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## Effect of Steam Pressure and Conditioning Temperature During the Pelleting Process on Phytase Stability

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## Effect of Steam Pressure and Conditioning Temperature During the Pelleting Process on Phytase Stability

### Abstract

This experiment was designed to evaluate the effects of steam pressure and conditioning temperature on the stability of microbial phytase. Treatments were arranged as a 2 × 3 factorial of steam pressure (24 and 44 psi) and conditioning temperature (170, 180, and 190°F). Phytase was added to a corn-soybean meal-based diet and mash samples were collected for phytase analysis. The diet was pelleted via steam conditioning (10 × 55 in Wenger twin staff pre-conditioner, Model 150) and using a pellet mill (CPM Model 1012-2) with a 3/16 × 1 1/4 in pellet die (L:D 6.7). Conditioner retention time was set at 30 sec and production rate was set at 33 lb/min, approximately 100% of the rated throughput for the pellet mill. All treatments were replicated on 3 separate days. For each treatment, pellet and conditioned mash samples were composited such that 2 samples of each were analyzed for phytase activity and pellet durability index (PDI). Moisture analysis was conducted on initial mash, conditioned mash, hot pellet, and cooled pellet samples. Conditioning temperature, hot pellet temperature (HPT), and production rate were recorded throughout each processing run. 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 ( $P > 0.17$ ) for a steam pressure × conditioning temperature interaction for HPT, phytase stability, moisture, or PDI. Increasing conditioning temperature from 170 to 190°F increased (linear,  $P < 0.01$ ) HPT. There was no evidence for difference ( $P = 0.80$ ) in HPT between steam pressures. Phytase stability of conditioned mash decreased (linear,  $P < 0.01$ ) with increasing conditioning temperature. In cooled pellets, phytase stability decreased (linear,  $P < 0.01$ ) with increasing conditioning temperature. Cooled pellets tended ( $P = 0.08$ ) to have greater phytase stability when steam pressure was set at 44 psi compared to 24 psi. Moisture of conditioned mash and pellets increased (linear,  $P \leq 0.05$ ) with increasing conditioning temperature, and PDI tended (linear,  $P = 0.06$ ) to increase with increasing conditioning temperature. There was no evidence ( $P > 0.35$ ) that steam pressure affected feed moisture or PDI.

Results of this experiment show that phytase stability in conditioned mash and pellets decreases linearly when the conditioning temperature rises above 170°F and HPT above 179°F. As expected, HPT increased and feed moisture tended to increase with increasing conditioning temperature. Increasing steam pressure from 24 to 44 psi resulted in tendencies for greater phytase stability in pellets and had no effect on HPT or feed moisture.

### Keywords

conditioning temperature, pelleting, phytase stability, steam pressure

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### Cover Page Footnote

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

### Authors

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## Effect of Steam Pressure and Conditioning Temperature During the Pelleting Process on Phytase Stability<sup>1</sup>

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### Summary

This experiment was designed to evaluate the effects of steam pressure and conditioning temperature on the stability of microbial phytase. Treatments were arranged as a  $2 \times 3$  factorial of steam pressure (24 and 44 psi) and conditioning temperature (170, 180, and 190°F). Phytase was added to a corn-soybean meal-based diet and mash samples were collected for phytase analysis. The diet was pelleted via steam conditioning (10 × 55 in Wenger twin staff pre-conditioner, Model 150) and using a pellet mill (CPM Model 1012-2) with a 3/16 × 1 1/4 in pellet die (L:D 6.7). Conditioner retention time was set at 30 sec and production rate was set at 33 lb/min, approximately 100% of the rated throughput for the pellet mill. All treatments were replicated on 3 separate days. For each treatment, pellet and conditioned mash samples were composited such that 2 samples of each were analyzed for phytase activity and pellet durability index (PDI). Moisture analysis was conducted on initial mash, conditioned mash, hot pellet, and cooled pellet samples. Conditioning temperature, hot pellet temperature (HPT), and production rate were recorded throughout each processing run. 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 ( $P > 0.17$ ) for a steam pressure × conditioning temperature interaction for HPT, phytase stability, moisture, or PDI. Increasing conditioning temperature from 170 to 190°F increased (linear,  $P < 0.01$ ) HPT. There was no evidence for difference ( $P = 0.80$ ) in HPT between steam pressures. Phytase stability of conditioned mash decreased (linear,  $P < 0.01$ ) with increasing conditioning temperature. In cooled pellets, phytase stability decreased (linear,  $P < 0.01$ ) with increasing conditioning temperature. Cooled pellets tended ( $P = 0.08$ ) to have greater phytase stability when steam pressure was set at 44 psi compared to 24 psi. Moisture of conditioned mash and pellets increased (linear,  $P \leq 0.05$ ) with increasing conditioning temperature, and PDI tended (linear,  $P = 0.06$ ) to increase with increasing conditioning temperature. There was no evidence ( $P > 0.35$ ) that steam pressure affected feed moisture or PDI.

