Effects of conditioning temperature and pellet mill die speed on pellet quality and relative stabilities of phytase and xylanase[1]

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ABSTRACT: The objective of this experiment was to determine the effect of conditioning temperature and die speed on pellet quality and enzyme stability of phytase and xylanase. Treatments were initially arranged as a 2 × 3 factorial of conditioning temperature (74 and 85 °C) and die speed (127, 190, and 254 rpm); however, when conditioning at 85 °C, it was not possible to pellet at 127 rpm. Thus, data were analyzed in two different segments using the GLIMMIX procedure of SAS. First, linear and quadratic contrasts were utilized to test the response to increasing die speed at 74 °C. Second, the data was analyzed as a 2 × 2 factorial of conditioning temperature (74 and 85 °C) and die speed (190 and 254 rpm). Treatments were arranged in a completely randomized design and replicated three times. Diets were conditioned for approximately 30 s and pelleted with a 4.8-mm-diameter × 44.5-mm-effective length die at a rate of 4.5 MT/h. Pellet durability index (PDI) was determined using the tumble box and Holmen NHP 100 methods. Samples of the unconditioned mash (M), conditioned mash (CM), and pellets (P) were collected and analyzed for phytase and xylanase concentration. Relative enzyme stabilities were expressed as CM:M, P:CM, and P:M. Stabilities expressed as P:M were used an indication of enzyme stability through the entire pelleting process. Diets conditioned at 74 °C showed no evidence of difference in phytase or xylanase P:M stability when decreasing die speed from 254 to 127 rpm. However, when conditioning diets at 74 °C, decreasing die speed increased (linear, $P < 0.001$) PDI. There was no conditioning temperature × die speed interaction for overall xylanase P:M stability or PDI. However, there was a conditioning temperature × die speed interaction ($P < 0.01$) for phytase P:M stability. When conditioning diets at 85 °C, increasing die speed decreased phytase P:M stability. However, when conditioning at 74 °C, increasing die speed did not influence phytase P:M stability. For main effects of conditioning temperature, increasing temperature improved ($P < 0.001$) PDI with no evidence of difference for xylanase P:M stability. For the main effects of die speed (254 vs. 190 rpm), decreasing die speed decreased ($P < 0.001$) the P:M xylanase stability, but there was no evidence of difference for PDI. The results of this trial indicate that die speed should be taken into consideration when evaluating enzyme stability of both phytase and xylanase as pellet mill models may be operating at different speeds. Additionally, increasing conditioning temperature will improve PDI but may result in decreased phytase stability.

Key words: die speed, enzyme stability, pellet, pellet durability, pellet mill

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INTRODUCTION

Pelleting properties of mash feed can be influenced by a range of variables, some better understood than others. For decades, researchers have explored the relationship of feed conditioning and die specifications on optimized pellet quality (Mohensin and Zaske, 1976; Thomas et al., 1997; Behnke, 2001; Cutlip et al., 2008). In more recent years, greater reliance on exogenous enzymes in animal nutrition has broadened the scope of pelleting research to also include the effects on enzyme stability (Pope, 2019; Saensukjaroenphon, 2019, Truelock, 2020). Little attention, however, has been focused on understanding the influence of equipment parameters, such as horsepower, roller assembly (e.g., roll number and size), and die speed on pellet quality or enzyme stability.

Pellet mill die speed is typically measured at the outside diameter of the die. It is a product of the main drive speed, whether gear or belt driven, and any subsequent gear or belt reducers. In general, increased rotational speed not only maximizes throughput but also reduces the accumulation of conditioned mash in front of the die rolls. Turner (2013) stated that this leads to increased manufacturing stability as realized through greater possible conditioning temperatures and reduced energy input. Slower speeds, however, may be necessitated by quality concerns with cubes or from high die discharge rates resulting in pellet collision with the interior walls of the pellet mill chamber. Leaver (1988) suggested a peripheral die speed of 610 m/min as the optimum speed for pellets ranging from 3.2 to 6.4 mm in diameter, while reduced speeds between 366 and 396 m/min are preferred for pellet diameters exceeding 16 mm. Similarly, Turner (2013) recommended a die speed of 540 m/min when manufacturing smaller diameter pellets. It remains unclear how these recommendations were derived and validated, whether based on throughput, quality, or a combination of both. Application of Leaver and Turner's guidance is further complicated by the various operating die speeds observed throughout the industry.

