

## LCA Case Studies

# Environmental Assessment of Ronozyme® P5000 CT Phytase as an Alternative to Inorganic Phosphate Supplementation to Pig Feed Used in Intensive Pig Production

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## Preamble

2. Nielsen PH, Wenzel H (2006): Environmental Assessment of Ronozyme® P5000 CT Phytase as an Alternative to Inorganic Phosphate Supplementation to Pig Feed Used in Intensive Pig Production. Int J LCA, OnlineFirst (DOI: <http://dx.doi.org/10.1065/lca2006.08.265.2>)

1. Nielsen PH, Oxenbøll KM, Wenzel H (2006): Cradle-to-Gate Environmental Assessment of Enzyme Products Produced Industrially in Denmark by Novozymes A/S. Int J LCA, OnlineFirst (DOI: <http://dx.doi.org/10.1065/lca2006.08.265.1>)

The present paper is the second in a series of two and focuses on application of the enzyme product Ronozyme Phytase in pig production. The previous paper addressed environmental impacts of industrial enzyme production.

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## Abstract

**Goal, Scope and Background.** Ronozyme® P5000 CT is an industrially produced enzyme product (phytase) which is able to degrade naturally occurring phytate in animal feed and release the phytate's content of phosphorus for pig's growth. Ronozyme P5000 CT (hereafter called Ronozyme Phytase) can be used as an alternative to inorganic phosphorus supplementation to feed and the study addresses the environmental implications of substituting inorganic phosphorus with Ronozyme Phytase in intensive pig production in Denmark.

**Methods.** Life cycle assessment is used as an analytical tool, and modelling of the two considered systems is facilitated in SimaPro 6.0 software. The study addresses changes induced by switching from the one alternative to the other, and all significant processes influenced by the change are included in the study.

**Results and Conclusions.** Application of Ronozyme Phytase in intensive pig production is justified by major advantages in terms of avoided contributions to global warming, acidification, photochemical ozone formation and particularly nutrient enrichment and by significant energy savings and particularly phosphate savings. A single trade-off in terms of agricultural land use for enzyme production is small and unimportant unless use of agricultural land is given very large relative weight.

**Recommendations and Perspectives.** Hundreds of enzyme products are commercially available on the market today, each with a range of different applications. There are several indications that enzymes like Ronozyme Phytase can play an important role in a transition to a more sustainable society, and more focus should be addressed to the evolving enzyme technology in environmental research.

**Keywords:** Environmental assessment; enzyme technology; eutrophication; monocalcium phosphate; nutrient enrichment; phosphorus; phytase; pig production; white biotechnology

## Introduction

Enzymes are biological catalysts with an enormous capacity to accelerate biochemical reactions and make processes possible which would otherwise not occur under given conditions (Berg et al. 2002). Enzymes are produced industrially and used in a great variety of industries, and due to their high specificity and efficiency they are often superior to their conventional alternatives in terms of raw material and energy consumption and in terms of yield and quality of the final product (Ullmann's 2003).

These qualities of enzymatic processes indicate that industrial enzymes can support a sustainable development of our society by contributing to lowering environmental impacts per produced unit in industry. To gain the best possible foothold of this general description of enzymatic processes as a candidate for a more sustainable development, however, concrete justification is needed. Industrial production of enzymes requires energy and raw materials (Nielsen et al. 2006) and there may be areas in which the enzyme assisted processes are environmentally advantageous and there may be areas, where they are not.

Lifecycle assessment addresses all processes in the product chain of a product, and LCA is an appropriate tool to compare the environmental implications of conventional processes and enzyme assisted processes. Such comparisons have not previously received much attention in the literature, except by Fu et al. (2005), who made a preliminary assessment of environmental benefits of enzyme bleaching for pulp and paper making.

