Stability of four commercial phytase products under increasing thermal conditioning temperatures[1]

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ABSTRACT Phytase is a feed-grade enzyme frequently added to swine diets to help improve the digestibility of phytate phosphorus. However, like any enzyme, it may be subject to heat damage when exposed to thermal processing. Therefore the objective of this experiment was to determine the stability of 4 commercial phytase products exposed to increasing thermal conditioning temperatures in the pelleting process. The 4 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 a corn-soybean meal-based swine diet at a concentration recommended by the manufacturer to provide a 0.12% aP release. Diets were exposed to each of 4 thermal conditioning temperatures (65, 75, 85, and 95°C) and the entire process repeated on 4 consecutive days to create 4 replicates. Samples were taken while feed exited the conditioner and before entering the pellet die. Samples were cooled to room temperature before being stored in plastic bags until analysis. Phytase stability was measured as the residual phytase activity (% of initial) at each conditioning temperature. There were no product × temperature interactions observed for conditioning temperature, conditioner throughput, or residual phytase activity. As target temperature increased, conditioner throughput decreased (linear; $P < 0.001$) and phytase activity decreased (linear; $P < 0.001$) for each product. Residual phytase activity decreased as conditioning temperature increased from 65 to 95°C at a rate of –1.9% for every 1°C increase in conditioning temperature. There was a significant phytase product ($P < 0.001$) main effect which was mainly driven by Microtech 5000 Plus having decreased ($P < 0.05$) phytase activity when compared to all other products at 65, 75, and 85°C. However at 95°C Axtra Phy TPT had greater ($P < 0.05$) residual phytase activity compared with Microtech 5000 Plus, with Quantum Blue G and Ronozyme Hi Phos intermediate. Increasing target conditioning temperatures decreased phytase stability regardless of product. In addition, Microtech 5000 Plus had decreased residual phytase activity (% of initial) when compared to all other products at 65, 75, and 85°C.

Key words: conditioning temperature, pelleting, phytase stability

Transl. Anim. Sci. 2017.1:255–260
doi:10.2527/tas2017.0030

INTRODUCTION

Phytase is an enzyme that breaks down phytate phosphorus and when included in swine diets will increase the amount of phosphorus available to the pig (Simons et al., 1990; Lei et al., 1993; Harper et al., 1997). However, phytase, like any catalytic protein, is subject to damage when exposed to numerous feed processing criteria, including the pelleting process (Jongbloed and Kemme, 1990). Thermal processing affects stability of phytase and each manufacturer has different recommendations for the amount of residual phytase after pelleting.

Several commercial phytase products are available for use in livestock diets. While previous research (Wyss

[1]Contribution no. 214–17-J from the Kansas Agric. Exp. Stn., Manhattan, KS 66506–0210.

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Received April 11, 2017.
Accepted May 10, 2017.

et al., 1998) has shown the heat stability of some phytase products, the heat stability of next generation products that are currently available to the market have not been compared. Thus, the objective of the current study was to evaluate 4 current commercial phytase products when exposed to increasing conditioning temperatures.

MATERIALS AND METHODS

Experimental Design and Diets

The 4 commercial phytase products used were: Quantum Blue G (declared potency of 5,000,000 FTU/kg; AB Vista, Plantation, FL); Ronozyme Hi Phos GT (declared potency of 2,699,282 FYT/kg; DSM Nutritional Products, Parsippany, NJ); Axtra Phy TPT (declared potency of 2,500,000 FTU/kg; Dupont, Wilmington, DE), and Microtech 5000 Plus (declared potency of 5,000,000 FTU/kg; Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China). One phytase unit (FTU or FYT) was defined as the amount of enzyme that catalyzes the release of 1 μmol of inositol P per minute from 5.1 mM sodium phytate in pH 5.5 buffer at 37°C (AOAC, 2009). Phytases were included as part of a corn-soybean meal-based complete swine diet. Concentrations used in formulation were determined by manufacturer recommendations from each product to release 0.12% available P (aP). Commercial phytase products were all obtained from a third party distributor.

Each product was initially mixed with 91 kg of soybean meal using a Wenger (Wenger Manufacturing Inc., Sabetha, KS) 91 kg double ribbon mixer. This was done to ensure proper mixing of the phytase throughout the subsequent 454 kg batches of complete feed used for the experiment (Table 1). The phytase-soybean meal mix was then bagged in 22.7 kg bags and hand added during the batching of complete diets used in the experiment. Complete feed was sacked into 22.7 kg bags after mixing and samples were taken from 10 separate bags to form a composite sample. This composite sample was used to provide initial concentrations of phytase in the diet. The phytase activity from the initial samples was used as a baseline for comparison of all subsequent samples taken during the experiment

