Kansas Agricultural Experiment Station Research Reports

Volume 6
Issue 10 *Swine Day*

Article 30

2020

# Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality

M. Saensukjaroenphon
*Kansas State University, Manhattan Kansas*, maruts@ksu.edu

C. E. Evans
*Kansas State University*, caitlinevans@k-state.edu

C. R. Stark
*Kansas State University, Manhattan*, crstark@ksu.edu

*See next page for additional authors*



## Recommended Citation

Saensukjaroenphon, M.; Evans, C. E.; Stark, C. R.; and Paulk, C. B. (2020) "Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality," *Kansas Agricultural Experiment Station Research Reports*: Vol. 6: Iss. 10. https://doi.org/10.4148/2378-5977.8011

# Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality


## Abstract

The addition of water to the mixer prior to pelleting is sometimes necessary to reach the target moisture content at the end of the conditioning process. However, there are limited data to demonstrate the impact of water addition in the mixer on phytase stability during the pelleting process. In addition, the variation of phytase analysis method may lead to incorrect or biased conclusions for research on industrial phytase stability. Therefore, the objective of this experiment was to determine the effect of water added in the mixer, feed holding time, and phytase analysis method on phytase stability and pellet quality. Treatments were arranged in a 2 × 2 × 2 factorial with main effects of added water (0% or 1%), holding time (0 or 2 h), and phytase analysis method (ELISA or EN ISO), respectively. For the 0% added water treatment, a 210-lb basal feed and 0.03-lb phytase were mixed for 5 min. For the 1% added water treatments, a 208-lb basal feed and 0.03-lb phytase were mixed for 120 s followed by the addition of 2-lb water and then the mixture was mixed for 180 s wet mix time. The water was applied to dry feed in the mixer using a hand-held sprayer (Country Tuff model 26329, Sedalia, MO) with a flat spray tip nozzle (TeeJet model TP11006, Glendale Heights, IL). After the diets were mixed, treatments were immediately pelleted or held in a closed container for 2 h before pelleting. Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model CI-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.87 in die. Samples were collected during discharge of the mixer, after conditioning and after pelleting. The conditioned mash and pelleted samples were cooled for 10 min using an experimental counterflow cooler. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. The results demonstrated that there was no evidence of three-way or two-way interactions among added water, holding time, and analysis method on phytase stability for mash samples, conditioned mash samples, and pellets. The added water and holding time did not impact phytase stability for mash samples, conditioned mash samples, and pellets. The ELISA method had greater ($P$ = 0.004) phytase activity than the EN ISO method for the pellet samples. The phytase activity was similar between the two analytical methods for mash samples and conditioned mash samples. For pellet quality, there was no evidence of interaction between added water and holding time. Added water and holding time did not impact pellet durability index. Therefore, the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when feed was stored in a bin up to 2 h prior to pelleting. The added water in mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei* phytase stability. Increasing moisture content of mash feed by 0.6% did not improve pellet quality.



## Keywords

phytase, pellet quality, phytase stability





## Authors

M. Saensukjaroenphon, C. E. Evans, C. R. Stark, and C. B. Paulk

K-STATE Research and Extension

Swine Day 2020

# Effect of Added Water, Holding Time, or Phytase Analysis Method on Phytase Stability and Pellet Quality

*Marut Saensukjaroenphon,[1] Caitlin E. Evans,[1] Charles R Stark,[1] and Chad B. Paulk[1]*

