# Phosphorus in Pig Diets

## Effect of Liquid Feeding, Phosphorus Levels and Phytase Supplementation on Digestibility and Performance

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# Abstract

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Pig feed is mainly based on cereals where phosphorus (P) is mostly present in inositol hexaphosphate ($IP_6$), which is not readily available to monogastric animals. More available P sources are often added to ensure that pigs' requirements are fulfilled; this results in high excretion levels of P. The digestibility of P depends on phytase activity and amount of $IP_6$ in feedstuffs. The overall aim was to study effects of liquid feeding, P levels and phytase supplementation on digestibility and performance. Effects of soaking and P levels on digestibility were studied by total collection in metabolic cages, and effects on performance were studied in 192 growing pigs. Effects of soaking fermentation and phytase supplementation on ileal and total tract digestibility were studied with indicator technique on pigs surgically fitted with PVTC cannulas. P levels and phytase supplementation were studied in 104 pregnant sows for two reproduction cycles. All diets were cereal based and included wheat. Basic properties of a cereal mix fermented with whey, wet wheat distillers' grain and water in different temperatures were also studied.

Soaking reduced the level of $IP_6$, whereas apparent digestibility of P was not significantly improved. Soaking increased average daily weight gain, carcass weights and improved the energy conversion ratio in pigs fed a low P diet to the same level as pigs fed high P diets. Low P diets resulted in lower femur density than high P diets. However, soaking of a low P diet resulted in increased femur density. Fermentation degraded $IP_6$ efficiently and improved ileal apparent digestibility of P, organic matter, nitrogen, amino acids and total tract apparent digestibility of organic matter. Microbiological and biochemical properties of fermented liquid diets are strongly affected by feed components and temperature used. Phytase supplementation slightly affected apparent digestibility of P. Supplementing a low P gestation diet with phytase did not significantly affect sow performance. The slight effects of phytase supplementations found may depend on high levels of intrinsic phytase in the diets, and possibly suggest that the provided P level in the sows may have been sufficient. Under typical Swedish conditions of sow management, reduced total P level in gestation diets seems not to negatively affect performance.

*Keywords:* liquid diets, intrinsic phytase, phytase supplementation, apparent digestibility, ileal, inositol hexaphosphate, phytate

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*'The only true wisdom is in knowing you know nothing.'*

Socrates

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**I.** Lyberg, K., Simonsson, A. & Lindberg, J.E. 2005. Influence of phosphorus level and soaking of food on phosphorus availability and performance in growing-finishing pigs. *Animal Science 81: 375-381.*

**II.** Lyberg, K., Lundh, T., Pedersen, C. & Lindberg, J.E. 2006. Influence of soaking, fermentation and phytase supplementation on nutrient digestibility in pigs fed a grower diet based on wheat and barley. *Submitted.*

**III.** Lyberg, K., Andersson, H.K., Simonsson, A., & Lindberg, J.E. 2006. Influence of different phosphorus levels and phytase supplementation in gestation diets on sow performance. *Submitted.*

**IV.** Lyberg, K., Olstorpe, M., Passoth, V., Schnürer, J. & Lindberg J.E. 2006. Nutritional and microbiological properties of a cereal mix fermented with whey, wet wheat distillers' grain and water at different temperatures. (Manuscript).

# Introduction

In many west-European countries with areas of high densities of pigs, problems have arisen during the last decades because high amounts of phosphorus (P) have spilled into the environment (Poulsen et al., 1999; van der Peet-Schwering and den Hartog, 2000). High amounts of P accumulate in soils and contribute to eutrophication of fresh waters and brackish waters, as well as marine environments (Daniel, et al., 1998; Karlson et al., 2002). Pig feed is mainly based on cereals and P in cereals is mostly present in the form called phytate or inositol hexaphosphate ($IP_6$), which is not readily available to monogastric animals such as pigs (Pointillart et al, 1984). To ensure that the P requirements of pigs are fulfilled other more available P sources are added to the diets, resulting in high P excretion levels (Poulsen, 2000; Jongbloed et al., 2001). Digestibility of P in different feedstuffs varies, and depends on enzyme activity of intrinsic phytase and the amount of $IP_6$ in feedstuffs (Pointillart et al., 1987; Jongbloed and Kemme, 1990). Intrinsic phytase activity depends on the composition of a diet and on feed treatment (Jongbloed et al., 1991). Supplementation with microbial phytases is one technique that has been used to degrade $IP_6$ and improve P digestibility (Poulsen, 1996; Kemme et al., 1999; Rodehutscord et al., 1999; Näsi et al., 1999). Different techniques with liquid diets have also been used to, for example, degrade $IP_6$ and increase digestibility of P (Näsi et al., 1995; Liu, et al., 1997; Skoglund et al., 1997b; Bergman et al., 2001).