There remains no standard operating die speed for pellet mills due to differences in equipment sizing and horsepower requirements. One manufacturer reports what appears to be a decreasing range of recommended die speeds as machine size and horsepower increase. Based on this manufacturer, a 150-hp pellet mill equipped with a 40.6-cm-diameter die should operate at a die speed of 254 rpm, while a larger 500-hp pellet mill equipped with a 91.4-cm-diameter die should operate at 211 rpm. Taking into account the die circumferences, this would correspond to approximately 324 and 606 m/min die peripheral velocities, respectively. These differences in die speed may offer yet another variable to consider when comparing results across various pelleting units.

Furthermore, changes in die speed may be a contributing factor to differences observed between pilot scale research and industry application. Pilot research trials have reported lower enzyme recoveries relative to industry and manufacturer reports (Pope, 2019 and Truelock, 2020). Pope (2019) suggests that these differences may be due to changes in production rate, die working area, and cooling protocols. It is hypothesized that differences in die speed may also be a contributing factor to variations in observed enzyme stability.

Though measurable, die speed remains an inconsistent target across pellet mills with no clear understanding of its role in subsequent pellet quality or enzyme stability. Thus, the objective of this trial was to evaluate the effects of conditioning temperature and die speed on pellet quality and enzyme stability of exogenous enzymes with varying heat tolerances (phytase and xylanase).

MATERIALS AND METHODS

Feed Manufacturing

A total of 41 MT of a swine finishing diet (Table 1) containing commercial phytase (Quantum Blue 5G, AB Vista Inc., Plantation, FL) and xylanase (Econase XT, AB Vista Inc., Plantation, FL) was pelleted to determine the effect of conditioning

Table 1. Diet composition for finishing swine

Ingredient	Inclusion, %
Corn[a]	76.05
Soybean meal	20.05
Soy oil	1.50
Limestone	1.10
Sodium chloride	0.35
Mono-calcium phosphate, 21% P	0.33
L-lysine HCl	0.26
Trace mineral premix[b]	0.13
Vitamin premix[c]	0.13
L-threonine	0.05
DL-methionine	0.02
Phytase[d]	0.02
Xylanase[e]	0.01
Total	100.00

[a] Ground corn was analyzed according to ASAE (1995) s319.2 for geometric mean diameter (568 μm) and SD (2.83).

[b] Composition per kilogram of premix: 73 g iron, 73 g zinc, 22 g manganese, 11 g copper, 0.2 g iodine, and 0.2 g selenium.

[c] Composition per kilogram of premix: 1,653,439 IU vitamin A, 661,376 IU vitamin D_3, 17,637 IU vitamin E, 13.3 mg vitamin B_{12}, 1,323 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.

[d] Quantum Blue 5G (AB Vista Inc., Plantation, FL) provided 1000 FTU/kg feed.

[e] Econase XT (AB Vista Inc., Plantation, FL) provided 16,000 BXU/kg of feed.

temperature and die speed on pellet quality and enzyme stability. Mash feed was conditioned at 74 or 85 °C and subsequently pelleted at a die speed of 127, 190, or 254 rpm.

Feed was mixed in 909-kg batches in a 1.63-m^3 twin shaft counterpose mixer (Hayes and Stolz, model TRDB63-0152, Fort Worth, TX). Dry ingredients were mixed for 60 s prior to the addition of liquid fat and then mixed for an additional 120 s. There were three 909-kg batches of feed per treatment replicate, yielding 2.7 MT of feed per pelleting run. Upon mixer discharge, mash samples were taken at regular intervals with five total samples for each replicate.

