SEGES INNOVATION

MEDDELELSE

No. 1304

7 November 2024

Heat stability of phytase products

Sally Veronika Hansen and Sabine Stoltenberg Grove

SEGES Innovation P/S

SUPPORTED BY

Danish Pig Levy Fund

Main conclusion

The heat stability of six phytase products was tested at 70, 85, 92 and 100 °C. All six phytase products proved heat-stable up to 85 °C, but for three of them, the phytase activity decreased at 92 °C. At 100 °C, a drop in phytase activity was observed for all six products.

Abstract

Analysis of the heat stability of the phytase products: Ronozyme HiPhos, HiPhorious, Axtra Phy Gold, Natuphos E, Quantum Blue and Optiphos Plus G confirmed that all six were heat-stable up to 85 °C.

At 92 °C - after correction was made for lost innate phytase from the grain - results showed activity levels of 90-100% for Natuphos E, Axtra Phy Gold and HiPhorious, and approx. 85% for Ronozyme HiPhos, Optiphos Plus G and Quantum Blue compared to the activity at 70 °C. Activity at 92 °C was significantly lower for Ronozyme HiPhos, Optiphos Plus G and Quantum Blue compared to the activity at 70 °C.

At 100 °C, analyses found a statistically significant decrease in phytase activity for all six products with the largest drop seen for Quantum Blue and Optiphos Plus G and the smallest drop seen for Axtra Phy Gold.

On feed mills where processing temperatures can be kept at maximum 92 °C, all products will maintain their activity level if an appropriate safety margin is applied. If the temperature routinely exceeds 92 °C, a large safety margin should be applied and/or production should only include the most heat-stable products.

The heat stability of the six phytase products was tested in the temperature interval 70-100 °C at a trial facility at the Danish Technological Institute in Kolding, where the temperature and duration of pelleting could be carefully controlled.

Background

The addition of phytase to pig feed is essential to the release of phosphorus in the feed ingredients, which is bound in phytate, thereby improving the utilization of phosphorus in the feed and lowering phosphorus emissions from pig production. However, phytase activity is affected by processing temperature, which can be a problem with pelleted feed where the processing temperature must be

minimum 81 °C to eliminate Salmonella. A test made at a Danish feed mill found that temperature during pelleting varied from 84 to 92 °C [1], and it is therefore crucial that the added phytase can maintain its activity despite high temperatures.

A wide range of phytase products is available for use in finished feed, but in 2023 a study revealed variations in phytase activity in finished feed [2]. The phytase activity is adversely affected by an increase in pelleting temperature but is dependent on the heat stability of the phytase product. Studies made in the period 2008-2010 found that at a pelleting temperature of 93 °C, phytase activity dropped by up to 35% compared to the phytase activity in the meal feed [3], and by about 10% when the temperature increased 80 to 95 °C for the phytases Ronozyme NP and Phyzyme XP [4].

Furthermore, the resting time in the pelleting process can also affect phytase activity [5]. Inactivation of phytase is mainly caused by steam during pelleting [6], which is why several phytase products are protected against the steam – either naturally or chemically – by, for instance, a water-repellent coating [7], changed amino acid structure [8] or by enzymes that are naturally more heat-stable.

The purpose of this project was to test the heat stability of different phytase products to establish recovery rates of the added phytase after heat-treatment during the pelleting process.

Materials and methods

The test was conducted by the Danish Technological Institute in Kolding at a mini-feed production facility, where feed was pelleted at different, controlled temperatures. Production of the meal diet, mixing technique, resting time, capacity and cooling time were identical for all products. Only the addition of phytase and the addition of steam to the cascade mixer to reach the desired pelleting temperatures of 70, 85, 92 and 100 °C varied.