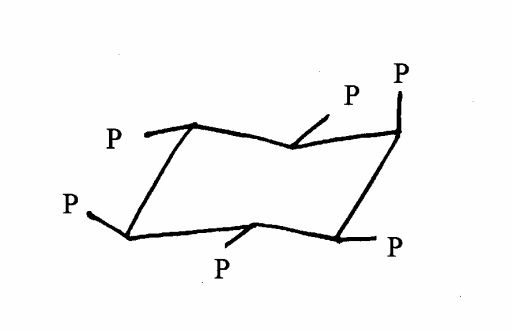


Fig 1. Structure of myo-inositol hexaphosphate, where P represents phosphate groups

## Structure and occurrence of $IP_6$

Inositol phosphates consist of a six-carbon-atom ring esterified with up to six phosphoric acid groups. The most abundant form in nature, and the form referred to in this thesis as $IP_6$, is the myo-inositol hexaphosphate, where six phosphoric acid groups are bound to the inositol ring, and myo- refers to the inositol stereoisomer with one axial group (Fig 1). $IP_6$ represents the main storage form of P in plants and occurs as an insoluble mineral complex in physiological pH. The molecules have high chelating capacity for multivalent cations e.g. $Zn^{2+}$, $Ca^{2+}$, $Fe^{2+}$ (Fig 2), which affects their bioavailability as well. The cations can chelate

strongly between two phosphate groups, but weakly within a phosphate group or between two different $IP_6$ molecules. The extent and strength of mineral binding can therefore vary and depends on the $IP_6$ to mineral ratio and pH. A reduction in phosphate groups attached to the inositol ring increases solubility, decreases mineral binding strength and improves mineral bioavailability (Türk, 1999). About 60 to 70% of the P in wheat and barley is stored as $IP_6$ and is mainly located in the outer layers of the seeds, in the pericarp and aleurone layers (Reddy et al., 1982).

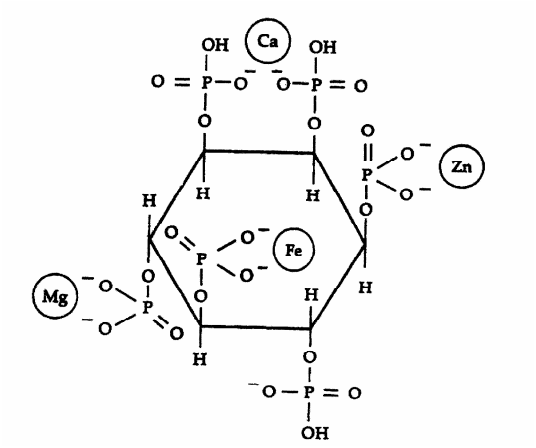


Fig 2. Example of the chelating capacity of insositol hexaphosphate ($IP_6$)

## Sources of phytase

Phosphatases with ability to hydrolyse $IP_6$ can by definition be considered to be phytases (Türk, 1999). Phytases produce myo-inostiol phosphates with decreasing number of phosphoric acid groups, with some differences in which order the dephosphorylation occurs depending on the phytase involved. Thus, isomers formed during $IP_6$ degradation differ, depending on phytase origin, which may affect nutrition (Irving, 1980; Greiner, 1993; Brearley and Hanke, 1996; Skoglund, 1998). Phytase activity is found to some extent in most plants, a variety of microorganisms can produce phytase and some phytase activity is also found in intestinal mucosa. Intestinal phytase activities are for example found in laboratory rats, humans and in pigs, but at such low levels that they have negligible effect (Pointillart, et al., 1984; Williams and Taylor, 1985; Iqbal et al., 1994). The phytase activity in plant feedstuffs varies considerably; high activities are found in for instance wheat, rye, and triticale, but much lower activities are found in maize and oats (Eeckhout and de Paepe, 1994). Dietary intrinsic phytase activity was found to be linearly related with P digestibility by Pointillart (1994), in a pooled data set over digestibility of plant P in pigs fed cereal-based diets. However, phytase activity in one feedstuff can also affect P availability in the rest of a diet

(Jongbloed et al., 1991). Plant phytases are activated when the feed is ingested (Moore, 1955; Sandberg et al., 1993) and they can also be activated before ingestion through feed treatments such as soaking (Skoglund et al., 1997bb; Larsen et al., 1999; Carlson and Poulsen, 2003). Phytase activities are found in many microorganisms, such as several fungi, bacteria and yeast species. Most commercially produced phytase products are based on the phytase-encoding gene from the mycelial fungi *Aspergillus niger* (Maenz, 2001).

