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Effect of a Novel Dietary Antioxidant on Growth Performance and Antioxidant Status of Nursery Pigs

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Summary

To evaluate the effect of dietary S-ascisic acid (S-ABA) supplementation on the growth performance and antioxidant status of pigs, 320 nursery pigs (DNA 241 × 600; initially 12.0 ± 1.13 lb) were weaned at approximately 18 d of age and assigned to pens in a generalized randomized block design with gender and weight category as blocking factors. Pigs were fed a common phase 1 diet from weaning to d 7. On d 8 post-weaning, pen of pigs (14.2 ± 1.25 lb) within gender × weight blocks were randomly allotted to one of four dietary treatments. Treatments included a conventional nursery diet (Control) and three diets that used the Control formulation with increasing S-ABA (0.5, 1.0 and 5.0 ppm). Treatments were provided during phases 2 (d 0 to 14) and 3 (d 14 to 35). Growth performance was measured weekly. Additionally, 32 pigs on d 0 and two pigs per pen on d 14 and 35 were bled to assess the erythrocytes' total glutathione (GSH+GSSG), reduced glutathione (GSH), oxidized to reduced glutathione ratio (GSSG:GSH), serum total antioxidant capacity (TAC), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS). At the termination of the trial, two pigs in each pen were euthanized and duodenal, jejunal and ileal mucosa were collected to measure GSH+GSSG, GSH and GSSG:GSH. No interactive effect on growth performance between S-ABA and gender was observed throughout the study. Additionally, increasing S-ABA did not influence growth performance. There was also no interaction between S-ABA and collection day on antioxidant parameters. Erythrocyte GSH+GSSG tended to increase with higher S-ABA in the diet (linear, $P = 0.056$) on d 14, while GSH showed a similar trend at d 14 (linear, $P = 0.096$) and d 35 (quadratic, $P = 0.100$). The d 35 GSSG:GSH ratio decreased with increasing S-ABA in the diet (quadratic, $P = 0.022$). Serum TAC, SOD and TBARS were not influenced by S-ABA in the diet. Similarly, dietary S-ABA had no effect on GSH+GSSG, GSH and GSSG:GSH in the intestinal mucosa. In conclusion, supplementation of S-ABA

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in the diet improved the antioxidant status of nursery pigs by reducing the erythrocyte GSSG:GSH ratio without negative effects on growth performance.

Introduction

Retinoids, which include retinol, are important in pig nutrition and health. Retinol (vitamin A), besides its crucial role for normal growth, reproduction and vision, possesses antioxidant properties.[5] Retinol scavenges reactive oxygen species (ROS), boosts antioxidant enzyme activities, and promotes antioxidant defense mechanisms. While mammals have the potential to synthesize vitamin A from carotenoids that are present in plant-based ingredients, its efficacy is limited.

S-ascisic acid (S-ABA) is a direct derivative of carotenoid and shares a structural similarity with retinoid, which is known for its antioxidant properties. In animals, it has been demonstrated that S-ABA plays a key role in resistance against different microbial pathogens, on inflammatory response, and as a potent antioxidant molecule. Soti et al. (2019) reported that S-ABA was able to reduce oxidative stress in rat brains as evident with reduced malondialdehyde (MDA) concentration, reduced H_2O_2 levels in rat diencephalon, and increased antioxidant enzyme catalase and peroxidase activities.[6]

To our knowledge, no study has been conducted evaluating S-ABA supplementation in swine diets. Thus, this study aimed to evaluate the effect of dietary S-ABA supplementation on the growth performance and antioxidant status of nursery pigs.

Procedures

Study facility

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The facility was completely enclosed and environmentally controlled. Pigs were placed in 4 × 4 ft pens that contained a four-hole dry self-feeder (Thorp Equipment, Thorp, WI) and a nipple drinker for *ad libitum* access to feed and water.

