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Stability of Four Commercial Microbial Phytase Sources Under Increasing Conditioning Temperatures and Conditioner Retention Times During Pelleting¹

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Summary

This experiment was designed to evaluate the effects of conditioning temperature and retention time on the stability of 4 commercially available microbial phytases in a corn-soybean meal diet. Treatments were arranged in a $4 \times 3 \times 2$ factorial of phytase source (A, B, C, and D), conditioning temperature (180, 190, and 200°F), and conditioner retention time (30 and 60 s). Diets were formulated to release 0.15% phosphorus. A total of 5 mash samples from each treatment were analyzed for phytase. Diets were pelleted via steam conditioning (10 × 55 in. Wenger twin shaft pre-conditioner, Model 150) using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 × 2 in. pellet die. Production rate was set at 10 lb/min, approximately 30% of the rated throughput for the pellet mill. All treatments were replicated on 3 separate days. Retention time was randomized within day and phytase sources were randomized within retention time. Pellets were collected after the die and cooled for 10 min. Five cooled pellet samples per treatment per day were analyzed for phytase. Data were analyzed using the GLIMMIX procedure in SAS 9.4, with pelleting run as the experimental unit and day as the blocking factor.

There was no evidence for a source × conditioning temperature × retention time interaction for hot pellet temperature or phytase stability. Increasing conditioning temperature (180, 190, and 200°F) increased (quadratic, $P < 0.03$) hot pellet temperature (203, 208, and 211°F, respectively). There was no evidence of difference in hot pellet temperature due to retention time. There was a phytase source × conditioning temperature interaction ($P = 0.01$) for phytase stability. At conditioning temperatures of 180, 190, and 200°F, phytase stabilities were 33.7, 17.5, and 16.3% for A; 13.0, 8.3, and 9.0%

¹Appreciation is expressed to DSM Nutritional Products for partial financial support of this project.

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for B; 24.2, 11.2, and 11.8% for C; and 20.7, 11.5, and 9.7% for D; respectively. At conditioning temperatures of 180 and 190°F, phytase A had greater ($P < 0.05$) stability compared to all other sources. At 200°F, there was no evidence of difference between phytase A and C stability, but stability of phytase A was greater ($P < 0.05$) than phytase B and D. Phytase stability of B was less ($P < 0.05$) than that of the other sources when pelleted at 180°F. When pelleted at a conditioning temperature of 200°F, phytase stability was similar between phytase B, C, and D. There was no evidence of difference in phytase stability due to retention time.

Microbial phytase stability was reduced by increasing conditioning temperature, although the amount of reduction was dependent on phytase source. Additionally, a maximum phytase stability of 33% was observed with the equipment used in this study, indicating severe consequences of achieving hot pellet temperatures above 200°F.

Introduction

Phosphorus is stored in plant tissues as phytic acid, a cyclic structure that is not easily digested by animals that lack the phytase enzyme. Limited bioavailability of phytate-bound phosphorus requires nutritionists to over-formulate for phosphorus. However, microbial phytase can be used to release phytate-bound phosphorus within plant-based feed ingredients, resulting in increased phosphorus digestion and decreased phosphorus excretion.⁵ Improved phosphorus utilization requires less phosphorus to be added to diets, simultaneously decreasing feed costs and improving performance.

Due to the growing use of phytase in pelleted diets, it is important to evaluate the effect of thermal processing on phytase stability. It is known that enzyme activity is inhibited by heat,⁶ suggesting that steam pelleting at high conditioning temperatures may damage phytase. Therefore, this experiment was designed to evaluate the effect of conditioning temperature and retention time on the stability of 4 commercially available microbial phytase sources.

Procedures

Treatments were arranged in a $4 \times 3 \times 2$ factorial with phytase source (A, B, C, and D), conditioning temperature (180, 190, and 200°F), and conditioner retention time (30 and 60 s). To ensure diet uniformity between treatments, 8 batches of 2,000 lb of a corn-soybean meal-based diet (Table 1) without phytase were mixed in a 1-ton mixer (Hayes & Stolz, Fort Worth, TX). The first and last 100 lb of each batch were discarded to derive an 1,800-lb batch. All batches were then blended to form 8 separate 1,800 lb batches for phytase source $\times$ retention time treatments. All 3 temperature treatments were derived within each batch of feed. Each 1,800-lb batch was then mixed with the appropriate amount of phytase to release 0.15% phosphorus and sacked.

Diets were steam conditioned (10 $\times$ 55 in. Wenger twin shaft pre-conditioner, Model 150) and pelleted on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 3/16 $\times$ 2 in. pellet die. Production rate was set

⁵Selle, P. H. and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Technol.* 135:1-41.

