



Difference in heat stability of phytase and xylanase products in pig feed

Trial report 875
September 1, 2010

Danish Agriculture
& Food Council
Pig Research Centre

Axelborg, Axeltorv 3
DK 1609 Copenhagen V
Denmark

T +45 3339 4000
F +45 3311 2545
E vsp-info@lf.dk
W www.vsp.lf.dk

Dorthe K. Rasmussen
Pig Research Centre, Danish Agriculture and Food Council

Abstract

The heat stability of two phytase and four xylanase products was investigated, and the results demonstrated that enzyme activity dropped under the highest conditioning temperatures.

For the phytase products, Ronozyme NP and Phyzyme XP TPT, and for the xylanase products, Ronoxyme WX, Econase XT 25 and Danisco Xylanase 8000 G, more than 80% of the enzyme activity was preserved up to 95°C. All these products were comparable in terms of heat stability. Porzyme 9302 stood out by being less heat stable; at 90°C xylanase activity was reduced to half of the original level.

Conditioning and pelleting processes at a feed mill normally do not exceed 95°C and should therefore not cause problems to the activity of the studied enzymes except for Porzyme 9302.

Enzyme activity in this study demonstrates only heat stability and not the effect in the pig. To ensure that the feed contains the declared levels of enzymes, it is possible to analyse this for phytase, whereas for xylanase it is a matter of trust.

The effect of Ronozyme WX and Porzyme 9302 is currently being investigated in different dosages in finisher feed.

The heat stability of the products in this study was studied in a trial facility at the Technological Institute in Kolding where it was possible to control temperature and duration of conditioning and pelleting. The study included two commercial phytase and four xylanase products conditioned for 30 seconds in the temperature interval 80-100°C prior to pelleting.

The study was financially supported by the Pig Levy Fund and the EU and the Rural Development Programme under the Danish Ministry of Food, Agriculture and Fisheries and has project ID DSP09/10/58 and file no. 3663-D-07-00234.

The Danish Agriculture & Food Council represents the agricultural and food industry of Denmark and is the result of a merger between the *Danish Agricultural Council, Danish Bacon and Meat Council, Danish Pig Production and Danish Agriculture*. The organisation also undertakes a range of key tasks for the *Danish Dairy Board*.

Agriculture and food are Denmark's largest industry and innovation grouping employing some 150,000 people and exporting agricultural products and equipment to a value of around €15 billion.

Background

The addition of phytase to feed is essential to obtain optimum utilization of the feed's content of phosphorus and is an important tool in reducing the emission of phosphorus from livestock production [1], [2], [3], [4], [5]. Several studies have also demonstrated an increase in daily gain and an improved feed conversion when phytase is added to pig feed [4], [27]. In practice, it is often questioned whether pig feed contains enough digestible phosphorus and whether the feed contains the level of phytase required when calculating the content of digestible phosphorus per feed unit (FU_{gp}).

A study made by the Danish Plant Directorate in 2007 revealed a phytase deficiency in 13 of 42 samples, corresponding to 31% [6]. In 2008, a corresponding study revealed a 14% deficiency in the analysed samples [7].

In the feed season 2008/2009, it was standard procedure to use double dosage phytase. When the value of phytase is included in relation to the minimum standard dosage of phosphorus, it is essential that the phytase is still present in the feed after heat-treatment and pelleting. Natural phytase also disappears during strong heat-treatment.

Phytase is naturally present in many types of grain such as rye, wheat, triticale and barley, and can also affect the utilization of phosphorus. In 2003, grain analyses revealed a phytase level of 1050 FTU per kg wheat and 580 FTU per kg winter barley [8]. These results are comparable with another analysis that revealed 1193 units per kg wheat and 582 units per kg barley [9].

The use of the carbohydrate splitting enzyme xylanase may improve daily gain and feed conversion in pigs, which has been demonstrated in several weaner and finisher trials [28], [15], [16]. However, other studies demonstrated no effect of the addition of xylanase to feed for finishers [10], [17].

Enzymes are affected by temperature and pressure, and that may become a problem during the production of pelleted feed where the minimum temperature must be 81°C to counteract Salmonella problems. An investigation of the temperature during conditioning at a Danish feedstuff factory demonstrated variations in temperature from 84°C to 92°C [10].

Trials with carbohydrate splitting xylanase have revealed highly varying results depending on the product in question. Results of trials conducted at temperatures between 90°C and 95°C ranged from no enzyme activity to 90% activity after pelleting [11], [12], [13]. Another trial conducted at 80°C demonstrated an enzyme activity of 85%, 55% and 33%, respectively, for three different products after pelleting [14]. Several other studies have also demonstrated large variations in enzyme activity in feed to which xylanase was added. One study found a 50% reduction in enzyme activity after pelleting [15]; other studies found less activity than expected [16], [10], and some found large variations [10], [17].