## Phosphorus function in the body

P is an essential mineral which all animals need to function. Almost all (about 80%) P taken up in the body is found in the skeleton. P is important in bone formation, not only in the mineralisation of the bone matrix, but also in the formation of organic matrix. The other 20% is found in different soft tissues and body fluids because P takes part in many metabolic functions. P is involved in energy metabolism and the transfer of energy via AMP, ADP and ATP, with implications on glucogenesis, fatty acid transport, amino acid and protein synthesis and activity of the sodium $Na^+$/potassium $K^+$ pump. Since P is a component in DNA and RNA, it is important for cell growth and differentiation. Cell membrane fluidity and integrity is due to phospholipids and phosphate helps to maintain osmotic and acid-base balance (Underwood and Suttle, 2001).

## P absorption in monogastric animals

The basic mechanisms of gastrointestinal P metabolism differ substantially between ruminants and non-ruminants (Breves and Schröder, 1991). Ruminants have microflora of the rumen that can breakdown $IP_6$, which the monogastric species lack. Some microbial degradation of $IP_6$ takes place in the large intestine of monogastric animals, that degradation is however considered to have a limited effect on digestibility. The major site for P absorption in monogastric species is the small intestine and the transport is stimulated by vitamin $D_3$ (cholecalciferol). The quantitative P absorption differs along the small intestine; in pigs and rats, the highest absorption rate is in the jejunum. For the total P absorption, the large intestine is of minor importance (Breves and Schröder, 1991). Monogastrics have a much lower flow of salivary secretion than ruminants, and therefore a much lower contribution of P from saliva to the gastrointestinal system. Small amounts of P are secreted via gastric and pancreatic juice, bile and intestinal fluid, but their contribution to the gastrointestinal P turnover is low.

The regulation of P homeostasis in monogastrics depends on renal P secretion and intestinal absorption. During low P intake the P absorption increases and the renal P reabsorption from the tubuli increases to minimise the P output in the urine. The regulation of calcium (Ca) and P homeostasis are closely connected although plasma P concentration is not as strictly regulated as Ca and may fluctuate throughout the day, especially after meals. In short, the regulation involves mainly three hormones: parathyroid hormone (PTH), calcitonin and 1.25-dihydroxycholecalciferol (1.25-$(OH)_2D_3$) or calcitriol. Absorbed vitamin $D_3$ is

converted into 25-hydroxycholecalciferol in the liver and then into 1.25-$(OH)_2D_3$ by the kidneys. Low Ca levels in the blood stimulate PTH secretion, which controls the 1.25-$(OH)_2D_3$ production. 1.25-$(OH)_2D_3$ is transported via the circulatory system to various target tissues and acts primarily through regulation of the transcription of various proteins. Synthesis of several proteins that enhance Ca absorption in the duodenum is increased and synthesis of a phosphate transport protein is stimulated, thus increasing P absorption in the small intestine. Both PTH and 1.25-$(OH)_2D_3$ stimulate renal reabsorption of Ca and P. The direct effect of 1.25-$(OH)_2D_3$ on bone is an increased number of osteoclasts and thereby a promoted reabsorption of Ca and P from bones. The main effects of 1.25-$(OH)_2D_3$ on bones are, however, via the indirect actions on the small intestine and kidneys, generating increased Ca and P levels available for bone mineralisation. Elevated Ca levels in the blood also have a stimulatory effect on secretion of calcitonin, which have an inhibitory effect on the activity of osteoclasts and slows the rate of bone turnover (Barrett and Barrett, 2003).