⁶Spring, P. K. E. Newman, C. Wenk, R. Messikommer, and M. Vukic Vranjes. 1996. Effect of pelleting temperature on the activity of different enzymes. *Poult. Sci.* 75:357-361.

at 10 lb/min, approximately 30% of the rated throughput for the pellet mill. All 24 treatments were pelleted on 3 separate days, thus achieving 3 replications per treatment. Conditioner retention times were calculated by adjusting the conditioner screw speed and dividing the amount of feed in the conditioner by the production rate. Retention time was randomized within day to ensure there were no effects of time due to environmental temperature or humidity. Phytase sources were randomized within retention time to ensure no effects of pelleting order. To begin each run, a corn-soybean meal flush diet was used to warm the mill up to 180°F. Six hundred lb of the first treatment was pelleted at all 3 conditioning temperatures (180, 190, and 200°F, respectively) for one of the two retention times. The pellet mill was then flushed again with the common corn-soybean meal diet. Once all treatments had been pelleted at the first retention time, the pellet mill was adjusted and all diets were pelleted at the second retention time. Conditioning temperature, hot pellet temperature, ambient temperature, ambient humidity, and production rate were recorded at 3 time points during each run (Table 3). Conditioning temperatures were as expected for each treatment. Average ambient temperature and humidity were 86°F and 42%, respectively, and average production rate was 10 lb/min.

Prior to pelleting, a total of 10 mash samples (500 g each) per treatment were collected from all but the first and last bag for each treatment via a grain probe. During each processing run, pellet samples were collected throughout the run and immediately placed in an experimental counter-flow cooler for 10 min. Once pellets were cool, 10 pellet samples (500 g each) per treatment were collected for analysis of phytase. All samples were sent to DSM Nutritional Products (Parsippany, NJ) for phytase analysis. Prior to analysis, mash and pellet samples were composited such that there were 5 mash and 5 pellet samples per treatment analyzed in duplicate for phytase activity according to the official AOAC method⁷ by incubation with sodium phytate. Phytase stability was calculated as the percentage of phytase remaining in pelleted diets averaged across 5 pelleted samples compared to the initial phytase (FYT) averaged across 5 mash samples (Table 2).

Data were analyzed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC), with pelleting run as the experimental unit and day as the blocking factor. Main effects included phytase source, conditioning temperature, and retention time. Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature. Results were considered significant if $P \leq 0.05$ and were considered marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

There was no evidence for a source $\times$ conditioning temperature $\times$ retention time interaction for hot pellet temperature, initial phytase, final phytase, or phytase stability. A source $\times$ conditioning temperature interaction did not exist ($P > 0.11$) for hot pellet temperature, initial or final phytase levels.

Hot pellet temperature increased (quadratic, $P = 0.03$) with increasing conditioning temperature. At conditioning temperatures of 180, 190, and 200°F, hot pellet tempera-

⁷AOAC. 2001. AOAC Official Method 2000.12 Phytase Activity in Feed. J. AOAC Int. 84:629.

tures were 202.5, 207.9, and 211.0°F, respectively. There was no evidence of difference in hot pellet temperature due to retention time or phytase source.

Main effects of phytase source were significant ($P < 0.01$) for initial and final phytase (Table 4). Initial phytase level in mash diets prior to pelleting was greatest for phytase B (1,131 FYT/kg) followed by phytase C (939 FYT/kg) and phytase D (790 FYT/kg) with phytase A (439 FYT/kg) being the lowest. After pelleting, phytase levels were greatest for phytase C (150 FYT/kg) and not different for phytase A, B, and D (99, 116, and 113 FYT/kg, respectively). Initial and final phytase levels were greater ($P < 0.01$) for 30 s treatments than 60 s treatments. By design, initial phytase levels were not different ($P = 1.00$) between conditioning temperatures.

There was a source $\times$ conditioning temperature interaction ($P = 0.01$; Figure 1) for phytase stability. At conditioning temperatures of 180 and 190°F, phytase A had greater stability compared to all other phytase sources (Table 3). At 200°F, phytase A stability was not different than phytase C, but greater than phytase B and D. At 180°F conditioning temperature, phytase stability of phytase B was less than that of the other phytase sources and not different than the stability of phytase C or D at 190 and 200°F. As conditioning temperature increased, phytase stability across all phytase sources decreased (quadratic, $P < 0.01$), with a majority of the decrease occurring from 180 to 190°F. Main effect of phytase source was significant ($P < 0.01$) for phytase stability. Across all conditioning temperatures and retention times, phytase stability was greatest for phytase A (22.5%) and least for phytase B (10.1%), with phytase C and D being intermediate (15.7 and 13.9%, respectively; Table 4). There was no evidence of difference in phytase stability ($P = 0.11$) due to retention time (Table 5).