Studies on the addition of phytase to feed have demonstrated that under pelleting temperatures of 93°C and double dosage phytase, the enzyme activity of two different phytase products was reduced to 80% and 70% compared with prior to pelleting [18]. Another study found an enzyme activity of 67% and 60%, respectively, following pelleting temperatures of 80°C and 90°C [19]. The sensitivity to temperature may vary greatly depending on the type of phytase used [20].

Enzyme activity is also influenced by the duration of conditioning. One study revealed no enzyme activity at all at temperatures above 80°C during heating for 55 seconds, but during heating for 140 seconds enzyme activity was completely lost at 75°C [21]. It is likely that this difference in duration explains the varying levels of enzyme activity found in different studies. Cooling time after heat-treatment may also greatly affect enzyme activity.

Enzyme activity also depends of the protection - natural or chemical - of the enzymes against heat-treatment. Deactivation of enzymes is primarily caused by steam during conditioning and pelleting [22]. Enzymes are therefore often coated with a water-repellent material that provides protection against the steam and prevents penetration of water [23]. Other enzymes are "designed" thermostable by altering their amino acid structure. One final option is using enzymes that are naturally thermostable [11]. A study of different types of coating of xylanase pelleted at 80°C found

levels of enzyme activity varying from 6% to 42% [24], and in another study enzyme activity increased from 12% to 34% during conditioning at 95°C with the use of coating [14].

Coating of enzymes increases their heat stability, but it may also complicate the release of enzyme in the animals [22]. One study demonstrated that thick coating improved the stability during pelleting, but reduced the effect in the animal by reducing the release of enzyme in the intestinal tract [25].

Porzyme 9302 from Danisco is the dominant xylanase product in the Danish market, where it is estimated to have a market share of approx. 80%. Ronozyme WX produced by Novozymes and supplied by DSM is believed to have a market share of approx. 20%, ie. these two products dominate the market. The dominant phytase products in the Danish markets are Natuphos from BASF, Ronozyme P from DSM and Phyzyme XP TPT from Danisco. BASF recommends spraying Natuphos onto the feed after pelleting as the product is not heat stable under temperatures above 85°C. In the future, Ronozyme P will be replaced by Ronozyme NP.

The aim of this study was to investigate the heat stability of phytase and xylanase products to be able to rank them according to heat stability.

Materials and method

The heat stability was studied in a miniature feed mill at the Technological Institute in Kolding where conditioning and pelleting was performed under 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 enzymes and the addition of steam in the cascade mixer to reach the desired conditioning temperature varied. The feed mill is described in Appendix 1.

The formulation of the diet corresponded to a regular standard diet for pig finishers and is shown in Appendix 2. The same feed was used throughout the entire study. Water percentage in the meal feed was standardised to 12.1%.

In table 1, the enzyme products used in this study are shown. The study comprised two phytase products: Phyzyme XP TPT from Danisco and Ronozyme NP from DSM; and four xylanase products: Porzyme 9302 and Danisco Xylanase 8000 G both from Danisco, Ronozyme WX from DSM and Econase XT 25 from AB Vista. Ronozyme NP will replace Ronozyme P; Econase XT 25 is a new, recently registered product; and Danisco Xylanase 8000 G is a newly developed product similar to Porzyme 9302, but for which it should be possible to retrieve more xylanase than is the case with the current product. Danisco 8000 G is not commercially available yet. Natuphos from BASF was not tested as this product is not heat stable at temperatures higher than 85°C and is sprayed onto the feed after pelleting.

Table 1. Enzyme products

Product	Supplier
Phytase products	
Phyzyme XP TPT	Danisco
Ronozyme NP	DSM
Xylanase products	
Porzyme 9302	Danisco
Danisco Xylanase 8000 G	Danisco
Ronozyme WX	DSM
Econase XT 25	AB Vista

Each batch with enzymes contained 290 kg feed, and five temperatures were studied for each batch. A phytase product and a xylanase product were added to each batch. The combination of phytase and xylanase products in a batch was random, but it was ensured that all combinations occurred an equal number of times. Conditioning temperatures above 80°C were selected as a minimum temperature of 81°C during pelleting at the feed mill is required by law. The study comprised 14 replicates (batches) for each enzyme product at each temperature. The study included one control group at 80°C and four trial temperatures per product: 85°C, 90°C, 95°C, and 100°C. Further-

more, the same feed, but without the addition of enzyme, was studied for each temperature to investigate the heat stability of natural phytase.

Three times the minimum dosage recommended by the suppliers was used for both phytase products and xylanase products (table 2) to have a higher content of the product in the samples for analysis of enzyme activity. Natural enzymes thereby constitute a smaller part of the total phytase activity. All enzyme products were added to the feed in granulated form before conditioning and pelleting.

