K-STATE Research and Extension

SWINE DAY 2015

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2015

Stability of Four Commercial Phytase Products under Increasing Conditioning Temperature

J. A. De Jong, J. C. Woodworth, J. M. DeRouchey, M. D. Tokach, R. D. Goodband, S.S. Dritz[1], C. K. Jones[2], and C. R. Stark[2]

Summary

A study was conducted to determine the stability of four commercial phytase products exposed to increasing conditioning temperatures. The four commercial products used were: Quantum Blue G (AB Vista, Plantation, FL); Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (Dupont, Wilmington, DE); and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). The phytase products were mixed as part of corn-soybean meal-based swine diet at a concentration recommended to provide a 0.12% aP release. All four diets were analyzed for phytase activity to establish baseline phytase activity for each product. Diets were then conditioned at four temperatures (149, 167, 185, and 203˚F). The entire process was repeated on four consecutive days to create four replicates. Samples were taken while feed exited the conditioner and before entering the pellet die. Phytase stability was expressed as the residual phytase activity (% of baseline) at each conditioning temperature.

No product × temperature interactions were observed for actual conditioning temperature, conditioner throughput, or residual phytase activity. As the target temperature increased the conditioning temperature increased (linear; $P < 0.001$) and conditioner throughput decreased (linear; $P < 0.001$). No evidence was observed for effects of phytase product on conditioning temperature or conditioner throughput.

As target temperature increased, phytase activity decreased (linear; $P < 0.001$). Residual phytase activity decreased 1.07% for every 1˚F increase in conditioning temperature between the target temperatures of 149 to 203˚F. The product main effect was significant ($P < 0.001$). The Microtech 5000 Plus had decreased ($P < 0.05$) phytase activity when compared to all other products. There was no evidence for residual phytase differences between the Quantum Blue G, Ronozyme Hi Phos GT, or Axtra Phy TPT products.

In the current experiment, target conditioning temperatures had a significant effect on phytase stability regardless of product, resulting in linear decreases in residual phytase

[1] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.
[2] Department of Grain Science and Industry, College of Agriculture, Kansas State University.

activity as temperature was increased. However, Microtech 5000 Plus had decreased residual phytase activity (% of initial) when compared to all other products.

Key words: conditioning temperature, pelleting, phytase stability

Introduction

Phytase is an enzyme that breaks down phytate phosphorus and when included in swine diets will increase phosphorus digestibility. Several commercial phytase products are currently available in the US market. These phytase products all have different recommendations for heat stability during the pelleting process. However, as a safety net, most nutritionists use the lowest guaranteed values for phytase products in formulation to ensure survivability of phytase during pelleting. Phytase, as any enzyme, is subject to damage when exposed to the heat and pressure of many feed processing methods including the pelleting process (Jongbloed and Kemme, 1990)[3].

Currently, little published research exists evaluating commercial phytase products exposed to increasing conditioning temperatures during the pelleting process. The objective of this study was to evaluate four current commercial products of phytase during conditioning when exposed to increasing conditioning temperatures.

Procedures

The study was conducted at the O. H. Kruse Feed Technology and Innovation Center (Kansas State University) to determine the stability of four commercial phytase sources exposed to increasing conditioning temperatures. The four phytase sources used were: Quantum Blue G (minimum declared potency of 5,000 FTU/g); Ronozyme Hi Phos GT (minimum declared potency of 2,702 FYT/g); Axtra Phy TPT (minimum declared potency of 2,500 FTU/g) and Microtech 5000 Plus (minimum declared potency of 5,000 FTU/g). One phytase unit (FTU or FYT) was defined as the amount of enzyme that catalyzes the release of 1 μmol of iP per minute from 5.1 mM sodium phytate in pH 5.5 buffer at 37°C. Phytases were formulated as part of a corn-soybean meal-based swine diet. Concentrations used in formulation were determined by manufacturer recommendations from each product in order to release 0.12% aP. Phytase products were all obtained from a third party distributor.

Phytase from each product was initially mixed with 200 lb of soybean meal using a Wenger (Wenger, Sabetha, KS) 200-lb double ribbon mixer. This was done in order to ensure proper mixing of the phytase throughout the subsequent 1,000-lb batches of complete feed (Table 1) used for the experiment. The phytase-soybean meal mix was then sacked and hand-added during batching of the complete diets used for pelleting. Feed was sacked after mixing and samples were taken from 10 separate bags to form a composite sample which was used to provide initial phytase concentrations in the diet. The phytase activity from the initial samples was used as a baseline for comparison of all subsequent samples analyzed during the experiment.