Animals and diets

A total of 320 pigs (241 × 600: DNA, Columbus NE) with initial body weight (BW) of 12.0 ± 1.13 lb were weaned at approximately 18 d of age. Pigs were assigned to pens in generalized randomized block design with gender and weight categories that represent first to fourth quartile blocking factors. Pigs were fed a common phase 1 diet (NE: 1,170 kcal/lb and SID Lys: 1.50%) for seven days. On d 7 post-placement (considered d 0 of the study), pens of pigs (BW: 14.2 ± 1.25 lb) within gender and weight blocks were randomly allotted to one of four dietary treatments. Each pen had five pigs and there were 16 pens per treatment (two pens per gender in each weight category). Treatments included a conventional nursery diet (Control), and three diets based on the Control formulation with increasing S-ABA (0.5, 1.0, and 5.0 ppm S-ABA). The experimental period included phase 2 (d 0 to 14) and phase 3 (d 14 to 35) periods. For each phase, dietary treatments were derived from a single corn-soybean meal-specialty

[5] Shastak, Y., A. Gordillo, and W. Pelletier. 2023. The relationship between vitamin A status and oxidative stress in animal production. J. Appl. Anim. Res. 51:546–553. doi:10.1080/09712119.2023.2239319.
[6] Soti, M., M. Abbasnejad, R. Kooshki, and S. Esmaeili-Mahani. 2019. Central microinjection of phytohormone abscisic acid changes feeding behavior, decreases body weight, and reduces brain oxidative stress in rats. Nutr. Neurosci. 22:678–687. doi:10.1080/1028415X.2018.1431093.

ingredient-based diet and three premixes that contained 0.1, 0.2, and 1.0% S-ABA were incorporated into the feed at an inclusion rate of 0.05% to provide 0.5, 1.0 and 5.0 ppm dietary S-ABA, respectively. The S-ABA premixes were provided by Sumitomo Chemical Co. (Tokyo, Japan) and were composed of S-ABA and limestone as an inert carrier. Internal analysis conducted by Sumitomo Chemical Co. showed that the premix contained 0.107, 0.196 and 1.009% S-ABA. In place of the S-ABA premix, 0.05% ground corn was added to the Control diet. Phase 2 and 3 diets were formulated to contain 1,130 and 1,100 kcal NE/lb, 1.35 and 1.30% standardized ileal digestible (SID) Lys, 0.39 and 0.35% standardized total tract digestible (STTD) P, 1,000 and 800 IU/lb vitamin A, and 7.3 and 5.0 IU/lb vitamin E, respectively. All nutrients were set to meet or exceed nutrient recommendations established by the NRC (2012) to be consistent with commercial swine production practices (Table 1).

Basal and experimental diets were manufactured in meal form at O. H. Kruse Feed Technology Innovation Center at Kansas State University. Representative samples for each diet were collected and analyzed in triplicate for S-ABA concentration (ABA LC-MS/MS method) at Valent BioSciences, Libertyville, IL.

Measurements, sampling and analytical methods

Throughout the 7-d acclimatization and 35-d experimental periods, pig and feeder weights were measured every six to seven days to determine ADG, ADFI, and F/G.

To evaluate the antioxidant status of pigs in this study, the following key parameters were measured: GSH+GSSG, GSH, and the GSSG:GSH ratio in erythrocytes, along with serum TAC, SOD, and TBARS. On day 0, blood samples were collected from 32 median-weight pigs, with one pig of each gender per weight category in each treatment group. On d 14, blood samples were taken from two median-weight pigs per pen, and the same pigs were bled again at the end of the study (half on d 34 and another half on d 35).

Pigs bled at the termination of the study were also euthanized for the collection of intestinal mucosae from the duodenum, jejunum, and ileum for GSH+GSSG and GSH analysis and GSSG:GSH ratio calculation.

Statistical analysis

Statistical analyses were performed using SAS v.9.4 (SAS Institute, Inc., Cary, NC). Pen was used as the experimental unit. Additionally, pig was used as the observational unit for criteria where multiple pigs within a pen were sampled. For growth performance and intestinal mucosa glutathione criteria, S-ABA, gender, and its interaction were used as fixed effects and weight category (and pen for intestinal mucosa) was used as a random intercept. For erythrocyte glutathione parameters and serum TAC, SOD and TBARS, a constrained longitudinal model was fit following procedures described by Coffman et al., (2016)[7] where the statistical model included the S-ABA × day interaction, day, and gender as fixed effects. Weight category and pen were included as random intercepts, and repeated measures were modeled using an unstructured covariance matrix structure with the individual pig as the subject. Interaction of gender with other fixed effects was initially modelled and found not significant ($P > 0.10$), therefore

[7] Coffman, C. J., D. Edelman, and R. F. Woolson. 2016. To condition or not condition? Analysing 'change' in longitudinal randomised controlled trials. BMJ Open. 6:e013096. doi:10.1136/bmjopen-2016-013096.

removed from the model. Means were estimated using LSMEANS function with slice option to evaluate differences within each day of collection.