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

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Results of this experiment show that phytase stability in conditioned mash and pellets decreases linearly when the conditioning temperature rises above 170°F and HPT above 179°F. As expected, HPT increased and feed moisture tended to increase with increasing conditioning temperature. Increasing steam pressure from 24 to 44 psi resulted in tendencies for greater phytase stability in pellets and had no effect on HPT or feed moisture.

## 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.<sup>4</sup> Improved phosphorus utilization requires less phosphorus to be added to diets, simultaneously decreasing feed costs and improving performance.

Due to the increasing use of phytase in pelleted diets, it is important to evaluate the effect of thermal processing on phytase stability. Previous research at Kansas State University has shown that phytase activity is inhibited by heat addition through increasing conditioning temperature.<sup>5</sup> However, it is speculated that moisture may be a contributing factor in the phytase stability matrix; specifically, increasing moisture results in decreased phytase stability. Manipulations in steam pressure can alter the quality of steam, resulting in changes in moisture and frictional heat, and thus hot pellet temperature. Therefore, this experiment was designed to evaluate the effect of steam pressure and conditioning temperature on phytase stability, feed moisture, and pellet quality.

## Procedures

Treatments were arranged as a 2 × 3 factorial of steam pressure (24 and 44 psi) and conditioning temperature (170, 180, and 190°F). Phytase was added to a corn-soybean meal-based diet at 3 lb/ton (Table 1) and diets were mixed in a 1-ton horizontal, counterpoise mixer (Hayes & Stolz, Fort Worth, TX). Prior to pelleting, initial mash samples of the diet were collected for phytase analysis. Diets were steam conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 sec at 170, 180, or 190°F and subsequently pelleted using a 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 3/16 × 1 1/4 in pellet die (L:D 6.7). Conditioner retention time was calculated by adjusting the conditioner screw speed and dividing the amount of feed in the conditioner by the production rate. Production rate was set at 33 lb/min, approximately 100% of the rated throughput for the pellet mill. All treatments were replicated on 3 separate days, thus achieving 3 replications per treatment. Steam pressure was randomized across day to minimize the effects of pelleting order. A conventional corn-soybean meal flush diet without phytase was used

<sup>4</sup> Selle, P. H. and V. Ravindran. 2007. Microbial phytase in poultry nutrition. *Anim. Feed Sci. Technol.* 135:1-41.

<sup>5</sup> Truelock, C. N., A. D. Yoder, C. E. Evans, C. R. Stark, S. S. Dritz, J. W. Wilson, N. E. Ward, and C. B. Paulk. 2018. Stability of Four Commercial Microbial Phytase Sources Under Increasing Conditioning Temperatures and Conditioner Retention Times During Pelleting. *Kansas Agricultural Experiment Station Research Reports: Vol. 4: Iss. 9.*

to warm the mill up to 170°F before each day of pelleting. Six hundred lb of the diet with phytase was pelleted at all 3 conditioning temperatures (170, 180, and 190°F, respectively) at one of the two steam pressures. When pelleting at the first steam pressure was completed, the pellet mill was shut down, steam pressure was adjusted via the Masoneilan valve at the steam harness prior to the conditioner, and the diet with phytase was again pelleted at all 3 conditioning temperatures following warm-up of the pellet mill. Conditioning temperature, hot pellet temperature (HPT), and production rate were recorded at 5 time points during each run (Table 2).

