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Abstract

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in animal feed. However, pelleting is a thermal process that can denature phytase. It is hypothesized that there are many factors that can account for phytase denaturing during the pelleting process, such as pellet mill model, die length to diameter ratio (L:D), steam quality, and residence time in conditioner and die. Therefore, the objective of this experiment was to determine the effect of pellet mill model, die thickness, and die retention time on pellet quality and phytase stability. Treatments were arranged as a completely randomized design to determine the effect of die retention time (RT). Diets were pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 3/16 × 2 in (10.6 L:D), a 3/16 × 1 1/4 in (6.6 L:D) or a 3/16 × 1 3/4 in (9.3 L:D) with 30 sec conditioning retention time at 185°F with designated production rate. These processing conditions were used to create the following RT treatments: 10.6 L:D with 4.3 sec RT, 10.6 L:D with 2.9 sec RT, 9.3 L:D with 1.7 sec RT, 9.3 L:D with 1.1 sec RT, 6.6 L:D with 2.6 sec RT, and 6.6 L:D with 1.6 sec RT. The pellet mills were run 3 separate times to provide 3 replicates for each treatment. There was an overall effect ($P < 0.001$) of treatment on phytase stability in cooled pellets. When using the 1012 PM, phytase was more stable regardless of die retention time when diets were manufactured using the 6.6 L:D die compared to the 10.6 L:D die ($P < 0.05$). The hot pellet temperature of 10.6 L:D die was 195–211°F, while 6.6 L:D die was 184–189°F. However, the phytase stability was similar between the feed pelleted with 1012 PM equipped with 6.6 L:D die and the 3016 PM equipped with 9.3 L:D regardless of retention time ($P > 0.05$). The hot pellet temperature of feed pelleted with the 1012 PM equipped with 6.6 L:D die was 184–189°F, while the feed pelleted with the 3016 die equipped with 9.3 L:D die was 180–183°F. There was also a quadratic decrease in phytase stability as the die L:D increased ($P < 0.0001$). There- fore, the pellet mill size or die retention time did not affect phytase stability when the hot pellet temperature was less than 189°F. Pellet quality increased (linear; $P < 0.0001$ for standard pellet durability index (PDI) or quadratic; $P < 0.0001$ for modified PDI) as die L:D increased. The die L:D had greater effects on both PDI methods than the die retention time. However, increased die retention time improved ($P < 0.05$) pellet quality when the feed was pelleted with 6.6 L:D, but not when pelleted using the 9.3 or 10.6 L:D. In conclusion, the phytase that was produced by *Trichoderma reesei* strain could tolerate hot pellet temperatures up to 189°F, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures ranged from 195–211°F. Therefore, hot pellet temperatures should be measured to monitor phytase stability. Increasing the die L:D had the greatest effect on improving pellet quality.

Keywords

retention time, phytase stability, pelleting

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Effect of Die Retention Time on Pellet Quality and Phytase Stability of a Corn-Soybean Meal Swine Diet

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Summary

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in animal feed. However, pelleting is a thermal process that can denature phytase. It is hypothesized that there are many factors that can account for phytase denaturing during the pelleting process, such as pellet mill model, die length to diameter ratio (L:D), steam quality, and residence time in conditioner and die. Therefore, the objective of this experiment was to determine the effect of pellet mill model, die thickness, and die retention time on pellet quality and phytase stability. Treatments were arranged as a completely randomized design to determine the effect of die retention time (RT). Diets were pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 3/16 × 2 in (10.6 L:D), a 3/16 × 1 1/4 in (6.6 L:D) or a 3/16 × 1 3/4 in (9.3 L:D) with 30 sec conditioning retention time at 185°F with designated production rate. These processing conditions were used to create the following RT treatments: 10.6 L:D with 4.3 sec RT, 10.6 L:D with 2.9 sec RT, 9.3 L:D with 1.7 sec RT, 9.3 L:D with 1.1 sec RT, 6.6 L:D with 2.6 sec RT, and 6.6 L:D with 1.6 sec RT. The pellet mills were run 3 separate times to provide 3 replicates for each treatment. There was an overall effect ($P < 0.001$) of treatment on phytase stability in cooled pellets. When using the 1012 PM, phytase was more stable regardless of die retention time when diets were manufactured using the 6.6 L:D die compared to the 10.6 L:D die ($P < 0.05$). The hot pellet temperature of 10.6 L:D die was 195–211°F, while 6.6 L:D die was 184–189°F. However, the phytase stability was similar between the feed pelleted with 1012 PM equipped with 6.6 L:D die and the 3016 PM equipped with 9.3 L:D regardless of retention time ($P > 0.05$). The hot pellet temperature of feed pelleted with the 1012 PM equipped with 6.6 L:D die was 184–189°F, while the feed pelleted with the 3016 die equipped with 9.3 L:D die was 180–183°F. There was also a quadratic decrease in phytase stability as the die L:D increased ($P < 0.0001$). Therefore, the pellet mill size or die retention time did not affect phytase stability when the hot pellet temperature was less than 189°F. Pellet quality increased (linear; $P < 0.0001$ for standard pellet durability index (PDI) or quadratic; $P < 0.0001$ for modified PDI) as die L:D increased. The die L:D had greater effects on both PDI methods than the