The mash batches were conditioned for approximately 30 s at 74 and 85 °C in a single-pass conditioner with a steam pressure of 1.52 bar. Diets were pelleted on a 100-hp pellet mill (CPM, model 3016-4 Master, Crawfordsville, IN) equipped with a 4.8-mm-diameter × 44.5-mm-effective length die (Table 2) and a target production rate of 4.5 MT/h. There were three defined pelleting runs per treatment characterized by allowing the conditioner to empty and the pellet mill to enter automated shut down. Die speed was adjusted via a variable frequency drive located on the main motor. Thus, when operating at 100%, 75%, and 50% of motor hertz, resulting shaft speeds were 1,800, 1,350, and 900 rpm, respectively (Table 3). This yielded peripheral die speeds of 254, 190, and 127 rpm or, based on die circumference, 324, 243, and 162 m/min. Die rpm was confirmed via precision laser tachometer (Fisher Scientific, Hampton, NH) prior to each pelleting run. The pellet mill die was warmed with 909 kg of feed prior to proceeding with experimental batches. Pelleting order was randomized within conditioning temperature to minimize residual changes in die temperature. Once conditioner temperature and production rate stabilized, conditioned mash and pellets were collected every 4 min for enzyme analysis with a total of five samples each. The conditioned mash samples were cooled for 8 min using a laboratory cooler with a 153-mm axial fan, while the pellets were cooled using a laboratory counter-flow cooler for 10 min. Conditioned mash temperature and pellet die exit temperature (hot pellet) were measured twice during each pelleting run. The samples were placed into a prewarmed double-wall thermos equipped with a digital thermometer. After the end of each pelleting run, the die surface temperature was measured in two places along the outside die periphery via infrared digital thermometer (IR002, Ryobi Limited, Anderson, SC). Additional samples of the mash and pellets were taken to determine moisture content and pellet durability as described below.

Table 2. Die specifications

Die hole diameter, mm [D]	4.8
Effective length, mm [L]	44.5
Internal die surface area, cm^2 [a]	1477.5
Holes per cm^2 [h_d]	2.3
Effective volume per hole,[a] cm^3 [v]	0.8
Feed per die in effective length,[b] kg [f]	1.7

[a] The effective volume per hole equals $\pi D^2 L/4$.

[b] The amount of material in the effective length of the die equals total number holes [ah_d] × volume per hole [v] × material density [0.609 g/cm^3].

Table 3. Pellet mill speed definitions

Shaft speed[a], rpm	Die speed, rpm	Peripheral die speed, m/min
1,800[b]	254	324
1,350	190	243
900	127	162

[a] Main drive shaft speed set via a variable frequency drive to 100%, 75%, and 50%, respectively.

[b] Standard main drive shaft speed for 100-hp pellet mill model 3016-4 (CPM Co., Crawfordsville, IN).

Data Collection

Energy consumption. Pellet mill voltage and amperage was recorded every 5 s during each pelleting run with a data logger (Supco model DVCV, Allenwood, NJ). Motor amperage was averaged across the individual pelleting run once conditioning temperature and production rate stabilized. Specific energy consumption (SEC) was calculated (Eq. 1) according to Stark (1994):

$$SEC(kWh/MT) = \frac{I \times E \times PF \times 1.73}{PR \times 1000}$$

where I is average motor amperage, E is voltage, PF is power factor set to 0.85, and PR is production rate expressed in MT/h.

Moisture content. Mash samples were taken at the mixer (mash) and conditioner (conditioned mash), while pellet samples were taken at the die (hot pellet) and post cooler (cool pellet) for the determination of moisture content. Upon collection, sample bags were immediately sealed and placed in a freezer set at −18 °C to prevent any potential moisture loss. Duplicate samples were taken at even intervals and were analyzed in triplicate according to AOAC (2006) s930.15. Briefly, a 100-g sample of mash or pellets was ground to pass through a 1-mm screen. A 2-g subsample of the ground material was then weighed to the nearest 0.1-mg and dried in an air oven at 135 °C for 2 h. Once removed, the sample was placed into a desiccator to cool. The sample was reweighed and the moisture content calculated based on loss in weight.

