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Measurement of the Acid-Binding Capacity of Common Ingredients and Complete Diets Intended for Weanling Pigs

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Measurement of the Acid-Binding Capacity of Common Ingredients and Complete Diets Intended for Weanling Pigs

Abstract

Some ingredients bind more acid in the stomach than others which can increase gastric pH in weaned pigs, causing decreased protein digestion and allowing pathogenic microorganisms to proliferate. The objective of this experiment was to measure acid-binding capacity at a pH of 4 (ABC-4) of common nursery ingredients and determine additivity in diets. Ingredient categories included: cereal grains, vegetable proteins, animal proteins and milk, vitamin premixes and minerals, amino acids, and fiber sources. A 0.5 g sample of each ingredient was suspended in 50 mL of distilled deionized water and titrated with 0.1 N hydrochloric acid. Sample ABC-4 was calculated as the amount of acid in milliequivalents (meq) required to lower 1 kg of a sample to a pH of 4. Cereal grains were found to have lower ABC-4 compared to other ingredients. Vegetable proteins had higher ABC-4 with more variation than cereal grains. Soy protein concentrate and enzymatically treated soybean meal (ESBM) had higher ABC-4 compared to SBM while fermented soybean meal (FSBM) was lower. Zinc oxide (ZnO) and calcium carbonate ($CaCO_3$) had the highest ABC-4 among all ingredients. Following ingredient analysis, a series of complete diets were analyzed to determine ingredient additivity by comparing the differences between calculated and analyzed ABC-4. Perfect ABC-4 additivity was not found, with all diets having lower analyzed ABC-4 than calculated values; however, the analyzed ABC-4 followed dietary calculated values for higher or lower ABC-4 diet values. These data suggest that ABC-4 diet can be adjusted through selection of ingredients, but feeding trials are needed to determine the impact on pig performance.

Keywords

Acid-binding capacity, ingredients, diets, nursery pig

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Measurement of the Acid-Binding Capacity of Common Ingredients and Complete Diets Intended for Weanling Pigs

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Summary

Some ingredients bind more acid in the stomach than others which can increase gastric pH in weaned pigs, causing decreased protein digestion and allowing pathogenic microorganisms to proliferate. The objective of this experiment was to measure acid-binding capacity at a pH of 4 (ABC-4) of common nursery ingredients and determine additivity in diets. Ingredient categories included: cereal grains, vegetable proteins, animal proteins and milk, vitamin premixes and minerals, amino acids, and fiber sources. A 0.5 g sample of each ingredient was suspended in 50 mL of distilled deionized water and titrated with 0.1 N hydrochloric acid. Sample ABC-4 was calculated as the amount of acid in milliequivalents (meq) required to lower 1 kg of a sample to a pH of 4. Cereal grains were found to have lower ABC-4 compared to other ingredients. Vegetable proteins had higher ABC-4 with more variation than cereal grains. Soy protein concentrate and enzymatically treated soybean meal (ESBM) had higher ABC-4 compared to SBM while fermented soybean meal (FSBM) was lower. Zinc oxide (ZnO) and calcium carbonate ($CaCO_3$) had the highest ABC-4 among all ingredients. Following ingredient analysis, a series of complete diets were analyzed to determine ingredient additivity by comparing the differences between calculated and analyzed ABC-4. Perfect ABC-4 additivity was not found, with all diets having lower analyzed ABC-4 than calculated values; however, the analyzed ABC-4 followed dietary calculated values for higher or lower ABC-4 diet values. These data suggest that ABC-4 diet can be adjusted through selection of ingredients, but feeding trials are needed to determine the impact on pig performance.

Introduction

The postweaning period is a stressful and critical period of the young pig's life and much interest has been directed toward positively influencing the gastrointestinal tract (GIT) to maximize lifetime production and health status. At weaning, pigs are switched to solid feed with an underdeveloped GIT that is unable to produce enough hydrochloric acid, ultimately leading to an increase in gastric pH as high as 5.0 for several days post-weaning.[2] Low stomach pH is important for protein digestion and when stomach

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

[2] Kidder, D. E., and M. J. Manners. 1978. Digestion in the Pig. Bristol: Scientechnica.

pH increases above 3.5, pepsin activity rapidly declines.[3] Increased gastric pH allows opportunistic pathogens to survive and compromise the digestive tract leading to clinical infection, disease, and possible death.