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die retention time. However, increased die retention time improved ($P < 0.05$) pellet quality when the feed was pelleted with 6.6 L:D, but not when pelleted using the 9.3 or 10.6 L:D. In conclusion, the phytase that was produced by *Trichoderma reesei* strain could tolerate hot pellet temperatures up to 189°F, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures ranged from 195–211°F. Therefore, hot pellet temperatures should be measured to monitor phytase stability. Increasing the die L:D had the greatest effect on improving pellet quality.

Introduction

Phytase is a phosphohydrolytic enzyme that releases phosphorus from phytate in cereal grains, which are the main ingredients in animal feed. This enzyme can be used to reduce phosphorus content in manure by reducing inorganic phosphorus inclusion in feed. There are benefits of pelleted feed over mash feed such as improved handling characteristics, feed conversion ratio, and average daily gain.[3] However, the pelleting process is a thermal process that can denature phytase. This has led companies to produce phytase products that are classified as thermostable. Previous research has demonstrated different responses on stability of thermostable phytases when the feed was pelleted at a constant temperature. When pelleting diets containing *Trichoderma reesei* phytase at 185°F, percentage phytase stability has ranged from 38–74% depending on the research project. However, there is no clear explanation of the difference in stability between experiments. It is hypothesized that many factors may account for the differences in stability, such as phytase source, pellet mill model, die length to diameter ratio (L:D), steam quality, and residence time in conditioner and die. Therefore, the objective of this experiment was to determine the effect of die retention time on pellet quality and phytase stability.

Procedures

Treatments were arranged as a completely randomized design to determine the effect of die RT on pellet quality and phytase (*Trichoderma reesei* derived phytase, Quantum Blue 10G, AB Vista, Plantation, FL) stability. A corn-soybean meal swine finishing diet was used for the experiment. The ingredients were added to a 1.64 m^3 twin counterpoise mixer (Hayes and Stolz model TRDB63-0152, Fort Worth, TX) and mixed for 1 min dry mix and 2 min wet mix time. The mixture was then pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 3/16 × 2 in (10.6 L:D), a 3/16 × 1 ¼ in (6.6 L:D) or a 3/16 × 1 ¾ in (9.3 L:D) with a 30 sec conditioning retention time at 185°F(Table 1). The experiment was designed to use designated production rates to create the following RT treatments: 10.6 L:D with 4.2 sec RT, 10.6 L:D with 2.6 sec RT, 9.3 L:D with 1.6 sec RT, 9.3 L:D with 1.1 sec RT, 6.6 L:D with 2.6 sec RT, and 6.6 L:D with 1.6 sec RT. However, the actual production rates achieved resulted in the following RT treatments that will be discussed throughout: 10.6 L:D with 4.3 sec RT, 10.6 L:D with 2.9 sec RT, 9.3 L:D with 1.7 sec RT, 9.3 L:D with 1.1 sec RT, 6.6 L:D with 2.6 sec RT, and 6.6 L:D with 1.6 sec RT. The pellet mills (PM) were run 3 separate times to provide 3 replicates for each treatment. Samples were collected during discharge from mixer, after conditioning,

[3] Stark C.R. (2012) Feed processing to maximize feed efficiency. In: Patience J.F. (eds) Feed efficiency in swine. Wageningen Academic Publishers, Wageningen.

and after pelleting. The condition mash samples were cooled using experimental 153 mm axial fans and the pellet samples were cooled using an experimental counter flow cooler for 10 min. The mash samples were analyzed for phytase activity and the pellet samples were analyzed for phytase activity, pellet durability index, and modified pellet durability index.