## Degradation of $IP_6$ in the pig

In Moore and Tyler (1955), it was concluded that the effects of plant phytase are exerted in the stomach. Later Lantzsch et al. (1992) also reported a considerable degradation of $IP_6$ due to plant phytase in the stomach and proximal half of the small intestine. Jongbloed et al. (1992) studied the effect of supplementation of Aspergillus niger phytase on $IP_6$ degradation in cannulated pigs. They concluded that a substantial $IP_6$ degradation took place in the stomach-duodenum part, bearing in mind that the microbial phytase is most active at pH 2.5 and 5.5 (Simons et al., 1990) and that post duodenal pH is about 6, minimising phytase actions. Plant phytase have an activity optimum around pH 5 (Irving, 1980) implying minimal actions beyond duodenum and that the time for plant phytase to act would be during a retention time in the stomach. However, Kemme et al. (1998) found time periods with a for plant phytase optimal pH in the duodenum, at times when large amounts of digesta entered duodenum. They concluded that plant phytase hydrolyse substantial amounts of $IP_6$ in the stomach and that some degradation may also occur in the anterior part of the duodenum before neutralisation of gastric contents. Although, due to the limited retention time in that section, it is quantitatively unimportant. The efficacy of phytase degradation by both plant and microbial phytase is, in other words, to a large extent determined by pH and retention time in the stomach (Kemme et al., 1998). In the caecum, bacterial population may contribute to the $IP_6$ degradation although the significance of gut bacteria in the $IP_6$ degradation is not known (Maenz, 2001). The limited phytase activity in small intestinal mucosa is very different from plant and microbial phytase. The mucosal enzyme is fixed on the membrane and acts within a thin microenvironment water layer with a pH at 6. $IP_6$ degradation in that thin layer depends on substrate access and the degree of enzyme expression there (Maenz, 2001).

## Methods for determination of digestibility

The dietary digestibility of P is most often assessed by the apparent digestibility (Dellaert, 1990). Two main procedures can be used: quantitative collection and qualitative collection. Quantitative collection implies a total collection of faeces, whereas in qualitative collection grab samples (point-samples several times) are used. During total collection the animals are held in metabolic cages where all faeces and urine can be separated, collected and then analysed for e. g. P content. The coefficient of apparent digestibility (CAD) for P is determined by the equation:

$$CAD=(P_{intake} - P_{faeces}) / P_{intake}$$

In grab sampling, a marker such as chromium oxide or acid-insoluble ash is used and CAD of the diets is then calculated using the indicator technique (Sauer et al., 2000) according the equation:

$$CAD_D=1-(DC_F \times I_D)/(DC_D \times I_F)$$

Where $CAD_D$ is the coefficient of CAD of a dietary component, $DC_F$ is the dietary component concentration in digesta or faeces, $I_D$ is the indicator concentration in diet, $DC_D$ is the dietary component concentration in diet and $I_F$ is the indicator concentration in digesta or faeces. The total collection technique is more expensive and time consuming, but may be more accurate than the grab sampling technique.

To determine the digestibility of different feedstuffs, three different methods have been used: the direct method, the difference method and linear regressions. In the direct method apparent P digestibility is determined in the fed diet, whereas in the difference method P digestibility is determined in a basal diet and then in the basal diet supplemented with a P source. The apparent digestibility of the added P source is then calculated by (basal diet + test P source)-(basal diet) on the assumption that the added P source does not affect the digestibility of the basal diet. A linear regression is a third method to relate the net digested P to P intake. The method is similar to the difference method but with increasing levels of the test P source. P digestibility coefficient is given by the slope of the linear regression. For both the difference method and the linear regression it is important that the P content in the diets does not exceed P requirements of the pigs (Poulsen, et al, 1999). The endogenous contribution of P is generally very low, but with a high P intake there is an adaptation and the endogenous losses are higher, which gives lower absorption values and thus lower digestibility values.

## Methods for estimation of requirements

*Slaughter technique and balance technique*

The best and most accurate method for establishing the requirements of P in pigs is the comparative slaughter technique, where whole animals are ground, sampled

and analysed for P content. It is obviously only applicable when animals have had an adequate P intake. The comparative slaughter technique is, however, expensive, time-consuming and difficult to handle. The requirements can also be estimated by the balance technique, which involves comparing the effects of different dietary P supply, on faecal and renal excretion, and performance. However, establishing satisfactory adequacy criteria is problematic and besides performance, several other parameters are used in attempt of determining whether different supplies are adequate. Estimations of exact P requirements are difficult because they depend on many factors, such as physiological and health status of the animals, production level, diet composition and interactions, feeding strategy and management (Underwood and Suttle, 2001).

### *Blood and bone parameters*

Blood samples are often used to evaluate P status of animals. Serum or plasma P decreases with a low P intake, whereas the level of alkaline phosphatase increases. A low P intake slightly increases serum Ca as well. The P level in serum correlates with growth rate and can give a rough measure of P status of the animals. However besides dietary P level, P level in serum can be affected by sample time after feeding, site of sampling and interval between sampling (Teleni et al., 1976). Bone parameters such as bone weight, ash content, density and breaking load have also been used to determine P status of pigs. Ash content as parameter could however slightly underestimate a deprivation of P due to reduction in organic matter as well as in ash content of a bone (Koch and Mahan, 1985).