Poor acidification of the stomach can be attributed to high acid-binding capacity (ABC) of feed ingredients.[4] The use of low acid-binding ingredients (avoidance of high acid-binding ingredients) in the diets of newly weaned pigs could be utilized to maintain an acidic environment in the stomach and improve feed utilization.[5] Lawlor et al.[5] evaluated ingredients common in early nursery swine diets and reported minerals had the highest ABC values, specifically calcium carbonate and zinc oxide. Additionally, the authors reported vegetable proteins, such as soybean meal, were found to have a relatively high ABC when compared to cereal grains. Vegetable proteins also make up a large percentage of early nursery pig diets, thus heavily influencing the ABC. Ingredients evaluated by Lawlor et al.[5] are European sourced and ABC may not be fully representative of ingredients used in North America. Furthermore, little research has been conducted on additivity of ABC ingredient values in complete diets, which is important for ABC diet formulation.

The objective of this experiment was to evaluate the ABC-4 of common North American feed ingredients used in diets of nursery pigs and determine if ABC-4 values are additive when mixed to form complete diets.

Procedures

The experiment was conducted at the Kansas State University Swine Nutrition Laboratory in Manhattan, KS. Ingredients commonly used in early nursery diets were obtained from commercial sources.

Protocol

All ingredient samples and diets were ground to achieve a consistent particle size of approximately 400 µm using a Rancilio Rocky Doserless Grinder (Rancilio Group; Villastanza, Italy). Approximately 100 g of each sample was then placed into a sample bag and stored at -20°C (-4°F) until ABC-4 was determined using a modified procedure established by Lawlor et al.[5] Modifications include beaker size, pH meter, and ending equilibrium criteria. A sample of .5 g of each ingredient and diet was weighed on a weigh boat then suspended in 50 mL of distilled de-ionized water. All samples, apart from minerals, were placed in a 100-mL beaker with a magnetic stir bar. Minerals were placed in a 250-mL beaker with a magnetic stir bar, because of the large amount of acid required to lower pH. The beaker was placed on a stir plate and samples were stirred until suspended in solution. Initial pH of each sample was recorded using a Mettler Toledo SevenCompact pH/Ion S220 meter. Each day of analysis, the pH meter was calibrated using a Fisher calibrated buffer solution. Titrations were performed by the

[3] Yen, J. T. 2001. Anatomy of the digestive system and nutritional physiology, In: Lewis, A. J., Southern, L. L. (Eds.), Swine Nutrition, CRC Press, Boca Raton, FL, pp. 21-64.

[4] Batonon-Alavo, D. I., B. Bouza, J. C. G. Cholet, and Y. Mercier. 2016. A method for determination of the acidifying value of organic acids used in pigs diets in the acid binding capacity at pH 4 (ABC-4) system. Anim. Feed Sci. 216, 197-203. doi:10.1016/j.anifeedsci.2016.03.021.

[5] 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.

addition of 0.1 N hydrochloric acid (HCl) in increments of 0.1 to 5.0 mL depending on ingredient and stage of titration. Once a stable pH of 4.00 ± 0.04 was reached, ABC-4 was calculated as the amount of acid in milliequivalents (meq) required to achieve pH of 4 for 1 kg of sample. Samples with an initial pH of less than 4 were titrated using sodium hydroxide (NaOH) to raise the pH. Sample pH equilibrium was determined was once the meter indicated stabilization.

Ingredient analysis

Ingredients were obtained from various commercial feed mills in the United States or ingredient suppliers (Table 1). Ingredients were grouped into categories: cereal grains, vegetable proteins, animal proteins and milk, vitamin premixes and minerals, amino acids (AA), and fiber sources. Each sample was analyzed three separate times to observe any variation within source, and ABC-4 was calculated as the average of the triplicate analysis. This value was then used in statistical analysis.