## Summary

The addition of water to the mixer prior to pelleting is sometimes necessary to reach the target moisture content at the end of the conditioning process. However, there are limited data to demonstrate the impact of water addition in the mixer on phytase stability during the pelleting process. In addition, the variation of phytase analysis method may lead to incorrect or biased conclusions for research on industrial phytase stability. Therefore, the objective of this experiment was to determine the effect of water added in the mixer, feed holding time, and phytase analysis method on phytase stability and pellet quality. Treatments were arranged in a 2 × 2 × 2 factorial with main effects of added water (0% or 1%), holding time (0 or 2 h), and phytase analysis method (ELISA or EN ISO), respectively. For the 0% added water treatment, a 210-lb basal feed and 0.03-lb phytase were mixed for 5 min. For the 1% added water treatments, a 208-lb basal feed and 0.03-lb phytase were mixed for 120 s followed by the addition of 2-lb water and then the mixture was mixed for 180 s wet mix time. The water was applied to dry feed in the mixer using a hand-held sprayer (Country Tuff model 26329, Sedalia, MO) with a flat spray tip nozzle (TeeJet model TP11006, Glendale Heights, IL). After the diets were mixed, treatments were immediately pelleted or held in a closed container for 2 h before pelleting. Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.87 in die. Samples were collected during discharge of the mixer, after conditioning and after pelleting. The conditioned mash and pelleted samples were cooled for 10 min using an experimental counterflow cooler. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. The results demonstrated that there was no evidence of three-way or two-way interactions among added water, holding time, and analysis method on phytase stability for mash samples, conditioned mash samples, and pellets. The added water and holding time did not impact phytase stability for mash samples, conditioned mash samples, and pellets. The ELISA method had greater ($P = 0.004$) phytase activity than the EN ISO method for the pellet samples. The phytase activity was similar between the two analytical methods for mash samples and conditioned mash samples. For pellet quality, there was no evidence of interac-

[1] Department of Grain Science and Industry, College of Agriculture, Kansas State University.

tion between added water and holding time. Added water and holding time did not impact pellet durability index. Therefore, the stability of phytase produced by a strain of *Trichoderma reesei* was not affected when feed was stored in a bin up to 2 h prior to pelleting. The added water in mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei* phytase stability. Increasing moisture content of mash feed by 0.6% did not improve pellet quality.

## Introduction

Swine diets are pelleted to improve bulk density, transportation characteristics of feeds, reduce ingredient segregation during handling, decrease dust levels, reduce feed intake, increase weight gain, and improve feed utilization. However, in order to maintain this advantage pellets must be of high quality. Previous research demonstrated that increasing mash feed moisture up to 15% improved pellet quality and decreased energy consumption.[2] This is often accomplished by adding water to the mixer or conditioner in addition to the water added by steam in the conditioning process. The added water and steam have the potential to reduce enzyme stability as both temperature and moisture content are two factors that influence enzyme inactivation kinetics.[3] As nutritionists continue to rely on enzymes (phytase, xylanase, and protease) to reduce feed cost by increasing nutrient digestibility, enzyme stability is crucial to ensure nutrient contents and prevent deficiencies. There are limited data to demonstrate the impact of moisture addition in the mixer on phytase denaturation during the pelleting process. Increasing mash feed moisture and the interaction time between water and phytase may increase phytase degradation during the pelleting process.

European Union Feed Additives and Premixtures Association (FEFANA) developed a method to determine phytase activity regardless of phytase sources, so meaningful comparisons could be made across products.[4] Each phytase supplier typically has a rapid phytase method to provide a quick turnaround time for their customers to help ensure the phytase activity is at the correct concentration in their diets. However, for research projects, the rapid method may create unexplained bias due to variation and could lead to misinterpretation of the data. Therefore, the objectives of this experiment were to determine the effect of adding water in the mixer, mash holding time, and phytase analysis method on phytase stability, and to determine the effect of adding water in the mixer and mash holding time on pellet quality.

## Procedures

A corn and soybean meal-based diet was used for the experiment (Table 1). The basal diet was mixed in a 63 $ft^3$ counterpoise mixer (Hayes & Stolz, model TRDB63-0152, Fort Worth, TX) for 3 min. The basal diet and phytase produced by a strain of *Trichoderma reesei* were added to a 6 $ft^3$ paddle mixer (Davis model 2014197-SS-S1, Bonner

[2] Greer, D. 2013. Is feed moisture variable or controlled nutrient? Feedstuffs. 85(43):16.

[3] Perdana, J., M. B. Fox, M. A. I. Schutyser, and R. M. Boom. 2012. Enzyme inactivation kinetics: Coupled effects of temperature and moisture content. Food Chem. 133, 116-123.

[4] Gizzi, G., P. Thyregod, C. Von Holst, G. Bertin, K. Vogel, M. Faurschou-Isaksen, R. Betz, R. Murphy, and B. Andersen. 2008. Determination of phytase activity in feed: Interlaboratory study. J. AOAC Int. 91(2), 259-267.