Sample Collection and Chemical Analysis

Diets were conditioned at 4 temperatures (65, 75, 85, and 95°C) and the entire process repeated on 4 consecutive days to create 4 replicates. Diets were processed through a CL5 Laboratory Mill (California Pellet Mill; Crawfordsville, IN). Diets were steam conditioned in a 13 × 91 cm single screw conditioner. The mill was equipped with a 15 kg hopper located above the conditioner with a vibratory auger feeding the conditioner. A 45 s retention time was targeted for each diet. At the beginning of each day, flush feed

Table 1. Diet composition (as-fed basis)

Item	%
Ingredient	
Corn	61.37
Soybean meal (46.5% CP)	33.79
Choice white grease	1.50
Monocalcium phosphate (21% P)	1.05
Limestone	1.00
Salt	0.35
L-lysine HCl	0.30
DL-met	0.12
L-thr	0.12
Vitamin premix[1]	0.15
Trace mineral premix[2]	0.25
Phytase[3]	—[4]
Total	100
Calculated analysis	
Standard ileal digestible (SID) amino acids, %	
Lysine	1.24
Ile:lys	63
Leu:lys	128
Met:lys	33
Met & Cys:lys	57
Thr:lys	63
Trp:lys	18.7
Val:lys	68
Total lys, %	1.39
ME, kcal/kg[5]	3341
NE, kcal/kg[5]	2471
SID lys:ME, g/Mcal	3.71
CP, %	21.6
NDF, %	8.5
Ca, %	0.70
P, %	0.63
Available P, w/o phytase, %	0.30
Available P, %	0.42
Phytase, FTU/kg	—[6]

[1]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 1,764 mg vitamin K; 3,307 mg riboflavin; 11,023 mg pantothenic acid; 19,841 mg niacin; and 15.4 mg vitamin B12.

[2]Provided per kg of premix: 26.5 g Mn from manganese oxide; 110 g Fe from iron sulfate; 110 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[3]Phytase products were added at concentrations expected to release 0.12% available P based on manufacturer recommendations.

[4]Quantum Blue G (AB Vista, Plantation, FL) included at 0.015%; Ronozyme Ronozyme Hi Phos GT (DSM Nutritional Products, Parsippany, NJ) included at 0.022%; Axtra Phy TPT (Dupont, Wilmington, DE) included at 0.007%; Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China) included at 0.017%.

[5]NRC. (2012).

[6]Quantum Blue G (AB Vista, Plantation, FL), Ronozyme Ronozyme Hi Phos GT (DSM Nutritional Products), Axtra Phy TPT (Dupont) and Microtech 5000 Plus (Guangdong Vtr Bio-Tech Co., Ltd.) provided 350, 550, 375, and 850 FTU/kg, respectively.

containing no phytase was used to warm the pellet mill to the initial conditioning temperature (65°C) at which point 15 kg of feed from 1 of the 4 products was placed in the hopper above 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 time points during each run for each phytase product. Immediately after sampling, feed was transferred to a pilot scale cooler where sample temperatures were reduced to ambient levels (21°C) within 5 min. After cooling, the 4 subsamples were combined and stored in a plastic bag until analysis.

After feed from the first phytase product exited the conditioner, 15 kg of flush diet was again added to the hopper and used to flush the system. Flush feed contained titanium dioxide as a tracer to verify when flush feed had passed through the conditioner. While the pellet mill was still at the initial conditioning temperature, feed from the second phytase product was added to the hopper. This process continued until all 4 products were conditioned at the initial temperature. When all products had been processed at the initial temperature, flush feed was again added to the hopper and the temperature was increased to the second conditioning temperature (75°C) and stabilized before adding the first phytase treatment. All samples were again processed through the conditioner using procedures similar to those used for the initial temperature samples. This process was replicated for all phytase products at each conditioning temperature. On d 2 of the trial (the next replication), similar procedures were used. However, if a phytase 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 completed for all phytase products to minimize potential 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 of phytase activity (AOAC, 2009).

Statistical Analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. 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 (65, 75, 85, and 95°C). Preplanned contrasts were used to evaluate the interaction between phytase product and temperature, 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

The initial calculated and analyzed phytase activities and the ratio of analyzed to calculated of the complete feed prior to conditioning are reported (Table 2). Calculated values were determined from the minimum declared phytase concentrations as provided by each products manufacturer. The analyzed to calculated ratio was 1.17, 1.52, 1.00, and 1.66 for Quantum Blue G, Ronozyme Hi Phos GT, Axtra Phy TPT, and Microtech 5000 Plus, respectively. The variation present in the analyzed to calculated ratios may be a result of some manufacturers providing a greater concentration of phytase then the minimum declared amount listed for the product (Sulabo et al., 2011). The minimum declared concentrations by each manufacturer are determined using internal company assays which in general are variations from the official AOAC method used in the current experiment (AOAC, 2009). Thus, differences in phytase analysis may have been present when comparing the calculated to analyzed values for d 0.