Early nursery diets

Two different early nursery diets were formulated to determine if ABC-4 values for ingredients are additive by comparing calculated ABC-4 with analyzed values. All diets were mixed in the Kansas State University Applied Swine Nutrition Laboratory (Department of Animal Science, College of Agriculture, Manhattan, KS). Ingredients used to formulate diets were from the same samples used in the ingredient analysis to reduce any variation in ABC from ingredient source. The same procedures to analyze individual ingredients were used to determine analyzed ABC-4 of complete feeds.

The first series of diets analyzed were phase 1 nursery diets intended for pigs weighing 11 to 15 lb. A total of 10 diets were arranged in a 2 × 5 factorial with increasing levels of calcium carbonate with or without the inclusion of 3,000 ppm of Zn from ZnO (Table 2). Calcium carbonate and ZnO diets were selected because of their high ABC-4. Diets were formulated to contain 0, 0.45, 0.90, 1.35, and 1.80% calcium carbonate at the expense of corn. Diets without ZnO contained 110 ppm of Zn from the trace mineral premix.

The second series of diets analyzed were phase 2 nursery diets intended for pigs weighing 15 to 26 lb. A total of 3 diets were formed with different levels of soybean meal, feed-grade AA, and specialty soy proteins products (Table 3). The two soy products included fermented soybean meal (MEpro, Prairie AquaTech, Brookings, SD) and enzymatically treated soybean meal (HP 300, Hamlet Protein, Findlay, OH) because of their low and high ABC-4 values, respectively. The first diet consisted of low soybean meal and high feed-grade AA with 5% fermented soybean meal. The second diet consisted of low soybean meal and high feed-grade AA with 5% enzymatically treated soybean meal. The third diet consisted of high soybean meal and low feed-grade AA with 5% enzymatically treated soybean meal. These diets were selected because of the large difference in ABC-4 for different protein sources.

Statistical analysis

All data analysis was performed 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]. For ingredient analysis, descriptive statistics were generated using the summary function, with value reported as average of sample means ± standard deviation of sample means. For calcium carbonate and ZnO diet analysis, data were analyzed as a completely randomized design using the lm function with difference between calculated and analyzed ABC-4 as the experimental unit. The main effects of ZnO and calcium carbonate, as well as their interactions, were tested. For soybean meal and specialty protein analysis, data were analyzed as a completely randomized design using the lm function with difference between calculated and analyzed ABC-4 as the experimental unit. The main effects of diet were tested. For testing of all hypotheses, differences were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

Ingredient analysis

The mean ABC-4 values of each ingredient are reported as the average of sample means ± standard deviation of sample means (Table 1). Cereal grains, with respect to other ingredients, were found to have a low ABC-4. Corn was found to have an ABC-4 of 84 ± 18.6 meq, with barley having a similar value to corn. Distillers dried grains with solubles (DDGS), cereal blend, oat products, and sorghum had among the highest of ABC-4 within cereal grains, and wheat products were found to have the lowest values.

Vegetable proteins were among two ingredient categories with the most variation between ingredients and sources. Soybean meal was found to have an ABC-4 of 602 ± 28.2 meq. Other soy products had a wide range of ABC-4 values. Enzymatically treated soybean meal and soy protein concentrate had higher ABC-4 than soybean meal, whereas fermented soybean meal had a lower value. However, there was great variation between soy protein concentrate and fermented soybean meal with standard deviations of 165.0 and 100.0 meq, respectively. Fermented soy isolate had an ABC-4 of -13 meq that had to be titrated with NaOH. Finally, high protein distillers dried grains (HPDDGs) had a low ABC-4 relative to other vegetable proteins.

Animal proteins and milk collectively, except for crystalline lactose, were higher in ABC-4 than vegetable proteins. Fish meal had the highest ABC-4 of 1,380 ± 150.9 meq most likely due to its high Ca content. Spray-dried bovine plasma, poultry meal, spray-dried whey powder, and whey permeate had lower values than fish meal, but still relatively high values compared to other ingredients tested. Crystalline lactose had the lowest ABC-4 of all animal proteins and milk with a value of 53 meq.

