Kansas Agricultural Experiment Station Research Reports

Volume 6
Issue 10 *Swine Day*

Article 26

2020

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Recommended Citation

Saensukjaroenphon, M.; Evans, C. E.; Stark, C. R.; and Paulk, C. B. (2020) "Effect of Pellet Cooling Method, Sample Preparation, Storage Condition, and Storage Time on Phytase Activity of a Swine Diet," *Kansas Agricultural Experiment Station Research Reports*: Vol. 6: Iss. 10. https://doi.org/10.4148/2378-5977.8007

Effect of Pellet Cooling Method, Sample Preparation, Storage Condition, and Storage Time on Phytase Activity of a Swine Diet

Abstract

Temperature and moisture content have been identified as two factors that influence enzyme inactivation. Phytase may be further degraded in feed samples if there is moisture left in the sample and it is not properly stored prior to analysis. Therefore, the objective of this experiment was to determine the effect of cooling method, sample preparation, storage condition, and storage time on phytase stability. In Exp. 1, treatments were arranged in 2 × 2 factorial with main effects of sample preparation (none or freeze-dried) and storage condition (ambient storage or freezer storage). Diets were mixed 3 separate times to provide 3 replicates per treatment. The result of Exp. 1 demonstrated that there was no interaction between drying process and storage condition for mash samples collected from the mixer. The sample drying process and storage condition did not impact the phytase stability. In Exp. 2, treatments were arranged in a 2 × 3 factorial with main effects of cooling method (counterflow cooler or freezer) and sample preparation (non-dried then freezer storage, freeze-dried then freezer storage, freeze-dried then ambient storage). The diet was steam conditioned for approximately 45 s at 185°F using a 5.1- × 35.8-in single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) at a production rate of 2.2 lb/min by holding the feeder at a constant speed setting. The sample was collected at the end of the conditioner and did not pass the pellet die. The conditioner was run 3 separate times to provide 3 replicates for each treatment. The result of Exp. 2 demonstrated that there was no interaction between the cooling method and sample preparation for phytase stability of conditioned mash samples. The cooling method and sample preparation did not affect the phytase stability. In Exp. 3, treatments were arranged in a 5 × 3 × 2 factorial with main effects of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler, or freezer), storage condition (ziplock/ ambient, ziplock/ frozen, and vacuum/frozen), and storage time (1 or 3 wk.). The diet was steam conditioned for approximately 45 s at 185°F and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with 0.16- × 0.50-in die. The diet was pelleted at a production rate of 2.2 lb/ min by holding the feeder at a constant speed setting. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. The result of Exp. 3 demonstrated that there were no three-way and two-way interactions among cooling method, storage condition, and storage time ($P > 0.686$). The cooling method, storage condition, and storage time did not impact phytase stability ($P > 0.348$). Therefore, freeze-drying, vacuum sealing, and freezing were not required when the feed samples were analyzed within 3 weeks of production. However, conditioned mash and hot pellet samples should be dried prior to sending the samples to the lab to prevent mold growth.

Keywords

phytase activity, phytase stability, sample preparation, sample storage

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K-STATE Research and Extension

Swine Day 2020

Effect of Pellet Cooling Method, Sample Preparation, Storage Condition, and Storage Time on Phytase Activity of a Swine Diet