Pellet durability. Pellet samples were collected directly off of the pellet die and placed in a counter-flow laboratory cooler for 10 min. There were two pellet samples taken per treatment replicate. Pellets were packaged and stored in commercial tri-layer paper feed sacks and rested for 24 h prior to analysis. The pellet durability index (PDI) was assessed using the tumble box and Holmen forced-air methods. In the tumble box method, pellets were initially sifted with a U.S. No. 5 (3.9 mm) sieve for fines removal. A 500-g sample of sifted pellets was then placed in the tumble box and rotated at 50 rpm for 10 min. After tumbling, the sample was collected and sifted again to remove fines. PDI was calculated according to S269.5 (ASAE, 2012):

$$PDI(\%) = \frac{Recovered\ pellet\ weight}{Initial\ pellet\ weight} \times 100$$

This standard tumble box PDI procedure was then modified by adding three 19-mm hex nuts to the tumbling chamber to increase the agitation stress. For the Holmen method, pellets were sifted prior to analysis as outlined above. A 100-g sample of sifted pellets was then placed in the chamber of the Holmen NHP100 (TekPro Ltd, Norfolk, UK). The machine was set to 70-mbar air pressure and outfitted with a tissue filter. Pellets were agitated with forced air for 30 or 60 s, after which the sample was collected and sifted for the removal of fines. PDI was calculated according to Eq. 2, the same manner as the tumble box method. All samples were analyzed in duplicate and results averaged.

Enzyme activity. Mash (M), conditioned mash (CM), and pellet (P) samples were analyzed for phytase and xylanase content by the manufacturer according to the methods described by Pope and Fahrenholz (2020). Phytase content was determined using the QuantiPlate ELISA kit specific for Quantum Blue in accordance with ESC Standard Analytical Method SAM099 of AB Vista. Xylanase content was determined using the QuantiPlate ELISA kit specific for Econase in accordance with ESC Standard Analytical Method SAM115 of AB Vista. The percentage of phytase and xylanase stability of the conditioned mash samples (n = 5) were then expressed relative to the average mash recovery for each treatment replicate (CM:M) according to Eq. 3. The percentage of phytase and xylanase stability of the pellet samples (n = 5) were expressed relative to both the average recoveries of mash (P:M) and conditioned mash (P:CM) according to Eq. 3.

$$Stability(\%) = \frac{RS}{RAvg} \times 100$$

where R_S is the enzyme recovery of the individual sample and R_{Avg} is the average enzyme recovery of the desired reference sample group.

Statistical Analysis

Treatments were initially arranged as a 2 × 3 factorial of conditioning temperature (74 and 85 °C) and die speed (127, 190, and 254 rpm); however, during testing, conditioning at 85 °C and pelleting at 127 rpm was infeasible. Thus, data were analyzed in two different segments using the GLIMMIX procedure of SAS. First, linear and quadratic contrasts were utilized to test the response to increasing die speed at 74 °C. Second, the data was analyzed as a 2 × 2 factorial of conditioning temperature (74 and 85 °C) and die speed (190 and 254 rpm). Treatments were arranged in a completely randomized design and replicated three times each with the date of manufacture serving as a random effect. Results were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Feed Manufacturing

The effect of conditioning temperature and die speed on pelleting parameters are shown in Table 4. Conditioning temperatures remained comparable to their respective targets of 74 and 85 °C as indicated by conditioned mash temperatures. Measured peripheral die speeds were also closely aligned with their targets of 127, 190, and 254 rpm. Production rates remained consistent across treatments, though, when conditioning at 85 °C, it was impossible to produce pellets at 127 rpm due to instances of die choking and eventual plugging.