For all criteria measured, linear and quadratic contrasts were evaluated to determine the response to increasing concentration of S-ABA. Probability values less than 0.05 were considered statistically significant, and values between 0.05 and 0.10 were considered tendencies.

Results and Discussion

No interactive effect between S-ABA and gender on growth performance was observed throughout the study (Table 2). Additionally, no main effect of S-ABA was detected at any time point for growth performance.

For erythrocyte GSH+GSSG, GSH and GSSG:GSH, and serum TAC, SOD and TBARS, no interactive effect of S-ABA and day of collection was observed (Table 3). Similarly, serum TAC, SOD, and TBARS were not influenced by the level of S-ABA in the diet. Total antioxidant capacity measures the cumulative action of all the antioxidants present in plasma and body fluids and provides information on the balance between oxidants and antioxidants, thus the redox status.[8] Superoxide dismutase is a metalloenzyme that catalyzes the dismutation of the superoxide anion to molecular oxygen and peroxide and thus is an important part of the cellular antioxidant defense mechanism.[9] Malondialdehyde (MDA) is a lipid peroxidation product that reacts with thiobarbituric acid to form TBARS. Therefore, TBARS is a good indicator of the level of oxidative stress within biological samples.[10] On the other hand, GSH+GSSG tended to increase with increasing S-ABA in the diet (linear, $P = 0.056$) at d 14 of the trial but not on d 35. Reduced glutathione tended to increase with increasing S-ABA in the diet at d 14 (linear, $P = 0.096$) and d 35 (quadratic, $P = 0.100$). Glutathione is the main antioxidant that detoxifies electrophiles and alleviates oxidative stress caused by ROS. The GSSG:GSH ratio, which reflects the redox status of the cells, decreased with increasing S-ABA in the diet at d 35 (quadratic, $P = 0.022$). Additionally, erythrocyte GSH+GSSG and GSH were lower on d 35 of collection, while erythrocyte GSSG:GSH and serum TAC and SOD were higher at d 35 compared to d 14 of collection ($P < 0.001$).

Except for the tendency of reduced GSH in the duodenal mucosa with increasing S-ABA in the diet ($P = 0.054$), the levels of GSH+GSSG, GSH and the GSSG:GSH in the duodenal, jejunal and ileal mucosa were not influenced by the level of S-ABA in the diet (Table 4). These criteria were also not influenced by gender. It is important to note that consistent with the study of Huo et al. (2023), the level of glutathione was higher in duodenum than in ileum.[11]

[8] Ghiselli, A., M. Serafini, F. Natella, and C. Scaccini. 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic. Biol. Med. 29:1106–1114. doi:10.1016/S0891-5849(00)00394-4.

[9] Le, H. H., W. Zhao, J. B. Furness, M. Shakeri, K. DiGiacomo, E. Roura, D. Renaudeau, N. K. Gabler, B. J. Leury, F. R. Dunshea, G. Wijffels, and J. J. Cottrell. 2023. Using recombinant superoxide dismutase to control oxidative stress in the gastrointestinal tract of cyclic heat-stressed pigs. Animals. 13. doi:10.3390/ani13162681.

[10] Aguilar Diaz De Leon, J., and C. R. Borges. 2020. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. JoVE. 61122. doi:10.3791/61122.

[11] Hou, Y., J. Michiels, C. V. Kerschaver, M. Vandaele, M. Majdeddin, E. Vossen, and J. Degroote. 2023. The kinetics of glutathione in the gastrointestinal tract of weaned piglets supplemented with different doses of dietary reduced glutathione. Front. Vet. Sci. 10:1220213. doi:10.3389/fvets.2023.1220213.

In conclusion, supplementation of S-ABA in the diet improved the antioxidant status of nursery pigs by reducing the erythrocyte GSSG:GSH ratio without negative effects on growth performance.

