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Ethan B. Stas, Mike D. Tokach, Jason C. Woodworth, Joel M. DeRouchey, Robert D. Goodband, and Jordan T. Gebhardt[1]

Summary

A total of 360 pigs (200 × 400 DNA; initially 12.9 lb) were used to evaluate the impact of increasing the acid-binding capacity-4 (ABC-4) of the diet on nursery pig performance and fecal dry matter (DM). At weaning, pigs were allotted to 1 of 6 dietary treatments. There were 5 pigs per pen and 12 replications per treatment. Pigs were fed experimental diets in two phases with phase 1 being from d 0 to 10 post-weaning followed by phase 2 from d 10 to 23. Diets were formulated with increasing ABC-4 levels ranging from 150 meq/kg (diet 1, low ABC-4) to 312 meq/kg (diet 5, high ABC-4) in phase 1 and 200 meq/kg (diet 1, low ABC-4) to 343 meq/kg (diet 5, high ABC-4) in phase 2. For diet 1, the low ABC-4 diets were formulated using specialty soy protein concentrate (AX3 Digest; Protekta; Newport Beach, CA) at 12.50 and 10.00% of the diet in phase 1 and 2, respectively. The low ABC-4 diet also utilized fumaric acid and formic acid at 0.50 and 0.48% of the diet, respectively for both phase 1 and 2. For diets 2 (medium low), 3 (medium), 4 (medium high), and 5 (high), increasing ABC-4 of the diet was achieved by progressively decreasing the level of acidifiers and replacing specialty soy protein concentrate with enzymatically treated soybean meal (HP 300; Hamlet Protein; Findlay, OH) on an SID Lys basis. Diets 1 through 5 were formulated without the inclusion of ZnO. For diet 6, a positive control diet was utilized which had the same formulation as the highest ABC-4 diet but with the addition of pharmacological levels of Zn from ZnO. Following phase 2, all pigs were placed on a common diet until d 38 of the study. In the experimental period (d 0 to 23) and overall (d 0 to 38), a quadratic response was observed ($P \leq 0.030$) where BW and ADG were highest for pigs fed the medium low and medium ABC-4 diets. During the experimental period (d 0 to 23), pigs fed increasing ABC-4 levels had poorer (linear, $P = 0.002$) F/G. For overall F/G, a quadratic response was observed ($P = 0.023$) where F/G was most improved for pigs fed the medium low and medium ABC-4 levels. Pigs fed diets with ZnO had increased ($P \leq 0.038$) ADG compared to pigs fed diets without ZnO during the experimental period and overall. In summary, pharmacological levels of Zn improved nursery pig performance as expected. The medium low and medium ABC-4 levels improved performance compared to higher ABC-4 levels, suggesting an optimal ABC-4 level of the diet for this study would be at or below 256 and 295 meq/kg in phase 1 and 2, respectively.

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Introduction

Weanling pigs experience a dramatic change from a liquid diet in the form of sow's milk to a dry cereal-based diet. After weaning, pigs have a limited capacity to produce enough hydrochloric acid in the stomach to maintain an acidic environment.[2] Certain ingredients in the diet bind more acid than others which can further increase gastric pH. High gastric pH is associated with decreased nutrient utilization and increased undigested protein in the large intestine promoting post-weaning diarrhea. As a result, elevated stomach pH can lead to decreased performance, morbidity, and mortality.[3] The concept of acid-binding capacity-4 (ABC-4) involves incorporating low acid-binding ingredients in feed to help maintain an acidic stomach pH for the young pig and improve performance.[4] Acid-binding capacity-4 is measured as the amount of acid in milliequivalents (meq) required to lower 1 kg of an ingredient or diet to a pH of 4. Selection of ingredients with a low ABC-4 could assist newly weaned pigs in maintaining an acidic gastric environment.

