

Effects of Dietary Acidifiers and Low Acid-Binding Capacity-4 (ABC-4) Formulation Strategies on Nursery Pig Performance and Fecal Dry Matter

*Ethan B. Stas, Mike D. Tokach, Joel M. DeRouchey,
Jason C. Woodworth, Robert D. Goodband, and Jordan T. Gebhardt¹*

Summary

A total of 725 pigs (DNA 241 × 600, initially 13.0 lb) were used to determine the effect of dietary acidifiers and other low acid-binding capacity at a pH of 4 (ABC-4) formulation strategies on nursery pig performance and fecal dry matter (DM). At weaning, pigs were randomly allotted to one of five dietary treatments. There were five pigs per pen and 29 replications per treatment across two facilities. Pigs were fed experimental diets in two phases with phase 1 provided with a feed budget of 5 lb/pig followed by phase 2 diets fed until d 24 post-weaning. The first three diets were formulated to approximately 200 and 250 meq/kg in phases 1 and 2, respectively, by using three different formulation strategies. The three formulation strategies included: 1) lowering the Ca:P ratio by 0.20, 2) addition of 0.6% formic acid (Amasil NA; BASF; Florham Park, NJ), and 3) replacing whey permeate (Dairylac 80; International Ingredients Corporation; St. Charles, MO) with crystalline lactose. Fumaric acid (Primary Products Ingredients Americas LLC, Decatur, IL) was included at 0.46% for all low ABC-4 diets across both phases. Diet 4 was a high ABC-4 diet formulated to be 100 meq/kg greater than the low ABC-4 diets. The first four dietary treatments contained 110 ppm of Zn provided by the trace mineral premix. Diet 5 was diet 4 with the addition of 3,000 and 2,000 ppm of Zn from ZnO in phases 1 and 2, respectively. Following phase 2, all pigs were fed a common corn-soybean meal-based diet until the completion of the study on d 38 post-weaning. In the experimental period (d 0 to 24) and overall (d 0 to 38), pigs fed the lactose replacement strategy had decreased ($P < 0.05$) ADFI compared to pigs fed the other low ABC-4 formulation strategies. In the experimental period (d 0 to 24) and overall (d 0 to 38), pigs fed the low ABC-4 diets had improved ($P \leq 0.022$) F/G compared to pigs fed the high ABC-4 diet. In the experimental period (d 0 to 24), pigs fed the diet containing ZnO had increased ($P \leq 0.001$) ADG and ADFI compared to pigs fed the high ABC-4 diet without ZnO. On all fecal collection periods (d 8, 17, and 24), pigs fed the low ABC-4 diets had increased ($P \leq 0.024$) fecal DM compared to pigs fed the high ABC-4 diet. In summary, low ABC-4 diets improved feed efficiency and fecal dry matter regardless of the formulation strategy. However, replacing whey permeate with crystalline lactose decreased feed intake. In addition, pigs fed diets

¹ Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.

containing fumaric and formic acid had the same ending BW and ADG as those fed the high ABC-4 diet with ZnO.

Introduction

The acid-binding capacity at a pH of 4 (ABC-4) in nursery pig diets is a growing area of interest because of its positive benefits on gastrointestinal health. Previous research has shown low ABC-4 diets can improve nursery pig performance and fecal dry matter.² The use of dietary acidifiers is an easy way to lower the ABC-4 level of the diet without significantly affecting other ingredients or nutrients. However, using acidifiers as the only strategy to lower the ABC-4 level of the diet is often not enough to reach the desired level without the acidifiers negatively affecting diet palatability. Therefore, other dietary formulation strategies must be incorporated along with the use of acidifiers to reach a low ABC-4 level. Low ABC-4 formulation strategies include but are not limited to utilizing low Ca levels in the diet, adding a combination of different acidifiers, low ABC-4 lactose sources, and/or low ABC-4 specialty protein sources to replace soybean meal.

Therefore, the objective of this experiment was to evaluate various strategies to lower the ABC-4 level of the diet in combination with a dietary acidifier. The hypothesis of the experiment was that low ABC-4 formulation would result in improved growth performance and fecal dry matter regardless of the formulation strategy and each formulation strategy would perform similarly.