Vitamin premixes and minerals had the highest ABC-4 between categories and had the most variation, as expressed by the standard deviations. Calcium carbonate and ZnO had the highest ABC-4 of 18,384 ± 769.7 and 21,863 ± 598.7 meq, respectively. These were also the two ingredients with the highest ABC-4 overall. The vitamin premix, trace mineral premix, vitamin and trace mineral premix, manganese, calcium propionate, and dicalcium phosphate also had high ABC-4, but did not reach the level of calcium carbonate and ZnO. Monocalcium phosphate, sodium chloride, choline chloride, and sodium metabisulfite had low values among vitamin premixes and minerals.

Amino acids were more consistent compared to other ingredient categories. All feed-grade AA analyzed had an ABC-4 between 83 and 200 meq, with L-Lys and L-Ile having the lowest and highest value, respectively.

The fiber source tested was another ingredient low in ABC-4 compared to the other categories. Beet pulp shreds were found to have an ABC-4 of 151 ± 25.3 meq.

Calcium carbonate and ZnO diets

Calculated and analyzed ABC-4 increased with the amount of calcium carbonate and ZnO added in the diet. The difference between calculated and analyzed ABC-4 was used to test ZnO and limestone's additivity when mixed with other ingredients to form complete diets.

There was a ZnO × calcium carbonate interaction observed ($P = 0.015$) for difference between calculated and analyzed ABC-4 (Table 4). Within the interaction, the difference between calculated and analyzed ABC-4 increased (linear, $P < 0.001$) as calcium carbonate levels increased in diets without ZnO. The difference between analyzed and calculated ABC-4 at 0% calcium carbonate was 29 meq without ZnO. This difference increased along with the addition of calcium carbonate, with the largest difference of 185 meq observed at 1.80% calcium carbonate. However, the difference between calculated and analyzed ABC-4 did not significantly change ($P > 0.05$) as calcium carbonate increased in the diet when ZnO was present. The lowest and highest differences were observed at 0 and 1.35% of calcium carbonate, with the difference between analyzed and calculated ABC-4 being 116 and 157 meq, respectively.

Soybean meal and specialty protein diets

The inclusion of enzymatically treated soybean meal and high levels of soybean meal increased the calculated and analyzed ABC-4 of complete diets compared to a diet with fermented soybean meal and low levels of soybean meal (Table 5). Similar to the first series of diets analyzed, all diets had lower analyzed values than calculated values. All three diets had similar differences between calculated and analyzed ABC-4 values. As a result, the differences between calculated and analyzed ABC-4 of all three diets were not significant ($P = 0.640$). The average difference between calculated and analyzed ABC-4 was 107 meq.

In summary, complete additivity of ingredients into a complete diet can't be assumed and warrants further investigation. Reduced ABC-4 of the diet can still be successfully manipulated through careful selection of ingredients. Use of feeding strategies including low calcium carbonate levels, low soybean meal inclusion (high feed-grade AA), and low ABC-4 ingredient levels can be used to target a low ABC-4 value in complete diets. Targeting a low ABC-4 in the diets of nursery pigs immediately post-weaning may alleviate problems in the early nursery stage such as poor growth, impaired GIT health, and mortality. However, further investigation is warranted to determine the optimal ABC-4 in diets for early nursery pigs of a specific weight range to optimize performance.

Table 1. Acid-binding capacity at pH 4 (ABC-4) of ingredients common in early nursery diets[1]