Hu[4] calculated the residence time in the extruder by multiplying the extruder's volume by the degree of fill divided by volumetric throughput. Similar to Hu's theory, the following equation is proposed to calculate feed retention time in the pellet die. Feed RT in the die can be defined as the amount of material in the die divided by the material flow rate through the die. The amount of mixture in the die should account for only the effective thickness of the die.

$$RT\ (sec) = \frac{Amount\ of\ material\ in\ the\ effective\ length\ of\ the\ die\ (lb)}{Mass\ flow\ rate\ (lb/sec)}$$

The amount of material in the effective length of die (lb) equals multiplication of the internal die surface area (inch^2), number of holes per inch^2, effective volume per hole (inch^3), and material density (lb/inch^3).

Both mash and pellet samples were sent to AB Vista Inc. (Pantation, FL) for phytase analysis. The phytase stability of condition mash or cooled pellets was calculated by multiplying phytase level of the condition mash or cooled pellet sample by 100 then dividing by average phytase level of mixer samples, respectively.

To measure PDI, a sample was sifted by standard US No. 5 sieve. A 500-g sifted sample was placed in the tumble box. The tumble box was turned for 500 revolutions. The sample was poured out of the tumble box and sifted by a No. 5 sieve then the remaining sample was weighed on the sieve. The standard PDI was calculated by multiplying the remaining sample by 100 and then dividing by the initial 500 g.[5] The modified PDI was performed the same way as standard PDI, but three ¾ inch nuts were added to the tumble box.

Data were analyzed as a completely randomized design to determine the effect of die retention time on phytase stability and pellet quality. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS (v. 9.4, SAS Institute, Inc., Cary, NC). Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

The average phytase level was 6,495 FTU/g for the *Trichoderma reesei* phytase enzyme source and 568 FTU/lb for the mixed diets. Based on the phytase source concentration, the mixer sample should have been 589 FTU/lb. The condition mash samples were heated for 29–39 sec in the conditioner at 179–188°F, as reported in Table 2. Average

[4] Hu, G. H. Reactive Polymer Processing: Fundamentals of REX. Ed. K. H. Jürgen Buschow, et al. Oxford: Elsevier, 2001.http://www.sciencedirect.com/science/article/pii/B0080431526014467.

[5] ASAE S269.5 Pellets, and crumbles-definitions and methods for determining density, durability, and moisture content. Am. Soc. Agric. Eng., Oct. 2012 (2016) (St. Joseph, MI)

HPT were 187, 182, and 203 for diets pelleted on the 1012 PM with 6.6 L:D, 3016 PM with 9.3 L:D, and 1012 PM with 10.6 L:D, respectively.

There was no significant difference for the average phytase level of condition mash samples between the conditioner of the 3016 and 1012 PM, ($P > 0.05$; data not shown). The average phytase levels of condition mash samples were 494 and 520 FTU/lb for 1012 and 3016 PM, respectively. The phytase stability of condition mash samples were 86.96% and 91.50% for 1012 PM and 3016 PM, respectively. There was an overall effect ($P < 0.001$) of treatment on phytase stability in the cooled pellet. When using the 1012 PM, phytase was more stable regardless of die retention time when diets were manufactured using the 6.6 L:D die compared to the 10.6 L:D die ($P < 0.05$; Table 3). The hot pellet temperature of pellets from the 10.6 L:D die was between 195 and 211°F, while those produced using 6.6 L:D die were 184–189°F. However, the phytase stability was similar between the feed pelleted with 1012 PM equipped with 6.6 L:D die and pellets produced using the 3016 PM equipped with the 9.3 L:D die regardless of retention time ($P > 0.05$). The hot pellet temperature of feed pelleted with the 1012 PM equipped with 6.6 L:D die was 184–189°F, while the feed pelleted with the 3016 PM equipped with 9.3 L:D die was 180–183°F. There was also a quadratic decrease in phytase stability as the die L:D increased ($P < 0.0001$). The pellet mill size or retention time did not affect ($P > 0.05$) the phytase stability when the hot pellet temperature was less than 189°F. In addition, when the die retention time was approximately 2.6 sec, the 6.6 L:D treatment with hot pellet temperature of 184-189°F had greater phytase stability as compared to 10.6 L:D treatment with hot pellet temperature of 195-211°F. However, there was no difference for phytase stability between 6.6 L:D treatment and 9.3 L:D treatment when the die retention time was approximately 1.6 sec. The hot pellet temperature of feed pelleted with the 6.6 L:D die was 185–187°F and the 9.3 L:D die was 180–183°F.

Pellet quality increased (linear; $P < 0.0001$ for standard PDI or quadratic; $P < 0.0001$ for modified PDI) as die L:D increased (Table 3). The die L:D had greater effects on both PDI methods than the die retention time. However, increased die retention time improved ($P < 0.05$) pellet quality when the feed was pelleted with 6.6 L:D, but not when pelleted using the 9.3 or 10.6 L:D.