To expand our insight into the environmental implications of enzyme assisted production further, the present paper reports a life cycle assessment of a commercial enzyme product; a phytase product called *Ronozyme® P5000 CT* (here-

after referred to as Ronozyme Phytase). Ronozyme Phytase is an industrially produced enzyme product which makes phytate bound phosphorus naturally occurring in feed available to monogastric animals such as pigs, poultry and fish. The product can be used as an alternative to inorganic phosphate supplementation to the feed and hereby reduce excretion of phosphorus with manure. See Johansen and Poulsen (2003a) and Ravindran (1995). Novozymes produces Ronozyme Phytase by fungal fermentation (Nielsen et al. 2006) and the product considered here is distributed to the market by DSM Nutritional Products. The study addresses Ronozyme Phytase applied at intensive pig farms (1.4 livestock units per 10000 m<sup>2</sup>) in Denmark. Environmental implications of feed composition for pigs have previously been addressed in a detailed LCA study by Eriksson et al. (2005), but this study did not consider supplementation of feed phosphate or phytase to the feed.

## 1 Method

Life cycle assessment is used as an analytical tool (Wenzel et al. 1997) and modelling is facilitated in SimaPro 6.0. The study is a comparative analysis of two solutions providing the same function in pig production and modelling refers to processes responding to a change in demand for each of the two alternatives. Consequently, a marginal and market-oriented approach is taken, and co-product issues are handled by system expansion, see Wenzel (1998), Weidema et al. (1999) and Ekvall and Weidema (2004).

## 2 Scope

### 2.1 System boundaries

The study includes all environmentally significant processes which are induced or displaced as a result of Ronozyme Phytase application. The induced processes are mostly associated with Ronozyme Phytase production and the displaced processes are mostly associated with inorganic feed phosphate production (Monocalcium phosphate, MCP) because Ronozyme Phytase is seen as an alternative to inorganic phosphate supplementation to the feed. See Fig. 1.

### 2.1.1 Production of Ronozyme phytase

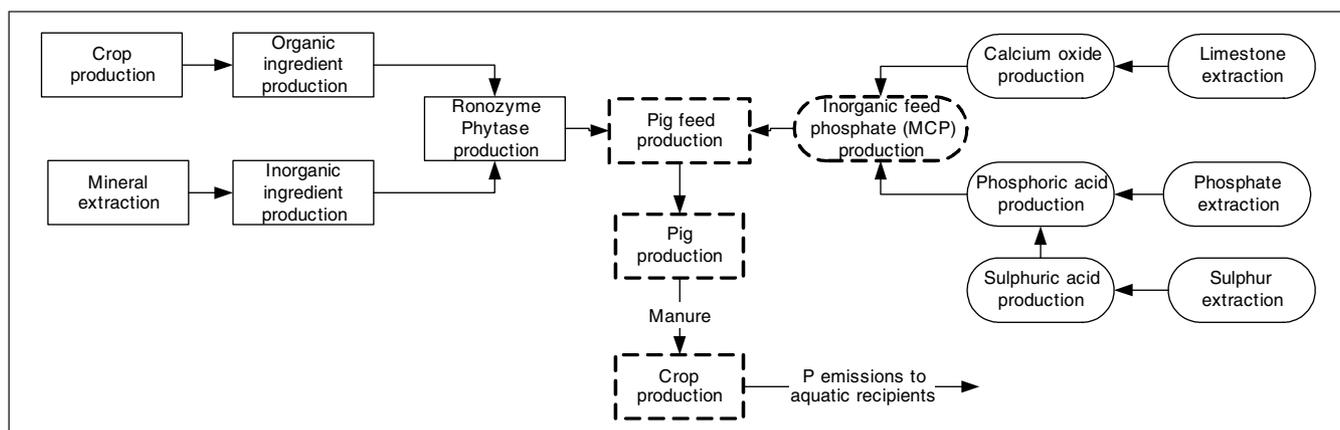
Ronozyme P5000 CT Phytase is a fungal phytase derived from *Peniophora lyci* found in nature on a dead tree. The phytase encoding gene has been transferred to *Apergillus oryzae* by gene technology. The enzyme is expressed in large scale in a fermentation process followed by a recovery process (filtration) and a granulation process. Electricity, steam and water are used in all processes and biomass and waste water is generated.

Data on Ronozyme Phytase production are representative for Novozymes' production in 2004. Data are provided at a detailed level and all inputs in terms of ingredients, energy and water for production and outputs in terms of products, wastewater and biomass are taken into account in the study, see Nielsen et al. (2006). Transport of Ronozyme Phytase is estimated to 120 km and is included in the assessment.

