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Summary

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 (165 and 185°F) and die speed (127, 190, and 254 rpm); however, when conditioning at 185°F it was not possible to pellet at 127 rpm. Thus, data were analyzed in 2 different segments using the GLIMMIX procedure of SAS. First, linear and quadratic contrasts were utilized to test the response to increasing die speed at 165°F. Second, the data were analyzed as a 2 × 2 factorial of conditioning temperature (165 and 185°F) and die speed (190 and 254 rpm). Treatments were arranged in a completely randomized design and replicated 3 times. Diets were conditioned for approximately 30 s and pelleted with a 3/16 in. diameter × 1 3/4 in. effective length die at a rate of 5 ton/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 and P:M. Stabilities expressed as P:M were used an indication of enzyme stability through the entire pelleting process. Diets conditioned at 165°F 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 165°F, 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 185°F, increasing die speed decreased phytase P:M stability. However, when conditioning at 165°F, 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.

Introduction

Pelleting properties of mash feed can be influenced by a range of variables, with some being better understood than others. For decades researchers have explored the relationship of feed conditioning and die specifications on optimized pellet quality. 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. Little attention, however, has been focused on understanding the influence of equipment parameters such as 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. There is no standard operating die speed for pellet mills due to differences in equipment sizing and horsepower requirements. In general, increased rotational speed not only maximizes throughput, but also reduces the accumulation of conditioned mash in front of the die rolls. 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.

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 study 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 45 tons 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 temperature and die speed on pellet quality and enzyme stability. Mash feed was conditioned at 165 or 185°F and subsequently pelleted at a die speed of 127, 190, or 254 rpm.

Feed was mixed in 2000 lb batches in a 57.6 ft^3 twin shaft counterpoise 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 2000 lb batches of feed per treatment replicate, yielding 3 tons of feed per pelleting run. Upon mixer discharge, mash samples were taken at regular intervals with 5 total samples for each replicate.

The mash batches were conditioned for approximately 30 s at 165 and 185°F in a single pass conditioner with a steam pressure of 22 psi. Diets were pelleted on a 100 HP pellet mill (CPM, model 3016-4 Master, Crawfordsville, IN) equipped with a 3/16 in. diameter × 1 3/4 in. effective length die and a target production rate of 5 ton/h. There were 3 defined pelleting runs per treatment, characterized by allowing the conditioner to empty and the pellet mill to enter automated shutdown. 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 1800, 1350, and 900 rpm, respectively. This yielded peripheral die speeds of 254, 190, and 127 rpm; or, based on die circumference, 1064, 796, and 532 ft/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 2000 lb 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 5 samples each. The conditioned mash samples were cooled for 8 min using a laboratory cooler with a 6.0 in. axial fan, while the pellets were cooled using a laboratory counterflow 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 pre-warmed double-wall thermos equipped with a digital thermometer. After the end of each pelleting run, the die surface temperature was measured in 2 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 pellet durability index (PDI) as described below.

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 according to Stark.[1]

Pellet durability index

Pellet samples were collected directly from the pellet die and placed in a counterflow laboratory cooler for 10 min. Two pellet samples were 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 (0.16 in.) sieve for fines removal. A 1.1-lb 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. The PDI was calculated according to ASAE S269.5. This standard tumble box PDI procedure was then modified by adding three 3/4 in. 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 0.22 lb sample of sifted pellets was then placed in the chamber of the Holmen NHP100 (TekPro Ltd, Norfolk, UK). The machine was set to 1.02 psi 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 removal of fines. The PDI was calculated similarly to 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. Phytase content was determined using the QuantiPlate ELISA kit specific for Quantum Blue in accordance with ESC Standard

[1] Stark, C. R. Pellet quality. I. Pellet quality and its effect on swine performance. II. Functional characteristics of ingredients in the formation of quality pellets. 1994. Kansas State University, Department of Grain Science, PhD dissertation.

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 percent 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). The percent 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).

$$\text{Stability (\%)} = \frac{R_s}{R_{Avg}} \times 100$$

where R_S is the enzyme recovery of the individual sample, 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 (165 and 185°F) and die speed (127, 190, and 254 rpm); however, during testing, conditioning at 185°F and pelleting at 127 rpm was infeasible. Thus, data were analyzed in 2 different segments using the GLIMMIX procedure of SAS (v. 9.4, SAS Inst., Cary, NC). First, linear and quadratic contrasts were utilized to test the response to increasing die speed at 165°F. Second, the data were analyzed as a 2 × 2 factorial of conditioning temperature (165 and 185°F) and die speed (190 and 254 rpm). Treatments were arranged in a completely randomized design and replicated 3 times each with date of manufacture serving as a random effect. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Feed manufacturing

Conditioning temperatures remained comparable to their respective targets of 165 and 185°F as indicated by conditioned mash temperatures (Table 2). Measured peripheral die speeds were also closely aligned with their targets of 127, 190, and 254 rpm. Production rates remained consistent across treatments, although when conditioning at 185°F 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. 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 specific 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 when there was a quadratic increase (quadratic, $P < 0.001$) in SEC as die speed decreased from 254 to 127 rpm when conditioning at 165°F. 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 ($P = 0.106$).

Pellet durability

Pellet durability index was assessed using both mechanical (tumble box) and pneumatic (NHP 100) agitation (Table 3). 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 165°F, 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.