Previous studies have shown a benefit to low ABC-4 diets on nursery pig performance compared to a high ABC-4 diet.[5] However, to our knowledge, there are no studies evaluating the optimal ABC-4 level in the diet for a specific weight range, or at what point nursery pig performance starts to diminish as ABC-4 changes in the diet. Therefore, the objective of this study was to determine the influence of increasing ABC-4 levels of the diet on nursery pig performance and fecal dry matter.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. Each pen was equipped with a 4-hole, dry self-feeder and nipple waterer to provide *ad libitum* access to feed and water.

Animals and diets

A total of 360 pigs (200 × 400 DNA; initially 12.9 lb) were used in a 38-d nursery trial across two barns. Pigs were weaned at approximately 21 d of age and placed in pens of 5 pigs each based on initial weight. At weaning, pigs were randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment. Pigs were fed experimental diets in two phases with phase 1 from d 0 to 10 postweaning, followed by phase 2 from d 10 to 23. Phase 1 and 2 diets were formulated to contain 1.36 and 1.35% SID Lys, respectively, and met or exceeded other nutrient requirement estimates established by

[2] Pluske, J. R. 2016. Invited review: Aspects of gastrointestinal tract growth and maturation in the pre- and postweaning period of pigs. J. Anim. Sci. 94:399-411. doi:10.2527/jas2015-9767.

[3] Bolduan, G., H. Jung, R. Schneider, J. Block, and B. Klenke. 1988. Influence of fumaric-acid and propandiol-formiat on piglets. J. Anim. Physiol. Anim. Nutr. 59, 72-78. doi:10.1111/j.1439-0396.1988.tb00057.x

[4] Lawlor, P. G., P. B. Lynch, P. J. Caffrey, J. J. O'Reilly, and M. K. O'Connell. 2005. Measurements of the acid-binding capacity of ingredients used in pig diets. Ir. Vet. J. 58, 447-452. doi:10.1186/2046-0481-58-8-447.

[5] Stas, E. B., A. J. Warner, C. W. Hastad, M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and J. T. Gebhardt. 2022. Effects of varying the acid-binding capacity-4 in diets utilizing specialty soy products with or without pharmacological levels of zinc on nursery pig performance," Kansas Agri. Exp. Station Research Reports: Vol. 8: Iss. 10. doi:10.4148/2378-5977.8370

the NRC.[6] Following the feeding of phase 2 diets, all pigs were placed on a common corn-soybean meal-based diet until d 38 of the trial.

The six dietary treatments consisted of increasing levels of ABC-4 (Table 1). Diet 1, the low ABC-4 diet, was formulated to a value of 150 and 200 meq/kg in phase 1 and 2, respectively. To achieve the low ABC-4 diet, specialty soy protein concentrate (AX3 Digest; Protekta; Newport Beach, CA) with an individual ingredient ABC-4 of -13 meq/kg was included at 12.5 and 10.0% of the diet in phase 1 and 2, respectively with fumaric acid and formic acid also included at 0.50 and 0.48% of the diet, respectively for both phase 1 and phase 2 diets. Fumaric acid and formic acid had individual ABC-4 values of -10,873 and -8,287 meq/kg, respectively. For diets 2 (medium low), 3 (medium), 4 (medium high), and 5 (high), increasing the ABC-4 level of the diets was achieved by progressively replacing specialty soy protein concentrate with enzymatically treated soybean meal (753 meq/kg; HP 300; Hamlet Protein; Findlay, OH) on an SID Lys basis and decreasing the levels of fumaric acid and formic acid proportionally for each diet. Diets 1 through 5 contained 110 ppm of Zn provided by the trace mineral premix and did not contain any added ZnO. Diet 6 was considered a positive control diet and utilized the same formulation as the high ABC-4 diet except with the inclusion of 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2, respectively. The addition of ZnO increased the ABC-4 of the diet by 87 and 55 meq/kg for phase 1 and 2, respectively. The ABC-4 of the diet was increased by approximately 50 meq/kg as diets increased from the lowest to the highest ABC-4 levels for each diet in both phase 1 and 2. Individual pig weights and feed disappearance were measured on d 10, 17, 23, 31, and 38 to determine ADG, ADFI, and F/G.