Table 2. Inclusion of enzyme		
	Enzyme units, per kg feed	Inclusion of product, g/t
Phytase products		
Phyzyme XP TPT	1500 FTU	450
Ronozyme NP	6000 FYT	600
Xylanase products		
Porzyme 9302	9000 U	1125
Danisco Xylanase 8000 G	9000 U	1125
Ronozyme WX	600 FXU	600
Econase XT 25	72000 BXU	450

Production of feed and pelleting processes

A basic diet was produced at the feed mill at the Technological Institute in Kolding (see Appendix 2 for a detailed description of the diet). One load was produced of the meal diet, and all ingredients originated from the same batches.

For each production of a batch of trial feed, a premix was produced in a paddle mixer with 10 kg of the meal diet and a weighed amount of enzymes. Subsequently, 280 kg of the meal feed was mixed with the premix for ten minutes in the horizontal mixer in the feed mill.

The trial feed was then conditioned in the cascade mixer until the desired temperature between 80°C and 100°C was reached (see figure 1 and Appendix 3) and subsequently pelleted. The amount of steam was regulated with an expansion valve and a collecting pipe.

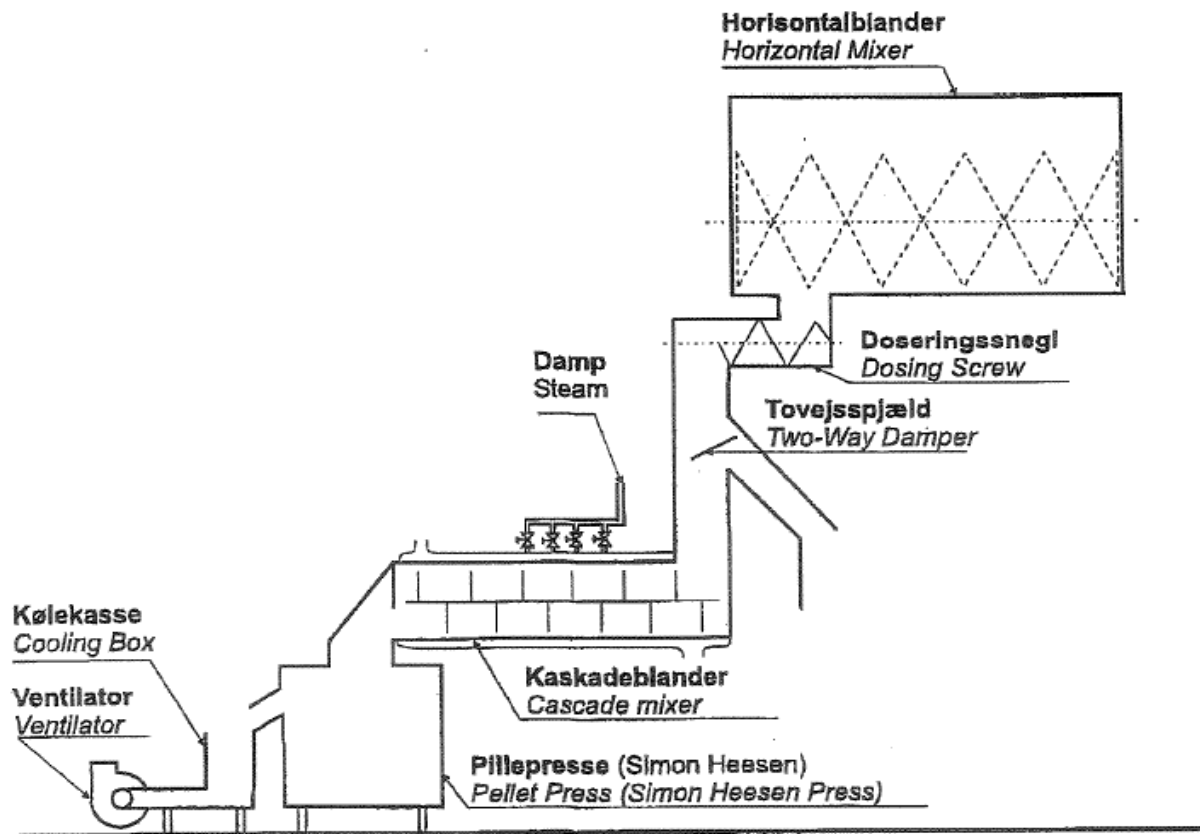


Figure 1. Mini feed mill used for pelleting feed containing enzymes.

The feed was mixed in the cascade mixer for 30 seconds and the temperature was routinely recorded. Pelleting at the given temperature lasted 8-10 minutes before samples were taken to ensure that the temperature was stable at the desired level. Sampling took place over approx. two minutes during which the samples, at the given temperature, were moved to a cooling box 10-15 seconds after leaving the pellet press. The temperature was then increased and the process repeated with the same trial feed. In the cooling box, the pelleted trial feed was cooled for 15 minutes with a ventilator. All sample material was divided into subsamples of 500 g each according to the TOS principle (Theory Of Sampling), labelled and shipped for analysis of enzyme activity. A sampling process with 290 kg trial feed and five different temperature levels took approx. 70 minutes.