There were no product × temperature interactions observed for actual conditioning temperature, conditioner throughput, or residual phytase activity (Table 3). As the target temperature was increased, conditioning temperature increased (linear; $P < 0.001$) and conditioner throughput (kg feed/h) decreased (linear; $P < 0.001$). There was no evidence for effects of phytase product on conditioning temperature or conditioner throughput.

As target temperature increased, phytase activity decreased (linear; $P < 0.001$) for all products. Residual phytase activity decreased at a rate of –1.9% for every increase in conditioning temperature of 1°C

Table 2. Calculated and analyzed phytase values of initial feed samples[1]

Item	Calculated, PU[2]/kg	Analyzed, PU/kg	Ratio[3]
Quantum Blue G[4]	350	409	1.17
Ronozyme Hi Phos GT[5]	550	835	1.52
Axtra Phy TPT[6]	375	376	1.00
Microtech 5000 Plus[7]	850	1410	1.66

[1]Values represent means of 2 replicate samples each analyzed in duplicate (AOAC (2009); New Jersey Feed Labs, Trenton, NJ).

[2]PU = phytase units.

[3]Analyzed to calculated ratio.

[4]AB Vista, Plantation, FL.

[5]DSM Nutritional Products, Parsippany, NJ.

[6]Dupont, Wilmington, DE.

[7]Guangdong Vtr Bio-Tech Co., Ltd., Guangdong, China.

Table 3. Effect of target conditioning temperature and phytase product on actual conditioning temperature, throughput, and residual phytase activity[1]

	Conditioning temperature, °C					Probability, $P <$		
Item	65	75	85	95	SEM	Product × temperature	Linear temperature	Product main effect
Conditioning temperature, °C								
Quantum Blue G[2]	66.8	75.2	85.5	93.8	1.11	0.992	0.001	0.761
Ronozyme Hi Phos GT[3]	66.1	75.4	85.2	93.4				
Axtra Phy TPT[4]	66.4	74.7	85.5	93.2				
Microtech 5000 Plus[5]	66.6	75.8	85.3	93.7				
Throughput, kg/h								
Quantum Blue G	65	61	55	57	3.9	0.621	0.001	0.916
Ronozyme Hi Phos GT	63	65	58	52				
Axtra Phy TPT	66	57	59	50				
Microtech 5000 Plus	64	57	62	57				
Residual phytase activity,[6]%								
Quantum Blue G	99.0[a]	78.2 [a]	37.9 [a]	21.1[ab]	8.80	0.385	0.001	0.001
Ronozyme Hi Phos GT	87.5[a]	59.7 [a]	43.3 [a]	22.9[ab]				
Axtra Phy TPT	80.6[a]	62.0 [a]	36.2 [a]	33.1[a]				
Microtech 5000 Plus	37.6[b]	21.4[b]	3.5[b]	3.5[b]				

[a,b]Means within a column with different superscripts differ (P < 0.05).

[1]Four replicate conditioning runs were completed for each product at each temperature. Within conditioning run, a composite sample consisting of 4 subsamples was used for analysis. Samples were taken as feed exited the conditioner.

[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.

between 65 and 95°C. There was a phytase product main effect ($P < 0.001$) which was mainly driven by Microtech 5000 Plus having decreased ($P < 0.05$) phytase activity when compared to all other products with the exception at 95°C where the Quantum Blue G, and Ronozyme Hi Phos GT were intermediate to Axtra Phy TPT and Microtech 5000. There was no evidence for a difference in residual phytase between the Quantum Blue G, Ronozyme Hi Phos GT, or Axtra Phy TPT products at 65, 75, and 85°C.

DISCUSSION

Phytate is the primary storage form for phosphorous in most cereal grains and is unavailable to the growing pig. Of the total P in most plants, 60 to 90% is stored as phytate (Reddy et al., 1982). Phytate P is low in digestibility and, thus, increased amount of P is present in the feces which can lead to increased amounts of P when manure is applied to soil (Greiner and Konietzny, 2006). Phytates have also been shown to interfere with the availability of other nutrients in the diet including, Ca, Zn, Fe, and proteins (Cheryan and Rackis, 1980). To supply the pig with an adequate amount of aP in the diet, inorganic products of P are used in formulation. The phytase enzyme is another option to use in diet formulation and is capable of breaking down phytate and releasing P to the animal. Phytase can be categorized by the site of hydrolysis of the phytate molecule as a 3-phytase or a 6-phytase, which breaks the inositol phytate ring at the 3 or 6 carbon, respectively (Selle and Ravindran, 2007). Not only does phytase work to improve P availability in the diet, it also reduces the amount of P in the feces and subsequent phosphate present in the soil.