### 2.1.2 Displacement of inorganic feed phosphate

MCP is generally used as an inorganic phosphorus source in Danish pig production and displacement of inorganic feed phosphate as a result of Ronozyme Phytase application refers to this substance (Johansen and Poulsen 2003b). The chemical formula of MCP is  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  and the phosphorus content varies slightly between different commercial products. The present study refers to a commercially available MCP product with 22.7% phosphorus.

The phytase requirement varies from feed type to feed type: 1) because crops contain different amounts of endogenous phytase and 2) because plant phytase is denatured during heat treatment in industrial feed production, whereas it remains intact in farm made feed produced without heat treatment (Johansen and Poulsen 2003a,b and Tybirk 2002). 'The National Committee for Pig Production' provides standards for pig feed production in Denmark and the standards are to a large extent followed by feed producers and pig producers. Ronozyme Phytase's activity is quantified in the activity unit FYT, and the organisation recommends displac-



**Fig. 1:** Main system boundaries of the study. Processes indicated with rectangular boxes are induced and processes indicated with rounded boxes are displaced when Ronozyme Phytase displaces inorganic phosphorus supplementation to the pig feed. Pig production and crop production at the manure receiving fields (marked with dotted boxes) are not changed when Ronozyme Phytase displaces inorganic feed phosphate and are not included in the assessment. The phosphorus content of the manure is reduced when inorganic feed phosphate supplementation is avoided and reduced emissions of phosphorus from manure spread on farmland is included. The final production of inorganic phosphate (MCP) is not included because data on the process are missing

**Table 1:** Displacements of phosphorus and MCP in pig feed and estimated reduction of P emissions to aquatic recipients from manure spread on agricultural land. All data are provided per kg Ronozyme Phytase applied. Data on P emissions refer to the 5% P-loss scenario, except data in brackets which refer to the 0% and 100% P loss scenario, respectively

	Phosphorus (P) displacement	MCP displacement	Reduced P emission to aquatic recipients
Quantity	6.7 kg	29 kg	0.33 kg (0–6.6 kg)

ing 1 g P in MCP with 750 FYT for industrially produced feed pills (Tybirk 2002) and 0.6 g P with 450 FYT for feed mixed on the farm. I.e. 1.33 mg P displacement per FYT in both cases. Based on the provided recommendations, Ronozyme Phytase's feed phosphate displacement capacity is shown in Table 1.

### 2.1.3 Secondary effects of Ronozyme phytase supplementation

Phytase increases digestibility of calcium and a number of other minerals (Windisch and Kirchgessner, 1996 and Tybirk 2002). Such secondary effects are considered negligible from an environmental impact point of view and hence ignored in the assessment. Phytase has furthermore shown minor positive side effects on amino acid digestion and hence growth of animals (Tybirk 2002). The effect is, however, uncertain and the possible positive effect of phytase supplementation on pig's growth is disregarded.

### 2.1.4 Production of inorganic feed phosphate

MCP can be produced by treating burnt chalk ( $\text{CaO}$ ) or  $\text{Ca}(\text{OH})_2$  with phosphoric acid ( $\text{H}_2\text{PO}_4$ ) (Ullmann's 1979). The exact inputs and outputs are unknown due to confidentiality in business, and data on MCP production are generated by combining stoichiometric quantities of  $\text{CaO}$  and  $\text{H}_3\text{PO}_4$ , see Nielsen et al. (2003). Thus, data on MCP production includes mining processes of phosphate rock and limestone and production phosphoric acid and burnt chalk, but not the final production of MCP (see Fig. 1). Data on all processes are derived from Ecoinvent (2003) except sulphur for sulphuric acid production, which refers to primary sulphur (Patyk and Reinhardt 1997), because primary sulphur is considered the marginal source of sulphur even though secondary sulphur from refineries have increased their importance significantly during the past years (USGS 2005). Transportation distance of MCP from producer to pig farm is unknown, but has been included conservatively in the assessment using the same transportation distance as for Ronozyme Phytase (120 km, see above).