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Summary

Temperature and moisture content have been identified as two factors that influence enzyme inactivation. Phytase may be further degraded in feed samples if there is moisture left in the sample and it is not properly stored prior to analysis. Therefore, the objective of this experiment was to determine the effect of cooling method, sample preparation, storage condition, and storage time on phytase stability. In Exp. 1, treatments were arranged in 2 × 2 factorial with main effects of sample preparation (none or freeze-dried) and storage condition (ambient storage or freezer storage). Diets were mixed 3 separate times to provide 3 replicates per treatment. The result of Exp. 1 demonstrated that there was no interaction between drying process and storage condition for mash samples collected from the mixer. The sample drying process and storage condition did not impact the phytase stability. In Exp. 2, treatments were arranged in a 2 × 3 factorial with main effects of cooling method (counterflow cooler or freezer) and sample preparation (non-dried then freezer storage, freeze-dried then freezer storage, freeze-dried then ambient storage). The diet was steam conditioned for approximately 45 s at 185°F using a 5.1- × 35.8-in single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) at a production rate of 2.2 lb/min by holding the feeder at a constant speed setting. The sample was collected at the end of the conditioner and did not pass the pellet die. The conditioner was run 3 separate times to provide 3 replicates for each treatment. The result of Exp. 2 demonstrated that there was no interaction between the cooling method and sample preparation for phytase stability of conditioned mash samples. The cooling method and sample preparation did not affect the phytase stability. In Exp. 3, treatments were arranged in a 5 × 3 × 2 factorial with main effects of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler, or freezer), storage condition (ziplock/ambient, ziplock/frozen, and vacuum/frozen), and storage time (1 or 3 wk.). The diet was steam conditioned for approximately 45 s at 185°F and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with 0.16- × 0.50-in die. The diet was pelleted at a production rate of 2.2 lb/min by holding the feeder at a constant speed setting. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. The result of Exp. 3 demonstrated that there were no three-way

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and two-way interactions among cooling method, storage condition, and storage time ($P > 0.686$). The cooling method, storage condition, and storage time did not impact phytase stability ($P > 0.348$). Therefore, freeze-drying, vacuum sealing, and freezing were not required when the feed samples were analyzed within 3 weeks of production. However, conditioned mash and hot pellet samples should be dried prior to sending the samples to the lab to prevent mold growth.

Introduction

Exogenous phytase is commonly added in non-ruminant feed to increase phosphorus release from plant-based ingredients, which reduces the amount of phosphorus in the manure. Exogenous phytases were developed to tolerate high temperatures during pelleting and low pH in the stomach. *Trichoderma reesei* phytase is one of the more heat-tolerant phytases on the market. However, the research conducted on the stability of phytases after conditioning and pelleting at a similar temperature is highly variable. There are additional factors that may account for the differences in stability, such as pellet mill size, die length to diameter ratio (L:D), steam quality, or residence time in the conditioner. In addition, the moisture content can influence inactivation of phytase.[2] Water molecules around phytase may change hydrogen bonding within the three-dimensional structure of phytase, which may alter the shape of the active site. However, to our knowledge, there are no data on how sample handling could affect the stability of phytase. Phytase may be further degraded in feed samples based on sample moisture content and storage conditions during the time prior to analysis. Therefore, the objective of this experiment was to determine the effect of pellet cooling method, sample preparation, storage condition, and storage time on phytase stability.

Procedures

Experiment 1

Treatments were arranged in a 2 × 2 factorial with main effects of sample preparation (none or freeze-dried) and storage condition (ambient storage or freezer storage at -9°F) to determine the effect on phytase activity. A swine finishing feed was used for the experiment (Table 1). The ingredients were added to a 2-ft^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX). The feed was mixed for 3 min. Two 0.88-lb samples were collected from the mixer discharge. Diets were mixed 3 separate times to provide 3 replicates per treatment. Each sample was randomly assigned into two different sample preparations. A sample was dried using a freeze-dryer (Labconco model FreeZone 12, Kansas City, MO) for 8 h and another was kept at room temperature. Next, the samples were split into 2 samples and placed in a plastic bag. They were randomly assigned to either ambient storage or freezer storage for 1 week before being sent to the laboratory for phytase activity. The sample from each drying process was analyzed for moisture content.

Experiment 2

Treatments were arranged in a 2 × 3 factorial with main effects of cooling method (counterflow cooler or freezer) and sample preparation (non-dried then freezer storage at -9°F, freeze-dried then freezer storage at -9°F, or freeze-dried then ambient storage)