Materials and Methods

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 Swine Teaching and Research Center and the Kansas State 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 725 pigs (DNA 241 × 600, initially 13.0 lb) were used in a 38-d nursery trial across two research facilities. The first facility utilized a single nursery room whereas the second facility had two identical nursery rooms. Pigs were weaned at approximately 21-d of age into pens of five pigs each based on initial weight and sex. Pens were randomly assigned to one of five dietary treatments with 29 replications per treatment. Pigs were fed experimental diets in two phases with phase 1 fed according to a feed budget of 5 lb/pig. Following the completion of phase 1 diets, pigs transitioned to phase 2 diets until d 24 post-weaning. Phase 1 and 2 diets were formulated to contain 1.35% SID Lys. Following phase 2, all pigs were fed a common corn-soybean meal-based diet until d 38 of the trial. The common diet was formulated to contain 1.30% SID Lys and met or exceeded all other nutrient requirements established by the NRC (2012).³

² Stas, E. B., M. D. Tokach, J. C. Woodworth, J. M. DeRouchey, R. D. Goodband, and J. T. Gebhardt. 2023. Dietary acid-binding capacity influences nursery pig performance and fecal dry matter. Kansas Agricultural Experiment Station Research Reports. Vol 9. Iss 7. doi:10.4148/2378-5977.8506.

³ NRC. 2012. Nutrient Requirements of Swine. 11th ed. Natl. Acad. Press, Washington, DC.

The first three dietary treatments were low ABC-4 diets using a dietary acidifier (0.46% fumaric acid, Primary Products Ingredients Americas LLC, Decatur, IL) and three different formulation strategies to achieve an ABC-4 level of approximately 200 and 250 meq/kg in phase 1 and 2, respectively. The first strategy consisted of low Ca levels by decreasing the amount of limestone in the diet. The low Ca diets were formulated to have a Ca:P ratio 0.20 lower than all other dietary treatments. The second strategy added 0.6% formic acid (Amasil NA, BASF; Florham Park, NJ) in both phases, in addition to the 0.46% fumaric acid. The last strategy to lower ABC-4 consisted of replacing whey permeate (Dairylac 80; International Ingredients Corporation; St. Charles, MO) with crystalline lactose based on lactose equivalence, but this strategy led to an ABC-4 of 193 and 262 meq/kg in phases 1 and 2, respectively. Ultimately, a complete replacement of whey permeate with crystalline lactose resulted in an ABC-4 diet slightly below target for phase 1 and slightly above target for phase 2. However, the average ABC-4 value across the two phases is similar to the other low ABC-4 formulation strategies. The lactose replacement strategy also adjusted the inclusion of microbially enhanced soybean meal (MEPRO; Prairie Aquatech; Brookings, SD) to maintain the same level of conventional soybean meal across all dietary treatments. Diets 4 and 5 were two high ABC-4 diets that were formulated to be 100 meq/kg higher in ABC-4 than the low ABC-4 diets. The high ABC-4 diets did not contain any acidifiers, utilized only whey permeate, and had a Ca:P ratio of 0.9:1 and 1.0:1 in phases 1 and 2, respectively. The difference between the two high ABC-4 diets was the presence or absence of ZnO. Diet 5 contained pharmacological levels of Zn from ZnO and was formulated to contain 3,000 and 2,000 ppm of Zn in phases 1 and 2, respectively. All other dietary treatments contained 110 ppm of Zn provided by the trace-mineral premix. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Individual pig weight and feed disappearance were measured on d 8, 17, 24, 31, and 38 to determine ADG, ADFI, and F/G.

Fecal samples were collected on d 8, 17, and 24 to determine fecal DM 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 for 48 h, and the ratio of dried-to-wet fecal weight determined the fecal percentage DM.