### 2.1.5 Spreading of phosphorus with manure

Pig manure is spread on farmland and phosphorus in manure is partly taken up by the crops, partly absorbed in the soil matrix and partly lost to aquatic recipients by leaching and erosion processes. Agricultural land at farms with high animal density is usually rich in phosphorus, and phosphorus spread with manure is in excess. The study refers to Ronozyme Phytase application at farms with 1.4 livestock units per 10000  $\text{m}^2$ , where

phosphorus spread with manure is in excess compared with the plant uptake. These farms are significant in Denmark (Statistics Denmark 2004) and the surplus phosphate can be reduced to about zero if phytase is applied as an alternative to inorganic phosphate (Pedersen 2002).

### 2.1.6 Emissions of phosphorus to aquatic recipients

Phosphorus spread with manure on farmland at farms with 1.4 livestock units per 10000  $\text{m}^2$  is on average in the order of 30 kg P per 10000  $\text{m}^2$  per year (DIAS 2003) while the plant uptake is about 20 kg P per 10000  $\text{m}^2$  per year (Kronvang et al. 2001). The net-surplus of about 10 kg P per 10000  $\text{m}^2$  per year will be subject to various loss processes to aquatic recipients and sorption to the soil matrix. The average annual phosphorus emissions to aquatic recipients is about 0.4–0.5 kg per 10000  $\text{m}^2$  per year (DIAS 2003) and this figure has been used to roughly estimate the current average phosphorus loss from fields to 5% of the net-surplus application due to lack of more accurate models. The importance of sorption processes and loss to aquatic recipients is determined by local conditions such as agricultural practice, weather conditions, field conditions, soil conditions, proximity to surface waters, etc. and the estimated P-loss is uncertain and covers a broad variation. Therefore, in addition to the 5% P-loss scenario, a 0% P-loss scenario (all P is fixed in the soil matrix) and a 100% P-loss scenario (no P is fixed in the soil matrix) have been included in the assessment to provide an impression of the entire range of possible impacts of phosphorus emissions. The applied relations between Ronozyme Phytase application and emission to aquatic recipients are shown in Table 1.

Phosphorus is the limiting factor for algal growth in most Danish lakes and in parts of the year Danish fiords and coastal waters, and the loss of phosphorus from agriculture constitutes presently more than 50% of the total phosphorus emission to Danish surface waters, see Kronvang et al. (2001). Hence, reduced emission of phosphorus from intensive pig production as a result of phytase application will contribute to alleviating eutrophication pressure on the aquatic environment.

## 2.2 Impact categories

The following environmental impact categories are included in the study: global warming, acidification, nutrient enrichment and photochemical ozone formation. Stratospheric ozone degradation is disregarded because no significant emissions of ozone degrading gasses appear in the considered system. Toxicity is disregarded because the available data basis is considered too incomplete. Application of phytase in pig production has a significant influence on phosphorus consumption and dispersion hereof in the environment. Phosphorus is essential to life and necessary for intensive crop production as practised in more and more areas of the world, and the resource 'phosphate rock' (which cannot be substituted) has therefore been considered in the assessment. Enzyme production is an energy intensive process and use of energy plays an important role in the considered system. Thus, energy consumption has been considered in terms of MJ primary energy carriers (low heat value,

LHV). Industrial production of enzymes is a biotechnological process, the substrate of which derives from agriculture. Therefore, any environmental benefits derived from using enzymes instead of alternative chemicals or other items, may happen at the expense of agricultural land. For this reason, use of agricultural land has been included in the study ( $\text{m}^2 \cdot \text{year}$ ). Use of land for other purposes (mines, infrastructure, production facilities, etc.) is not included because the available data basis is considered too inhomogeneous and incomplete. Ronozyme P5000 CT is authorised according to EU Regulation (EC) No 1831/2003 on additives for use in animal nutrition, and the above is considered covering the essential environmental issues.

Characterisation of environmental impacts is based on Eco-indicator 95 v2.1. Normalisation is based on Danish/international normalisation references derived from Stranddorf et al. (2005) except energy (BP 2003), phosphate rock (global yearly consumption data (USGS 2005)) and global area of land occupied by agriculture (FAOSTAT 2004). Population data for normalisation of resources are derived from PRB (2003).

### 2.3 Functional unit

The function of phytase is to break down phytate and, thus, make phytate bound phosphorus in the feed available to the pigs and the functional unit of the study is  $5 \cdot 10^6$  FYT, corresponding to one kg of the considered Ronozyme Phytase product (DSM 2004) and 29 kg of the considered MCP product as explained above.