Springs, KS). For the 0% added water treatment, a 210-lb basal feed and 0.03-lb phytase were mixed for 5 min. For the 1% added water treatments, a 208-lb basal feed and 0.03-lb phytase were mixed for 120 s followed by the addition of 2-lb water, and then the mixture was mixed for 180 s wet mix time. The water was applied to dry feed in the mixer using a hand-held sprayer (Country Tuff model 26329, Sedalia, MO) with a flat spray tip nozzle (TeeJet model TP11006, Glendale Heights, IL). After the diets were mixed, treatments were immediately pelleted or held in a closed container for 2 h before pelleting. Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.87 in die. The feeder setting was held constant at approximately 2.2 lb/min. There were 3 replicates for each treatment. Samples were collected during discharge of the mixer, after conditioning and after pelleting. The conditioned mash and pelleted samples were cooled for 10 min using an experimental counterflow cooler. All the samples were analyzed for moisture content, then kept at room temperature before being sent to the laboratory for analysis. For moisture content, treatments were arranged in 3 × 2 × 2 factorial of sample type (mixer, conditioned mash, or cooled pellet), added water (0% or 1%), and holding time (0 or 2 h) to determine the effect on sample moisture. Phytase activity was determined by ELISA and EN ISO method. For phytase, treatments were arranged in 2 × 2 × 2 factorial of added water (0% or 1%), feed holding time (0 or 2 h), and phytase analysis method (ELISA and EN ISO) to determine the effect on phytase stability of the mash, conditioned mash, and pellet samples. The pelleted samples from each pellet run were analyzed for pellet durability index (PDI) using the Holmen NHP 100 for 120 s at 60 bars. Treatments were arranged in 2 × 2 factorial of added water (0% or 1%) and holding time (0 or 2 h) to determine the effect on pellet quality.

For analysis of moisture content, an aluminum tray weight was recorded then a 2-gram sample was placed on the tray.[5] The sample was dried in the oven at 275°F for 2 h, then the sample tray was placed in the desiccator for 30 min. The moisture was calculated by dividing the difference between the sample tray and the empty tray by the sample weight and then multiplying by 100.

The supplier's ELISA QuantiPlate Kit for Quantum Blue was used for both mash and pellet samples. The color reaction was measured by plate reader at 450/630 nm. The amount of color was used to evaluate the phytase activity based on a calibration curve. For the EN ISO method (European Standard and International Organization for Standardization, EN ISO, 2009), a feed sample was extracted with 0.01% polysorbate 20 then incubated with 5 mM phytate substrate in pH 5.5 acetate buffer at 210°F for 30 min. A 0.8-mL stop agent (molybdate/vanadate) was added, resulting in formation of phosphomolybdate-vanadate, which produced a yellow complex. The yellow complex was measured at 415 nm then compared to a phosphate standard curve. The phytase results were reported as FTU/lb and % phytase stability. The phytase stability (%) of the conditioned mash sample or cooled pellets was calculated by dividing phytase activity of the conditioned mash sample of cooled pellets by the average phytase activity of the mash samples then multiplying by 100.

[5] AOAC. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists; Arlington, VA, USA: 1990.

For PDI, the sample was sifted with a U.S. No. 6 sieve. A 100-gram sample of sifted pellets was placed in the Holmen NHP 100 chamber. The Holmen NHP 100 was run for 120 s. The PDI was calculated by dividing the remaining sample weight by initial weight and then multiplying by 100.