The heat stability of the innate phytase in feed and of the six phytase products was analyzed (Table 1). The feed in each trial group was subject to four pelleting temperatures, after which the mini-feed production mill was cleaned before the next phytase product was added to the pelleting process. The pelleting process was performed only once for each group and samples were collected as the temperature increased – with a short resting time at each temperature level. The feed production mill is described in Appendix 1.

Table 1. Tested phytase products.

Producer	Phytase product	Concentration, FTU/g product[1]	Dosage of product, g/t feed
Innate phytase	-	-	-
AB Vista	Quantum Blue	10,000	200
BASF	Natuphos E	10,000	200
Danisco	Axtra Phy Gold	10,000	200
DSM	HiPhorious	10,000	200
DSM	Ronozyme HiPhos	20,000	100
HuvePharma	Optiphos Plus G	10,000	200

[1] Declared concentration.

The same diet was used throughout the test and the water content in the meal feed was 12.1%. The feed composition corresponded to standard finisher feed and is described in Appendix 2. The six phytase products were added in the dosage necessary to reach a concentration of 2,000 FTU/kg feed. All the phytase products were added to the feed in granulated form before pelleting.

Feed production and pelleting

A basic diet (see Appendix 2) was produced at the Danish Technological Institute's trial facility. The meal feed was produced in one go and all ingredients originated from the same batch. Prior to

production, mill and mixing equipment were cleaned of all feed residues and the mixer was vacuumed. The mini-feed production mill was cleaned before production of feed for each trial group: mixer and dosing screw were vacuumed, the cascade mixer was self-emptying, and residues were cleaned from the cooling box manually.

The feed for each trial group consisted of 280 kg meal feed from which 10 kg was used for mixing the specific amount of phytase in a paddle mixer. The paddle mixer was emptied and cleaned between each production round. Subsequently, the 10 kg meal containing phytase was mixed with the remaining 270 kg meal feed for 10 minutes in the horizontal mixer of the mini-feed mill. One sample of 1 kg was collected directly from the horizontal mixer.

The feed was subsequently heated with steam in the cascade mixer to reach the desired temperature between 70 and 100 °C (Figure 1 and Appendix 3). The amount of steam was regulated by an expansion valve and a collecting pipe. The resting time in the cascade mixer was 60 seconds, after which the feed was pelleted. The temperature of the meal feed in the cascade mixer was continuously recorded, and feed that was pelleted at a temperature lower than the desired temperature was discarded. When the temperature in the cascade mixer reached the desired level, the pelleted feed was collected during the following 90 seconds to a cooler, where it was cooled for 15 minutes with a ventilator. When sampling at the given temperature was complete, the temperature in the cascade mixer was increased to reach the next level, after which the sampling process was repeated. Appendix 4 provides an outline of the temperatures achieved in the cascade mixer during pelleting, i.e. the temperature in the cascade mixer was controlled, whereas the temperature in the pellet press was not recorded.

All samples were divided into sub-samples of 500 grams according to the TOS principle (Theory Of Sampling) [9], labelled and forwarded for analysis for phytase activity.