## 3 Results

### 3.1 Environmental impact assessment

Characterised environmental impact potentials induced by application of Ronozyme Phytase, or MCP, are provided in Table 2. The table shows that the environmental impacts

**Table 2:** Environmental impacts induced by Ronozyme Phytase or inorganic phosphate (MCP) application. Figures are provided per functional unit ( $5 \cdot 10^6$  FYT, i.e. one kg Ronozyme Phytase and 29 kg MCP) and refer generally to the 5% P-loss scenario, except figures in brackets which refer to the 0% and 100% P-loss scenarios, respectively

Impact category	Ronozyme Phytase <sup>1</sup>	MCP	MCP/Ronozyme
Glob. warming, g CO <sub>2</sub> eq.	1900	32000	17
Acidification, g SO <sub>2</sub> eq.	4.8	530	110
Nutrient enrichment, g PO <sub>4</sub> eq.	2.2	1500 (480–21000)	700 (220–9500)
Photochemical ozone formation, g C <sub>2</sub> H <sub>4</sub> eq.	1.5	12	8.0
Phosphate rock, g	< 0.1	24000	> 240000
Primary energy, MJ	26	400	15
Agricultural land, m <sup>2</sup> · year	0.15	–	–

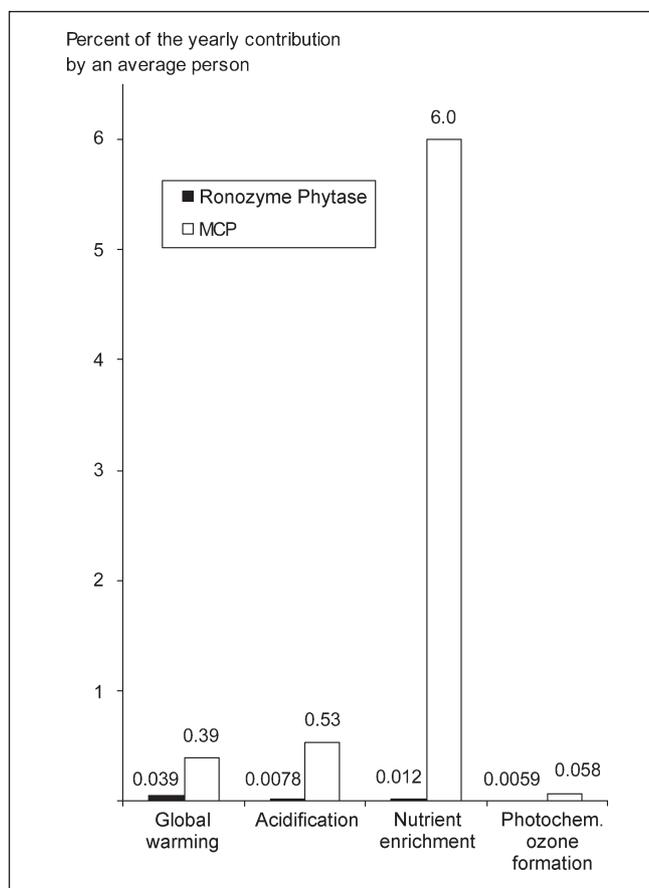
<sup>1</sup> Data for Ronozyme Phytase are slightly lower than recorded in the cradle-to-gate study (Enzyme C in Nielsen et al. 2006) because the feed value of digestible formulation ingredients is accounted for in the present study by crediting avoided impacts from alternative feed to the enzyme

associated with Ronozyme Phytase application are generally very low compared with the avoided impacts obtained by MCP displacement.

Avoided contributions to nutrient enrichment as a result of MCP displacement vary significantly depending on the P-loss scenario considered (0, 5 or 100% P-loss). In the realistic 5% P-loss scenario, a small 'investment' in terms of nutrient enrichment from Ronozyme Phytase production is 'paid back' by a factor of about 700 as a result of MCP displacement. In the 100% P-loss scenario, the 'pay-back' factor is in the order of 10000. Even in the 0% P-loss scenario application of Ronozyme Phytase is still justified by large reductions of nutrient enrichment as a result of MCP displacement. The latter is explained primarily by avoided emissions of phosphorus during phosphoric acid production for MCP production (see Fig. 1). Thus, it can be concluded that in any of the considered P-loss scenarios, application of Ronozyme Phytase can be justified by significant reductions of environmental impact due to MCP displacement.