Before production of feed started, the feed mill was cleaned of feed remnants and the mixer was vacuum cleaned. The miniature feed mill was cleaned before and after each replicate. Mixer and dosing equipment were vacuum cleaned, and the cascade mixer was self-emptying. The small mixer for premix and the cooling box were cleaned after each replicate.

Analyses of feed

The enzyme activity was analysed for all products and for natural phytase at all tested temperatures. Phytase was analysed at the Eurofins Steins analysis lab. Xylanase was analysed at laboratories selected by the company supplying the enzyme, ie. they were basically analysing their own product. Before shipment, the samples were blinded in terms of temperature.

Statistics

The difference in per cent enzyme activity of phytase and xylanase compared with the control temperature (80°C) and the difference in survival of enzyme activity between the products at each temperature level were subjected to a proc mixed analysis in SAS. The statistical model included temperature as systematic effect and replicate as variable. Significant differences are stated at 5% level. A linear regression was also made of the enzyme activity under increasing temperatures.

Results and discussion

Identical analysis methods were used for all products for analysis of enzyme activity for phytase, whereas for xylanase many different methods exist and each laboratory uses their own method. Furthermore, the inclusion rates of the products differ. As a result, the exact levels of enzyme activity for the different xylanases are not directly comparable.

The lowest level of enzyme activity for both phytase and xylanase products were found at the highest temperatures. However, an increase in temperature did not trigger a significant linear drop in percentage activity for neither phytase products nor xylanases.

Phytase

Figure 2 shows the average enzyme activity for Ronozyme NP, Phyzyme XP TPT and natural phytase at different temperatures in per cent of the activity at 80°C. The enzyme activity for Ronozyme NP and Phyzyme XP TPT is the recorded activity for that product from which the activity of natural phytase is deducted, i.e. the actual activity of the product is shown.

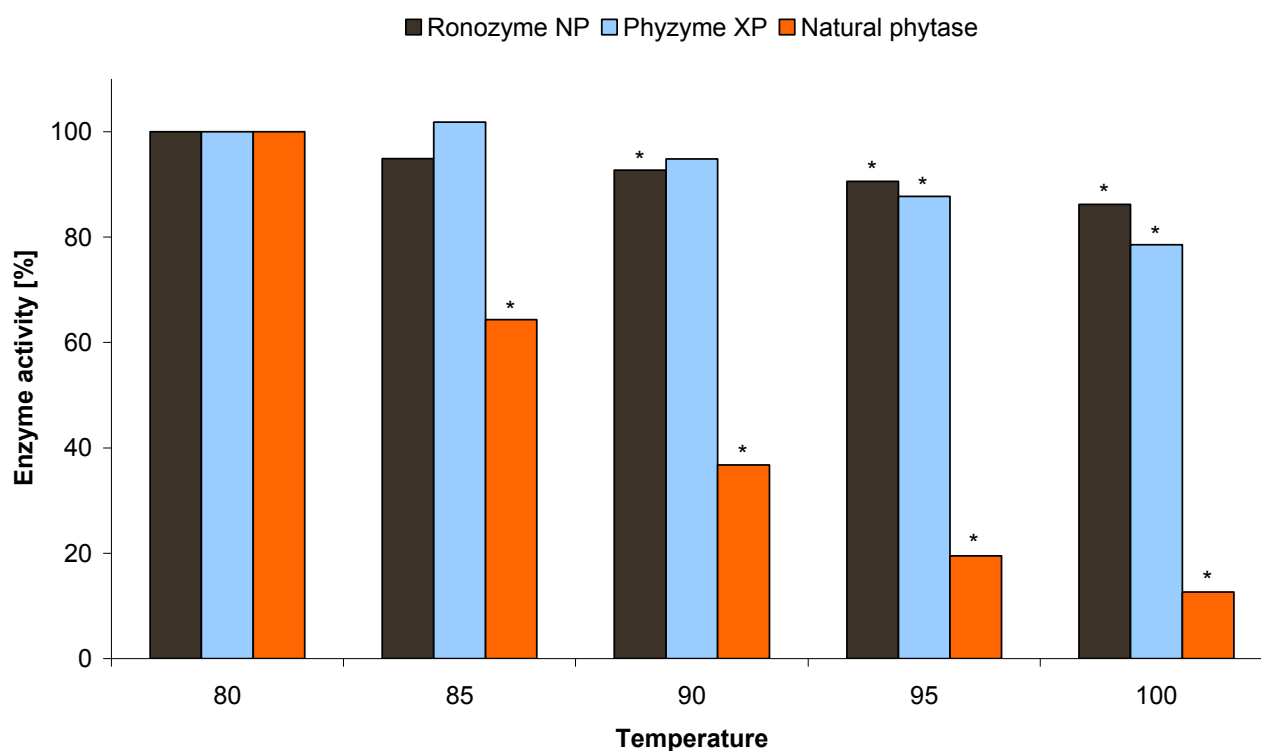


Figure 2. Average phytase activity in per cent compared with activity at 80°C. * illustrates significant difference at 5 per cent level for each product compared with control at 80°C.