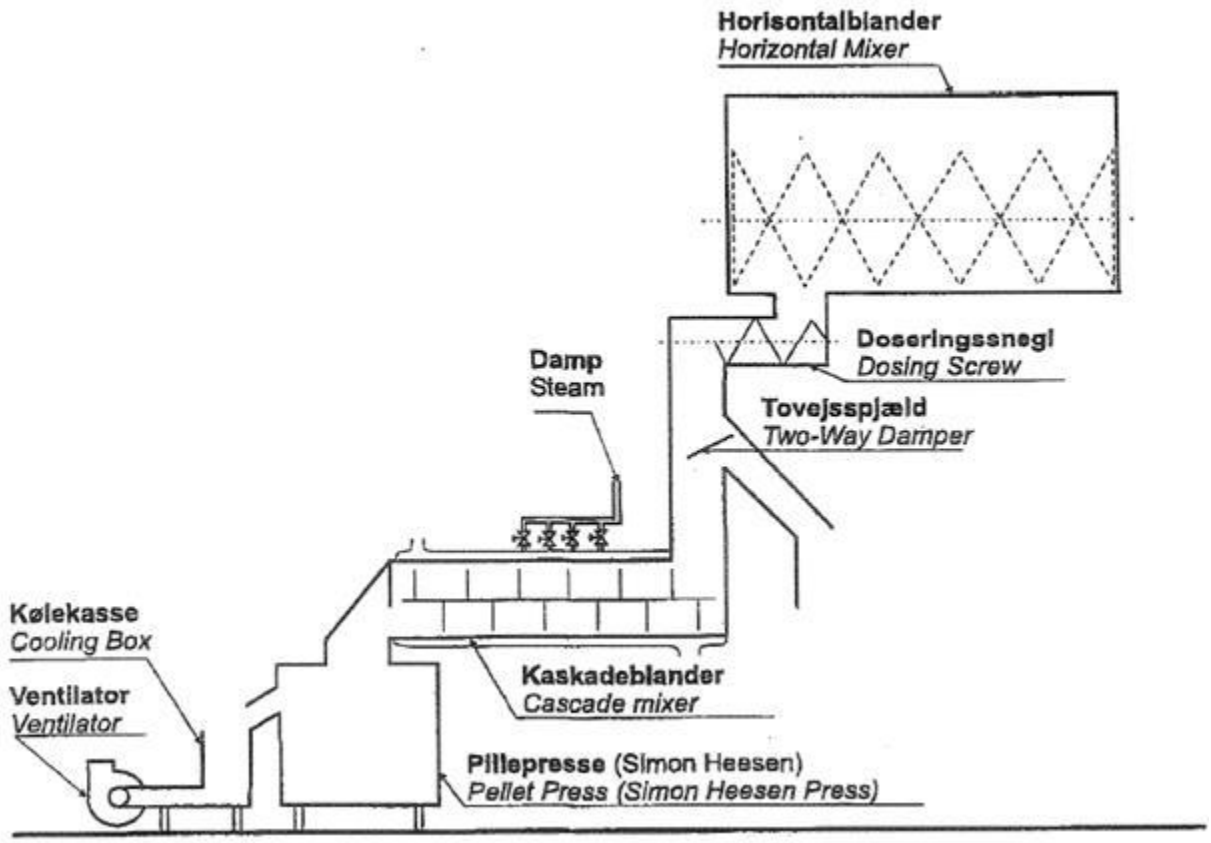

Figure 1. Illustration of the facility used at the Danish Technological Institute for pelleting.

Feed analyses

Analyses of the phytase activity for all products (including the remaining innate phytase) and the innate phytase in feed without added phytase at all tested temperatures including in the meal feed were made at the Eurofins Steins Laboratory. Three analyses/group were made of the meal feed and five analyses/group were made of the pelleted feed for each temperature level.

Statistical analyses

The percentage decrease in phytase activity between the control temperature (70 °C) and each of the measured temperatures (85, 92 and 100 °C) for each group was subject to analysis with a t-test in R. Phytase activity was not subject to statistical analysis before pelleting, and the decrease in phytase activity was not compared across the phytase products. Statistically significant differences are stated at the 5 per cent level.

Results and discussion

The innate phytase activity in the feed was 287 FTU/kg before pelleting, and it was numerically similar at 70 °C (Table 2), but when the temperature reached to 85 °C or more, the activity dropped below the detection limit of 180 FTU/kg. This was below the level recorded in a similar Danish study that found a phytase activity of 431 FTU/kg at 80 °C [4]. However, there are some similarities between the two studies, as the activity at 100 °C was approx. 12% of the level at 80 °C [4], which corresponds to the activity below the detection limit in this study. The relative phytase activity compared with the level in the meal feed decreased with increasing temperature and differed at 100°C for the six products (Appendix 5).