### *Renal excretion*

Elevated P levels in the urine may indicate excessively high dietary P levels. It is known that when P requirements are just fulfilled, faecal endogenous excretion is limited and most of any surplus P is excreted in the urine (Finco, 1989, Breves and Schröder, 1991; Underwood and Suttle, 1999). Renal P excretion is regulated by an overflow mechanism, where all P is reabsorbed in the glomerular filtrate when the plasma P concentration is below a critical value (1 mmol/l in humans), but above this value the rate of P loss in urine is directly proportional to additional increases (Guyton and Hall, 2000). Endogenous loss of P in the urine is estimated to about 1 mg per kg live weight (LW) per day for all categories of pigs (Jongbloed and Everts, 1992). There are however limitations in using urinary P excretion as a parameter to determine whether P requirements are met since urinary P excretion not only depends on dietary supply and requirements, but also depends on renal function, intrarenal Ca concentrations, acid-base balance, presence of steroids, parathyroid hormone and vitamin $D_3$ levels (Jongbloed, 1987).

## Influence of diet physical form

Physical form of a diet can affect its nutritional properties. Pelleting and heat treatment of a diet may for example prevent actions of intrinsic phytases in a diet (Skoglund, 1997; Kornegay, 2001). The use of liquid diets have increased during

the last decade in European countries, with a wide variety of different feed and fermentation techniques and fermentation of both individual feedstuffs or complete compound diets are used (Scholten et al., 1999). Hygienic properties and nutritional effects can vary depending on the fermentation process involved (Brooks et al., 2003). Soaking and fermentation is often pooled under the label of liquid or wet feed, and there is not always clearly differentiated. A fermented feed have in general a low pH (<4.5), high levels of lactic acid, volatile fatty acids and high amounts of lactic acid bacteria (Jensen and Mikkelsen, 1998; Scholten, 2001). Some studies suggest that pigs find wet-feed tastier and more acceptable than dry feed (Mikkelsen and Jensen, 2001). Villus height and form is positively affected by wet-feed, which influences utilisation of the feed (Hurst et al., 2001; Mikkelsen and Jensen, 2001; Pluske, 2001). When wet-feed is mixed in advance, fermentation occurs. Based on a limited number of studies, fermented diets can reduce gastric pH and the number of coliform bacteria in the gastrointestinal tract (Scholten et al., 1999). Daily weight gain and feed conversion ratios have, like gastrointestinal health, been positively affected by fermented wet-feed (Jensen and Mikkelsen, 1998; Schloten et al., 1999; Brooks, 2003). Both the biochemical properties and microbial populations developed in fermented feed affect hygienic quality, intestinal health, performance (Scholten et al., 1999) as well as nutritional aspects of the diet. Pedersen and Lindberg (2003) found an improvement of in vitro digestibility of organic substance by 3.4% and protein by 2.5% due to fermentation of a liquid diet. Lactic acid bacteria and yeast populations developed in a fermented feed (Jensen and Mikkelsen, 1998) could among other things be capable of degrading $IP_6$ (Lopez et al., 2001). The degradation of $IP_6$ due to both activation of intrinsic phytase and microbial activity are of interest for wet-feeding systems. The increased use of liquid diets prompt a need to better understand nutritional differences of soaking and fermentation of complete pig diets, as well as influences of liquid co-products from human food industry on fermentation processes.

# Objectives

The overall aim of this thesis was to study P in pig diets, the effects of liquid feeding, phytase supplementation and P levels on digestibility and performance.

The specific objectives were to study:

- Effects of soaking in relation to dietary P levels on degradation of $IP_6$, apparent digestibility of P and performance in growing-finishing pigs.

- Effects of reducing dietary P levels for growing-finishing pigs in relation to Swedish feeding recommendations.

- Effects of the different feed treatments: soaking, fermentation and phytase supplementation on $IP_6$ degradation, and ileal and total tract apparent digestibility in growing pigs.

- Different P levels and phytase supplementation in diets for pregnant sows in connection to their requirements, and to evaluate reduction potential in dietary P levels for pregnant sows in relation to Swedish feeding recommendations.

- Basic nutritional and microbiological properties of a cereal mix fermented with whey, wet wheat distillers' grain and water at different temperatures.