For natural phytase, enzyme activity was significantly lower already at 85°C compared with 80°C and thereby also at higher temperatures. At 100°C, the enzyme activity was only 12% compared with the level at 80°C, which corresponded to averagely 431 FTU per kg natural phytase at 80°C and 51 FTU per kg at 100°C. This drop corresponds to approx. 0.3 g digestible phosphorus per feed unit. Results from a trial with a not-heat-treated basic diet containing 51% barley and 20% wheat demonstrated the same levels of phytase as found in this present study at 80°C. At heat-treatment slightly above 81°C, the enzyme activity was reduced by 25% [5], which corresponds with the slightly greater loss at 85°C found in this study. Another study revealed a 56% reduction in the activity of natural phytase after pelleting [9].

Ronozyme NP had a tendency to a lower enzyme activity at 85°C than at 80°C ($p=0.067$) and a significantly lower activity at high temperatures (see figure 2). Phyzyme XP TPT tended to have lower enzyme activity at 90°C than at 80°C, and from 90°C to 100°C the activity was significantly lower than the level at 80°C. Ronozyme NP had an 86% enzyme activity at 100°C and Phyzyme XP TPT had a 79% enzyme activity compared with the level at 80°C.

Compared with 80°C, the enzyme activity was identical for Phyzyme XP TPT and Ronozyme NP up to and including 95°C, but at 100°C the activity of Ronozyme NP was significantly higher than for Phyzyme XP TPT. The activity of Ronozyme NP dropped with the same intervals as the temperature increased, whereas Phyzyme XP TPT had the same enzyme activity at low temperatures and then dropped faster than Ronozyme NP.

The heat stability of both Ronozyme NP and Phyzyme XP TPT must be considered sufficiently high that pelleting should not cause problems. Up to and including 95°C, the heat stability of the two products was fully comparable.

At all temperatures, Ronozyme NP had a higher content of enzyme compared with the declared value; 30% and 12% at 80°C and 100°C, respectively, above declaration (see Appendix 4). The enzyme activity for Phyzyme XP TPT at 80°C was equal to the expected level.

Xylanase

Figure 3 shows the percentage of enzyme activity of xylanase products compared with the activity during the control temperature of 80°C.