[3] Jongbloed, A.W.,Kemme, P.A., 1990. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Anim. Feed Sci. Technol. 28, 233–242.

Diets were conditioned at four temperatures (149, 167, 185, and 203°F) and the entire process repeated on four consecutive days to create four replicates. Diets were processed through a CL5 Laboratory Mill (California Pellet Mill; Crawfordsville, IN). Each day flush feed containing no phytase was used to warm the pellet mill to the initial conditioning temperature (145°F). At that point 30 lb of feed from one of the four products was placed in the hopper above the conditioner. Flush feed contained titanium dioxide as a tracer to determine when flush feed was still present in the conditioner.

Feed was processed through the conditioner and samples were taken between the conditioner and pellet die. Temperature of the hot mash exiting the conditioner was used to determine conditioning temperature. Samples were taken at 4 points during each run for each phytase source. Immediately after sampling, feed was transferred to a cooling apparatus capable of cooling feed in less than 5 minutes. After cooling the 4 sub-samples were combined for analysis.

After feed from the first product exited the conditioner, 30 lb of flush diet was added to the hopper and used to flush the system. While the pellet mill was still at the initial conditioning temperature, feed from the second product was added to the hopper. This process continued until all four products were conditioned at the initial temperature. When all products had been processed at the initial temperature, flush feed was added to the hopper, and the temperature was increased to the second conditioning temperature (167°F) and stabilized before adding the first phytase treatment. All samples were processed through the conditioner using procedures similar to those used for the initial temperature samples. This process was replicated for the phytase products at each conditioning temperature. On d 2 of the trial (the next replication), similar procedures were used. However, if a product had previously been conditioned first for all temperatures, it was rotated and conditioned second on d 2, third on d 3, and fourth on d 4. This was done for all phytase products so that there would be no effects from conditioning order during each replication. All samples were sent to New Jersey Feed Lab (New Jersey Feed Lab Inc., Trenton, NJ) for analysis (AOAC 30024:2009) of phytase activity.

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC), with conditioning run as the experimental unit. Treatments were analyzed as a 4 × 4 factorial with the main effect of phytase product (Quantum Blue G, Ronozyme Hi Phos GT, Axtra Phy TPT, and Microtech 5000 Plus) and temperature (149, 167, 185 and 203°F). Preplanned contrasts were used to evaluate the interaction between phytase product and temperature, temperature linear and quadratic effects within product, linear and quadratic temperature effect, and the product main effect. Pairwise comparisons were also used to determine differences between products for residual phytase activity. Treatment differences were considered significant at $P < 0.05$ and were considered tendencies between $P > 0.05$ and $P < 0.10$.

Results and Discussion

There were no product by temperature interactions for actual conditioning temperature, throughput, or phytase activity. As the target temperature increased, conditioning temperatures increased (linear; $P < 0.001$; Table 2) and were within +/- 3.3°F of target temperature. This indicates target temperatures were achieved in the current

experiment and target conditioning temperatures were consistent across phytase products. As conditioning temperature increased as expected, throughput decreased (linear; $P < 0.001$; Table 3). There was no evidence for differences between products on throughput.

As target temperature increased, phytase activity decreased (linear; $P < 0.001$) for all products with no evidence for differences in the degradation rate across the products. Regardless of product, residual phytase activity decreased from 149 to 203˚F by 1.07% for every 1˚F conditioning temperature increase. However, there was a significant ($P < 0.001$) phytase product effect. The Microtech 5000 Plus had decreased ($P < 0.05$) phytase activity when compared to all other products. There was no evidence for residual phytase differences between the Quantum Blue G, Ronozyme Hi Phos GT, or Axtra Phy TPT products.

Increasing conditioning temperature reduced residual activity, regardless of product, in a linear manner. Also, the Microtech 5000 Plus had significantly less activity after conditioning compared to other products, with no differences among the other three products.

Conditioning temperatures may vary from 149 to 185˚F under normal commercial mill conditions depending on the type of diet. The current data suggest that conditioning temperatures above 149˚F may negatively affect the phytase activity of each product used in the experiment. It should be noted that data from the current trial were taken from a lab scale pellet mill. Additional research utilizing each product may be necessary in different commercially operated pellet mills to further understand effects of thermal feed processing on phytase stability.