Table 2. Mean values (± the standard deviation) of phytase activity (FTU/kg) in feed without the addition of phytase (control) or with one of the six phytase products added to the meal feed before pelleting and at the four pelleting temperatures.

Phytase product	Meal feed	70 °C (control temperature)	85 °C	92 °C	100 °C
Innate phytase	287 ± 5	256 ± 9	U.D.[1]	U.D.	U.D.
Quantum Blue	3,291 ± 1,299	2,390 ± 333	2,652 ± 331	1,850 ± 75*	U.D.
Natuphos E	3,185 ± 195	2,588 ± 324	2,300 ± 377	2,243 ± 351	1,233 ± 49*
Axtra Phy Gold	2,205 ± 425	1,951 ± 352	1,902 ± 72	1,878 ± 303	1,315 ± 142*
HiPhorious	3,038 ± 559	2,965 ± 476	2,445 ± 328	2,551 ± 508	1,429 ± 372*
Ronozyme HiPhos	2,766 ± 351	2,635 ±,347	2,405 ± 596	2,059 ± 635*	1,059 ± 236*
Optiphos Plus G	2,857 ± 77	2,911 ± 107	2,506 ± 245	2,363 ± 166*	356 ± 51*

[1] Below the detection limit of 180 FTU/kg.

* Statistically significant decreases in phytase activity at the 5 per cent level compared with the control temperature (70°C).

The phytase activity in the meal feed in the six trial groups was 10-65% higher than the expected 2,000 FTU/kg (Table 2) and this is attributed to the fact that most producers apply a safety margin of 3,000-4,000 FTU/g product. In addition, the feed contained 287 FTU/kg of innate phytase and as expected the phytase activity was unaffected by pelleting at 70 °C. Analyses found no difference in phytase activity at 70 °C and 85 °C for any of the six phytase products, and thus they can all manage at a minimum of 81 °C which is required to eliminate Salmonella. Phytase activity decreased by approximately 20% for Ronozyme HiPhos, Quantum Blue and Optiphos Plus G at 92 °C compared to the control temperature ($P \leq 0.04$). However, when corrected for loss of innate phytase the drop was about 15% whereas the remaining three products showed no significant decrease in phytase activity.

Pelleting at 100 °C generated a decrease in phytase activity for all phytase products of minimum 30% ($P \leq 0.03$), with Quantum Blue falling below the detection limit and with only 10-15% activity remaining for Optiphos Plus G.

Thus, all the tested phytase products should be heat-stable during the pelleting process, provided the temperature is kept below approx. 92 °C. Other studies have also found phytase activity to be relatively unaffected up to around 85-90 °C [5,6,10] and for that activity to drop at temperatures above 90 °C [5].

The calculated relative phytase activity at the four temperatures compared with the level in meal feed is shown in Appendix 5, where correction is made for the content of innate phytase. All phytase products maintain a minimum of 70% relative activity up to 85 °C, but at 100 °C the activity of Quantum Blue and Optiphos Plus G drops below 15% of the activity in the meal feed and for Axtra Phy Gold it drops to 68%. This is followed by HiPhorious, Natuphos E and Ronozyme HiPhos with recovery rates of 52, 43 and 42%, respectively. However, these differences may be coincidental, as more replicates would be required to be able to separate them.

This study confirms existing knowledge that temperatures below 90 °C during the pelleting process have minimal impact on the phytase activity [5,6,10] of commercial phytase products that are reportedly heat-stable while significant differences are found between products at 100 °C.

Conclusion

Overall, Ronozyme HiPhos, HiPhorious, Axtra Phy Gold, Natuphos E, Quantum Blue, and Optiphos Plus G are heat-stable during the pelleting process at 85 °C, but Ronozyme HiPhos, Quantum Blue and Optiphos Plus G stand out by being less heat-stable at 92 °C, with a drop in activity of approx. 20%, but only 15% when correction is made for the innate phytase content.

