedensted, Denmark) who manufactures the Cabanin CSD.

Literature Cited

Ao, W., M. Cheng, Y. Chen, J. Sun, C. Zhang, X. Zhao, M. Liu, and B. Zhou. 2022. Fermented apple pomace improves plasma biochemical and antioxidant indicators and fecal microbiota of weaned pigs. *Agriculture* 12:1603. doi: 10.3390/agriculture12101603

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* 71:188–198. doi: 10.1016/j.cyto.2014.10.030

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. *J. Anim. Sci. Biotechnol.* 4:19. doi: 10.1186/2049-1891-4-19

Chen, C., Z. Wang, J. Li, Y. Li, P. Huang, X. Ding, J. Yin, S. He, H. Yang, and Y. Yin. 2019. Dietary vitamin E affects small intestinal histomorphology, digestive enzyme activity, and the expression of nutrient transporters by inhibiting proliferation of intestinal epithelial cells within jejunum in weaned piglets1. *J. Anim. Sci.* 97:1212–1221. doi: 10.1093/jas/skz023

Chen, J., D. Chen, B. Yu, Y. Luo, P. Zheng, X. Mao, J. Yu, J. Luo, Z. Huang, H. Yan, et al. 2022. Chlorogenic acid attenuates oxidative stress-induced intestinal mucosa disruption in weaned pigs. *Front. Vet. Sci* 9:806253. doi: 10.3389/fvets.2022.806253

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

Dell'Anno, M., M. Hejna, S. Sotira, V. Caprarulo, S. Reggi, R. Pilu, F. Miragoli, M. L. Callegari, S. Panseri, and L. Rossi. 2020. Evaluation of leonardite as a feed additive on lipid metabolism and growth of weaned piglets. *Anim. Feed Sci. Technol.* 266:114519. doi: 10.1016/j.anifeedsci.2020.114519

Faccin, J. E. G., M. D. Tokach, R. D. Goodband, J. M. DeRouchey, J. C. Woodworth, and J. T. Gebhardt. 2023. Industry survey of added vitamins and trace minerals in US swine diets. *Transl. Anim. Sci.* 7:txad035. doi: 10.1093/tas/txad035

Faria, A., I. Fernandes, S. Norberto, N. Mateus, and C. Calhau. 2014. Interplay between anthocyanins and gut microbiota. *J. Agric. Food Chem.* 62:6898–6902. doi: 10.1021/jf501808a

Fiesel, A., D. K. Gessner, E. Most, and K. Eder. 2014. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. *BMC Vet. Res.* 10:196. doi: 10.1186/s12917-014-0196-5

Flohr, J., J. DeRouchey, J. Woodworth, M. Tokach, R. Goodband, and S. Dritz. 2016. A survey of current feeding regimens for vitamins and trace minerals in the US swine industry. *J. Swine Health Prod.* 24:290–303.

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)* 101:605–628. doi: 10.1111/jpn.12579

Guo, Z., X. Chen, Z. Huang, D. Chen, J. Yu, H. Yan, H. Chen, J. He, P. Zheng, Y. Luo, et al. 2022. Apple polyphenols improve intestinal barrier function by enhancing antioxidant capacity and suppressing inflammation in weaning piglets. *Anim. Sci. J.* 93:e13747. doi: 10.1111/asj.13747

Hackman, R. M., J. A. Polagruto, Q. Y. Zhu, B. Sun, H. Fujii, and C. L. Keen. 2008. Flavanols: digestion, absorption and bioactivity. *Phytochem. Rev.* 7:195–208. doi: 10.1007/s11101-007-9070-4

Han, X., T. Shen, and H. Lou. 2007. Dietary polyphenols and their biological significance. *Int. J. Mol. Sci.* 8:950–988. doi: 10.3390/i8090950

Hao, Y., M. Xing, and X. Gu. 2021. Research progress on oxidative stress and its nutritional regulation strategies in pigs. *Animals (Basel)* 11:1384. doi: 10.3390/ani11051384