Acknowledgments

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Table 1. Composition, calculated analysis, and analyzed composition of experimental diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient			
Corn	44.90	55.70	66.50
Soybean meal, 47.7% CP	21.30	25.80	29.70
Blood plasma	2.50	--	--
Specialty soy product[2]	6.00	3.75	--
Whey powder	20.00	10.0	--
Soybean oil	2.00	1.00	--
Calcium carbonate	0.60	0.60	0.80
Monocalcium phosphate, 21% P	0.60	0.90	1.00
Salt	0.30	0.60	0.60
L-Lys-HCl	0.45	0.47	0.50
DL-Met	0.28	0.23	0.16
L-Thr	0.17	0.18	0.20
L-Trp	0.03	0.05	0.04
L-Val	0.13	0.12	0.13
Trace mineral premix	0.15	0.15	0.15
Zinc oxide	0.40	0.26	--
Vitamin premix	0.125	0.125	0.125
Vitamin A (454,000,000 IU/lb)	0.00004	0.00004	--
Vitamin E (227,000 IU/lb)	0.00101	0.00101	--
Phytase[3]	0.08	0.08	0.08
S-ABA[4,5]	--	+/-	+/-
			continued
Calculated analysis			
SID AA, %			

Table 1. Composition, calculated analysis, and analyzed composition of experimental diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Lys	1.50	1.35	1.30
Ile:Lys	56	57	55
Leu:Lys	111	114	116
Met:Lys	38	37	33
Met+Cys:Lys	60	58	54
Thr:Lys	62	62	62
Trp:Lys	19	20	19
Val:Lys	70	70	70
SID Lys:NE, g/Mcal	5.83	5.41	5.34
NE, kcal/lb	1,168	1,132	1,103
STTD P, %	0.40	0.39	0.35
Vit. A, IU/lb	1,000	1,000	800
Vit. E, IU/lb	7.3	7.3	5.0
S-ABA, ppm	--	0 to 5.0	0 to 5.0
Chemical analysis			
Total Ca, %	--	0.74	0.68
Total P, %	--	0.62	0.53
S-ABA, ppm[6]			
Control	--	--	--
Control + 0.5 ppm S-ABA	--	0.66	0.64
Control + 1.0 ppm S-ABA	--	1.21	1.14
Control + 5.0 ppm S-ABA	--	5.65	5.36

[1]Phase 1 was fed from d 0 to 7 post-weaning. Phases 2 and 3 were fed from d 7 to 21 and d 21 to 42 post-weaning, respectively (or d 0 to 14 and d 14 to 35 of experimental period).
[2]HP 300, Hamlet Protein, Findlay, OH.
[3]Ronozyme HiPhos 2,700, DSM Nutritional Products Canada Inc., Ontario, Canada.
[4]Sumitomo Chemical Co., Tokyo, Japan. Three premixes containing 0.1, 0.2 and 1.0% S-ABA were added to the basal diet at 0.05% resulting in dietary S-ABA of 0.5, 1.0 and 5.0 ppm.
[5]Internal analysis of Sumitomo Chemical Co. showed that the premixes contained 0.107, 0.196 and 1.009% S-ABA.
[6]S-ABA levels were calculated by subtracting the S-ABA level of the control diet with the detected S-ABA of the fortified diets.

Table 2. Effect of S-ABA on growth performance of nursery pigs[1,2]

	S-ABA, ppm					*P*[3]-value	
	0	0.5	1.0	5.0	SEM	Linear	Quadratic
Body weight, lb							
d 0	14.2	14.3	14.2	14.2	1.24	0.918	0.936
d 14	25.7	26.6	25.6	26.3	1.90	0.467	0.761
d 35	53.2	54.1	52.9	54.3	2.97	0.393	0.680
Day 0 to 35							
ADG, lb	1.13	1.15	1.12	1.15	0.054	0.478	0.690
ADFI, lb	1.63	1.66	1.60	1.66	0.079	0.530	0.396
F/G	1.44	1.44	1.43	1.44	0.013	0.941	0.302

[1]A total of 320 pigs were used in 7-d acclimatization and 35-d experimental feeding trial, with five pigs per pen and 16 replicate pens per treatment (two pens per gender in each weight category). Experimental diets were provided 7 d post weaning, which was considered d 0 of the study.

[2]ADG = average daily gain, ADFI = average daily feed intake, F/G = feed to gain ratio.

[3]For the analysis, pen was considered the experimental unit, with diet (S-ABA), gender and S-ABA × gender interaction as fixed effects and weight group as a random effect. No interactive effect of diet and gender was observed ($P > 0.05$).