Fecal samples were collected on d 10, 17, and 23 to determine fecal dry matter percentage from the same three medium weight pigs from each pen. After collection, fecal samples were dried at 131°F (55°C) in a forced air oven and the ratio of dried to wet fecal weight determined the fecal percentage dry matter.

Statistical analysis

Data were analyzed as a completely randomized design using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) using R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen as the experimental unit. Barn was included in the model as a random effect. Linear and quadratic effects of diet ABC-4 were tested using the lmer function. The effect of ZnO was tested by a pairwise comparison of the high ABC-4 diets with and without ZnO. Fecal DM samples were analyzed using the fixed effects of day, treatment, and the associated interaction, accounting for repeated measures over time. Differences between treatments and day (where appropriate) as well as their interaction were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

All analyzed ABC-4 diet values were less than calculated values across both phases. Analyzed ABC-4 increased with decreasing acidifiers and the replacement of specialty

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

soy protein concentrate with enzymatically treated soybean meal (Table 1). Analyzed values were on average 35 and 63 meq/kg lower than calculated values for phase 1 and 2, respectively. A previous study by Stas et al.[7] also reported lower analyzed ABC-4 diet values compared to calculated ABC-4 dietary values. It is possible that ingredients (such as limestone, ZnO, and premixes) with a very high ingredient ABC-4 value, with a low addition to the diet could be contributing to the discrepancy. Another possible explanation for the discrepancy between analyzed and calculated ABC-4 values is that additivity may not be linear, and rather interactions between different ingredients may influence the analyzed ABC-4 values of complete diets.

For increasing ABC-4 levels, during phase 1 (d 0 to 10) there were no differences in growth performance (Table 3). In phase 2 (d 10 to 23), a quadratic response was observed ($P \leq 0.045$) where ADG and ADFI were highest for pigs fed the medium low and medium ABC-4 diets. Pigs fed increasing ABC-4 had poorer (linear, $P = 0.001$) F/G. For the entire experimental period (d 0 to 23), a quadratic response was observed ($P = 0.030$) where BW and ADG were highest for pigs fed the medium low and medium ABC-4 diets. Pigs fed increasing ABC-4 had poorer (linear, $P = 0.002$) F/G. In the common period (d 23 to 38), a quadratic response was observed ($P \leq 0.043$) where ADG and F/G were most improved for pigs previously fed the medium low and medium ABC-4 diets. Overall (d 0 to 38), a quadratic response was observed ($P \leq 0.046$) where BW, ADG, ADFI, and F/G were most improved for pigs fed the medium low and medium ABC-4 diets. For fecal DM, there were no significant treatment × day interactions ($P > 0.05$). On d 10 and 17, fecal DM percentage decreased (linear, $P \leq 0.036$) as dietary ABC-4 increased. A day effect was observed ($P < 0.001$) where fecal DM progressively increased from d 10 to 23.

During phase 1 (d 0 to 10), phase 2 (d 10 to 23), and the experimental period (d 0 to 23), pigs fed diets with ZnO had increased ($P \leq 0.034$) BW, ADG, and ADFI compared to pigs fed diets without ZnO. In the common period (d 23 to 38), there were no differences between pigs previously fed diets with or without ZnO ($P > 0.10$). Overall (d 0 to 38), pigs fed diets with ZnO had increased ($P = 0.038$) ADG compared to pigs fed diets without ZnO. In the experimental period (d 0 to 23) and overall (d 0 to 38), pigs fed diets with ZnO tended to have improved ($P \leq 0.078$) F/G compared to pigs fed diets without ZnO. For fecal DM, there were no differences between pigs fed diets with or without ZnO for the duration of the experimental period (d 0 to 23, $P > 0.10$).