For each treatment, 3 conditioned mash samples were collected as feed exited the conditioner prior to the pellet die, and immediately cooled in an experimental counter-flow cooler for 15 min. Conditioned mash samples were then composited into 2 samples for phytase analysis. Likewise, 5 pellet samples per treatment were collected as feed exited the pellet die, cooled for 15 min, and composited into 2 samples for phytase analysis. All samples were sent to DSM Nutritional Products (Parsippany, NJ) for phytase analysis. Samples were analyzed in duplicate for phytase activity according to the official AOAC method<sup>6</sup> by incubation with sodium phytate. Phytase stability was calculated as the percentage of phytase (FYT) remaining in the pelleted diets compared to the initial mash samples.

Composite samples of initial mash, conditioned mash, and cooled pellets were analyzed for moisture and pellet durability index (PDI). Additionally, a single hot pellet sample per treatment was collected as pellets exited the pellet die and was immediately frozen for moisture analysis. Samples for moisture analysis were ground with a mortar and pestle, weighed to 1 g, and placed in a forced air oven for 24 h at 105°C. Moisture was calculated as the percentage of weight loss after drying. For analysis of PDI, fines were sifted off from cooled pellets using a U.S. No. 5 (4 mm) sieve. One hundred g of the sifted pellets were placed into a Holmen 100 pellet tester and agitated with air for 60 sec. Following agitation, the sample was again sifted through a No. 5 sieve and the remaining pellets were weighed. Pellet durability index was calculated as the percentage of the initial pellet sample remaining after agitation with air.

Data were analyzed using the GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC), with pelleting run as the experimental unit and day as the blocking factor. Main effects included steam pressure and conditioning temperature. 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 ( $P > 0.17$ ) for a steam pressure  $\times$  conditioning temperature interaction for HPT, phytase stability, moisture, or PDI (Table 2).

Production rate and conditioning temperature were as expected for each treatment. Increasing conditioning temperature from 170 to 190°F increased (linear,  $P < 0.01$ ) HPT, and there was no evidence of difference ( $P = 0.80$ ) in HPT between steam pressures.

<sup>6</sup> AOAC. 2001. AOAC Official Method 2000.12 Phytase Activity in Feed. J. AOAC Int. 84:629.

Phytase concentrations (FYT/kg) of conditioned mash and cooled pellets were reduced by increasing conditioning temperature (linear,  $P < 0.01$ ). Additionally, there was a tendency ( $P = 0.07$ ) for greater phytase concentrations in cooled pellets when steam pressure was set at 44 psi compared to 24 psi. Steam pressure had no effect ( $P = 0.29$ ) on phytase concentrations in conditioned mash. Phytase concentrations were used to calculate phytase stability, and the resulting stability data closely mirrored that which was previously mentioned. Increasing conditioning temperature from 170 to 190°F decreased (linear,  $P < 0.01$ ) phytase stability of conditioned mash. In cooled pellets, phytase stability was also decreased (linear,  $P < 0.01$ ) with increasing conditioning temperature. Cooled pellets tended ( $P = 0.08$ ) to have greater phytase stability when steam pressure was set at 44 psi compared to 24 psi.

Average moisture of initial mash prior to pelleting was 13.6%. Differences in moisture between the two steam pressure treatments were not observed ( $P > 0.35$ ) in conditioned mash, hot pellets, or cooled pellets. Moisture of conditioned mash and pellets increased (linear,  $P \leq 0.05$ ) with increasing conditioning temperature. There was no evidence of difference ( $P > 0.47$ ) in moisture of hot pellets due to conditioning temperature or steam pressure.

Pellet durability index tended to increase (linear,  $P = 0.06$ ) with increasing conditioning temperature. There was no evidence for difference ( $P = 0.48$ ) in PDI when feed was steam conditioned at 24 or 44 psi.

Similar to previous data, results of this experiment demonstrate that phytase stability in conditioned mash and pellets decreases linearly when conditioning temperature rises above 170°F and HPT above 179°F. With parameters used in this study, phytase stability averaged 109.6, 67.8, and 32.1% in conditioned mash and 51.8, 24.7, and 8.0% in pellets at conditioning temperatures of 170, 180, and 190°F and HPT of 179.2, 186.0, and 192.3°F, respectively. As expected, HPT and feed moisture increased and PDI tended to increase with increasing conditioning temperature. Steam pressure had no effect on feed moisture or HPT. Increasing steam pressure from 24 to 44 psi had no effect on phytase stability in conditioned mash and resulted in tendencies for greater phytase stability in pellets.