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

to determine the effect on phytase activity. A swine finishing feed was used for the experiment (Table 1). The ingredients were added to a 2-ft^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX) and mixed for 3 min. The diet was steam conditioned for approximately 45 s at 185°F using a 5.1- × 35.8-in single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) at a production rate of 2.2 lb/min by holding the feeder at a constant speed setting. The sample was collected at the end of the conditioner and did not pass the pellet die. A 1.8 lb sample was collected from the mixer discharge and four 2.2 lb samples were collected after conditioning. The conditioner was run 3 separate times to provide 3 replicates for each treatment. Each sample was randomly assigned into 2 different cooling methods. Two samples were cooled using the counterflow experimental cooler for 10 min, while the other 2 samples were cooled in the freezer (Criterion model CCF50M2W, Medley, FL) for 1 hour. Each set of 2 samples were randomly assigned into 2 drying processes: non-dried or freeze-dried. For the freeze-dried treatment, the samples were dried using a freeze-dryer (Labconco model FreeZone 12, Kansas City, MO) for 8 h. After that, the samples were split into 2 samples and placed in a plastic bag. They were randomly assigned to either ambient storage or freezer storage. For the non-dried treatment, the samples from both cooling methods were stored in the freezer. After 1 wk storage, the samples from 3 sample preparation methods (non-dried then freezer storage at -9°F, freeze-dried then freezer storage at -9°F, or freeze-dried then ambient storage) were sent to the laboratory for phytase activity. Both non-dried and freeze-dried samples from each cooling method were analyzed for moisture content.

Experiment 3

Treatments were arranged in 5 × 3 × 2 factorial with main effects of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler or freezer at -9°F), storage condition (ziplock/ambient, ziplock/frozen, or vacuum/frozen) and storage time (1 wk or 3 wk) to determine the effect on phytase activity. A swine finishing feed was used for the experiment (Table 1). The ingredients were added to a 2-ft^3 double ribbon mixer (Hayes & Stolz model HP2SSS-0106, Fort Worth, TX) and mixed for 3 min. The diet was steam conditioned for approximately 45 s at 185°F and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with a 0.16 × 0.50 in die. Diets were pelleted at a production rate of 2.2 lb/min by holding the feeder at a constant speed setting. The pellet mill was run 3 separate times to provide 3 replicates for each treatment. A 0.55 lb sample was collected from the mixer discharge and thirty 0.55 lb samples were collected after pelleting. Each sample was randomly assigned into 5 different temperature reduction methods: none (sample placed directly in a sample bag); heat diffusion (sample placed on 11.8 in paper plate for 30 min); experimental fan cooler or counterflow cooler (sample cooled with a 6 in axial fan or a counterflow cooler for 10 min); and freezer (sample placed in a freezer at -9°F, Criterion model CCF50M2W, Medley, FL, for 1 hour). Six cooled samples from each method were randomly assigned to 3 different storage conditions: ziplock/ambient—placed in a 4.6 × 7.4 in ziplock seal top plastic bag and ambient storage; ziplock/frozen—placed in a 4.6 × 7.4 in ziplock seal top plastic bag and freezer storage; and vacuum/frozen—vacuum sealed by a vacuum sealer (Ziploc® model V203, Racine, WI) and freezer storage. Two packed samples from each cooling method and storage condition were randomly assigned to 2 different storage times: 1 or 3 weeks. The

samples were analyzed for phytase activity. The samples from each cooling method were analyzed for moisture content.

Data Collection

Both mash and pellet samples were analyzed by using the QuantiPlateTM Kit for Quantum Blue® (AB Vista Inc, Plantation, FL). The color reaction was measured by the plate reader at 450/630 nm. The color was used to evaluate the phytase activity based on a calibration curve. The phytase results were reported as FTU/lb and percent phytase stability. The percentage phytase stability of the conditioned mash sample or cooled pellets was calculated by dividing the phytase activity of the conditioned mash sample or cooled pellets by the average phytase activity of the mash samples, then multiplying by 100.

For moisture content (AOAC 930.15, 1990), an aluminum tray weight was recorded then a 2-g sample was placed on the tray. 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 content was calculated by dividing the difference between the sample tray and the empty tray by sample weight then multiplying by 100.