In conclusion, pharmacological levels of Zn from ZnO improved nursery pig performance as expected. The medium low and medium ABC-4 diets improved performance compared to the low, medium high and high ABC-4 diets. Decreased performance in the low ABC-4 level may be due to decreased palatability from the level of acidifiers as ADFI was lowest for this diet. This study also suggests that formulating diets to an ABC-4 level of 203 to 256 meq/kg in phase 1 and 247 to 296 meq/kg in phase 2 can result in numerically similar performance to pigs fed high ABC-4 diets containing pharmacological levels of Zn from ZnO.

[7] Stas, E. B., M. D. Tokach, J. M. DeRouchey, R. D. Goodband, J. C. Woodworth, J. T. Gebhardt. 2022. Evaluation of the acid-binding capacity of ingredients and complete diets commonly used for weanling pigs. Trans. Anim. Sci. doi:10.1093/tas/txac104.

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Table 1. Phase 1 diet composition (as-fed basis)[1]

	ABC-4					
ABC-4, meq/kg:	**Low**	**Medium low**	**Medium**	**Medium high**	**High**	**High[2]**
ZnO:	-	-	-	-	-	+
Ingredients, %						
Corn	51.71	51.30	50.88	50.36	49.91	49.52
Soybean meal	11.95	11.95	11.95	11.95	11.95	11.95
Crystalline lactose	15.00	15.00	15.00	15.00	15.00	15.00
Specialty soy protein concentrate[3]	12.50	9.38	6.25	3.13	---	---
Enzymatically treated soybean meal[4]	---	3.80	7.55	11.40	15.20	15.20
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50	2.50
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.35	0.35	0.36	0.37	0.38	0.38
Monocalcium phosphate	1.00	0.95	0.90	0.88	0.83	0.83
Salt	0.65	0.68	0.73	0.75	0.80	0.80
L-Lys	0.44	0.44	0.44	0.44	0.44	0.44
DL-Met	0.18	0.19	0.19	0.19	0.20	0.20
L-Thr	0.22	0.22	0.23	0.23	0.24	0.24
L-Trp	0.08	0.08	0.07	0.08	0.08	0.08
L-Val	0.06	0.07	0.07	0.08	0.08	0.08
Fumaric acid	0.50	0.38	0.25	0.13	---	---
Formic acid	0.48	0.36	0.24	0.12	---	---
ZnO	---	---	---	---	---	0.40
Vitamin premix with phytase[5]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100

Table 1. Phase 1 diet composition (as-fed basis)[1]

	ABC-4					
ABC-4, meq/kg:	**Low**	**Medium low**	**Medium**	**Medium high**	**High**	**High[2]**
ZnO:	-	-	-	-	-	+
						continued
Calculated analysis						
SID amino acids, %						
Lys	1.36	1.36	1.36	1.36	1.36	1.36
Ile:Lys	57	57	57	57	57	57
Leu:Lys	118	116	115	114	113	113
Met:Lys	34	34	34	34	34	34
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	66	66	66	66	66	66
Trp:Lys	22.2	22.2	22.3	22.4	22.4	22.4
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
Total Lys, %	1.51	1.52	1.52	1.52	1.52	1.52
NE, kcal/lb	1,182	1,184	1,184	1,186	1,187	1,182
SID Lys:NE, g/Mcal	5.22	5.21	5.20	5.20	5.20	5.22
CP, %[6]	21.2	21.2	21.1	21.1	21.1	21.1
Ca, %	0.50	0.50	0.50	0.51	0.51	0.51
P, %	0.52	0.52	0.53	0.53	0.54	0.54
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Calculated ABC-4, meq/kg	150	203	256	311	365	452
Analyzed ABC-4, meq/kg	113	173	233	280	333	393

[1] Phase 1 diets were fed from d 0 to 10 post-weaning.
[2] Diet contained 3,000 ppm of Zn from ZnO.
[3] AX3 Digest; Protekta; Newport Beach, CA.
[4] HP 300; Hamlet Protein; Findlay, OH.
[5] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 567 FYT/lb.
[6] CP = crude protein.