Phytase stability

Enzyme stability results are shown in Table 3. When diets were conditioned at 165°F, 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 185°F, increasing die speed from 190 to 254 rpm decreased phytase stability, while increasing die speed did not influence phytase stability when conditioning at 165°F. 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 for any treatment, indicating that the conditioning temperature (up to 185°F) alone was least likely to influence the change in phytase stability in the experiment conducted herein. When conditioning diets at 185°F, increasing die speed from 190 to 254 rpm decreased phytase stability, while increasing die speed did not influence phytase stability when conditioning at 165°F. 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. The authors hypothesized 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 2) would indicate that the exit temperature of pellets exceeded the recommended temperature for phytase preservation. 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 which occur during the pressing process make it difficult to come to a definitive conclusion in this regard, therefore, further research is needed.

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 165°F with increasing die speed. There was no interaction ($P = 0.283$) between conditioning temperature and die speed (190 and 254 rpm 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 165°F with increasing die speed. There was, however, an interaction ($P = 0.026$) between conditioning temperature and die speed (190 and 254 rpm only) for CM:M, where xylanase stability was poorer at the slower die speed when conditioning at 185°F compared to 165°F. There was no interaction ($P = 0.283$) between conditioning temperature and die speed (190 and 254 rpm only) for P:M, 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 (165°F) decreasing die speed will improve pellet durability, while high conditioning temperatures (185°F) 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 185°F due to increased phytase degradation. The mode of action is unclear for this response, 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. 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.

Table 1. Diet composition for finishing swine (as-is)

Ingredient	Inclusion, %
Corn[1]	76.05
Soybean meal	20.05
Soy oil	1.50
Limestone	1.10
Sodium chloride	0.35
Monocalcium phosphate, 21% P	0.33
L-lysine HCl	0.26
Trace mineral premix[2]	0.13
Vitamin premix[3]	0.13
L-threonine	0.05
DL-methionine	0.02
Phytase[4]	0.02
Xylanase[5]	0.01
Total	100.00

[1] Ground corn was analyzed for geometric mean diameter (568 µm) and standard deviation (2.83).
[2] Composition per kg of premix: 73 g iron, 73 g zinc, 22 g manganese, 11 g copper, 0.2 g iodine, and 0.2 g selenium.
[3] Composition per kg 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.
[4] Quantum Blue 5G (AB Vista Inc., Plantation, FL) provided 1000 FTU/kg feed.
[5] Econase XT (AB Vista Inc., Plantation, FL) provided 16,000 BXU/kg of feed.

Table 2. The effect of conditioning temperature and die speed on pelleting parameters[1]

	Conditioning temperature, °F				
	165°F			185°F	
Die speed, rpm:	**127**	**190**	**254**	**190**	**254**
Actual die peripheral speed[2]	130	195	261	194	260
Die roll contact, hits/min	260	390	522	388	520
Prod. rate, ton/h	5.0	5.1	4.8	4.6	4.7
Temperature, °F					
Conditioning mash	164.8	165.0	164.8	181.8	185.4
Hot pellet	169.2	170.8	171.7	184.3	187.5
Die	153.5	156.4	153.9	172.9	179.1

[1] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 0.19 × 1.75 in. die with 3 replications per treatment.
[2] Peripheral die speed (rpm) measured via laser tachometer prior to each pelleting run.

Table 3. The effect of conditioning temperature and die speed on specific energy consumption (SEC), pellet durability index (PDI) and stabilities of phytase and xylanase[1]

	Conditioning temperature, °F						Probability, $P <$				
	165°F			185°F			165°F, die speed[2]		2 × 2 factorial[3]		
Die speed, rpm	127	190	254	190	254	SEM	Linear	Quad	Temp	RPM	Temp × RPM
SEC,[4] 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[5]	85.8	83.9	83.3	91.2	91.2	0.58	0.001	0.082	0.001	0.245	0.313
Mod tumble[5]	68.6	64.1	61.7	80.3	80.6	1.84	0.001	0.262	0.001	0.198	0.103
NHP 30s[6]	78.1	73.1	70.8	87.7	87.8	1.51	0.001	0.207	0.001	0.214	0.175
NHP 60s[6]	56.6	46.3	43.3	78.7	78.2	2.46	0.001	0.063	0.001	0.257	0.433
Phytase stability[7]											
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: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[8]											
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:M	93.2	89.6	94.9	81.6	87.3	7.79	0.611	0.103	0.542	0.019	0.283

[1] Diets were conditioned for approximately 30 s prior to pelleting (Model 3016-4 CPM Co., Crawfordsville, IN) on a 3/16 × 1 3/4 in. die. Treatments were replicated 3 times. Date of production served as a random effect to account for any environmental changes that may have influenced pelleting parameters.

[2] Linear and quadratic contrasts testing the effect of increasing die speed when conditioning at 165°F.

[3] Factorial analysis consisted of two conditioning temperatures (165 and 185°F) and two die speeds (190 and 254 rpm).

[4] Specific energy consumption was calculated according to Stark, 1994 (Stark, C. R. Pellet quality. I. Pellet quality and its effect on swine performance. II. Functional characteristics of ingredients in the formation of quality pellets. 1994. Kansas State University, Department of Grain Science, PhD dissertation).

[5] Standard and modified tumble box methods with three 3/4 in. hex nuts used for modification.

[6] Holmen NHP100 (TekPro Ltd, Norfolk, UK) pneumatic pellet tester set at 1.1 psi forced air with 30 or 60 s run time.

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

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