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. Effects of formulation strategy, ABC-4 level, and ZnO were tested by using pre-planned contrast comparisons. The formulation strategy effect was tested by a contrast comparison of the three low ABC-4 diets with different formulation strategies and excluded the high ABC-4 diets. The ABC-4 level effect was tested by a comparison of the mean of the three low ABC-4 diets against the high ABC-4 diet without added ZnO. The ZnO effect was tested by a pairwise comparison of the high ABC-4 diets with or without added ZnO. Fecal DM 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

In diets not containing added ZnO, analyzed ABC-4 values were on average 33 and 20 meq/kg lower than calculated ABC-4 values for phases 1 and 2, respectively. The analyzed ABC-4 values for the high ABC-4 diet with ZnO was 74 and 44 meq/kg lower than calculated values for phases 1 and 2, respectively.

There were no differences in growth performance between pigs fed the three low ABC-4 formulation strategies for phase 1 (d 0 to 8). In phase 2 (d 8 to 24) and the experimental period (d 0 to 24), pigs fed the lactose replacement strategy had decreased ($P < 0.05$) ADFI compared to pigs fed the other strategies. In the common period (d 24 to 38) and overall (d 0 to 38), there was a tendency observed ($P \leq 0.094$) where pigs fed the combination of acidifiers had the greatest ADG. Overall (d 0 to 38), pigs fed the lactose replacement strategy had decreased ($P < 0.05$) ADFI compared to pigs fed the other strategies. In addition, there was a tendency observed ($P = 0.068$) where pigs fed the combination of acidifiers had the greatest BW. On d 8, pigs fed the lactose replacement strategy had increased ($P < 0.05$) fecal DM compared to pigs fed the combination of acidifiers with the low Ca strategy intermediate.

In phase 1 (d 0 to 8), pigs fed low ABC-4 diets had improved ($P = 0.006$) F/G and tended to have increased ($P \leq 0.099$) BW and ADG compared to pigs fed the high ABC-4 diet without ZnO. In phase 2 (d 8 to 24), there were no differences between pigs fed diets with low or high ABC-4 levels. In the experimental period (d 0 to 24), pigs fed low ABC-4 diets had improved ($P = 0.022$) F/G compared to pigs fed the high ABC-4 diet without ZnO. In the common period (d 24 to 38), pigs previously fed low ABC-4 diets tended to have improved ($P = 0.062$) F/G compared to pigs previously fed the high ABC-4 diet without ZnO. Overall (d 0 to 38), pigs fed low ABC-4 diets had improved ($P = 0.005$) F/G and tended to have increased ($P = 0.073$) BW compared to pigs fed the high ABC-4 diet without ZnO. For all fecal collection periods (d 8, 17, and 24), pigs fed low ABC-4 diets had increased ($P \leq 0.024$) fecal DM compared to pigs fed the high ABC-4 diet without ZnO.

In phase 1 (d 0 to 8), pigs fed the diet containing ZnO had improved ($P \leq 0.003$) BW, ADG, ADFI, and F/G compared to pigs fed the high ABC-4 diet without ZnO. In phase 2 (d 8 to 24) and the experimental period (d 0 to 24), pigs fed the diet containing ZnO had increased ($P \leq 0.003$) BW, ADG, and ADFI compared to pigs fed the high ABC-4 diet without ZnO. In addition, in the experimental period (d 0 to 24), pigs fed the diet containing ZnO tended to have improved ($P = 0.054$) F/G compared to pigs fed the high ABC-4 diet without ZnO. In the common period (d 24 to 38), there were no differences in growth performance of pigs previously fed diets with or without added ZnO. Overall (d 0 to 38), pigs fed the diet containing ZnO tended to have increased ($P = 0.068$) ADG compared to pigs fed the high ABC-4 diet without ZnO. There were no differences in pigs fed diets with or without ZnO for fecal DM.