Hein, E. -M., K. Rose, G. van't Slot, A. W. Friedrich, and H. -U. Humpf. 2008. Deconjugation and degradation of flavonol glycosides by pig cecal microbiota characterized by fluorescence in situ hybridization (fish). *J. Agric. Food Chem.* 56:2281–2290. doi: 10.1021/jf073444o

Hu, R., Z. He, M. Liu, J. Tan, H. Zhang, D. Hou, J. He, and S. Wu. 2020. Dietary protocatechuic acid ameliorates inflammation and up-regulates intestinal tight junction proteins by modulating gut microbiota in LPS-challenged piglets. *J. Anim. Sci. Biotechnol.* 11:92. doi: 10.1186/s40104-020-00492-9

Hu, R., S. Wu, B. Li, J. Tan, J. Yan, Y. Wang, Z. Tang, M. Liu, C. Fu, H. Zhang, et al. 2022. Dietary ferulic acid and vanillic acid on inflammation, gut barrier function and growth performance in lipopolysaccharide-challenged piglets. *Anim. Nutr*. 8:144–152. doi: 10.1016/j.aninu.2021.06.009

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

Jiang, X. R., H. J. Zhang, G. Mantovani, G. L. Alborali, J. M. Caputo, G. Savoini, V. Dell'Orto, and V. Bontempo. 2014. The effect of plant polyphenols on the antioxidant defence system of weaned piglets subjected to an Escherichia coli challenge. *J. Anim. Feed Sci*. 23:324–330. doi: 10.22358/jafs/65668/2014

Kim, J. C., B. P. Mullan, J. L. Black, R. J. E. Hewitt, R. J. van Barneveld, and J. R. Pluske. 2016. Acetylsalicylic acid supplementation improves protein utilization efficiency while vitamin E supplementation reduces markers of the inflammatory response in weaned pigs challenged with enterotoxigenic E coli. *J. Anim. Sci. Biotechnol*. 7:58. doi: 10.1186/s40104-016-0118-4

Landete, J. M. 2013. Dietary intake of natural antioxidants: vitamins and polyphenols. *Crit. Rev. Food Sci. Nutr*. 53:706–721. doi: 10.1080/10408398.2011.555018

Lauridsen, C. 2010. Evaluation of the effect of increasing dietary vitamin E in combination with different fat sources on performance, humoral immune responses and antioxidant status of weaned pigs. *Anim. Feed Sci. Technol*. 158:85–94. doi: 10.1016/j.anifeedsci.2010.03.015

Lindholm-Perry, A. K., L. A. Kuehn, J. E. Wells, L. A. Rempel, C. G. Chitko-McKown, B. N. Keel, and W. T. Oliver. 2021. Hematology parameters as potential indicators of feed efficiency in pigs. *Transl. Anim. Sci*. 5:txab219. doi: 10.1093/tas/txab219

Lipiński, K., M. Mazur, Z. Antoszkiewicz, and C. Purwin. 2017. Polyphenols in monogastric nutrition – A review Ann. *Anim. Sci*. 17:41–58. doi: 10.1515/aoas-2016-0042

Liu, X. Z., Y. Ju, N. Bao, Y. L. Luo, L. L. Huang, N. X. Cao, M. Z. Liu, J. N. Bo, S. Zhang, and Y. Yan. 2021. Effects of polyphenol-rich Aronia melanocarpa pomace feeding on growth performance, biochemical profile, and meat quality in pigs at weaned and finishing stages. *Livest. Sci*. 252:104674. doi: 10.1016/j.livsci.2021.104674

Lü, J. M., P. H. Lin, Q. Yao, and C. Chen. 2010. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J. Cell. Mol. Med*. 14:840–860. doi: 10.1111/j.1582-4934.2009.00897.x

Naczk, M., and F. Shahidi. 2006. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J. Pharm. Biomed. Anal*. 41:1523–1542. doi: 10.1016/j.jpba.2006.04.002

NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed. National Academic Press, Washington, DC.