All products lose a significant part of their activity at 100 °C and for Optiphos Plus G and Quantum Blue recovery rates are below 15%. The recovery rates for Ronozyme HiPhos, Natuphos E and HiPhorious average 40-55% after correction for loss of innate phytase content, while Axtra Phy Gold retained 68% of activity at 100 °C. If the temperature often exceeds 90 °C, a large safety margin should be applied and/or production should only include the most heat-stable products, while feed mills that can stay below 90 °C have a wider range of products to choose from and are able to apply a smaller safety margin when dosing phytase.

This analysis concludes only on the heat stability of the phytase products, and not the effect in pigs. For confirmation that feed contains the declared amounts of phytase, analyses of the phytase activity in the feed will be necessary.

References

[1] Hansen, S.; Rasmussen, D.K. (2009). Afprøvning af Ronozyme WX til slagtesvin. Meddelelse 848. Dansk Svineproduktion, Den rullende Afprøvning.

[2] Grove, S. S. og Tybirk, P. (2023): Kontrol af færdigfoder. Meddelelse 1287. SEGES Innovation P/S.

[3] Timmons, J.R.; Angel, R.; Harter-Dennis, J.M.; Saylor, W.W.; Ward, N.E. (2008). Evaluation of heatstable phytases in pelleted diets fed to broilers from day zero to thirty-five during the summer months. Journal of Applied Poultry Research 17, 482-489.

[4] Rasmussen, D. K. (2010). Forskel i varmestabilitet for fytase- og xylanaseprodukter i foder. Meddelelse 875. Dansk Svineproduktion, Den rullende Afprøvning.

[5] Promdonkoy, P.; Tang, K.; Sornlake, W.; Harnpicharnchai, P.; Kobayashi, R.S.; Ruanglek, V.; Upathanpreecha, T.; Vesaratchavest, M.; Eurwilaichitr, L.; Tanapongpipat, S. (2009). Expression and characterization of *Aspergillus* thermostable phytases in *Pichia pastoris*. FEMS Microbiology Letters, 290,18–24, https://doi.org/10.1111/j.1574-6968.2008.01399.x

[6] Wang, Y; Zhao, F; Zhang, H; Zhang, Q; Zhao, W; Sa, R; Xie, J. 2023. Effects of Phytase Source and Dose on Its Stability during Pelleting Process under Different Conditioning Temperatures. Animals 13, no. 23: 3741. https://doi.org/10.3390/ani13233741

[7] Yang, E; Dong, H; Khongkomolsakul, W; Dadmohammadi, Y; Abbaspourrad, A. 2023. Improving the thermal stability of phytase using core-shell hydrogel beads. Food Chem X 16, no. 21: 101082. https://doi.org/10.1016/j.fochx.2023.101082

[8] Xing, H; Wang, P; Yan, X; Yang, Y; Li, X; Liu, R; Zhou, Z. 2023. Thermostability enhancement of Escherichia coli phytase by error-prone polymerase chain reaction (epPCR) and site-directed mutagenesis. Frontiers in Bioengineering and Biotechnology. 11. 10.3389/fbioe.2023.1167530.

[9] Jørgensen, L.; Fisker, B. (2006). Udtagning af foderprøver. Info Svin, Videncenter for Svineproduktion.

[10] De Jong J.A; Woodworth, J.C.; DeRouchey, J.M.; Goodband, R.D.; Tokach, M.D.; Dritz, S.S.; Stark, C.R.; Jones, C.K. (2017). Stability of four commercial phytase products under increasing thermal conditioning temperatures. Translational Animal Science, 1, 255–260, https://doi.org/10.2527/tas2017.0030.

Participants

Technician: Linda Sandberg Pedersen
Statistician: Mai Britt Friis Nielsen
Other participants: Danish Technological Institute

Other information

Trial no. 1942
Project no.: 101128

//JAHP//

Appendix 1 - Description of mini-feed mill

The feed mill consists of:

Horizontal mixer: 700 L volume and a speed of 48 rpm (rounds per minute) with a mixing capacity of 80 to 300 kg.