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**Table 1. Diet composition<sup>1</sup>**

Ingredient	%, as-is
Ground corn	69.07
Soybean meal, 47% crude protein	26.48
Choice white grease	1.50
Monocalcium phosphate	0.55
Limestone	1.13
Salt	0.35
L-Lysine HCl	0.31
DL-Methionine	0.07
L-Threonine	0.09
Trace mineral premix	0.15
Vitamin premix	0.15
Phytase <sup>2</sup>	0.15

<sup>1</sup>Diets were mixed in a 1-ton Hayes & Stolz horizontal counterpoise mixer with a 60 sec dry mix time and 120 sec wet mix time.

<sup>2</sup>HiPhos 2700 was superdosed in the diet to minimize analytical variation due to small inclusion levels.

**Table 2. Effect of steam pressure and conditioning temperature on hot pellet temperature, phytase stability, and pellet durability index of a finishing swine diet<sup>1</sup>**

Target conditioning temp, °F:	Steam pressure, psi						SEM <sup>2</sup>	Probability, <			
	24			44				psi	Linear <sup>3</sup>	Quadratic	psi × temp
	170	180	190	170	180	190					
Conditioning temp, °F	170.2	180.3	190.1	170.3	180.8	190.5	0.21	0.08	<0.01	0.10	0.69
Production rate, lb/min	34.0	34.2	34.0	33.8	33.9	34.2	0.18	0.42	0.28	0.66	0.46
Hot pellet temp, °F	179.1	186.2	192.0	179.3	185.9	192.6	0.88	0.80	<0.01	0.72	0.85
Phytase, FYT/kg											
Initial mash	4,790	4,790	4,790	4,836	4,867	4,821	163.4	0.61	0.95	0.85	0.98
Conditioned mash	4,961	3,115	1,654	5,310	3,493	1,737	296.4	0.29	<0.01	0.67	0.86
Cooled pellet	2,492	1,090	395	2,487	1,509	706	186.0	0.07	<0.01	0.11	0.35
Phytase stability, <sup>4</sup> %											
Conditioned mash	106.0	64.2	31.5	113.1	71.4	32.7	8.66	0.38	<0.01	0.62	0.88
Cooled pellet	52.4	20.3	4.7	51.2	29.0	11.4	4.09	0.08	<0.01	0.07	0.26
Moisture, <sup>5</sup> %											
Conditioned mash	15.9	17.5	18.2	17.5	16.6	18.1	0.63	0.65	0.05	0.48	0.17
Hot pellet	17.5	17.4	17.6	17.4	17.7	17.8	0.39	0.67	0.47	0.94	0.84
Cooled pellet	14.3	14.5	14.8	14.5	14.6	14.7	0.20	0.35	0.03	0.96	0.61
Pellet durability index, %	78.7	83.3	84.1	80.9	83.3	86.5	2.58	0.48	0.06	0.74	0.87

<sup>1</sup>A corn-soybean meal finishing swine diet was mixed in a 1-ton Hayes & Stolz horizontal counterpoise mixer. HiPhos 2700 was added to the diet at 3 lb/ton and steam pelleted (10 in width × 55 in length Wenger twin staff pre-conditioner, Model 150) for approximately 30 sec at 2 steam pressures (24 and 44 psi) and 3 conditioning temperatures (170, 180, and 190°F) on a 1-ton, 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) with a 3/16 × 1 1/4 in pellet die (L:D 6.7).

<sup>2</sup>Pooled standard error of least squares means (*n* = 3).

<sup>3</sup>Linear and quadratic contrasts were used to evaluate the effect of conditioning temperature.

<sup>4</sup>Phytase stability was calculated as the percentage of phytase (FYT) remaining in pelleted diets compared to initial phytase analyzed in the mash samples.

<sup>5</sup>Average initial moisture of mash feed was 13.6%.