Primary energy used to produce Ronozyme Phytase is about 26 MJ per kg at the factory's gate, while consumption is about 400 MJ for MCP; about fifteen times more. Contributions to global warming follow the same pattern because contribution to a large extent is driven by CO<sub>2</sub> emissions from energy conversion processes (electricity and heat production). However, the relative contribution from MCP is slightly higher, because an additional quantity of CO<sub>2</sub> is emitted to air during calcium oxide production from lime stone ( $\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2$ ) (see Fig. 1). A significant quantity of SO<sub>2</sub> is emitted during sulphuric acid production for MCP (see Fig. 1) and this explains MCP's relatively large contribution to acidification. Contributions to photochemical ozone formation are to a large extent driven by non-methane volatile organic compound (NMVOC) emissions from energy conversion processes. Relatively large NMVOC emissions from transportation of ingredients for Ronozyme Phytase production and electricity production for fermentation process explains the relatively high contribution to photochemical ozone formation from Ronozyme Phytase. Transportation processes are insignificant for the other indicators considered.

Phosphorus is used to produce Ronozyme Phytase (fertiliser for carbohydrate production), but most of it is returned to agricultural land (where it displaces artificial fertiliser, see Nielsen et al. 2006) and the net consumption is limited to the loss from agricultural fields due to erosion and leaching. The exact magnitude of phosphorus loss is difficult to assess, but it is estimated (by mass balances) to be below 0.1 g per kg Ronozyme Phytase. The phosphorus consumption induced by enzyme production is, therefore, clearly justified by the significant savings resulting from MCP displacement. Production of one kg Ronozyme Phytase requires 0.15 m<sup>2</sup> · year agricultural land, about the same quantity of land as required to produce 150 g bread (see Nielsen et al. 2003) while no agricultural land is used to produce MCP.

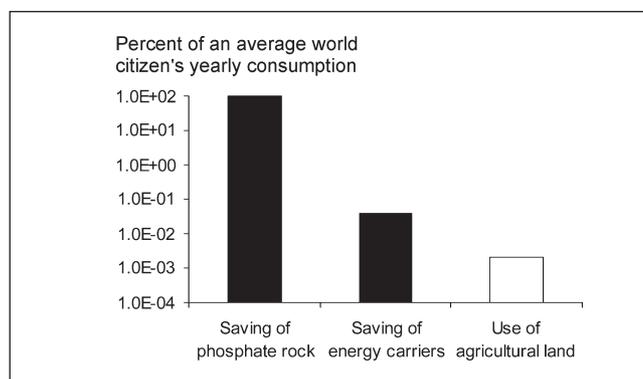


**Fig. 2:** Normalised environmental impact potentials for Ronozyme Phytase and MCP application (5% P-loss scenario). All data are provided per functional unit

Normalised environmental impact potentials induced by Ronozyme Phytase or MCP application are shown in Fig. 2. The figure shows that saved nutrient enrichment is by far the most significant effect of Ronozyme Phytase application and that one kg of the enzyme product can reduce contribution to nutrient enrichment corresponding to six percent of an average Dane's yearly contribution. Similar figures (not shown) indicate that 80% of an average Danes contribution to nutrient enrichment can be avoided if all excess phosphate is assumed to be lost to aquatic recipients (100% scenario) while it is less than 0.2% if all phosphorus is bound in the soil matrix (0% P-loss scenario). Application of Ronozyme Phytase is, thus, increasingly justified by avoided contributions to global warming and acidification when phosphorus loss is approaching zero (e.g. on clayish soils with low animal density).

Normalised resource consumptions induced and displaced by Ronozyme Phytase application as an alternative to MCP are shown in Fig. 3.

Fig. 3 shows that the normalised savings of phosphate rock and energy carriers are significant compared with use of land and that application of Ronozyme Phytase is clearly justified by significant overall resource savings unless use of agricultural land is given very high weight compared with the savings of phosphate and energy carriers and also reductions of environmental impacts



**Fig. 3:** Normalised savings of phosphate rock and energy carriers and use of agricultural land per functional unit. Note that the y-axis is logarithmic

### 3.2 Sensitivity analysis

The most important assumptions and simplifications made to accomplish the present study have been subject to sensitivity analyses. One sensitivity analysis related to the animal density at pig farms using either MCP or Ronozyme Phytase pointed to a serious issue for the interpretations of the results and is described in detail below. Other sensitivity analyses which contributed to a better understanding of the system without changing the overall interpretation are summarised briefly in Table 3.