Results of this experiment show that microbial phytase levels and phytase stability is reduced by increasing conditioning temperature, although the amount of reduction is dependent on phytase source. Additionally, there was no evidence that retention time affected phytase stability. The maximum phytase stability of 33% observed with equipment and production rate used in this study indicates severe consequences of achieving hot pellet temperatures above 200°F.

Table 1. Composition of diets (as-fed basis)¹

Ingredient, %	Diet
Corn	54.99
Soybean meal, 47% crude protein	37.60
DDGS ²	4.00
Choice white grease	0.75
Defluorinated phosphate	0.66
Limestone	0.95
Salt	0.35
L-Lysine HCl	0.19
DL-Methionine	0.17
Choline chloride	0.10
Vitamin trace mineral premix ³	0.25

¹Phytase sources were added to the diet in place of corn and formulated to release 0.15% nPP. Phytase A was added at 20.6 g/ton, phytase B at 45.9 g/ton, phytase C at 9.5 g/ton, and phytase D at 41.2 g/ton.

²DDGS = distillers dried grains with solubles.

³Provided per lb of diet; vitamin A, 1,400,000 IU; vitamin D₃, 500,000 IU; vitamin E, 3,000 IU; vitamin B₁₂, 2 mg; menadione, 150 mg; riboflavin, 1,200 mg; thiamine, 200 mg; D-pantothenic acid, 1,200 mg; niacin, 5,000 mg; vitamin B₆, 250 mg; folic acid, 125 mg; choline, 70,000 mg; biotin, 6 mg.

Table 2. Analyzed phytase levels in mash samples of diets containing 4 commercially available microbial phytase sources prior to pelleting¹

Retention time:	30 s				60 s			
Phytase source:	A	B	C	D	A	B	C	D
Initial phytase, ² FYT/kg	426	1,217	1,058	854	453	1,045	820	725
CV, ³ %	17.6	27.3	14.0	43.4	17.4	26.1	19.8	43.5

¹Four commercial microbial phytase sources (A, B, C, and D) were added to a corn-soybean meal diet. Diets were mixed as 8 separate 1,800 lb batches.

²Phytase sources were added to the diet in place of corn and formulated to release 0.15% nPP.

³A total of 30 mash samples (500 g each) were collected within each batch. Pairs of mash samples were combined such that 15 separate mash samples for each batch were analyzed for initial phytase levels according to the official AOAC method. Phytase results were averaged within source × retention time treatments (total of 15 samples). CV was then calculated using the following equation, CV = (FYT standard deviation of 15 samples ÷ mean FYT of 15 samples) × 100.

Table 3. Phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 3 conditioning temperatures¹

	Phytase source				SEM ²	Probability, <i>P</i> <
Item	A	B	C	D		Source × temp
Hot pellet temperature, °F						
180	204.5	202.2	202.7	200.7	0.83	0.11
190	207.3	208.3	208.2	207.7		
200	211.2	211.5	210.0	211.3		
Initial phytase, FYT/kg						
180	439	1,131	939	790	98.2	1.00
190	439	1,131	939	790		
200	439	1,131	939	790		
Final phytase, FYT/kg						
180	148	150	231	170	22.1	0.34
190	77	98	105	92		
200	73	100	113	76		
Phytase stability, ³ %						
180	33.7 ^a	13.0 ^{def}	24.2 ^b	20.7 ^{bc}	1.86	0.01
190	17.5 ^{cd}	8.3 ^f	11.2 ^f	11.5 ^{ef}		
200	16.3 ^{cde}	9.0 ^f	11.8 ^{ef}	9.7 ^f		

¹Four commercial microbial phytase sources (A, B, C, and D) were added to a corn-soybean meal diet and steam pelleted (10 in. width $\times$ 55 in. length Wegner twin shaft pre-conditioner, Model 150) at 3 conditioning temperatures (180, 190, and 200°F) and 2 retention times (30 and 60 s) on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 in. $\times$ 2 in. pellet die over 3 days of replication.

²Pooled standard error of least squares means ($n = 3$).

³Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

^{a,b,c,d,e,f}Means without a common superscript differ ($P < 0.05$).

Table 4. Main effects of phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 2 retention times and 3 conditioning temperatures¹

Item	Phytase source				SEM ²	Probability, $P <$
	A	B	C	D		Source
Hot pellet temperature, °F	207.7	207.3	206.9	206.6	0.48	0.39
Initial phytase, FYT/kg	439 ^d	1,131 ^a	939 ^b	790 ^c	86.6	0.01
Final phytase, FYT/kg	99 ^b	116 ^b	150 ^a	113 ^b	17.5	0.01
Phytase stability, ³ %	22.5 ^a	10.1 ^c	15.7 ^b	13.9 ^b	1.21	0.01

¹Four commercial microbial phytase sources (A, B, C, and D) were added to a corn-soybean meal diet and steam pelleted (10 in. width × 55 in. length Wegner twin staff pre-conditioner, Model 150) at 3 conditioning temperatures (180, 190, and 200°F) and 2 retention times (30 and 60 s) on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 in. × 2 in. pellet die over 3 days of replication.