Statistical Analysis

Data were analyzed as a completely randomized design for the 3 experiments. Exp. 1 treatments were arranged in a 2 × 2 factorial design of sample preparation (none or freeze-dried) and storage condition (ambient storage or freezer storage) to determine the effect on phytase activity. For Exp. 2, treatments were arranged in a 2 × 3 factorial of cooling method (counterflow cooler or freezer) and sample preparation (non-dried then freezer storage, freeze-dried then freezer storage, or freeze-dried then ambient storage) to determine the effect on phytase activity. For moisture content in Exp. 2, treatments were arranged in 2 × 2 factorial of cooling method (counterflow cooler or freezer) and sample preparation (non-dried or freeze-dried) to determine the effect on moisture content. For Exp. 3, treatments were arranged in a 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler, or freezer), storage condition (ziplock/ambient, ziplock/frozen, or vacuum/frozen) and storage time (1 wk or 3 wk) to determine the effect on phytase activity. For moisture content in Exp. 3, treatments were arranged to determine the effect of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler, or freezer) on moisture content. There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. Means were separated by least squares means. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Experiment 1

There was no interaction between drying process and storage condition (Table 2) for the mash samples collected from the mixer. The sample drying process and storage condition did not impact the analyzed phytase activity. The analyzed phytase activity was similar between samples that were stored under room temperature and in a freezer at -9°F for 1 wk before they were sent to the laboratory. The moisture content was 9.38 and 9.28% for non-dried and freeze-dried samples, respectively. The freeze-dryer

pulled out only 0.1% moisture from the mash sample after 8 h of operation. The lower moisture content of the initial sample may have reduced the efficiency of the drying process. Thus, when the feed moisture was lower than 9.4% and stored for 1 wk, drying or freezing the sample before sending the samples for phytase analysis did not affect the degradation of *Trichoderma reesei* phytase.

Experiment 2

There was no interaction between the cooling method and sample preparation (Table 3) for phytase stability of the conditioned mash samples. The cooling method and sample preparation did not affect the phytase stability of the conditioned mash samples. The phytase stability was similar among the 3 sample preparation methods regardless of cooling method. The phytase stability was 81.4% when the sample was freeze-dried then stored at room temperature for 1 wk. There was no evidence of difference for phytase stability between non-dried sample and freeze-dried sample when they were stored in a freezer at -9°F for 1 wk. The conditioned mash sample that was cooled by the counterflow experimental cooler for 10 min had a similar phytase stability as compared to the samples that were cooled in the freezer at -9°F for 1 hour. The analyzed phytase activity decreased between 7 and 31% from the mash sample when the mash feed was conditioned at 185°F for 45 s and then pelleted at a production rate of 2.2 lb/min. The results demonstrated that using different sample handling procedures after the feed was conditioned did not influence phytase stability. There was an interaction between cooling method and sample preparation ($P < 0.012$; Table 4) for moisture content of the conditioned mash samples. The freeze-dried sample had significantly lower moisture content as compared to the non-dried sample when they were cooled in the freezer at -9°F for 1 hour. However, there was no significant difference in moisture content between the freeze-dried sample and non-dried sample when they were cooled by the experimental counterflow cooler for 10 min. The freeze-dryer pulled out 2% moisture from the sample cooled in the freezer at -9°F but only 0.3% moisture from the sample that was cooled by a counterflow cooler. The counterflow cooler decreased the sample temperature and took away moisture, while the freezer only cooled the samples. Thus, when the moisture of the sample was lower than 11% and stored for 1 wk, freezing the samples before sending them for phytase analysis did not affect the degradation of *Trichoderma reesei* phytase. Though moisture level did not appear to influence phytase stability under the constraints of this trial, overall sample quality should still be considered. Increased moisture levels in samples such as conditioned mash can lead to reduced shelf-life of the sample and lead to bacterial and fungal growth, resulting in mold.