Data were analyzed as a completely randomized design. For phytase stability, treatments were arranged in 2 × 2 × 2 factorial of added water (0% or 1%), holding time (0 or 2 h), and phytase analysis method (ELISA or EN ISO) to determine the effect on phytase stability of mash, conditioned mash, and cooled pellet samples. For moisture, treatments were arranged in 3 × 2 × 2 factorial of sample type (mixer, conditioned mash, or cooled pellet), added water (0% or 1%) and holding time (0 and 2 h) to determine the effect on moisture. For PDI, treatments were arranged in 2 × 2 factorial of added water (0% or 1%) and holding time (0 and 2 h) to determine the effect on pellet quality. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS version 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 pellet mill throughput and retentions time were similar across treatments (Table 2). For the moisture content of the three sample types (Table 3), there were no three-way and two-way interactions observed among sample type, added water level, and holding time. There was difference for moisture content among sample types ($P < 0.001$). The conditioned mash sample had the greatest moisture content followed by cooled pellets and mash sample. The feed with 1% added water had greater moisture than feed without added water ($P = 0.001$). The holding time did not affect moisture content of the feed. For the mash samples (Table 4), there were no three-way and two-way interactions among added water, holding time, and analysis method. The added water, holding time and analysis method did not impact phytase stability in mash feed prior to conditioning and pelleting. For conditioned mash samples (Table 5), there were no three-way and two-way interactions among added water, holding time, and analysis method for phytase activity and phytase stability. The added water, holding time, and analysis method did not impact phytase activity and phytase stability. The phytase stability of conditioned mash was similar between the mash feed with no-added and 1% added water. For the cooled pellets (Table 6), there were no three-way and two-way interactions among added water, holding time and analysis method for phytase activity and phytase stability. The added water and holding time did not impact phytase activity and phytase stability. When the cooled pellets were analyzed by two methods, the EN ISO method had decreased phytase activity and phytase stability compared to the ELISA method ($P = 0.023$). These data indicated that there was no evidence of a difference between the ELISA method and EN ISO method in mash and conditioned mash samples. However, there was a difference in phytase activity when analyzing cooled pellets. The difference in phytase activity between methods in cooled pellets did not create a three-way interaction or two-way interactions between added water and method or holding time and method. Thus, both methods could be used in the laboratory to determine *Trichoderma reesei* phytase activity. In the current study, adding 1% water or increasing the holding time by 2 h resulted in a similar phytase activity as compared to feed that had no water addition or that was immediately pelleted.

For pellet quality (Table 7), there was no interaction between added water and holding time. Both added water and holding time did not impact PDI ($P = 0.163$). For analyzed moisture content, adding 1% water in the mixer increased the moisture content of mash feed from 12.2 to 12.8%. When the mash feed was pelleted with a 5.6 L:D die at 185°F, the PDI was similar between mash feed with 12.2 and 12.8% moisture content. Adding water to mash feed in the mixer may have a greater benefit on pellet quality when the initial moisture content of the mash feed is less than 12.0%.

## *Conclusion*

The results of this study indicate that the stability of *Trichoderma reesei* phytase was not affected when feed was stored in a bin up to 2 h prior to pelleting. The added water in mash feed did not affect the degradation of *Trichoderma reesei* phytase when the feed moisture did not exceed 13%. Additionally, the ELISA or EN ISO method could be used in the laboratory to determine *Trichoderma reesei* phytase stability. Finally, increasing moisture content of mash feed by 0.6% did not improve pellet quality.

*Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.*

**Table 1. Diet compositions of starter diet**

| Ingredients | Quantity (%) |
|---|---|
| Corn | 54.24 |
| Soybean meal | 36.00 |
| Soy oil | 4.50 |
| Defluorinated phosphate | 0.95 |
| Limestone | 1.55 |
| Poultry mineral and vitamin premix[1] | 0.25 |
| Choline chloride | 0.10 |
| L-lysine HCl | 0.20 |
| DL-methionine | 0.20 |
| L-threonine | 0.10 |
| Salt | 0.50 |
| Xylanase | 0.01 |
| Inert filler[2] | 1.40 |
| Total | 100.00 |

[1]Composition per kilogram: 20 g iron, 40 g zinc, 40 g manganese, 4.5 g copper, 0.6 g iodine, and 0.06 g selenium, 3,080,000 IU vitamin A, 1,100,000 IU vitamin D3, 6,600 IU Vitamin E, 4.4 mg vitamin B12, 330 mg menadione, 2,640 mg riboflavin, 2,640 mg d-pantothenic acid, and 11,000 mg niacin.
[2]Silicon dioxide.

**Table 2. Pellet mill processing parameters[1]**

| Treatment | | | | |
|---|---|---|---|---|
| Added water level, % | Holding time, h | Production rate, lb/h | Retention time, s | Condition mash temperature, °F |
| 0 | 0 | 139.6 | 29.1 | 183.2 |
| 0 | 2 | 137.1 | 28.7 | 184.6 |
| 1 | 0 | 138.7 | 31.0 | 183.0 |
| 1 | 2 | 135.1 | 31.6 | 182.5 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.