Dosing screw: Skjold TR with variable speed used for emptying the mixer and for dosing the meal feed.

Cascade mixer: KAHL mixer with a length of 130 cm and a diameter of 30 cm. The mixer has a speed of 155 rpm and 37 adjustable pallets. The resting time in the cascade mixer is approximately 30 seconds at a production of 300 kg per hour.

A collecting pipe is mounted on the side of the cascade mixer with a water supply and 3 steam valves from which steam is added to the meal feed.

High-pressure boiler: Dan Stroker with a maximum capacity of 400 kg steam per hour. The steam is added to the meal feed via an expansion valve, which controls the supply of steam to the cascade mixer. Three valves on the collecting pipe fine-tune the desired temperature in the meal feed. When 1% steam is added, the temperature of the meal feed increases by 14 °C.

The temperature of the meal feed is measured with a digital Testo 925 thermometer with a Pt 100 sensor. The sensor is located at the mouth of the cascade mixer. The thermometer is calibrated with an approved mercury Goldbrand/39 Q9732-818 thermometer.

Pellet press: Simon Hessen of the type labor (mono roll) with a 7.5 kW motor. The matrix has an inside diameter of 173 mm with a 3.5 x 35 mm screen. The press roll is 50 mm high and has a diameter of 140 mm.

The samples are cooled in a compartmentalized cooler with a perforated bottom, where the feed is ventilated with a fan with a capacity of 1,500 m^3 of air/hour.

Appendix 2 - Compound feed

Ingredients	%
Wheat	40.00
Barley	38.23
Soybean meal	17.00
Rapeseed oil	2.00
Vitamin and mineral premix	2.77

Appendix 3 – Mini feed mill

1 – Horizontal mixer
2 – Cascade mixer
3 – Pellet press
4 – Cooling box

Appendix 4 – Recorded temperatures

The recorded temperatures for each of the seven groups in the cascade mixer (°C)

Producer	Phytase product	Desired temperatures			
		70 °C	85 °C	92 °C	100 °C
Innate phytase	-	69.9-70.3	84.6-84.9	92.0-92.2	100.0-100.2
AB Vista	Quantum Blue	69.9-70.2	85.1-85.3	92.0-92.2	99.9-100.2
BASF	Natuphos E	69.7-70.0	85.0-85.2	92.0-92.2	100.0-100.2
Danisco	Axtra Phy Gold	69.8-70.0	84.9-85.2	92.0-92.2	99.9-100.1
DSM	HiPhorious	69.9-70.2	84.9-85.2	92.0-92.2	100.0-100.2
DSM	Ronozyme HiPhos	69.9-70.2	84.9-85.2	92.0-92.2	100.1-100.3
HuvePharma	Optiphos Plus G	70.0-70.3	84.9-85.1	92.0-92.2	99.9-100.1

Appendix 5 – Relative phytase activity

The relative phytase activity or the recovery rate of phytase at 70, 85, 92 and 100 °C in relation to the activity in the meal feed is calculated using the following formula:

$$\frac{Phytase\ activity\ in\ product_x\ at\ temperature_i - Innate\ phytase\ activity\ at\ temperature_i}{Phytase\ activity\ in\ product_x\ in\ meal\ feed - Innate\ phytase\ activity\ in\ meal\ feed} * 100$$

Table A. Phytase activity below the detection limit (180 FTU/kg) are estimated from previous experiments (red figures).