Experiment 3

There was no three-way and two-way interaction among cooling method, storage condition, and storage time for phytase stability of cooled pellets (Table 5). The cooling method, storage condition, and storage time did not impact phytase stability. The phytase stability was similar among the 5 different cooling methods regardless of storage condition and storage time. There was no difference for phytase stability between the samples stored at room temperature and -9°F when they were packed in a 4.6 × 7.4-in ziplock seal top plastic bag. For storage time, the phytase stability was similar between the samples stored for 1 wk and 3 wk regardless of cooling method and storage condition. The results of this experiment demonstrated that the cooling method, storage

condition and storage time did not influence phytase stability. The cooling method resulted in different ($P < 0.001$; Table 6) moisture content in the cooled pellets. The non-dried samples had increased moisture content compared to all other samples. Samples cooled using the counterflow cooler had decreased moisture content compared to non-dried, freezer, and heat diffusion. There was no difference in pellet moisture content between the experimental fan cooler and experimental counterflow cooler. Therefore, when the moisture of the sample was lower than 16.3% and stored up to 3 wk, vacuum sealing and freezing did not prevent the degradation of *Trichoderma reesei* phytase. The results of this experiment suggest that the experimental counterflow cooler and fan cooler could be used to cool down the conditioned mash sample to reduce the possibility of mold in a sample stored at room temperature. There was no evidence that the added moisture from the conditioning step, the efficiency of cooling step, and 3-wk holding period prior to phytase analysis affected the phytase stability.

Conclusion

The results of these experiments suggest that freeze-drying, vacuum sealing, and freezing were not required when samples are analyzed within 3 weeks of production. However, conditioned mash and hot pellet samples should be dried prior to sending the samples to the laboratory. Though moisture level did not appear to influence phytase stability under the constraints of this trial, overall sample quality should still be considered. Increased moisture levels in samples such as conditioned mash can lead to reduced shelf-life of the sample and lead to bacterial and fungal growth, resulting in mold.

Table 1. Diet composition (as-fed basis)

Ingredients	%
Corn	78.42
Soybean meal	19.20
Monocalcium phosphate, 21% P	0.33
Limestone	1.10
Swine vitamin premix[1]	0.13
Swine trace mineral premix[2]	0.13
L-lysine HCl	0.25
DL-methionine	0.02
L-threonine	0.05
Salt	0.35
Phytase[3]	0.02
Total	100.00

[1]Composition per kilogram: 73 g iron, 73 g zinc, 22 g manganese, 11 g copper, 0.2 g iodine and 0.2 g selenium.
[2]Composition per kilogram: 1,653,439 IU vitamin A, 661,376 IU vitamin D3, 17,637 IU vitamin E, 13.3 mg vitamin B12, 1,323 mg menadione, 3,307 mg riboflavin, 11,023 mg d-pantothenic acid, and 19,841 mg niacin.
[3]Quantum® Blue 5G (AB Vista Inc, Plantation, FL) provided 1,000 phytase units (FTU)/kg with a release of 0.195% available P.

Table 2. The effect of the drying process and storage condition on phytase activity in the mash sample (Exp. 1)[1]

Drying process	Storage condition	Phytase activity, FTU/lb
Interaction effects		
None	Ambient storage	386
None	Freezer	449
Freeze-dried[2]	Ambient storage	443
Freeze-dried	Freezer	449
SEM		47.9
Main effect		
None		418
Freeze-dried		391
SEM		30.9
	Ambient storage	410
	Freezer	399
	SEM	30.9
Source of variation		*P*-value
Drying process × storage condition		0.122
Drying process		0.539
Storage condition		0.798

[1]Treatments were arranged in a 2 × 2 factorial with main effects of sample preparation (none or freeze-dried) and storage condition (ambient storage or freezer storage at -9°F) to determine the effect on phytase activity. Diets were mixed 3 separate times to provide 3 replicates per treatment.
[2]Freeze-dried – sample was dried with freeze-dryer for 8 h.

Table 3. The effect of cooling method and sample preparation on phytase activity, and phytase stability of conditioned mash samples (Exp. 2)[1]

Cooling method[2]	Sample preparation[3]	Phytase activity, FTU/lb	Phytase stability, %
Interaction effects			
Experimental cooler	Non-dried and freezer storage	305	74.6
Experimental cooler	Freeze-dried and ambient storage	286	70.0
Experimental cooler	Freeze-dried and freezer storage	353	86.2
Freezer	Non-dried and freezer storage	323	78.8
Freezer	Freeze-dried and ambient storage	379	92.7
Freezer	Freeze-dried and freezer storage	283	69.1
SEM		46.3	11.32
Main effect			
Experimental cooler		313	76.4
Freezer		327	79.9
SEM		26.8	6.54
	Non-dried and freezer storage	314	76.7
	Freeze-dried and ambient storage	333	81.4
	Freeze-dried and freezer storage	318	77.7
	SEM	29.9	7.31
Source of variation		*P*-value	
Cooling method × sample preparation		0.144	0.144
Sample preparation		0.879	0.879
Cooling method		0.686	0.686