# Material and methods

This research is based on five studies, presented in four papers. Balance studies in Paper **I** and **II** were performed at the experimental unit at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden. Performance studies in Paper **I** and **III** were performed at the Swedish Pig Centre of Lantmännen, Svalöv, Sweden. All studies involving animals were approved by local animal ethics committees. The in vitro study in Paper **IV** was performed at the Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

## Balance studies (Paper I and II)

Castrated specific pathogen-free male pigs (Swedish Landrace x Yorkshire) were used in the balance studies (Paper **I** and **II**). All were housed in individual pens and water was available ad libitum. Animals studied in Paper **I** had an average weight at start of 26 kg. Those used in Paper **II** were surgically fitted PVTC cannulas (van Leeuwen et al., 1991), at an average weight of 25 kg, used for another study, and introduced into the study of Paper **II** at an average weight of 65 kg. In Paper **I**, total collection of faeces and urine was conducted and in Paper **II**, the indicator technique was used. During the collection periods of Paper **I**, the animals were kept in metabolic cages.

All diets (Paper **I** and **II**) were non-heat-treated meal diets based on wheat and barley, formulated with respect to nutrient requirements according to Swedish feed recommendations (Simonsson, 1994) except for P. Calculated energy content of diets were 12.5-12.6 MJ ME/kg. Dry diets with low P and no monocalcium phosphate contained 4.1 g P $kg^{-1}$ feed in Paper **I** and 4.4 g total P $kg^{-1}$ feed in Paper **II**, whereas the high P diet (only in Paper **I**) contained 6.8 g P $kg^{-1}$ feed due to addition of monocalcium phosphate. Ca in the diets was 7-8 g $kg^{-1}$ feed. All

pigs were fed twice daily with a daily feed allowance of approximately 4% of their live weight (LW).

The experiment in Paper **I** was a 4×4 Latin square designed with factorial (P level and soaking) arrangement of treatments and with four animals. The four treatments were low P, dry or soaked and high P, dry or soaked. Each trial period consisted of 12 days, 7 adaptation days and 5 collection days. In Paper **II**, the experiment was as a two-period changeover design, with eight animals and four treatments. A low P diet was used and the four treatments were dry feed, dry feed supplemented with phytase (phytase; Bio-feed® Phytase CT, Novozymes), soaked feed and fermented feed. Water to feed ratio in liquid diets was 3:1. Soaking was for 1 h in room temperature, with initial water temperature of 10°C. Fermented feed had an initial fermentation of 5 days, and then 50% was replaced daily at feeding, resulting in 23.5 h of fermentation, with the use of two fermentation tanks. In Paper **II** each experimental period lasted 14 days, 7 adaptation days, 4 days of faeces collection and 2 days of ileal digesta collection. Feed samples were collected during each period (Paper **I** and **II**). Total collection (Paper **I**) of faeces and urine was performed twice daily. In Paper **II**, faeces was collected twice daily, whereas quantitative collection of ileal digesta through the PVTC cannula was carried out during 1-h periods, on day 12 from 8:30-9:30, 10:30-11:30, 12:30-13:30 and 14:30-15:30, and on day 14 from 9:30-10:30, 11:30-12:30, 13:30-14:30 and 15:30-16:30.

Samples were freeze-dried, ground through a 1-mm mesh screen, analysed for dry matter (DM), ash and nitrogen (Nordic Committee on Food Analysis, 2003). Mineral contents were analysed in ash samples of feed and faeces (Nordic Committee on Food Analysis, 1991), as well as in wet digesta samples and urine (Nordic Committee on Food Analysis, 1998). Phytase activity (Engelen et al., 1994) and inositol phosphates (Skoglund et al., 1997a) were analysed in the diets. In Paper **II**, amino acids were determined with the performic acid oxidation with acid hydrolysis-sodium metabisulfite method (Llames and Fontaine, 1994) and neutral detergent fibre (NDF) were determined (Weizhong and Udén (1998) as well. Microbial counts of lactic acid bacteria (LAB) were determined in fermentation samples. Samples were diluted in peptone media, homogenised in a stomacher blender for 2 min, plated on selective media; de Man, Rogosa Sharp agar, (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with 100 µg $ml^{-1}$ delvocid (Gist-brocades B.V., Netherlands) and incubated anaerobically at 37°C for 48 h. Acid-insoluble ash was used as marker for calculation of digestibility in Paper **II** and was analysed according to (McCarthy, 1974).