In conclusion, an ABC-4 level of 200 and 250 meq/kg in phases 1 and 2, respectively, resulted in improved feed efficiency and fecal dry matter and tended to increase final pig BW compared to diets 100 meq/kg higher in ABC-4. Replacing whey permeate with crystalline lactose decreased feed intake but did improve d 8 fecal DM. The combination of acidifiers used to lower the ABC-4 level of the diet provided some numerical benefits in growth performance compared with pigs fed the other low ABC-4 formu-

lation strategies. As expected, the addition of ZnO in the high ABC-4 diets improved growth performance particularly in the experimental period. At the end of the study, the combination of acidifiers treatment without ZnO had the same ending BW and ADG as the high ABC-4 diet with ZnO. Overall, it appears that the combination of fumaric and formic acid may be the optimal strategy to achieve the target ABC-4 level.

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

	Acidifier + low Ca	Combination of acidifiers	Acidifier + lactose replacement	High	High
Low ABC-4 Strategy: ABC-4, meq/kg:	200	200	193	300	387
ZnO:	-	-	-	-	+
Ingredients, %					
Corn	57.65	56.78	59.21	57.84	57.44
Soybean meal	12.00	12.00	12.00	12.00	12.00
Whey permeate ²	15.00	15.00	---	15.00	15.00
Crystalline lactose	---	---	12.00	---	---
Microbially enhanced soybean meal ³	7.50	7.50	8.10	7.50	7.50
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50
Fumaric acid ⁴	0.46	0.46	0.46	---	---
Formic acid ⁵	---	0.60	---	---	---
Corn oil	2.00	2.00	2.00	2.00	2.00
Limestone	0.01	0.28	0.28	0.28	0.28
Monocalcium phosphate	0.75	0.75	0.95	0.75	0.75
Salt	0.40	0.40	0.78	0.78	0.78
L-Lys	0.55	0.55	0.55	0.55	0.55
DL-Met	0.24	0.24	0.24	0.24	0.24
L-Thr	0.24	0.24	0.24	0.24	0.24
L-Trp	0.08	0.08	0.09	0.08	0.08
L-Val	0.16	0.16	0.16	0.16	0.16
L-Ile	0.02	0.02	0.02	0.02	0.02
ZnO	---	---	---	---	0.40
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase ⁶	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100

continued

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

Low ABC-4 Strategy:	Acidifier + low Ca	Combination of acidifiers	Acidifier + lactose replacement	High	High
ABC-4, meq/kg:	200	200	193	300	387
ZnO:	-	-	-	-	+
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	52	52	53	52	52
Leu:Lys	112	111	113	112	112
Met:Lys	36	36	36	36	36
Met and Cys:Lys	56	56	56	57	57
Thr:Lys	64	64	64	64	64
Trp:Lys	21.1	21.1	20.8	21.1	21.1
Val:Lys	70	70	70	70	70
His:Lys	33	33	34	33	33
Total Lys, %	1.50	1.50	1.50	1.50	1.50
NE, kcal/lb	1,205	1,194	1,190	1,207	1,202
SID Lys:NE, g/Mcal	5.08	5.12	5.14	5.07	5.07
CP, %	19.4	19.3	19.5	19.4	19.4
Ca, %	0.36	0.46	0.46	0.36	0.36
P, %	0.51	0.51	0.51	0.51	0.51
STTD P, %	0.46	0.46	0.46	0.46	0.46
Ca:P	0.70	0.90	0.90	0.90	0.90
Analyzed ABC-4, meq/kg	170	167	163	263	313

¹Phase 1 diets were fed according to a feed budget of 5 lb/pig.

²Dairylac 80; International Ingredients Corporation; Fenton, MO.

³MEPRO; Prairie Aquatech; Brookings, SD.

⁴Primary Products Ingredients Americas LLC, Decatur, IL

⁵Amasil NA; BASF; Florham Park, NJ.

⁶Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 674 FYT/lb.