Table 3. Effect of S-ABA on nursery pigs' erythrocyte GSH+GSSG, GSH and GSSG:GSH and serum TAC, SOD and TBARS[1,2]

	S-ABA, ppm					*P*-value[3,4]		
							Diet	
	0	0.5	1.0	5.0	SEM	Day	Linear	Quadratic
Erythrocytes								
GSH+GSSG, nmol/mL								
Baseline	54.36				--	<0.001	--	--
Day 14	72.25	71.35	76.91	79.35	4.060		0.056	0.455
Day 35	41.26	44.26	47.51	49.22	4.129		0.348	0.440
GSH, nmol/mL								
Baseline	39.39				--	<0.001	--	--
Day 14	61.12	59.62	63.58	65.90	3.297		0.096	0.720
Day 35	32.11	36.06	40.74	40.84	3.911		0.130	0.100
GSSG:GSH								
Baseline	0.187				--	<0.001	--	--
Day 14	0.101	0.103	0.107	0.109	0.0154		0.618	0.787
Day 35	0.274	0.191	0.130	0.166			0.298	0.022
Serum								
TAC, mmol - CRE								
Baseline	565.8				--	<0.001	--	--
Day 14	545.8	568.3	558.2	577.1	32.56		0.312	0.725
Day 35	627.6	636.1	611.9	645.9	32.37		0.380	0.454
SOD, U/mL								
Baseline	2.49				--	<0.001	--	--
Day 14	3.91	3.66	3.55	3.88	0.317		0.669	0.243
Day 35	4.58	4.26	4.17	4.31	0.345		0.805	0.294
TBARS, μmol MDA								
Baseline	7.55				--	0.180	--	--
Day 14	7.48	7.85	7.52	7.47	0.337		0.730	0.828
Day 35	7.01	6.94	7.25	7.39	0.331		0.354	0.748

[1]Blood samples were collected from 32-median-weight pigs at d 0 of the experiment to represent baseline levels for the different antioxidant parameters. The 32 pigs were balanced across four treatments within each gender × weight category blocks. On d 14, blood samples were taken from two median-weight pigs per pen, and the same pigs were bled again at the end of the study, resulting in 32 observations per treatment.

[2]GSH+GSSG = total glutathione, GSH = reduced glutathione, GSSG = oxidized glutathione, TAC = total antioxidant capacity, expressed as mmol Cu-reducing equivalent, SOD = superoxide dismutase, expressed as enzyme activity (U) per mL, and TBARS = thiobarbituric reactive substance, expressed as μmol malondialdehyde.

[3]A constrained longitudinal model was fitted with diet (S-ABA) × collection day, day of collection and gender as fixed effects, and weight category and pen as random effects. Repeated measures were modelled using an unstructured covariance matrix structure with individual pig as subject.

[4]No interactive effect of gender with other fixed effects were observed ($P > 0.05$).

Table 4. Effect of S-ABA on duodenal, jejunal and ileal GSH+GSSG, GSH, and GSSG:GSH of nursery pigs[1,2]

	S-ABA, ppm					P^3-value	
	0	0.5	1.0	5.0	SEM	Linear	Quadratic
Duodenum							
GSH+GSSG, µmol/g	1.30	1.24	1.21	1.25	0.055	0.705	0.119
GSH, µmol/g	1.10	1.03	0.99	1.02	0.044	0.452	0.054
GSSG:GSH	0.09	0.11	0.12	0.11	0.010	0.412	0.140
Jejunum							
GSH+GSSG, µmol/g	0.98	1.06	1.00	1.08	0.067	0.269	0.868
GSH, µmol/g	0.85	0.91	0.85	0.94	0.067	0.242	0.945
GSSG:GSH	0.08	0.08	0.10	0.08	0.010	0.839	0.389
Ileum							
GSH+GSSG, µmol/g	0.65	0.63	0.66	0.63	0.054	0.705	0.879
GSH, µmol/g	0.59	0.57	0.58	0.55	0.048	0.476	0.867
GSSG:GSH	0.06	0.06	0.08	0.08	0.020	0.210	0.543

[1]Two median-weight pigs in each pen (same pigs that were bled) were euthanized for the collection of intestinal mucosae from duodenum, jejunum and ileum.

[2]Levels of GSH+GSSG, GSH and GSSG:GSH are different among sections of intestine ($P < 0.001$).

[3]For the analysis, pen was considered as experimental unit and pig as observational unit, with diet (S-ABA), gender, and S-ABA × gender interactions as fixed effect and weight group and pen as random effects. No interactive effect of diet and gender and main effect of gender was observed ($P > 0.05$).