## Performance studies (Paper I and III)

In Paper **I**, 192 growing pigs (Swedish Landrace x Yorkshire) with an average LW of 25 kg at start to 110 kg at slaughter, were studied. The pigs were housed with 4 animals in each pen, resulting in 12 pens per treatment. They were fed three times a day, according to average weight in each pen using a linear scale (17 to 35 MJ ME/pig and day), from an LW of 25 kg up to 60 kg and then at 35 MJ ME/pig

and day, until slaughter. The experiment was designed as a 2×2 factorial (P level and soaking). The same feed and treatments as in the balance study of Paper **I** were used. Weight of the animals was recorded, daily weight gains calculated and feed samples were collected throughout the study. Blood serum samples from 16 animals per treatment, representing 6 pens per treatment, were collected, at the start of the experiment, at 60 kg LW and before slaughter. At slaughter, measurements were made of warm carcass weight, grading quality and left ham weight with and without subcutaneous fat and femur. The left femur was stored, soft tissue was removed through dissection and density was then measured (Helander and Partanen, 1997). Serum was analysed for P and Ca (Konelab 60i analyser, Thermo Clinical Labsystems, Finland).

The study of Paper **III** lasted for two gestation periods and a total of 104 sows (Swedish Landrace x Yorkshire) of different parities was introduced in the study. They were randomly allotted to four different experimental gestation diets and received the same lactation diet. They were housed in a total of seven groups in large pens with deep straw bedding and with individual feeding stalls during insemination and gestation, and moved to individual pens before farrowing. The sows were not restrained during lactation, cross fostering were practiced and piglets were weaned at an average of 34 days of age. A diet based on wheat, oats and barely was used in all treatments, and was formulated according to Swedish feeding standards (Simonsson, 1994), except for P and addition of phytase. The different treatments were low P (3.7 g P $kg^{-1}$ feed), low P with phytase (Ronozyme® P, DSM Nutritional Products), medium P (4.5 g P $kg^{-1}$ feed) and high P (6.0 g P $kg^{-1}$ feed). Daily feed allowance was 2.6 kg during gestation and 9.2 kg during lactation.

LW of sows and piglets were recorded at the beginning of gestation, at farrowing, 3 weeks after farrowing and at weaning, and number of born, stillborn, and live-born piglets was registered. Blood serum samples were collected from 12 sows in each treatment, before and after each gestation and lactation period. Serum was also collected from three piglets at three weeks of age, from each of the sampled sows. Milk samples were collected from 12 sows in each treatment 3 weeks after farrowing, obtained by hand milking after i.m. injection of 2 ml (10 IU/ml) of oxytocin (Partoxin, Pharmacia & Upjohn Animal Health AB). A total of 13 to 16 piglets per treatment (from different litters) that died during the first 24 h after birth, and with a body weight of 1-2 kg, was collected. They were ground twice through a 5-mm mesh screen in a meat grinder, samples were freeze-dried and ground through a 1-mm mesh screen. Feed samples from each batch were ground through a 1-mm mesh screen and frozen. Feed and freeze-dried piglet carcass samples were analysed for DM, ash, nitrogen (Nordic Committee on Food Analysis, 2003), minerals (Nordic Committee on Food Analysis, 1991) and fat content (Official Journal of the European Communities, 1984). Phytase activity (Engelen et al., 1994) and inositol phosphates (Skoglund et al., 1997a) were determined in the feed. P and Ca were analysed in serum (with a Konelab 60i analyser, Thermo Clinical Labsystems, Finland) and in milk samples (Nordic Committee on Food Analysis, 1998).

Estimated P requirements for conception products in Paper **III** were calculated based on nitrogen (N) in tissues × 0.06 g P (Jongbloed, 1987). N in tissues (placenta, fluids, empty uterus and udder) was estimated based on protein energy, on the assumption that N content of protein is 0.16 g $g^{-1}$ and the energy value of protein is 23.8 kJ $g^{-1}$ (Noblet et al., 1985). Estimated P requirements for growth were based on data of P (g) in pigs from the LW 1 to 110 kg (Jongbloed and Everts, 1992). Maintenance requirement were set to 7 mg P $kg^{-1}$ LW $day^{-1}$ (Jongbloed and Everts, 1992). Estimation of P for mobilisation during following lactation was based on LW in the study in Paper **III**, and data on proportion of P mobilized from bones in gilts (165 kg LW), between pregnancy day 105 and lactation day 56 (Farries et al., 1984).

## In vitro study (Paper IV)

In the in vitro study (Paper **IV**), a cereal mix (wheat, barley and triticale) was fermented with whey (W; Milko, Bollnäs, Sweden), wet wheat distillers' grain (WWDG; the Absolut Company, Åhus, Sweden) and water ($H_2O$). The feed components were mixed to obtain approximately 25% DM $kg^{-1}$ liquid feed. Liquid diets were incubated in 10°C, 15°C and 20°C in triplicates. After an initial five days of fermentation, 80% of the contents were replaced daily with fresh liquid feed mix (simulating feed outtakes and leaving 20% as inoculants for the fresh mix), during the following 14 days.