**Table 3. The effect of sample type, added water level, and holding time on moisture content**

| Sample type | Added water level, % | Holding time, h | Moisture, % |
|---|---|---|---|
| Interaction effects | | | |
| Condition mash | 0 | 0 | 16.95 |
| Condition mash | 0 | 2 | 17.16 |
| Condition mash | 1 | 0 | 17.72 |
| Condition mash | 1 | 2 | 17.19 |
| Cooled pellet | 0 | 0 | 13.45 |
| Cooled pellet | 0 | 2 | 13.33 |
| Cooled pellet | 1 | 0 | 14.27 |
| Cooled pellet | 1 | 2 | 14.18 |
| Mash | 0 | 0 | 12.15 |
| Mash | 0 | 2 | 12.28 |
| Mash | 1 | 0 | 12.89 |
| Mash | 1 | 2 | 12.71 |
| SEM | | | 0.31 |
| Main effect | | | |
| Condition mash | | | 17.26[a] |
| Cooled pellet | | | 13.81[b] |
| Mash | | | 12.51[c] |
| SEM | | | 0.15 |
| | 0 | | 14.22[x] |
| | 1 | | 14.83[y] |
| | SEM | | 0.12 |
| | | 0 | 14.57 |
| | | 2 | 14.48 |
| | | SEM | 0.12 |
| Source of variation | | | *P*-value |
| Sample type × water level × holding time | | | 0.602 |
| Water level × holding time | | | 0.300 |
| Sample type × holding time | | | 0.951 |
| Sample type × water level | | | 0.509 |
| Holding time | | | 0.567 |
| Water level | | | 0.001 |
| Sample type | | | 0.001 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.

[a-c]Means within a main effect of sample type followed by different letter are significantly different ($P \leq 0.05$).

[x-y]Means within a main effect of added water level by different letter are significantly different ($P \leq 0.05$).

**Table 4. The effect of added water level, holding time, and phytase analysis method on phytase activity of mash samples[1]**

| Added water level, % | Holding time, h | Method | Phytase activity, FTU/lb |
|---|---|---|---|
| Interaction effects | | | |
| 0 | 0 | ELISA[2] | 481 |
| 0 | 0 | EN ISO[3] | 492 |
| 0 | 2 | ELISA | 374 |
| 0 | 2 | EN ISO | 401 |
| 1 | 0 | ELISA | 407 |
| 1 | 0 | EN ISO | 499 |
| 1 | 2 | ELISA | 470 |
| 1 | 2 | EN ISO | 456 |
| SEM | | | 58.3 |
| Main effect | | | |
| 0 | | | 437 |
| 1 | | | 458 |
| SEM | | | 25.2 |
| | 0 | | 470 |
| | 2 | | 425 |
| | SEM | | 25.2 |
| | | ELISA | 433 |
| | | EN ISO | 462 |
| | | SEM | 25.2 |
| Source of variation | | | *P*-value |
| Water level × holding time × method | | | 0.405 |
| Water level × holding time | | | 0.147 |
| Water level × method | | | 0.779 |
| Method × holding time | | | 0.546 |
| Water level | | | 0.560 |
| Holding time | | | 0.233 |
| Method | | | 0.431 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.
[2]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.
[3]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity."

**Table 5. The effect of added water level, holding time, and phytase analysis method on phytase activity and phytase stability of conditioned mash samples**