Due to the proteolytic structure of phytase, it is susceptible to protein denaturation when exposed to heat (Yao et al., 2011). In the swine industry, pelleting of diets is a common practice to improve both ADG and feed efficiency in growing pigs (Stark et al., 1994; Potter et al., 2010; DeJong et al., 2013). Pelleting conditions can vary, but normally consist of conditioning temperatures ranging from 65 to 95°C, with temperatures varying depending on the feed mill equipment and diet type pelleted. The poultry industry utilizes high pelleting temperatures as a kill-step for bacterial and viral containment (Furuta et al., 1980).

To understand the influence of pelleting temperature on phytase stability, Wyss et al. (1998) investigated 3 acid-based phytases under 2 conditioning temperatures (75 and 85°C). Increasing the conditioning temperature from 75 to 85°C reduced the percentage recovery of all 3 phytase products by 20 to 40%. It was also noted that at 75°C, the recovery of the phytase products ranged from 60 to 70% when compared

to the baseline concentrations suggesting that the lower conditioning temperature was already degrading the phytases. This is similar to the current experiment where all phytase products showed decreased residual phytase activity at 75°C (21 to 78% residual activity) with phytase activities being further decreased as conditioning temperatures were increased. In addition to phytase, other in-feed enzymes may be degraded at increased conditioning temperatures.

In the current experiment, diets were formulated to allow phytase to release 0.12% aP. The decrease in phytase activity found in the current trial as conditioning temperature increased, especially at the highest conditioning temperature (95°C), would have resulted in a reduction of approximately 0.06, 0.07, 0.08, and 0.10% aP for Axtra Phy TPT, Ronozyme Hi Phos GT, Quantum Blue G, and Microtech 5000 Plus, respectively, if analyzed values truly correlate to changes in pig performance. These decreases in aP would have resulted in diets deficient in aP if no safety margin was considered in diet formulation. Feeding growing swine diets deficient in P not only reduces growth performance (Coalson et al., 1972; Mahan, 1982; Ruan et al., 2007) but it can also decrease plasma P concentrations and bone mineralization (Nicodemo et al., 1998). Understanding and accounting for the phytase activity of a diet post-pelleting is crucial to maximize performance of growing pigs and to ensure P deficiencies do not occur.

It should be noted that reductions in the phytase activity from conditioning in the current experiment were beyond what was expected based on manufacturers recommendations. Commercial feed mills currently utilize a wide variety of conditioning temperatures and retention times when manufacturing pelleted feeds (McCracken, 2002). In addition, feed mill equipment such as the specific types of conditioners and pellet mills will vary. The variation in equipment as well as mill ambient temperature can create a large amount of variance in the processing methods used to pellet swine feed. In the current experiment, a lab scale conditioner was utilized. Slominski et al. (2007) evaluated 2 phytase products at 2 separate feed mills in Canada. Hot pellet temperatures for the 2 mills were 67 and 70°C, respectively. There were no differences in the change in phytase activity of the pelleted feed between the 2 mills or between the 2 products. However, pelleting at 67 and 70°C reduced phytase activity from 50 to 63%, respectively, as compared to the baseline sample. In addition to the commercial study, they also conducted an in vitro study evaluating the same 2 phytase products at 60 and 70°C. Both phytase products had significantly reduced activity at the 70°C. Eeckhout (2002) evaluated the effects of die opening diameter, die channel length, and diet composition on phytase stability during pelleting. They observed that a small die opening reduced phytase activity when compared to a larger die opening which was most likely a result of the increased friction and heat produced from the smaller die. In addition, at moderate conditioning temperatures, a longer channel length within the die resulted in decreased phytase activity, which again, was most likely a result of the increase in friction and heat created by a long channel. Lastly, a diet with a greater percentage of fat (22%) and low crude fiber (6%) was also capable of retaining phytase activity during the pelleting process as compared to a diet with low fat (10%) and high crude fiber (16%) content (Eeckhout, 2002). Again, the added fat would have reduced the heat and friction at the die which could have been the reason for improved phytase activity. Data from Slominski et al. (2007) and Eeckhout (2002) both suggest that phytase products may respond differently in a commercially operated mill when compared to a lab scale pellet mill and may also respond differently across mills depending on pellet mill style, operation parameters, and diet composition. As previously stated, this should be taken into account when utilizing data from the trial herein where a single conditioner and diet composition was used in a lab scale pellet mill.

Conclusions

Increasing conditioning temperature linearly reduced residual activity regardless of product. Also, Microtech 5000 Plus had significantly less activity after conditioning compared with other products with no differences among the other 3 products at 65, 75, and 85°C. The current data suggests that conditioning temperatures at and above 65°C negatively affects the phytase activity of each product used in this experiment. While the present data were acquired in a lab scale pellet mill, additional research in different commercially operated pellet mills to further understand thermal feed processing on phytase stability is warranted.

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