The study of Ronozyme Phytase as an alternative to MCP has focused on pig farms with high livestock density where phosphate in pig manure is in excess compared with the crop's demand, and where application of phytase can reduce the surplus of phosphate to about zero (see above). However, less intensive pig farming is also relevant (Statistics Denmark 2004) and a scenario where livestock density is low and P-fertiliser value of manure is in harmony with the crop's demand has been addressed. At such farms (livestock density below 1.4 livestock units per ha) reduced phosphorus dispersal with manure induced by MCP displacement will most likely lead to a corresponding increase of artificial P fertilizer application in the field to maintain crop yield. Therefore, an assessment of the environmental effect of phytase application at farms with low animal density has been made, assuming that an equivalent quantity of P fertilizer (data from Patyk and Reinhardt 1997) is applied when MCP is displaced. The results show that the environmental differences between Ronozyme Phytase and MCP application becomes limited to the difference between Ronozyme Phytase and artificial fertilizer, on the one hand, and MCP production, on the other. In this situation, the advantages achieved by displacing MCP are to a large extent outweighed by disadvantages from additional artificial fertilizer usage. It is therefore stressed that the study cannot be extrapolated directly to non-intensive farms where spreading of phosphorus with manure does not lead to an excess of phosphorus in the field compared with the crop's requirements.

With this exception, sensitivity analysis have revealed that the observed environmental advantages associated with Ronozyme Phytase application as an alternative to MCP are robust, although exact magnitudes of results are associated with variation and uncertainty.

**Table 3:** Brief summary of sensitivity analyses which did not change the overall interpretation of the study

No.	Issue of concern	Sensitivity analysis performed	Main observations
1	Ronozyme Phytase is delivered in different strengths.	Another phytase product with a lower strength (Ronozyme P CT with $2.5 \cdot 10^6$ FYT/kg) has been assessed.	Increases impacts of the enzymatic solution by about 50%. Ronozyme P CT has largely been substituted by Ronozyme P5000 CT in the market.
2	Ronozyme Phytase is favoured by the use of natural gas as marginal source of electricity in Denmark (Nielsen et al. 2006).	The assessment has been performed with coal as the marginal source of electricity. See Weidema (2003)	Increases Ronozyme Phytase's impact by 10 to 40%, depending on impact category.
3	Ronozyme Phytase is favoured by steam supply from 'Symbiosis network' (see Nielsen et al. 2006).	Steam from 'symbiosis network' has been replaced with steam from oil combustion.	Increases Ronozyme's environmental impact by maximally 10%, depending on impact category.
4	Sodium sulphate is used as formulation agent in Ronozyme Phytase and it may displace sulphur amendment at agricultural fields when spread with manure.	Sodium sulphate was neglected in the assessment of Ronozyme Phytase.	Reduces impacts of the enzymatic solution by 0 to 25%, depending on impact category.
5	Impacts of MCP are underestimated because data on the MCP production process is missing and hence disregarded.	Energy consumption for the MCP production is simulated with average energy data for production of inorganic chemicals (ETH 1996).	Impacts induced by MCP increase by 20–60%, depending on impact category.
6	MCP displacement ratio has been fixed to 1 g P per 750 FYT, but may be as low as 0.6 g P per 750 FYT due to natural variation in feed composition, feed processing, genetic potential of animals and other factors (see Johansen and Poulsen 2003a,b).	The assessment has been performed with a displacement potential of Ronozyme Phytase of 0.6 g P in MCP per 750 FYT.	Impacts induced by MCP are reduced to 60% of the base scenario values.
7	According to Danish standards, digestibility of MCP is assumed to be 67%. Other countries use other digestibility ratios and the assessment would come out less favourable to Ronozyme Phytase if digestibility of MCP was considered higher.	The assessment has been performed with 100% digestibility of MCP representing the highest possible digestibility of MCP	Impacts of MCP are reduced to 67% of the base scenario values.
8	Sulphuric acid production is based on average data for Europe (Ecoinvent 2003).	Data on this particular process have been replaced with data from a plant with much focus on SO <sub>2</sub> -reductions (Tessenderlo 2002).	MCP's contribution to acidification decreases by about 30%.
9	Excess sulphur from refineries may be the marginal source of sulphur for sulphuric acid production in the near future (USGS 2005).	It has been assumed that marginal impacts induced by secondary sulphur production are 1) zero, as a possible future alternative is wasting the sulphur and 2) as reported in Ecoinvent (2003) (allocated data).	1) Use of sulphur from refineries which would otherwise be wasted reduces impacts of MCP by up to 15%. 2) Use of Ecoinvent (2003) data increases all impacts of MCP (80% for acidification).
10	Ronozyme's positive effect on pig's calcium uptake has been disregarded.	Calcium displacement has been roughly set to 0.8 g per 750 FYT.	No significant changes of impacts except limestone saving.
11	Dicalcium phosphate (DCP) is another important source of inorganic feed phosphate for pigs.	Based on similar principles, the entire assessment has been performed with DCP instead of MCP.	Contribution to global warming from the non-enzymatic solution increased by about 20%. Other impacts remained at the same level.
12	Toxicity aspects have been disregarded because of lack of appropriate data.	Available data and qualitative judgements have been used to address this issue using EDIP impact assessment method (Wenzel et al. 1997).	Normalised contributions to toxicity appear to be low compared with normalised contributions from other impact categories. Contributions to toxicity are to a large extent determined by energy consumption and it appears that inclusion of toxicity would sustain the observed differences between Ronozyme and MCP