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

	ABC-4					
ABC-4, meq/kg:	**Low**	**Medium low**	**Medium**	**Medium high**	**High**	**High[2]**
ZnO:	-	-	-	-	-	+
Ingredients, %						
Corn	58.76	58.50	58.16	57.89	57.59	57.32
Soybean meal	18.00	18.00	18.00	18.00	18.00	18.00
Crystalline lactose	7.50	7.50	7.50	7.50	7.50	7.50
Specialty soy protein concentrate[3]	10.00	7.50	5.00	2.50	---	---
Enzymatically treated soybean meal[4]	---	3.00	6.05	9.05	12.10	12.10
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.51	0.51	0.52	0.53	0.53	0.53
Monocalcium phosphate	1.05	1.00	0.98	0.95	0.93	0.93
Salt	0.68	0.73	0.75	0.78	0.80	0.80
L-Lys	0.52	0.52	0.52	0.52	0.52	0.52
DL-Met	0.20	0.20	0.20	0.21	0.21	0.21
L-Thr	0.22	0.22	0.22	0.22	0.23	0.23
L-Trp	0.09	0.09	0.09	0.09	0.09	0.09
L-Val	0.12	0.12	0.13	0.13	0.13	0.13
Fumaric acid	0.50	0.38	0.25	0.13	---	---
Formic acid	0.48	0.36	0.24	0.12	---	---
ZnO	---	---	---	---	---	0.25
Vitamin premix with phytase[5]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100

continued

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

	ABC-4					
ABC-4, meq/kg:	Low	Medium low	Medium	Medium high	High	High[2]
ZnO:	-	-	-	-	-	+
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	57	57	57	57	57
Leu:Lys	116	115	114	113	112	112
Met:Lys	36	36	36	36	36	36
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	63	63	63	63	63	63
Trp:Lys	22.5	22.5	22.2	22.3	22.3	22.3
Val:Lys	71	71	71	71	71	71
His:Lys	36	36	36	36	36	36
Total Lys, %	1.50	1.50	1.50	1.50	1.50	1.50
NE, kcal/lb	1,139	1,141	1,142	1,144	1,145	1,142
SID Lys:NE, g/Mcal	5.38	5.37	5.36	5.35	5.35	5.36
CP, %[6]	21.1	21.1	21.1	21.1	21.0	21.0
Ca, %	0.59	0.58	0.59	0.59	0.60	0.60
P, %	0.56	0.55	0.56	0.56	0.57	0.57
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Calculated ABC-4, meq/kg	200	247	296	343	390	445
Analyzed ABC-4, meq/kg	133	173	233	280	333	393

[1] Phase 2 diets were fed from d 10 to 23 post-weaning.
[2] Diet contained 2,000 ppm of Zn from ZnO.
[3] AX3 Digest; Protekta; Newport Beach, CA.
[4] HP 300; Hamlet Protein; Findlay, OH.
[5] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 567 FYT/lb.
[6] CP = crude protein.

Table 3. Effects of increasing dietary acid-binding capacity-4 (ABC-4) on nursery pig performance and fecal dry matter (DM)[1]