It is hypothesized that a combination of increased feed accumulation in front of the die rolls and moisture content were responsible for this failure. As conditioned mash is fed to the die rolls, it undergoes compaction to form a layer of feed commonly referred to as the feed pad. If the conditioned mash begins to accumulate too quickly in the die, the fixed die rolls may struggle to extrude feed through the die at the appropriate rate to sustain production. Thus, slowing die speeds, as Turner (2013) suggests, will result in increased accumulation of conditioned mash in front of the die rolls and a thicker feed pad. Once the feed pad becomes too thick for the die roll to overcome, roll slip force will increase allowing further accumulation of conditioned mash in the pelleting chamber until the die becomes choked and unable to rotate. While equipment failure is the greatest indicator of roll slip, increased pellet mill energy consumption (SEC) is also indicative of the roll's struggle to compensate for conditioned mash accumulation at the feed pad. This was evident in the current trial where there was a quadratic increase (quadratic, $P < 0.001$) in SEC as die speed decreased from 254 to 127 rpm when conditioning at 74 °C. There was no evidence of interaction ($P = 0.074$) between conditioning temperature and die speed (190 and 254 rpm only) for SEC. Additionally, there was no evidence of difference in SEC for increasing conditioning temperature ($P = 0.578$) or die speed ($P = 0.106$).

The theorized roll slip issues observed in this trial may have been amplified by increasing the conditioning temperature and would provide rationale for the ability to pellet at 127 rpm when conditioning at 74 °C as opposed to 85 °C. Based on previous research conducted using this pellet mill, increasing conditioning temperature from 74 to 85 °C increased the mash moisture content by 0.8% (Kort et al., 2020). Under the constraints of the current trial, however, only a 0.5% increase in moisture was observed when conditioning at 85 °C with no evidence of differences ($P > 0.495$) in conditioned mash moisture between treatments. The authors can only postulate what level of moisture is needed to induce roll slip and result in equipment failure; however, changes in the observed hot pellet exit temperatures (Table 4) appear to further support the theory that increased moisture content may have been a contributing factor when conditioning at 85 °C. The lower ΔT between conditioned mash and hot pellet exit temperature when

Table 4. The effect of conditioning temperature and die speed on pelleting paramaters[a]

	Conditioning temperature, °				
	74 °C			85 °C	
Die speed, rpm	127	190	254	190	254
Actual die peripheral speed[b]	130	195	261	194	260
Die roll contact, hits/min	260	390	522	388	520
Prod. rate, MT/h	4.5	4.6	4.4	4.2	4.3
Die retention,[c] s	1.4	1.3	1.4	1.4	1.3
Temperature, °C					
Cond. mash	73.8	73.9	73.8	83.2	85.2
Hot pellet	76.2	77.1	77.6	84.6	86.4
ΔT across die	2.4	3.2	3.8	1.4	1.2
Die	67.5	69.1	67.7	78.3	81.7

[a] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 4.8- × 44.5-mm die with three replications per treatment.

[b] Peripheral die speed measured via laser tachometer prior to each pelleting run.

[c] Calculated based on Saensukjaroenphon (2019) using die specifications (Table 2) and production rate.

Table 5. The effect of conditioning temperature and die speed on moisture content[a]

	Conditioning temperature, °						Probability, $P <$				
	74°C			85°C			74°C, die speed[b]		2 × 2 factorial[c]		
Die speed, rpm	127	190	254	190	254	SEM	Linear	Quad	Temp	RPM	Temp × RPM
Moisture,[d] %											
Mash	13.3	13.6	13.7	13.3	13.4	0.32	0.024	0.436	0.502	0.463	0.994
Condition mash	17.1	16.8	16.9	17.2	17.3	0.42	0.495	0.493	0.519	0.674	0.949
Hot pellet	16.2	16.8	16.6	16.3	17.1	0.26	0.057	0.017	0.969	0.037	<0.001
Cool pellet	13.6	13.7	13.9	13.6	13.5	0.27	0.045	0.908	0.532	0.932	0.216

[a] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 4.8- × 44.5-mm die. Treatments were replicated three times.

[b] Linear and quadratic contrasts testing the effect of increasing die speed when conditioning at 74 °C.

[c] Factorial analysis consisted of two conditioning temperatures (74 and 85 °C) and two die speeds (190 and 254 rpm).