Table 1. Diet composition (as-fed basis)

Item	Phytase product			
	Quantum Blue G[1]	Ronozyme Hi Phos GT[2]	Axtra Phy TPT[3]	Microtech 5000 Plus[4]
Ingredient, %				
Corn	61.36	61.36	61.37	61.36
Soybean meal (46.5% CP)	33.79	33.79	33.79	33.79
Choice white grease	1.50	1.50	1.50	1.50
Monocalcium phosphate (21% P)	1.05	1.05	1.05	1.05
Limestone	1.00	1.00	1.00	1.00
Salt	0.35	0.35	0.35	0.35
L-lysine HCl	0.30	0.30	0.30	0.30
DL-methionine	0.12	0.12	0.12	0.12
L-threonine	0.12	0.12	0.12	0.12
Vitamin premix	0.15	0.15	0.15	0.15
Trace mineral premix	0.25	0.25	0.25	0.25
Phytase[5]	0.007	0.022	0.015	0.017
Total	100	100	100	100
Calculated analysis				
Standard ileal digestible (SID) amino acids, %				
Lys	1.24	1.24	1.24	1.24
Ile:lys	63	63	63	63
Leu:lys	128	128	128	128
Met:lys	33	33	33	33
Met & cys:lys	57	57	57	57
Thr:lys	63	63	63	63
Trp:lys	18.7	18.7	18.7	18.7
Val:lys	68	68	68	68
Total lys, %	1.39	1.39	1.39	1.39
ME, kcal/lb[6]	1,516	1,516	1,516	1,516
NE, kcal/lb[6]	1,121	1,120	1,121	1,121
SID lys:ME, g/Mcal	3.71	3.71	3.71	3.71
CP, %	21.6	21.6	21.6	21.6
Crude fiber, %	2.5	2.5	2.5	2.5
Ca, %	0.70	0.70	0.70	0.70
P, %	0.63	0.63	0.63	0.63
Available P, w/o phytase, %	0.30	0.30	0.30	0.30
Available P, %	0.42	0.42	0.42	0.42
Phytase, FTU/kg	350	550	375	850

[1] Quantum Blue G (AB Vista, Plantation, FL).
[2] Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ).
[3] Axtra Phy TPT (Dupont, Wilmington, DE).
[4] Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China).
[5] Phytase products were added at levels expected to release 0.12% available P based on manufacturer recommendations.
[6] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

Table 2. Effect of target conditioning temperature and phytase product on actual conditioning temperature, throughput, and residual phytase activity[1]

	Conditioning temperature, ˚F					Probability, $P <$		
Item	149	167	185	203	SEM	Product × temperature	Linear temperature	Product main effect
Conditioning Temp, ˚F								
Quantum Blue G[2]	152.2	167.4	185.9	200.8	1.11	0.992	0.001	0.761
Ronozyme Hi Phos GT[3]	150.9	167.7	185.4	200.2				
Axtra Phy TPT[4]	151.6	166.5	185.9	199.7				
Microtech 5000 Plus[5]	151.8	168.5	185.5	200.6				
Throughput, lb/hr								
Quantum Blue G	144	135	121	126	8.5	0.621	0.001	0.916
Ronozyme Hi Phos GT	138	144	127	114				
Axtra Phy TPT	145	126	130	111				
Microtech 5000 Plus	141	126	137	126				
Residual phytase activity,[6] %								
Quantum Blue G	99.0	78.2	37.9	21.1	8.80	0.385	0.001	0.001
Ronozyme Hi Phos GT	87.5	59.7	43.3	22.9				
Axtra Phy TPT	80.6	62.0	36.2	33.1				
Microtech 5000 Plus	37.6	21.4	3.5	3.5				

[1] Four replicate pelleting runs were completed for each product at each temperature.
[2] Quantum Blue G (AB Vista, Plantation, FL).
[3] Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ).
[4] Axtra Phy TPT (Dupont, Wilmington, DE).
[5] Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China).
[6] Stability was measured as the analyzed phytase concentration divided by phytase concentration prior to conditioning. Four replicate pelleting runs were completed for each product at each temperature. Within pelleting run, a composite sample consisting of 4 subsamples was used for analysis. Samples were taken from the conditioner.