Item	N[2]	Average initial pH	Average ABC-4 (meq/kg)
Cereal grains			
Corn	5	6.50	84 ± 18.6
Sorghum	2	7.04	110 ± 14.1
Red winter wheat	2	6.70	43 ± 4.7
White wheat	2	6.67	60 ± 0.0
Barley	2	5.49	77 ± 4.7
Whole oats	3	6.33	122 ± 23.4
Oat groats	2	6.44	107 ± 37.7
Dried distillers grains with solubles	3	4.85	147 ± 43.7
Cereal blend	1	6.08	107
Vegetable protein			
Soybean meal	6	6.98	602 ± 28.2
Expelled SBM	1	7.13	567
Soy protein concentrate[4]	2	7.36	737 ± 165.0
Fermented SBM[5]	3	4.69	207 ± 100
Fermented soy isolate[6]	1	3.95	-13
Enzymatically treated SBM[7]	1	6.29	753
HPDDGs[8]	2	4.87	100 ± 84.9
Animal proteins and milk			
Fish meal[9]	2	6.32	1,380 ± 150.9
Spray-dried bovine plasma[10]	1	6.99	713
Poultry meal[11]	1	6.74	1,007
Spray-dried whey powder	1	6.29	440
Whey permeate	1	6.16	520
Crystalline lactose	1	7.09	53
Vitamins and minerals			
Calcium carbonate	3	9.62	18,384 ± 769.7
Zinc oxide	3	9.55	21,863 ± 598.7
Monocalcium phosphate	1	4.21	73
Dicalcium phosphate	3	5.54	2,693 ± 848.8
Calcium propionate	1	9.11	9,240
Sodium chloride	2	6.00	15 ± 5.0
Vitamin premix	2	6.99	10,767 ± 613.8
Trace mineral premix	2	5.37	7,867 ± 264.0
Vitamin trace mineral premix	1	4.67	1,727
Manganese	1	8.22	2,347
Sodium metabisulfite	1	4.00	0
Choline chloride	1	5.21	40

Table 1. Acid-binding capacity at pH 4 (ABC-4) of ingredients common in early nursery diets[1]

Item	N[2]	Average initial pH	Average ABC-4 (meq/kg)
Amino acids			
L-Lys	2	5.76	83 ± 4.7
DL-Met	2	5.54	137 ± 4.7
L-Thr	2	5.43	160 ± 0.0
L-Trp	1	5.11	120
L-Val	1	5.53	193
L-Ile	1	5.76	200
Fiber source			
Beet pulp	3	5.57	151 ± 25.3

[1] Acid-binding capacity-4 was determined by the amount of 0.1 N HCl required to lower the pH of a 1 kg sample to a stable pH of 4.00 ± 0.04. Each sample was analyzed three times and value reported is average of sample means ± standard deviation of sample means.
[2] Number of samples analyzed, with each sample analyzed in triplicate.
[3] Buffering capacity is calculated as ABC-4 divided by the total change in pH.
[4] Xsoy, CJ Bio, Seoul, South Korea; SoyTide, CJ Bio, Seoul, South Korea.
[5] MEpro, Prairie AquaTech, Brookings, SD; Fermex, Purina Animal Nutrition, Shoreview, MN; Proplex T, ADM Animal Nutrition, Quincy, IL.
[6] AX3, TripleA, Hornsyld, Denmark.
[7] HP 300, Hamlet Protein, Findlay, OH.
[8] High protein distillers dried grains. NexPro, Poet, Sioux Falls, SD; Protomax, ICM, Colwich, KS.
[9] TASA, Lima, Peru; Omega Protein, Houston TX.
[10] APC, Ankeny, IA.
[11] AV-E Digest, XFE Products, Des Moines, IA.

Table 2. Calcium carbonate titration with or without ZnO diet composition[1]

	No ZnO					Added ZnO				
Item $CaCO_3$:	**0%**	**0.45%**	**0.90%**	**1.35%**	**1.80%**	**0%**	**0.45%**	**0.90%**	**1.35%**	**1.80%**
Corn	52.50	52.05	51.60	51.15	50.70	52.10	51.65	51.20	50.75	50.30
Soybean meal	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60	15.60
Whey powder	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Enzymatically treated SBM	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Fish meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Bovine plasma	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	---	0.45	0.90	1.35	1.80	---	0.45	0.90	1.35	1.80
Monocalcium phosphate	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys-HCl	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Met	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
L-Thr	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-Trp	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L-Val	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Vitamin premix with phytase[2]	0.25	0.25	0.25	0.25	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	0.15	0.15	0.15	0.15
Zinc oxide	---	---	---	---	---	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100	100	100	100	100
Calculated ABC-4, meq[3]	316	398	481	563	645	403	486	568	650	733

[1] A total of 10 diets were formulated to contain increasing levels of calcium carbonate with or without 3,000 ppm of Zn from ZnO. Diets without ZnO contained 110 ppm of Zn from the trace mineral premix. Calcium carbonate was added to the diet at the expense of corn. Diets were formulated for pigs weighing 11 to 15 lb.