Phytase product	Meal feed	70 °C	85 °C	92 °C	100 °C
Innate phytase	287	256	118	48	3
Quantum Blue	3,291	2,390	2,652	1,850	100
Natuphos E	3,185	2,588	2,300	2,243	1,233
Axtra Phy Gold	2,205	1,951	1,902	1,878	1,315
HiPhorious	3,038	2,965	2,445	2,551	1,429
Ronozyme HiPhos	2,766	2,635	2,405	2,059	1,059
Optiphos Plus G	2,857	2,911	2,506	2,363	356

Due to analytical uncertainty, a relative phytase activity above 100% is achieved for Optiphos Plus at 70 °C, and a higher relative phytase activity is achieved for Quantum Blue, Axtra Phy Gold and HiPhorious at high temperatures (85°C, 90°C) compared to low temperatures (70°C, 85°C).

Table B. Relative phytase activity at 70, 85, 92 and 100 °C in relation to the activity in the meal feed (the activity in the meal feed is 100%).

Phytase product	70 °C	85 °C	92 °C	100 °C
Innate phytase	89.2%	41.1%	16.7%	1.0%
Quantum Blue	71.0%	84.4%	60.0%	3.2%
Natuphos E	80.5%	75.3%	75.7%	42.4%
Axtra Phy Gold	88.4%	93.0%	95.4%	68.4%
HiPhorious	98.5%	84.6%	91.0%	51.8%
Ronozyme HiPhos	96.0%	92.3%	81.1%	42.6%
Optiphos Plus G	103.3%	92.9%	90.1%	13.7%

The function of the relative phytase activity in response to the pelleting temperature in Figure A is adjusted because of the degree of accuracy of the analysis method. The calculated relative phytase activity from Table B is illustrated with a circle.

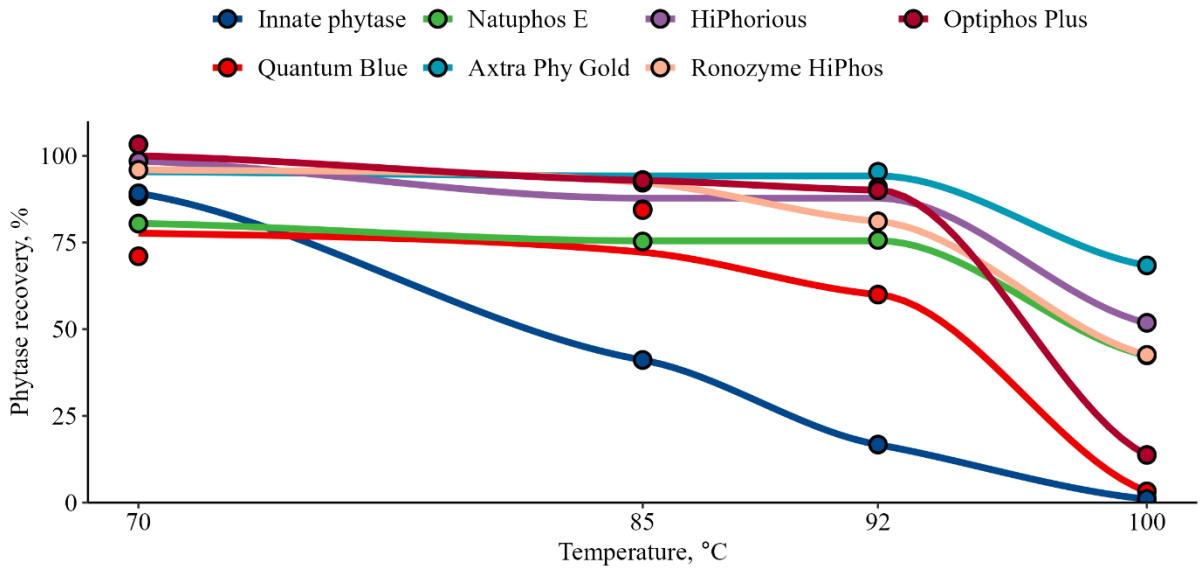

Figure A. Development in phytase activity in feed pelleted at increasing temperature relative to phytase activity in meal feed.