[1]Treatments were arranged in a 2 × 3 factorial with main effects of cooling method (counterflow cooler and freezer) and sample preparation (non-dried then freezer storage, freeze-dried then freezer storage, or freeze-dried then ambient storage) to determine the effect on phytase activity. The diet was steam conditioned for approximately 45 s at 185°F using a 5.1- × 35.8-in single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN). The sample was collected at the end of the conditioner and did not pass the pellet die. The conditioner was run 3 separate times to provide 3 replicates for each treatment.
[2]Counterflow cooler: sample was cooled with a counterflow cooler for 10 min; and freezer: sample was placed in a freezer for 1 hour.
[3]Freeze-dried: sample was dried with a freeze-dryer for 8 h; and freezer storage: sample was placed in a freezer at -9°F.

Table 4. The effect of cooling method and sample preparation on moisture content of pelleted samples (Exp. 2)[1]

Cooling method[2]	Sample preparation[3]	n	Moisture, %
Interaction effects			
Cooler	None	3	9.84[c]
Cooler	Freeze-dried	3	9.54[c]
Freezer	None	3	13.02[a]
Freezer	Freeze-dried	3	11.01[b]
SEM			0.263
Main effect			
Cooler		6	9.69[l]
Freezer		6	12.02[k]
SEM			0.186
	None	6	11.43[x]
	Freeze-dried	6	10.28[y]
	SEM		0.186
Source of variation			*P*-value
Cooling method × sample preparation			0.012
Sample preparation			0.002
Cooling method			<0.0001

[1]Treatments were arranged in a 2 × 3 factorial design of cooling method (counterflow cooler or freezer) and sample preparation (non-dried then freezer storage, freeze-dried then freezer storage, or freeze-dried then ambient storage) to determine the effect on phytase activity. The diet was steam conditioned for approximately 45 s at 185°F using a 5.1- × 35.8-in single shaft conditioner of a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN). The sample was collected at the end of the conditioner and did not pass the pellet die. The conditioner was run 3 separate times to provide 3 replicates for each treatment.

[2]Counterflow cooler: sample was cooled with a counterflow cooler for 10 min; and freezer: sample was placed in a freezer for 1 hour.

[3]Freeze-dried: sample was dried with a freeze-dryer for 8 h; and freezer storage: sample was placed in a freezer at -9°F.

[a-c]Means within an interaction effect between cooling method and sample preparation by different letters are significantly different ($P \leq 0.05$).

[k-l]Means within a main effect of cooling method by different letters are significantly different ($P \leq 0.05$).

[x-y]Means within a main effect of sample preparation by different letters are significantly different ($P \leq 0.05$).

Table 5. The effect of cooling method, storage condition, and storage period on phytase activity, and phytase stability of cooled pellet samples (Exp. 3)[1]