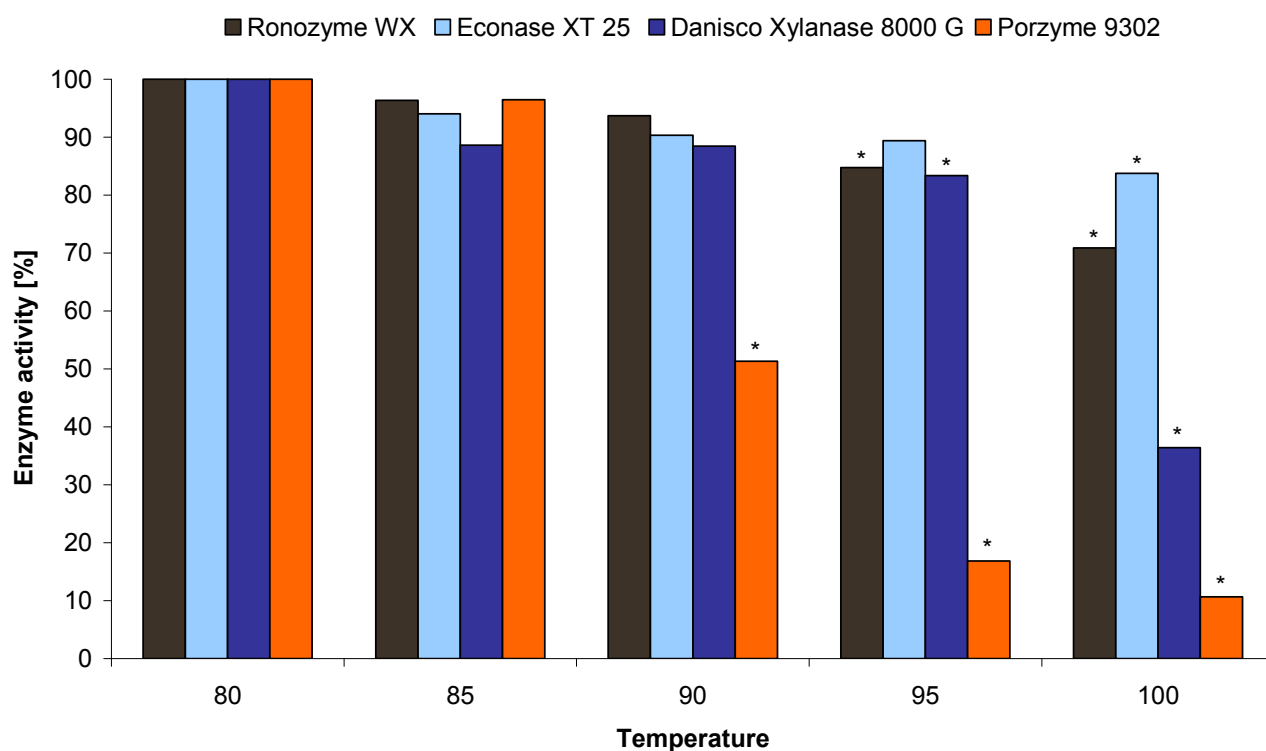


Figure 3. Average xylanase activity in per cent compared with activity at 80°C. * illustrates significant difference at 5 per cent level for each product compared with control at 80°C.

Enzyme activity of the products varied greatly. Econase XT 25 only had a significantly lower xylanase activity at 100°C compared with 80°C, but not at any other temperature. Ronozyme WX and Danisco Xylanase 8000 G had a significantly lower enzyme activity at 95°C and 100°C compared with 80°C, and Porzyme 9302 had a significantly lower activity at 90°C when compared with 80°C. Ronozyme WX, Econase XT 25, Danisco Xylanase 8000 G and Porzyme 9302 had an average xylanase activity at 100°C of 71%, 84%, 36% and 11%, respectively.

The enzyme activity of Econase XT 25 was 45% higher at 80°C and 21% higher at 100°C compared with the declared level. All the other products had a deficiency compared with the declared levels at 80°C of 14%, 6% and 87% for Ronozyme WX, Danisco Xylanase 8000 G and Porzyme 9302, respectively (see Appendix 5).

The enzyme activity of the xylanase products was analysed at different laboratories depending on the product, and the analysis results are therefore not directly comparable.

Ronozyme WX, Econase XT 25 and Danisco Xylanase 8000 G preserved more than 80% enzyme activity up to and including 95°C when compared with 80°C. Porzyme 9302 was less heat stable; at 90°C, activity had dropped by 50% compared with 80°C. As stated by the supplier, the percentage of xylanase retrieved during the subsequent analyses was higher for Danisco Xylanase 8000 G than for Porzyme 9302.

General discussion

The enzyme activity, particularly for the xylanase products, differs greatly between the products at high temperatures.

It is essential that enzymes have a good heat-stability during conditioning and that they are subsequently quickly released in the gastro-intestinal tract to preserve their effect in the animal. Coating of enzymes increases their heat stability, but may also complicate the excretion of enzyme in the animal [23]. The question is whether some of the enzymes are so heavily coated that the animals are unable to access them. This was not investigated in this study.

One study investigated the effect of the thickness of different coatings of phytase products. This study found that thicker coating improved stability during conditioning, but reduced the effect in the animal by reducing the release of enzyme in the intestinal tract [25]. The results of the present study show that Ronozyme WX is highly heat stable, but one study of Ronozyme WX in finisher feed demonstrated no effect on productivity [10]. It may be that the enzyme was so well coated that the pigs were unable to obtain an effect of the enzyme in the feed. The effect of Ronozyme WX and Porzyme 9302 is currently being investigated in different dosages in finisher feed.

Several international studies of xylanase for chickens demonstrate that the recorded enzyme activity cannot always be transferred directly to an effect on the animal's productivity [13], [24]. Danish investigations with xylanase for pigs have also demonstrated that the analysed enzyme activity does not always correspond to the effect seen in the animal. Porzyme 9302, which in this study had the lowest heat stability, improved feed conversion in two different finisher trials despite a much lower enzyme activity than expected [15]. A low enzyme activity in heat-treated feed does not necessarily demonstrate a significant breakdown of the enzyme. The reason for this contradiction is not known, but it may be triggered by incomplete extraction during analysis caused by binding of the enzyme to the substrate in the feed or the existence of "disrupting elements" in the feed. In addition, the enzyme may have been added in excess amounts, ie. there is still enough despite damage caused by the heat. It is thus not only important that enzyme activity remains high after pelleting, but also that the enzymes are active in the animal. The response recorded in the animals will always be the most sensitive measure of the effect of enzyme activity in feed.

No studies have indicated a positive effect on productivity of low phytase activity after pelleting. It seems that the effect of phytase depends primarily on the level of phytase recorded in the feed. This may be due to the fact that we have more knowledge on the amount of phytase required to find an effect in the animal, and we also operate with a standardised analysis for phytase.

Conclusion

Overall, Ronozyme NP, Phyzyme XP TPT (phytase products) and Ronozyme WX, Econase XT 25 and Danisco Xylanase 8000 G (xylanase products) all had a high heat stability; more than 80% of the enzyme activity was preserved following conditioning and pelleting up to 95°C compared with 80°C. All these products are comparable in terms of heat stability.