| Added water level, % | Holding time, h | Method | Phytase activity, FTU/lb | Phytase stability, % |
|---|---|---|---|---|
| Interaction effects | | | | |
| 0 | 0 | ELISA[2] | 356 | 81.1 |
| 0 | 0 | EN ISO[3] | 318 | 69.3 |
| 0 | 2 | ELISA | 409 | 93.3 |
| 0 | 2 | EN ISO | 336 | 73.2 |
| 1 | 0 | ELISA | 403 | 91.9 |
| 1 | 0 | EN ISO | 371 | 80.9 |
| 1 | 2 | ELISA | 402 | 91.6 |
| 1 | 2 | EN ISO | 385 | 84.0 |
| SEM | | | 47.1 | 10.47 |
| Main effect | | | | |
| 0 | | | 354 | 79.2 |
| 1 | | | 390 | 87.1 |
| SEM | | | 21.5 | 4.78 |
| | 0 | | 362 | 80.8 |
| | 2 | | 383 | 85.5 |
| | SEM | | 22.5 | 5.01 |
| | | ELISA | 392 | 89.5 |
| | | EN ISO | 352 | 76.8 |
| | | SEM | 21.5 | 4.78 |
| Source of variation | | | *P*-value | |
| Water level × holding time × method | | | 0.684 | 0.675 |
| Water level × holding time | | | 0.643 | 0.635 |
| Water level × method | | | 0.614 | 0.630 |
| Method × holding time | | | 0.872 | 0.859 |
| Water level | | | 0.263 | 0.265 |
| Holding time | | | 0.503 | 0.499 |
| Method | | | 0.215 | 0.086 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.
[2]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.
[3]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity."

**Table 6. The effect of added water level, holding time, and phytase analysis method on phytase activity and phytase stability of cooled pellet samples[1]**

| Added water level, % | Holding time, h | Method | Phytase activity, FTU/lb | Phytase stability, % |
|---|---|---|---|---|
| Interaction effects | | | | |
| 0 | 0 | ELISA[2] | 307 | 70.0 |
| 0 | 0 | EN ISO[3] | 285 | 62.1 |
| 0 | 2 | ELISA | 303 | 69.0 |
| 0 | 2 | EN ISO | 231 | 50.4 |
| 1 | 0 | ELISA | 265 | 60.4 |
| 1 | 0 | EN ISO | 243 | 52.9 |
| 1 | 2 | ELISA | 312 | 71.1 |
| 1 | 2 | EN ISO | 268 | 58.4 |
| SEM | | | 25.2 | 5.66 |
| Main effect | | | | |
| 0 | | | 281 | 62.8 |
| 1 | | | 272 | 60.7 |
| SEM | | | 12.6 | 2.83 |
| | 0 | | 275 | 61.3 |
| | 2 | | 278 | 62.2 |
| | SEM | | 12.0 | 2.69 |
| | | ELISA | 296[a] | 67.6[a] |
| | | EN ISO | 257[b] | 56.0[b] |
| | | SEM | 11.7 | 2.64 |
| Source of variation | | | *P*-value | |
| Water level × holding time × method | | | 0.677 | 0.711 |
| Water level × holding time | | | 0.059 | 0.062 |
| Water level × method | | | 0.680 | 0.673 |
| Method × holding time | | | 0.292 | 0.292 |
| Water level | | | 0.573 | 0.570 |
| Holding time | | | 0.835 | 0.817 |
| Method | | | 0.023 | 0.004 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.
[2]Phytase activity assay, ELISA specific for Quantum Blue, ESC Standard Analytical Method, SAM099.
[3]EN ISO 30024:2009 "Animal feeding stuffs-Determination of phytase activity."
[a-b]Means within a column follow by different letter are significantly different ($P \leq 0.05$).

**Table 7. The effect of added water level and holding time on pellet durability index (PDI) as determined by the Holmen method with 120 s analytical time[1]**

| Added water level, % | Holding time, h | PDI, % |
|---|---|---|
| Interaction effects | | |
| 0 | 0 | 78.4 |
| 0 | 2 | 76.7 |
| 1 | 0 | 80.5 |
| 1 | 2 | 79.5 |
| SEM | | 2.20 |
| Main effect | | |
| 0 | | 77.5 |
| 1 | | 80.0 |
| SEM | | 1.49 |
| | | |
| | 0 | 79.4 |
| | 2 | 78.1 |
| | SEM | 1.22 |
| Source of variation | | *P*-value |
| Water level × holding time | | 0.830 |
| Holding time | | 0.430 |
| Water level | | 0.163 |

[1]Treatments were steam conditioned at 185°F for approximately 30 s and pelleted using a pellet mill (California Pellet Mill Co. model Cl-5, Crawfordsville, IN). The pellet mill was equipped with a 0.16 × 0.874 in die. There were 3 replicates for each treatment.