In conclusion, the phytase that was produced by *Trichoderma reesei* stain could tolerate hot pellet temps up to 189°F, regardless of pellet mill model, die thickness, and die retention time. However, phytase stability was dramatically reduced when hot pellet temperatures ranged from 195–211°F. Therefore, hot pellet temperature should be measured to monitor phytase stability. Increasing die L:D had the greatest influence on improving pellet quality.

Table 1. Pellet mill parameters and calculated die retention

Pellet mill[1]:	1012	1012	3016	3016	1012	1012
Pellet die length:diameter:	6.6	6.6	9.3	9.3	10.6	10.6
Die retention time, sec:	1.6	2.6	1.1	1.7	2.9	4.3
Die hole diameter, in:	3/16	3/16	3/16	3/16	3/16	3/16
Pellet mill model	1012	1012	3016	3016	1012	1012
Internal die surface area, in^2	84.78	84.78	226.08	226.08	84.78	84.78
Effective length, in	1 1/4	1 1/4	1 3/4	1 3/4	2	2
Holes per in^2	14.00	14.00	14.67	14.67	14.00	14.00
Effective volume per hole, in^3 ($\pi D^2L/4$)	0.034	0.034	0.048	0.048	0.055	0.055
Density, lb/in^3	0.022	0.022	0.022	0.022	0.022	0.022
Feed per die in effective length,[2] lb	0.90	0.90	3.53	3.53	1.44	1.44
Production rate, lb/sec	0.56	0.35	3.25	2.07	0.50	0.34

[1]Diets were pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 30 sec conditioning retention time at a 185°F target conditioning temperature.
[2]The amount of material in the effective length of die (lb) equals multiplication of the internal die surface area ($inch^2$), number of holes per $inch^2$, effective volume per hole ($inch^3$), and material density ($lb/inch^3$).
[3]Die retention time (RT) was calculated by dividing the amount of material in the die (lb) by the production rate (lb/sec).

Table 2. Pellet mill processing parameters[1]

Pellet mill model[2]:	1012	1012	3016	3016	1012	1012
Pellet die length:diameter:	6.6	6.6	9.3	9.3	10.6	10.6
Die retention time, sec:	1.6	2.6	1.1	1.7	2.9	4.3
Die hole diameter, in:	3/16	3/16	3/16	3/16	3/16	3/16
Die effective length, in	1 1/4	1 1/4	1 3/4	1 3/4	2	2
Production rate, ton/h	1.01	0.63	5.85	3.72	0.90	0.62
Condition mash temperature, °F	183-187	184-188	179-183	179-183	184-188	184-188
Hot pellet temperature, °F	185-187	184-189	180-183	180-183	195-211	198-208

[1]Diets were pelleted 3 separate times to provide 3 replicates for each treatment.
[2]Diets were pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 30 sec conditioning retention time at a 185°F target conditioning temperature.

Table 3. The effect of pellet mill die length:diameter (L:D) ratio and die retention time on phytase stability and pellet durability index[1]

Pellet mill model[2]:	1012	1012	3016	3016	1012	1012		*P*-value		
Pellet die L:D:	6.6	6.6	9.3	9.3	10.6	10.6				
Die retention time, sec:	1.6	2.6	1.1	1.7	2.9	4.3	SEM	Treatment	Linear, L:D	Quadratic, L:D
Phytase stability, [3]%	80.65[a]	73.08[a]	81.49[a]	74.85[a]	9.85[b]	1.33[b]	5.191	0.001	0.001	0.001
Pellet durability index,%										
Standard	62.91[e]	74.81[d]	85.72[c]	87.49[b]	95.12[a]	95.42[a]	0.569	0.001	0.001	0.863
Modified	36.67[d]	52.21[c]	68.34[b]	70.15[b]	89.82[a]	90.77[a]	1.191	0.001	0.001	0.001

[1]Diets were pelleted 3 separate times to provide 3 replicates for each treatment.
[2]Diets were pelleted using either a 1012-2 HD California Pellet Mill (CPM) Master Model or a 3016-4 HD CPM Master Model equipped with a 30 sec conditioning retention time at a 185°F target conditioning temperature.
[3]Stability by mixer was calculated by dividing phytase level of cooled pellet sample by average phytase level of mixer samples and then multiplied by 100.
[a,b,c,d,e]Within a row, means without a common superscript differ ($P < 0.05$).