Pié, S., J. P. Lallès, F. Blazy, J. Laffitte, B. Sève, and I. P. Oswald. 2004. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *J. Nutr*. 134:641–647. doi: 10.1093/jn/134.3.641

Pistol, G. C., L. M. Palade, D. E. Marin, M. Stancu, and I. Taranu. 2019. The effect of grape wastes, wine industry byproducts, on inflammatory and antioxidant biomarkers in post-weaning piglets. *Lucrări Ştiinţifice - Universitatea de Ştiinţe Agricole şi Medicină Veterinară, Seria Zootehnie* 71:219–223.

Rao, Z. -X., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and J. T. Gebhardt. 2023. Evaluation of selenium source on nursery pig growth performance, serum and tissue selenium concentrations, and serum antioxidant status. *Transl. Anim. Sci*. 7:txad049. doi: 10.1093/tas/txad049

R Core Team. 2022. *R: A language and environment for statistical computing*., Vienna, Austria.

Rey, A., C. López-Bote, and G. Litta. 2017. Effects of dietary vitamin E (DL-α-tocopheryl acetate) and vitamin C combination on piglets oxidative status and immune response at weaning. *J. Anim. Feed Sci*. 26:226–235. doi: 10.22358/jafs/76595/2017

Silva-Guillen, Y. V., C. Arellano, R. D. Boyd, G. Martinez, and E. van Heugten. 2020. Growth performance, oxidative stress and immune status of newly weaned pigs fed peroxidized lipids with or without supplemental vitamin E or polyphenols. *J. Anim. Sci. Biotechnol*. 11:22. doi: 10.1186/s40104-020-0431-9

Song, R., C. Chen, L. J. Johnston, B. J. Kerr, T. E. Weber, and G. C. Shurson. 2014. Effects of feeding diets containing highly peroxidized distillers dried grains with solubles and increasing vitamin E levels to wean-finish pigs on growth performance, carcass characteristics, and pork fat composition. *J. Anim. Sci*. 92:198–210. doi: 10.2527/jas.2013-6334

Tsao, R. 2010. Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2:1231–1246. doi: 10.3390/nu2121231

Wei, X., L. Li, H. Yan, Q. Li, J. Gao, and R. Hao. 2022. Grape seed procyanidins improve intestinal health by modulating gut microbiota and enhancing intestinal antioxidant capacity in weaned piglets. *Livest. Sci*. 264:105066. doi: 10.1016/j.livsci.2022.105066

Wilburn, E. E., D. C. Mahan, D. A. Hill, T. E. Shipp, and H. Yang. 2008. An evaluation of natural (RRR-alpha-tocopheryl acetate) and synthetic (all-rac-alpha-tocopheryl acetate) vitamin E fortification in the diet or drinking water of weanling pigs. *J. Anim. Sci*. 86:584–591. doi: 10.2527/jas.2007-0377

Xu, Q., M. Cheng, R. Jiang, X. Zhao, J. Zhu, M. Liu, X. Chao, C. Zhang, and B. Zhou. 2022. Effects of dietary supplement with a Chinese herbal mixture on growth performance, antioxidant capacity, and gut microbiota in weaned pigs. *Front. Vet. Sci*. 9:971647. doi: 10.3389/fvets.2022.971647

Xu, X., X. Chen, Z. Huang, D. Chen, B. Yu, H. Chen, J. He, Y. Luo, P. Zheng, J. Yu, et al. 2019. Dietary apple polyphenols supplementation enhances antioxidant capacity and improves lipid metabolism in weaned piglets. *J. Anim. Physiol. Anim. Nutr. (Berl)* 103:1512–1520. doi: 10.1111/jpn.13152

Zhang, H. J., X. R. Jiang, G. Mantovani, A. E. V. Lumbreras, M. Comi, G. Alborali, G. Savoini, V. Dell'Orto, and V. Bontempo. 2014. Modulation of plasma antioxidant activity in weaned piglets by plant polyphenols. *Ital. J. Anim. Sci*. 13:424–430. doi: 10.4081/ijas.2014.3242

Zheng, L., M. E. Duarte, A. Sevarolli Loftus, and S. W. Kim. 2021. Intestinal health of pigs upon weaning: challenges and nutritional intervention. *Front. Vet. Sci*. 8:628258. doi: 10.3389/fvets.2021.628258