[d] Moisture content calculated based on AOAC 930.15 using duplicate samples from each replication analyzed in triplicate.

conditioning at 85 °C compared to 74 °C indicates that the difference in moisture content was great enough to increase lubrication and reduce die friction. The responses observed in this trial may have further been exacerbated by the pellet mill model and die size. Larger dies and rolls may be less sensitive to changes in feed pad thickness and moisture content, creating an advantage in overcoming increased nip angles at the roll–die interface.

Moisture results (Table 5) indicated a change in moisture between feed entering the die chamber and exiting the die. When feed was conditioned at 74 °C, decreasing die speed quadratically decreased (quadratic, $P = 0.017$) hot pellet moisture after the die. Additionally, there was an interaction ($P < 0.001$) between conditioning temperature and die speed (190 and 254 rpm) that demonstrated decreasing die speed lowered hot pellet moisture when conditioning at 85 °C compared to 74 °C. The authors can only speculate on the cause of this response, but perhaps differences in residence time in the feed pad was a factor. The slower die speeds potentially have thicker feed pads with lower instances of roll contact (Table 4) and, thus, longer residence time in the feed pad. This could lead to greater loss in moisture in the die chamber as feed dwell time increased in the feed pad, which would explain the increased moisture loss with the slower die speeds. The loss of moisture may further be increased at higher conditioning temperatures where the die and chamber temperature may influence evaporative moisture loss. This may explain the lack of change when conditioning at 74 °C and pelleting at 190 rpm compared to conditioning at 85 °C.

Pellet Durability

Pellet durability index was assessed using both mechanical (tumble box) and pneumatic (NHP 100) agitation (Table 6). Agitation stress was increased in the tumble box by adding hex nuts and in the NHP 100 by increasing pellet exposure time to the forced air stream. Though raw values differed numerically between the durability methods utilized in this trial, the interpretation of the effect between treatments remained the same. When conditioning diets at 74 °C, decreasing die speed from 254 to 127 rpm increased (linear, $P < 0.001$) PDI. There was no interaction ($P > 0.103$) between conditioning temperature and die speed (190 and 254 rpm) for PDI. For main effects, increasing conditioning temperature improved ($P < 0.001$) PDI, while there was no evidence of difference ($P > 0.198$) in PDI based on die speed.

Table 6. The effect of conditioning temperature and die speed on SEC of the pellet mill and pellet quality[a]

	Conditioning temperature, °						Probability, P <				
	74 °C			85 °C			74 °C, die speed[b]		2 × 2 factorial[c]		
Die speed, rpm	127	190	254	190	254	SEM	Linear	Quad	Temp	RPM	Temp × RPM
SEC,[d] kWh/MT	10.9	8.9	9.0	9.6	9.1	0.41	0.001	0.001	0.578	0.106	0.074
PDI, %											
Std tumble[e]	85.8	83.9	83.3	91.2	91.2	0.58	0.001	0.082	0.001	0.245	0.313
Mod tumble[e]	68.6	64.1	61.7	80.3	80.6	1.84	0.001	0.262	0.001	0.198	0.103
NHP 30 s[f]	78.1	73.1	70.8	87.7	87.8	1.51	0.001	0.207	0.001	0.214	0.175
NHP 60 s[f]	56.6	46.3	43.3	78.7	78.2	2.46	0.001	0.063	0.001	0.257	0.433

[a] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 4.8- × 44.5-mm die. Treatments were replicated three times. Date of production served as a random effect to account for any environmental changes that may have influenced pelleting parameters.

[b] Linear and quadratic contrasts testing the effect of increasing die speed when conditioning at 74 °C.

[c] Factorial analysis consisted of two conditioning temperatures (74 and 85 °C) and two die speeds (190 and 254 rpm).

[d] Specific energy consumption was calculated according to Stark (1994) based on production rate and average pellet mill motor amperage over the run.

[e] Standard and modified tumble box methods according to ASAE S269.4 with three 19-mm hex nuts used for modification.

[f] Holmen NHP100 (TekPro Ltd, Norfolk, UK) pneumatic pellet tester set at 70 mbar forced air with 30 or 60 s run time.