Cooling method[2]	Storage condition	Storage time, wk	Phytase activity, FTU/lb	Phytase stability, %
Interaction effects				
Fan cooler	Ziplock/ambient	1	244	52.7
Fan cooler	Ziplock/ambient	3	198	42.9
Fan cooler	Vacuum/frozen	1	191	41.1
Fan cooler	Vacuum/frozen	3	226	48.8
Fan cooler	Ziplock/frozen	1	212	45.8
Fan cooler	Ziplock/frozen	3	274	59.2
Freezer	Ziplock/ambient	1	229	49.5
Freezer	Ziplock/ambient	3	228	49.3
Freezer	Vacuum/frozen	1	230	49.8
Freezer	Vacuum/frozen	3	208	45.0
Freezer	Ziplock/frozen	1	282	61.0
Freezer	Ziplock/frozen	3	246	53.2
Heat diffusion	Ziplock/ambient	1	284	61.4
Heat diffusion	Ziplock/ambient	3	231	49.9
Heat diffusion	Vacuum/frozen	1	255	55.2
Heat diffusion	Vacuum/frozen	3	246	53.1
Heat diffusion	Ziplock/frozen	1	271	58.5
Heat diffusion	Ziplock/frozen	3	233	50.4
None	Ziplock/ambient	1	241	52.0
None	Ziplock/ambient	3	216	46.7
None	Vacuum/frozen	1	234	50.5
None	Vacuum/frozen	3	170	36.8
None	Ziplock/frozen	1	271	58.6
None	Ziplock/frozen	3	170	36.6
Counterflow cooler	Ziplock/ambient	1	250	54.1
Counterflow cooler	Ziplock/ambient	3	241	52.2
Counterflow cooler	Vacuum/frozen	1	253	54.6
Counterflow cooler	Vacuum/frozen	3	230	49.6
Counterflow cooler	Ziplock/frozen	1	227	49.1
Counterflow cooler	Ziplock/frozen	3	283	61.1
SEM			40.9	13.68

continued

Table 5. The effect of cooling method, storage condition, and storage period on phytase activity, and phytase stability of cooled pellet samples (Exp. 3)[1]

Cooling method[2]	Storage condition	Storage time, wk	Phytase activity, FTU/lb	Phytase stability, %
Main effect				
Fan cooler			224	48.4
Freezer			237	51.3
Heat diffusion			254	54.8
None			217	46.9
Counterflow cooler			247	53.5
SEM			22.0	4.75
	Ziplock/ambient		236	51.1
	Vacuum/frozen		224	48.5
	Ziplock/frozen		247	53.4
	SEM		16.7	3.62
		1	245	52.9
		3	227	49.0
		SEM	13.6	2.93
Source of variation			*P*-value	
Cooling method × storage condition × storage time			0.958	0.958
Storage condition × storage time			0.948	0.948
Cooling method × storage time			0.686	0.686
Cooling method × storage condition			0.999	0.999
Storage time			0.348	0.348
Storage condition			0.636	0.636
Cooling method			0.725	0.725

[1]Treatments were arranged in 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler or freezer), storage condition (ziplock/ambient, ziplock/frozen, or vacuum/frozen), and storage time (1 wk or 3 wk) to determine the effect on phytase activity. Diets were steam conditioned for approximately 45 s at 185°F and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with 0.16 in × 0.50 in die. Diets were pelleted 3 separate times to provide 3 replicates for each treatment.

[2]None: sample was placed directly in a sample bag; heat diffusion: sample was placed on 11.8 in paper plate for 30 min.; fan cooler or counterflow cooler: sample was cooled with a 6-in axial fan or a counterflow cooler for 10 min; and freezer: sample was placed in a freezer for 1 hour.

Table 6. The effect of cooling method on moisture content of pelleted samples (Exp. 3)[1]

Cooling method	Moisture, %
None	16.28[a]
Freezer	15.43[b]
Heat diffusion	14.75[b,c]
Fan cooler	14.38[c,d]
Counterflow cooler	13.97[d]
SEM	0.349
Source of variation	*P*-value
Cooling method	0.0001

[1]Treatments were arranged in 5 × 3 × 2 factorial design of cooling method (none, heat diffusion, experimental fan cooler, experimental counterflow cooler, or freezer), storage condition (ziplock/ambient, ziplock/frozen, or vacuum/frozen) and storage time (1 wk or 3 wk) to determine the effect on phytase activity. Diets were steam conditioned for approximately 45 s at 185°F and pelleted using a pellet mill (California Pellet Mill model Cl-5, Crawfordsville, IN) equipped with 0.16 × 0.50 in die. Diets were pelleted 3 separate times to provide 3 replicates for each treatment.

[2]None: sample was placed directly in a sample bag; heat diffusion: sample was placed on 11.8 in paper plate for 30 min; fan cooler or counterflow cooler: sample was cooled with a 6 in axial fan or a counterflow cooler for 10 min; and freezer: sample was placed in a freezer for 1 hour.

[a-d]Means within a column by different letters are significantly different ($P \leq 0.05$).