Porzyme 9302 stood out by being less heat stable; at 90°C xylanase activity had dropped by 50% compared with the original level.

Enzyme activity in this study only demonstrates heat stability and not the effect in the pig. To ensure that the feed contains the declared levels of enzymes, it is possible to analyse this for phytase, whereas for xylanase it is a matter of trust.

Conditioning and pelleting processes at a feed mill normally do not exceed 95°C and should therefore not cause problems to the activity of the studied enzymes except for Porzyme 9302.

References

- [1] Simons, P.C.M.; Versteegh, H.Q.J.; Jongbloed, A.W.; Kemme, P.A.; Slump, P.; Bos, K.D.; Wolters, M.G.E.; Beudeker, R.F.; Verschoor, G.J. (1990). Improvement of phosphorous availability by microbial phytase in broilers and pigs. *British Journal of Nutrition* 63, 525-540.
- [2] Jongbloed, A.W.; Kemme, P.A.; Mroz, Z.; V. Diepen, H.Th.M. (2000). Efficacy, use and application of microbial phytase in pig production: a review. In *Biotechnology in the Feed Industry, Proceedings of Alltech's sixteenth annual Symposium*, 111-129.
- [3] Johansen, K.; Poulsen, H.D. (2003). Svins fosforudnyttelse – hvilken effekt kan forventes ved fytasetilsætning. *Grøn viden - Husdyrbrug nr. 30, Danmarks JordbrugsForskning*.
- [4] Young, L.G.; Leunissen, M.; Atkinson, J.L. (1993). Addition of microbial phytase to diets of young pigs. *Journal of Animal Science* 71, 2147-2150.
- [5] Reduceret fosforudskillelse ved anvendelse af fytasetilsætning til svinefoder, Miljøprojekt 928, Miljøstyrelsen, juni 2004.
- [6] Plantedirektoratet. (2008). Fytase i foder. FO nr. 13/08.
- [7] Plantedirektoratet. (2009). Fytase i foder – resultater af kontrollen i 2008. FO nr. 08/09.
- [8] Vils, E.; Sloth, N.M.; Tybirk, P.; Poulsen, H.D. (2004). Fytaseaktivitet i korn, herunder variation i byg og hvede afhængig af kornsort, tørringstemperatur og gastæt oplagring. Notat 0441, Landsudvalget for Svin, Dansk landbrugsrådgivning og Landscentret Svin.
- [9] Eeckhout, W.; De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Animal Feed Science and Technology*, 47, Issues 1-2, 19-29.
- [10] Hansen, S.; Rasmussen, D.K. (2009). Afprøvning af Ronozyme WX til slagtesvin. Meddelelse 848, Dansk Svineproduktion, Den rullende Afprøvning.
- [11] Petterson, D.; Rasmussen, P.B. (1997). Improved heat stability of xylanases. *Proceedings of Australian Poultry Science Symposium* 9, 119-121.
- [12] Gibson, K. (1995). The pelleting stability of animal feed enzymes. In: *Proceedings of the 2nd European Symposium on Feed Enzymes*. Noordwijkerhout, NL., 157-162.
- [13] Bedford, M.R.; Pack, M.; Wyatt, C.L. (1997). Relevance of in feed analysis of enzyme activity for prediction of bird performance in wheat based diets. *Poultry Science* 76 (Suppl 1.), 39.
- [14] Pickford, J. R. (1992). Effects of processing on the stability of heat labile nutrients in animal feeds. In: *Recent Advances in Animal Nutrition*. P. C. Garnsworthy, W. Haresign, D. J. A. Cole, ed. Butterworth-Heinemann, Oxford, U.K., 177-192.
- [15] Callesen, J. (1998). Porzyme 9300 til slagtesvin. Meddelelse nr. 403, Landsudvalget for Svin.
- [16] Hansen, C.F.; Kjærsgaard, H.D.; Bach Knudsen, K.E. og Jensen, B.B. (2002). Effekt af melfoder og Porzyme 9300 på salmonella, mave-tarm-sundhed og produktivitet hos slagtesvin. Meddelelse 558, Landsudvalget for svin.
- [17] Rasmussen, D.K. (2008). Bergazym P i hjemmeblandet foder. Meddelelse 826, Dansk Svineproduktion, Den rullende Afprøvning.
- [18] Timmons, J.R.; Angel, R.; Harter-Dennis, J.M.; Saylor, W.W.; Ward, N.E. (2008). Evaluation of heat-stable 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.
- [19] Angel, R.; Wyatt, C. (2006). Effect of an evolved thermo-tolerant phytase on performance and bone ash in broiler chickens. *Poultry Science* 85 (Suppl. 1), 14.
- [20] Kjeldsen, N.; Ibsen, M.S. (2002). Enzymer tilsat svinefoder. Rapport nr. 20.
- [21] Silversides, F.G.; Bedford, M.R. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poultry Science* 78, 1184-1190.
- [22] Eeckhout, M.; DeSchrijver, M.; Vanderbeke, E. (1995). The influence of process parameters on the stability of feed enzymes during steam pelleting. *Proceedings of the 2nd European Symposium on Feed Enzymes*. Noordwijkerhout, The Netherlands, 163-169.
- [23] Bedford, M. (2008). Next generation thermo-tolerant enzymes for reducing feeding costs. 16th Annual ASA-IM SEA feed technology and nutrition workshop, Singapore, 1-8.
- [24] Francesch, M.; Perez-Vrendrell, A.M. (1997). Recent studies on enzyme application in animal feeding. *South European Feed Manufacturers Conference, Spain*, 149-159.
- [25] Klein Holkenburg, A.; Braun, J. (2001) Effect of a hydrophobic coating on phytase granulates on pelleting stability and bio-efficacy in broilers. 13th European Symposium on Poultry Nutrition: 341.