	ABC-4[2]							*P* =		
ABC-4, meq/kg[3,4]	Low	Medium low	Medium	Medium high	High	High[5]		ABC-4[6]		
ZnO:	-	-	-	-	-	+	SEM	Linear	Quadratic	ZnO[7]
BW, lb										
d 0	12.9	12.9	12.9	13.0	12.9	12.9	0.36	0.495	0.945	0.604
d 10	15.1	15.7	15.5	15.4	15.5	16.1	0.44	0.490	0.198	0.011
d 23	26.3	27.1	27.4	26.1	25.9	27.5	0.55	0.105	0.014	0.004
d 38	46.8	48.7	48.8	47.1	46.6	48.0	1.00	0.347	0.007	0.136
Phase 1 (d 0 to 10)										
ADG, lb	0.22	0.27	0.26	0.25	0.25	0.32	0.018	0.396	0.163	0.009
ADFI, lb	0.24	0.27	0.28	0.27	0.27	0.31	0.013	0.195	0.156	0.034
F/G	1.13	1.02	1.13	1.13	1.10	1.01	0.061	0.812	0.778	0.228
Phase 2 (d 10 to 23)										
ADG, lb	0.86	0.89	0.90	0.82	0.80	0.88	0.022	0.013	0.045	0.027
ADFI, lb	1.12	1.17	1.18	1.12	1.10	1.19	0.027	0.415	0.037	0.027
F/G	1.30	1.32	1.32	1.38	1.37	1.36	0.020	0.001	0.944	0.722
Experimental period (d 0 to 23)										
ADG, lb	0.58	0.61	0.61	0.57	0.56	0.63	0.015	0.144	0.030	0.002
ADFI, lb	0.73	0.77	0.78	0.75	0.74	0.81	0.017	0.921	0.299	0.009
F/G	1.27	1.26	1.27	1.32	1.32	1.28	0.015	0.002	0.462	0.063
Common period (d 23 to 38)										
ADG, lb	1.36	1.44	1.43	1.41	1.36	1.36	0.032	0.588	0.012	0.807
ADFI, lb	1.89	1.96	1.93	1.95	1.90	1.90	0.039	0.877	0.110	0.969
F/G	1.38	1.37	1.36	1.39	1.40	1.40	0.014	0.178	0.043	0.678
Overall (d 0 to 38)										
ADG, lb	0.89	0.94	0.93	0.90	0.88	0.92	0.018	0.253	0.004	0.038
ADFI, lb	1.18	1.24	1.23	1.22	1.20	1.24	0.022	0.884	0.046	0.157
F/G	1.34	1.32	1.32	1.36	1.37	1.34	0.010	0.003	0.023	0.078
Fecal DM, %[8]										
d 10	22.6	21.8	19.7	19.2	19.5	21.1	1.17	0.003	0.282	0.190
d 17	24.5	25.2	25.4	23.3	22.2	22.2		0.036	0.107	0.964
d 23	26.5	26.9	26.5	25.5	26.8	25.5		0.778	0.802	0.333

[1] A total of 360 pigs (DNA 200 × 400; initial BW of 12.9 ± 0.36 lb) across two barns were used in a 38-d nursery trial.
[2] Increasing the ABC-4 level of the diet was done by replacing specialty soy protein concentrate (-13 meq/kg; AX3 Digest; Protekta; Newport Beach, CA) with enzymatically treated soybean meal (753 meq/kg; HP 300; Hamlet Protein; Findlay, OH) and decreasing the levels of organic acids (fumaric acid and formic acid).
[3] Phase 1 diets fed from d 0 to 10 of the trial were formulated with increasing ABC-4 levels from 150, 203, 256, 311, and 365 meq/kg for the low, medium low, medium, medium high, and high diets, respectively.
[4] Phase 2 diets fed from d 10 to 23 of the trial were formulated with increasing ABC-4 levels from 200, 247, 296, 343, and 390 meq/kg for the low, medium low, medium, medium high, and high diets, respectively.
[5] Formulated the same as the high ABC-4 diet with the addition of 3,000 and 2,000 ppm of Zn from ZnO in phase 1 and 2, respectively. The addition of ZnO increased the ABC-4 of the diet by 87 and 55 meq/kg for phase 1 and 2, respectively.
[6] Excludes diet containing ZnO.
[7] Compares high ABC-4 diets while excluding all other treatments.
[8] Linear treatment × day, $P = 0.103$; Quadratic treatment × day, $P = 0.098$; Linear treatment, $P = 0.011$; Quadratic treatment, $P = 0.888$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day.