²Pooled standard error of least squares means ($n = 3$).

³Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

^{a,b,c,d}Means within a row without a common superscript differ ($P < 0.05$).

Table 5. Main effects of retention time and conditioning temperature on phytase stability of 4 commercially available microbial phytase sources in diets pelleted at 2 retention times and 3 conditioning temperatures¹

Item	Retention time, s		SEM ²	Conditioning temp, °F			SEM	Probability, $P <$		
	30	60		180	190	200		Retention	Linear	Quadratic
Hot pellet temperature, °F	207.1	207.1	0.34	202.5	207.9	211.0	0.41	0.95	0.01	0.03
Initial phytase, FYT/kg	889 ^a	761 ^b	83.5	825	825	825	85.1	0.01	1.00	1.00
Final phytase, FYT/kg	135 ^a	104 ^b	16.1	175	93	90	16.8	0.01	0.01	0.01
Phytase stability, ³ %	16.4	14.8	0.98	22.9	12.1	11.7	1.10	0.11	0.01	0.01

¹Four commercial microbial phytase sources (A, B, C, and D) were added to a corn-soybean meal diet and steam pelleted (10 in. width × 55 in. length Wegner twin staff pre-conditioner, Model 150) at 3 conditioning temperatures (180, 190, and 200°F) and 2 retention times (30 and 60 s) on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 in. × 2 in. pellet die over 3 days of replication.

²Pooled standard error of least squares means ($n = 3$).

³Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

^{a,b}Means within a row without a common superscript differ ($P < 0.05$).

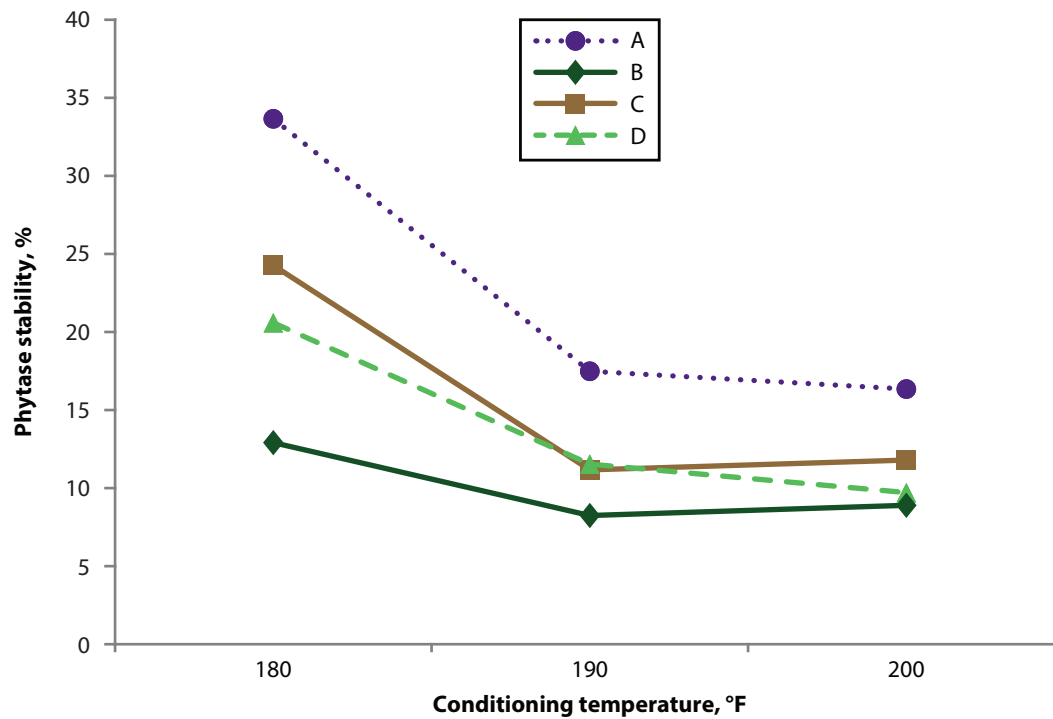


Figure 1. Interaction between phytase source and conditioning temperature in a corn-soybean meal diet containing 4 commercially available microbial phytase sources pelleted at 3 conditioning temperatures and 2 retention times ($P = 0.01$).