#### 4 Conclusions and Perspectives

Application of Ronozyme Phytase as an alternative to inorganic phosphate supplementation to pig feed at intensive pig farms in Denmark is justified by major advantages in terms of all studied environmental impact categories: global warming, acidification, nutrient enrichment and photochemical ozone formation, and by significant energy carrier savings and, particularly, phosphate rock savings.

The most significant environmental advantages are related to nutrient enrichment due to reduced phosphate content in pig

manure and following reduction of phosphate loss to aquatic environment from the agricultural fields receiving the manure.

The environmental advantages related to Ronozyme Phytase application are major for the above impact categories, even in scenarios where loss of phosphate to aquatic recipients from manure is neglected. This is primarily due to the fact that a small quantity of Ronozyme Phytase displaces a large quantity of MCP, and the environmental impacts induced by a large quantity of MCP is much higher than impacts induced by a small quantity of Ronozyme Phytase.

Ronozyme Phytase application causes use of agricultural land for carbohydrate production for the fermentation process. The normalised land use is, however, orders of magnitude lower than normalised energy carrier and phosphate rock saving, and the relatively small land application induced by Ronozyme Phytase production is clearly justified by the obtained environmental gains and other resource savings, unless use of agricultural land is given a very large relative weight.

Data and assumptions applied in the study have been subject sensitivity analysis, and it has been justified that the observed environmental advantages associated with Ronozyme Phytase application at intensive pig farms as an alternative to inorganic feed phosphate are robust, although the exact magnitudes are associated with variation and uncertainty.

Ronozyme Phytase is applied for other monogastric animals than pigs (e.g. poultry and farmed fish) and the present study can be seen as a first attempt to address this particular enzyme's environmental implications when applied as an alternative to inorganic phosphate. Non-published studies of phytase applied for broiler and trout based on the same principles and at the same level of detail as in the present study, show that environmental advantages of phytase are about the same for broiler and even larger for trout. Use of phytase for farmed fish is still in its infancy, and the larger environmental advantages of using it for trout, can be explained by two main factors: 1) larger MCP displacement ratio and 2) direct excretion in water.

Hundreds of enzyme products are available on the market today, each with a range of different applications. Much has been learned by studying one enzyme in one application in the present study, but much more remains to be learned about all the others. Based on the present study and a series of non-published assessments, we believe that enzymes offer an important contribution to a sustainable development of our society. LCI data for a small selection of characteristic enzymes is therefore provided by Nielsen et al. (2006) together with a methodological approach to analyse the products, in order to support other LCA practitioners with interest in this evolving field.

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