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

Low ABC-4 Strategy:	Acidifier +	Combination	Acidifier	High	High
	low Ca	of acidifiers	+ lactose replacement		
ABC-4, meq/kg:	250	250	262	350	404
ZnO:	-	-	-	-	+
Ingredients, %					
Corn	57.61	56.74	58.53	57.80	57.55
Soybean meal	22.70	22.70	22.70	22.70	22.70
Whey permeate ²	10.00	10.00	---	10.00	10.00
Crystalline lactose	---	---	8.00	---	---
Microbially enhanced soybean meal ³	5.00	5.00	5.40	5.00	5.00
Fumaric acid ⁴	0.46	0.46	0.46	---	---
Formic acid ⁵	---	0.60	---	---	---
Corn oil	1.00	1.00	1.00	1.00	1.00
Limestone	0.15	0.42	0.42	0.42	0.42
Monocalcium phosphate	0.85	0.85	1.00	0.85	0.85
Salt	0.55	0.55	0.55	0.55	0.55
L-Lys	0.53	0.53	0.53	0.53	0.53
DL-Met	0.23	0.23	0.23	0.23	0.23
L-Thr	0.24	0.24	0.24	0.24	0.24
L-Trp	0.08	0.08	0.08	0.08	0.08
L-Val	0.15	0.15	0.15	0.15	0.15
ZnO	---	---	---	---	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase ⁶	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100

continued

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

Low ABC-4 Strategy:	Acidifier + low Ca	Combination of acidifiers	Acidifier + lactose replacement	High	High
ABC-4, meq/kg:	250	250	262	350	404
ZnO:	-	-	-	-	+
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	55	55	55	55	55
Leu:Lys	113	113	114	113	113
Met:Lys	37	37	37	37	37
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	64	64	64	64	64
Trp:Lys	21.9	21.8	21.9	21.9	21.9
Val:Lys	70	70	70	70	70
His:Lys	35	35	36	35	35
Total Lys, %	1.49	1.49	1.49	1.50	1.50
NE, kcal/lb	1,150	1,140	1,139	1,153	1,150
SID Lys:NE, g/Mcal	5.32	5.37	5.37	5.37	5.37
CP, %	20.5	20.5	20.6	20.5	20.5
Ca, %	0.44	0.55	0.55	0.55	0.55
P, %	0.55	0.55	0.55	0.55	0.55
STTD P, %	0.46	0.46	0.46	0.46	0.46
Ca:P	0.80	1.00	1.00	1.00	1.00
Analyzed ABC-4, meq/kg	227	233	240	333	360

¹Phase 2 diets were fed from approximately d 10 to 24 post-weaning.

²Dairylac 80; International Ingredients Corporation; Fenton, MO.

³MEPRO; Prairie Aquatech; Brookings, SD.

⁴Primary Products Ingredients Americas LLC, Decatur, IL

⁵Amasil NA; BASF; Florham Park, NJ.

⁶Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 674 FYT/lb.

Table 3. Effects of dietary acidifiers and low ABC-4 formulation strategies on nursery pig performance and fecal dry matter (DM)¹

Low ABC-4 Strategy:	Acidifier +			High	High	SEM	P =		
	Acidifier + low Ca ²	Combination of acidifiers ³	Acidifier + lactose replacement ⁴				Strategy ⁵	ABC-4 ⁶	ZnO ⁷
Phase 1 ABC-4, meq/kg:	200	200	193	300	387				
Phase 2 ABC-4, meq/kg:	250	250	262	350	404				
ZnO:	-	-	-	-	+				
BW									
d 0	13.0	13.0	13.0	13.0	13.0	0.20	0.175	0.497	0.758
d 8	14.7	14.8	14.6	14.5	15.0	0.15	0.436	0.099	<0.001
d 24	27.7	28.0	27.4	27.3	29.1	0.65	0.132	0.268	<0.001
d 38	45.1	46.2	44.8	44.9	46.2	0.66	0.068	0.073	0.394
Phase 1 (d 0 to 8)									
ADG, lb	0.22	0.22	0.21	0.19	0.26	0.014	0.641	0.055	<0.001
ADFI, lb	0.27	0.26	0.25	0.25	0.30	0.013	0.492	0.611	<0.001
G:F	0.81	0.85	0.86	0.76	0.87	0.027	0.868	0.006	0.003
F/G ⁸	1.23	1.18	1.17	1.32	1.15	---	0.868	0.006	0.003
Phase 2 (d 8 to 24)									
ADG, lb	0.81	0.83	0.80	0.80	0.88	0.035	0.138	0.407	<0.001
ADFI, lb	1.13	1.13	1.06	1.11	1.20	0.037	0.024	0.973	0.003
G:F	0.72	0.74	0.75	0.72	0.73	0.029	0.165	0.126	0.345
F/G ⁸	1.39	1.36	1.33	1.38	1.36	---	0.165	0.126	0.345
Experimental period (d 0 to 24)									
ADG, lb	0.61	0.63	0.60	0.60	0.67	0.021	0.162	0.200	<0.001
ADFI, lb	0.84	0.84	0.80	0.82	0.89	0.026	0.031	0.926	0.001
G:F	0.73	0.75	0.76	0.73	0.75	0.025	0.202	0.022	0.054
F/G ⁸	1.37	1.33	1.31	1.37	1.34	---	0.202	0.022	0.054
Common period (d 24 to 38)									
ADG, lb	1.25	1.30	1.25	1.25	1.23	0.037	0.094	0.625	0.405
ADFI, lb	1.83	1.85	1.80	1.84	1.80	0.030	0.170	0.643	0.333
G:F	0.68	0.70	0.69	0.68	0.68	0.015	0.349	0.062	0.928
F/G ⁸	1.46	1.42	1.44	1.47	1.47	---	0.349	0.062	0.928