The increased PDI resulting from conditioning at a higher temperature has been well documented by other researchers (Cutlip et al., 2008; Stark and Ferket, 2011). Generally, this response to the addition of heat and moisture has been attributed to altered physico-chemical properties of the feed, typically leading to improved binding properties between particles (Thomas and van der Poel, 1996). Previous work examining the effects of die speed on pellet durability is more poorly documented. Stevens (1987) examined the effect of die speed on the pellet durability of a corn-based swine formulation after conditioning at 75 °C. The corn diet was pelleted at die speeds from 150 to 268 rpm or 143–256 m/min velocity based on die circumference. Results demonstrated improved pellet durability when utilizing the lowest die speed setting of 150 rpm, with no evidence of a difference between the other die speeds. This is similar to the findings of this trial where pelleting at 127 rpm or 162 m/min yielded the greatest PDI for feed conditioned at 74 °C.

Phytase Stability

One of the primary concerns for exogenous enzyme use in pelleted livestock feed is their ability to withstand the rigors of pelleting (Pope, 2019). Factors like temperature and moisture have been shown to influence enzyme inactivation (Bychkov et al., 2011; Perdana et al., 2012). Two commercial exogenous enzymes were chosen for testing in this experiment: a phytase produced by a strain of *Trichoderma reesi* reported to tolerate conditioning temperatures up to 90 °C and a xylanase reported to be intrinsically thermostable and tolerant of conditioning temperatures up to 95 °C. In an effort to understand how the processes of conditioning and pressing the pellets at different die speeds affect the enzyme stability, samples were taken directly after conditioning (CM) and as feed exited the pellet die (P).

Enzyme stability results are shown in Table 7. When diets were conditioned at 74 °C, there was no evidence of a difference ($P > 0.198$) in the phytase recovery in pellets relative to the initial mash (P:M). There was a conditioning temperature × die speed (254 and 190 rpm only) interaction ($P = 0.004$) for phytase stability of pellets relative to the initial mash. When conditioning diets at 85 °C, increasing die speed from 190 to 254 rpm decreased phytase stability, while increasing die speed did not influence phytase stability when conditioning at 74 °C. Focusing on the phytase activity in the conditioned mash relative to mash (CM:M) provides insight into losses in activity occurring due to conditioning temperature alone. In this study, there was no evidence of differences ($P > 0.086$) in phytase stability among any treatment, indicating that the conditioning temperature (up to 85 °C) alone was least likely to influence the change in phytase stability in the experiment conducted herein. Comparing the phytase activity in the pellets relative to conditioned mash (P:CM) represents changes in stability due to the pressing process. Under the constraints of this trial, there was no evidence of a difference ($P > 0.123$) in phytase stability with decreasing die speed when the feed was conditioned at 74 °C. There was a conditioning temperature × die speed (254 and 190 rpm only) interaction ($P = 0.004$) for phytase stability of pellets relative to the conditioned mash, which was similar to that observed in pellets relative to initial mash. When conditioning diets at 85 °C, increasing die speed from 190 to 254 rpm decreased phytase stability, while increasing die speed did not influence phytase stability when conditioning at 74 °C. These results would indicate that the greatest contributors to phytase degradation occur during the pressing process at the die. These authors recognize that conditioning temperature and moisture may also interact at the die interface causing degradation; however, these changes would again influence forces during the pressing process and not strictly conditioning. Pope (2019) had a similar conclusion based on his works where phytase denaturation was not simply a result of exposure to steam within the conditioner but was rather a complex response to the accumulation of forces necessary to bind particles within the pellet mill die, such as moisture, heat, and pressure.

The authors can only hypothesize that the reduced phytase stability at greater die speed when conditioning at a higher temperature is a result of a combination of several factors. Hot pellet temperatures (Table 4) would indicate that the exit temperature of pellets exceeded the recommended temperature for phytase preservation. This is a theory supported by Truelock (2020) who found that hot pellet temperature was a better indicator for phytase degradation than conditioning temperature alone. Perhaps die temperature or the amount of die to roll contact played some role in the observed results. Ultimately the complexities among factors and forces occurring during the pressing process make it difficult to come to a definitive conclusion in this regard and need further research.