[2] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ).

[3] Individual sample ABC-4 values were used for calculations and not ingredient mean values.

Table 3. Soybean meal and specialty protein diets[1]

Item	Low SBM, fermented SBM	Low SBM, enzymatically treated SBM	High SBM, enzymatically treated SBM
Corn	60.69	58.48	52.43
Soybean meal	20.42	22.78	29.49
Whey powder	10.00	10.00	10.00
Fermented SBM[2]	5.00	---	---
Enzymatically treated SBM[3]	---	5.00	5.00
Calcium carbonate	0.75	0.80	0.78
Monocalcium phosphate	1.05	0.90	0.80
Salt	0.58	0.55	0.55
L-Lys-HCl	0.51	0.51	0.30
DL-Met	0.21	0.20	0.15
L-Thr	0.20	0.20	0.10
L-Trp	0.04	0.03	---
L-Val	0.15	0.15	---
Vitamin premix with phytase[4]	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15
Total	100	100	100
Calculated ABC-4, meq[5]	352	408	443

[1] A total of three diets were formulated to contain different levels of soybean meal and synthetic amino acids. Two different soy products were included in the diet based on their analyzed ABC-4 values. The diets were formulated for pigs weighing 11 to 26 lb.
[2] MEpro, Prairie AquaTech, Brookings, SD.
[3] HP 300, Hamlet Protein, Findlay, OH.
[4] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ).
[5] Individual sample ABC-4 values were used for calculations and not ingredient mean values.

Table 4. Evaluation of calculated vs. analyzed ABC-4 on increasing calcium carbonate with or without the presence of ZnO[1]

	No ZnO					Added ZnO				
Calcium carbonate:	**0%**	**0.45%**	**0.90%**	**1.35%**	**1.80%**	**0%**	**0.45%**	**0.90%**	**1.35%**	**1.80%**
Calculated ABC-4 (meq)	316	398	481	563	645	403	486	568	650	733
Analyzed ABC-4 (meq)[2]	287 ± 11.5	333 ± 23.1	353 ± 23.1	393 ± 11.5	460 ± 20.0	287 ± 11.5	340 ± 20.0	433 ± 11.5	493 ± 11.5	600 ± 20.0
Difference[3]	29[c]	65[c]	128[b]	170[a]	185[a]	116[b]	146[ab]	135[b]	157[ab]	133[b]

[1] A total of 10 diets were analyzed in a 2 × 5 factorial design with increasing levels of calcium carbonate at the expense of corn with or without 3,000 ppm of Zn from ZnO. Each diet was sampled and analyzed three times.

[2] Analyzed ABC-4 values are reported as the diet average from 3 samples ± standard deviation within diet.

[3] ZnO × calcium carbonate interaction ($P = 0.015$). Dose effect of calcium carbonate when ZnO is not included at pharmacological levels, linear $P < 0.001$. Dose effect of calcium carbonate when ZnO is included at pharmacological levels, linear $P = 0.175$. Main effect of calcium carbonate (linear, $P < 0.001$). Main effect of ZnO ($P = 0.009$). SEM = 9.9. Difference values lacking common superscripts differ ($P < 0.05$).

Table 5. Evaluation of calculated vs. analyzed ABC-4 of diets formulated with different protein sources and levels of soybean meal[1]

Item	Low SBM, fermented SBM[2]	Low SBM, enzymatically treated SBM[3]	High SBM, enzymatically treated SBM[3]	SEM	*P* =
Calculated ABC-4 (meq)	403	457	487	---	---
Analyzed ABC-4 (meq)[4]	300 ± 20.0	353 ± 11.5	373 ± 11.5	---	---
Difference	103	104	114	8.6	0.637

[1] A total of three diets were analyzed three times with different levels of soybean meal and synthetic amino acids. Two different soy products were used based on their analyzed ABC-4. Each diet was sampled and analyzed three times.
[2] MEpro; Prairie Aquatech; Brookings, SD.
[3] HP 300; Hamlet Protein; Findlay, OH.
[4] Analyzed ABC-4 values are reported as the diet average from 3 samples ± standard deviation within diet.