continued

Table 3. Effects of dietary acidifiers and low ABC-4 formulation strategies on nursery pig performance and fecal dry matter (DM)¹

Low ABC-4 Strategy:	Acidifier +			High	High	SEM	P =		
	Acidifier + low Ca ²	Combination of acidifiers ³	Acidifier + lactose replacement ⁴				Strategy ⁵	ABC-4 ⁶	ZnO ⁷
Phase 1 ABC-4, meq/kg:	200	200	193	300	387				
Phase 2 ABC-4, meq/kg:	250	250	262	350	404				
ZnO:	-	-	-	-	+				
Overall (d 0 to 38)									
ADG, lb	0.85	0.87	0.84	0.84	0.87	0.018	0.079	0.288	0.068
ADFI, lb	1.20	1.21	1.16	1.19	1.23	0.023	0.048	0.887	0.209
G:F	0.70	0.72	0.72	0.70	0.71	0.007	0.789	0.005	0.128
F/G ⁸	1.42	1.39	1.38	1.43	1.41	---	0.789	0.005	0.128
Fecal DM, % ⁹									
d 8	22.9	21.7	24.3	21.5	22.7	1.70	0.001	0.024	0.150
d 17	24.9	24.7	24.8	23.2	24.1		0.913	0.018	0.288
d 24	24.5	25.1	24.9	23.1	24.2		0.784	0.009	0.179

¹ A total of 725 pigs (initial BW of 13.0 ± 0.20 lb) were used in a 38-d nursery trial across three rooms. A total of five dietary treatments were utilized with three low ABC-4 diets utilizing dietary acidifiers and low ABC-4 formulation strategies. In addition, two high ABC-4 diets were utilized with or without pharmacological levels on Zn from ZnO.

² Fumaric acid (Primary Products Ingredients Americas LLC, Decatur, IL) and reducing the Ca:P ratio by 0.20.

³ Fumaric acid and formic acid (Amasil NA; BASF, Florham Park, NJ).

⁴ Fumaric acid and replacing whey permeate (Dairylac 80; International Ingredients Corporation, St. Charles, MO) with crystalline lactose based on lactose equivalence.

⁵ Compares the three strategies to achieve a low ABC-4 level. Excludes high ABC-4 diets.

⁶ Compares the mean of the low ABC-4 diets and the high ABC-4 diet without ZnO. Excludes the high ABC-4 diet with ZnO.

⁷ Compares the two high ABC-4 diets. Excludes all low ABC-4 diets.

⁸ Calculated using the inverse of G:F. Therefore, no SEM is reported and P values are the same as G:F.

⁹ Treatment × day, P = 0.414; Treatment, P < 0.001; Day, P < 0.001. The P-values represented in the data table show the effect of treatment within day.