Table 7. The effect of conditioning temperature and die speed on relative phytase and xylanase stability during the pelleting process[a]

	Conditioning temperature, °						Probability, $P <$				
	74 °C			85 °C			74 °C, die speed[b]		2 × 2 factorial[c]		
Die speed, rpm	127	190	254	190	254	SEM	Linear	Quad	Temp	RPM	Temp × RPM
Phytase stability[d]											
CM:M	84.1	87.7	85.0	84.8	85.8	3.74	0.086	0.663	0.769	0.810	0.612
P:CM	103.6	99.5	97.7	87.9	70.8	3.01	0.123	0.699	0.019	0.001	0.004
P:M	90.9	88.0	86.6	75.8	61.1	3.09	0.198	0.799	0.035	0.001	0.004
Xylanase stability[e]											
CM:M	102.9	101.4	95.1	88.0	94.9	5.49	0.077	0.504	0.430	0.906	0.026
P:CM	92.8	89.0	94.1	89.8	96.1	5.30	0.692	0.122	0.861	0.020	0.806
P:M	93.2	89.6	94.9	81.6	87.3	7.79	0.611	0.103	0.542	0.019	0.283

[a] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 4.8- × 44.5-mm die. Treatments were replicated three times. Date of production served as a random effect to account for any environmental changes that may have influenced pelleting parameters.

[b] Linear and quadratic contrasts testing the effect of increasing die speed when conditioning at 74 °C.

[c] Factorial analysis consisted of two conditioning temperatures (74 and 85 °C) and two die speeds (190 and 254 rpm).

[d] Relative phytase stability calculated as the percentage of FTUs remaining in conditioned mash (CM) or pellet (P) samples compared to the initial mash (M) or CM.

[e] Relative xylanase stability calculated as the percentage of BXUs remaining in conditioned mash (CM) or pellet (P) samples compared to the initial mash (M) or CM.

Xylanase Stability

Comparatively, conditioning temperature and die speed seem to have had a reduced effect on the stability of the more thermal tolerant xylanase in this trial. There was no evidence of differences ($P > 0.103$) in xylanase stability in pellets relative to the initial mash (P:M) when conditioning at 74 °C with increasing die speed. There was no interaction ($P = 0.283$) between conditioning temperature and die speed (190 and 254 only); however, there was a main effect of die speed in which increased die speed resulted in greater xylanase stability. This is in direct opposition to the response of phytase to increased die speed. When comparing the xylanase recovery in the conditioned mash relative to the mash (CM:M), there was no evidence of a difference ($P > 0.077$) in xylanase stability when conditioning at 74 °C with increasing die speed. There was, however, an interaction ($P = 0.026$) between conditioning temperature and die speed (190 and 254 only) where xylanase stability was poorer at the slower die speed when conditioning at 85 °C compared to 74 °C. Similar to P:M, pellets relative to the conditioned mash (P:CM) had no evidence of differences ($P > 0.122$) in xylanase stability with increasing die speed when conditioning at 74 °C. There was no interaction ($P = 0.283$) between conditioning temperature and die speed (190 and 254 only); however, there was a main effect of die speed in which increased die speed resulted in greater xylanase stability.

CONCLUSIONS

The results of this trial indicate that conditioning temperature and die speed can influence pellet quality. When conditioning at lower temperatures (74 °C), decreasing die speed will improve pellet durability, while high conditioning temperatures (85 °C) will yield greater durability regardless of die speed. However, reducing die speed resulted in increased specific energy consumption. Regarding enzyme stability, die speed should be considered when conditioning feed at 85 °C due to increased phytase degradation. The mode of action behind this response is unclear, which warrants further exploration into the role of temperature, moisture, and friction at the mash–die interface. Additionally, when pelleting more heat-tolerant enzymes like the xylanase used in this trial, conditioning temperature and die speed may be of less concern in preserving activity.

Most importantly, because pellet mill models may be operating at different die speeds, care should be taken when interpreting or applying pelleting research. This may be especially true when comparing small pellet mills with lower die peripheral speeds and velocities to larger-industry-sized equipment.

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