- [26] Jørgensen, L.; Fisker, B. (2006): Udtagning af foderprøver. Info Svin, Videncenter for Svineproduktion.
- [27] Sloth, N.M.; Tybirk, P. (2009). Fosforniveau I foderblandinger med højt indhold af plantefosfor til slagtesvin. Meddelelse 812, Den rullende Afprøvning.
- [28] Van Lunen, T.A.; Schulze, H. (1996). Influence of *Trichoderma longibrachiatum* xylanase supplementation of wheat and corn based diets on growth performance of pigs. *Canadian Journal of Animal Science* 76, 271-273.

Participants

Technological Institute, Kolding

Danisco

DSM

AB Vista

Eurofins

Statistician Mai Britt Nielsen

Trial no. 1070

Appendix 1

Description of feed mill

The feed mill consists of:

- A horizontal mixer with a volume of 700 l, a speed of 48 rpm and a mixing capacity of 80-300 kg.
- A dosing screw of the type Skjold TR with variable speed used for emptying the mixer and for dosing the meal feed.
- A cascade mixer of the type KAHL with a length of 130 cm and a diameter of 30 cm. It has a speed of 155 rpm and 37 adjustable pallets. Resting time in the cascade mixer is approx. 30 seconds at a production of 300 kg/hour.
- A collecting pipe on one side of the cascade mixer with a water supply and 3 steam valves from which steam is added to the meal feed.

Steam is added by a high-pressure boiler of the type Dan Stroker with a maximum capacity of 400 kg steam/hour. The steam is added to the meal feed with an expansion valve controlling the addition of steam to the cascade mixer. The three valves on the collecting pipe are used for fine-tuning the desired temperature in the meal diet. If 1% steam is added, the temperature of the meal increases by 14°C.

The temperature of the meal is recorded with a digital thermometer of the type Testo 925 with a Pt 100 sensor. The sensor is placed by the mouth of the cascade mixer. The thermometer is calibrated with an approved mercury thermometer of the type Goldbrand/39 Q9732-818.

The pellet press is a 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 cooling box divided into compartments with perforated bottom through which the meal feed is cooled with a ventilator with a capacity of 15000 m³ air/hour.

Appendix 2

Composition of the feed

Ingredient	%
Wheat	54.18
Soybean meal, flaked	20.54
Barley	10.00
Wheat bran	5.00
Rapeseed cake, 5 % fat	5.00
Palm oil	2.50
Feed lime	1.34
Mono calcium phosphate	0.69
Salt	0.35
Vitamin premix	0.20
Lysine 99%	0.13
Threonine 98.5%	0.04
DL-methionine	0.03

Appendix 3

Mini feed mill with ventilator, cooling box, pellet press, steam and cascade mixer



Mini feed mill with pellet press, steam, cascade mixer and horizontal mixer.



Appendix 4

Average enzyme activity for phytase products and natural phytase, FTU per kg

Temperature, °C	Ronozyme NP ¹	Phyzyme XP TPT ²	Natural phytase
80	7779	1544	431
85	7378	1561	282
90	7202	1452	160
95	7036	1350	83
100	6697	1204	51

Note: The levels of enzyme activity in the products are not directly comparable as they were added in different inclusion rates.

¹ Declared value: 6000 FYT per kg

² Declared value: 1500 FTU per kg.

Appendix 5

Average enzyme activity for xylanase products

Temperature, °C	Ronozyme WX ¹ , FXU	Econase XT 25 ² , BXU	Porzyme 9302 ³ , U	Danisco Xylanase 8000 G ³ , U
80	519	104522	1167	8424
85	497	97850	1066	7330
90	483	93892	543	7309
95	438	93019	182	6851
100	366	87219	111	3045

Note: The levels of enzyme activity in the products are not directly comparable as they were added in different inclusion rates.

¹ Declared value: 600 FXU

² Declared value: 72000 BXU

³ Declared value: 9000U