Sampling of diets was performed at start, after 3, 5, 7, 10, 13, 17 and 19 days of fermentation, and pH was measured at start, day 3 and daily from day 5 to 19. Aliquots of fresh samples for microbial quantification and samples for nutritional evaluation were taken and immediately frozen. Freeze-dried samples were ground through a 1-mm mesh screen and analyzed for DM, ash, minerals (Nordic Committee on Food Analysis, 1991) and in vitro digestibility (Boisen and Fernández, 1997). Dry cereal mix was analysed for phytase activity (Engelen et al., 1994) and inositol phosphates (Skoglund et al., 1997a). Wet samples were centrifuged (5 min at 13,000*g), 250 µl supernatant was mixed with 1000 µl of the internal standard solution (0.5 g/l of pivalic acid in 0.09 M sulphuric, centrifugated and analysed for organic acids and ethanol (Andersson and Hedlund, 1983). Fresh samples (20 g) for the microbial quantification were diluted with 180 ml sterile peptone water [bacteriological peptone 2 g $l^{-1}$ (Oxoid) supplemented with 0.15 g $l^{-1}$ Tween 80 (Kebo)], homogenised for 2 min in a stomacher blender, serially diluted in peptone water and 100 µl portions were spread on different agar plates. LAB were quantified on de Man Rogosa Sharp agar (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with 100 µg $ml^{-1}$ delvocid (Gist-brocades B.V., Netherlands) and were incubated anaerobically at 37°C for 48 h. Heat-treated (80 °C for 13 min) samples were quantified for Clostridia on reinforced clostridium agar (Oxoid LTD, Basingstoke, Hampshire, England) supplemented with 0.2 mg $ml^{-1}$ cycloserine (Sigma Aldrich Inc, St Louis, USA), and incubated anaerobically at 37°C for 48 h. A GasPack system (Becton Dickinson; Sparks, Md.) was used throughout the study to obtain an anaerobic environment. Yeast was enumerated on malt extract agar (Oxoid LTD,

Basingstoke, Hampshire, England) plates supplemented with 100 µg $ml^{-1}$ chloramphenicol (Sigma-Aldrich Inc, St Louis, USA), and incubated at 25°C for 2 to 4 days. Moulds were quantified on dichloran-glycerol (DG18) agar base and incubated in 25°C for 5 days. Enumeration of *Enterobacteriaceae* was made in violet red bile agar (Oxoid LTD, Basingstoke, Hampshire, England) incubated in 30°C for 24 hours. Total amount of aerobic bacteria was quantified in tryptone glucose extract agar (Oxoid LTD, Basingstoke, Hampshire, England) incubated in 30°C for 3 days.

# Results

## Digestibility

In Paper **I**, the effect of P level and 1 hour of soaking in cold (10 °C) water, on degradation of $IP_6$, apparent digestibility of P and performance in growing-finishing pigs was studied. Soaking reduced the level of P bound to inositol penta- and hexaphosphate ($IP_5$-$IP_6$) in the feed with 10% ($P < 0.05$). Apparent digestibility of P was higher in the high P treatments ($P < 0.01$), and soaking did not significantly affect the apparent digestibility of P. However, apparent digestibility of P did not differ between high P dry and low P soaked. Retention of P was affected by P level ($P < 0.001$) and soaking tended to increase retention of P ($P < 0.10$). Digestibility of Ca was highest in soaked low P diet ($P < 0.05$). Retention of Ca was affected by P level ($P < 0.05$) and was highest in high P treatments. Apparent digestibility of nitrogen and organic matter did not differ between treatments.

Influence of soaking (as in Paper **I**), fermentation and phytase supplementation of a similar feed as in Paper **I**, is described in Paper **II**. The fermented feed (pH 4.4; LAB 9.2 log cfu $g^{-1}$), gave a higher ileal apparent digestibility ($P < 0.05$) of organic matter (OM), N, and most indispensable amino acids. The ileal apparent digestibility of P was higher ($P < 0.01$) in the fermented feed compared with the dry and soaked feed, and tended ($P = 0.065$) to be higher than in the phytase feed. At the total tract level only apparent digestibility of OM differed ($P > 0.05$) among treatments, with higher digestibility in fermented feed. However, the total tract apparent digestibility of P was higher ($P < 0.05$) in treated feeds compared with dry feed. Total tract apparent digestibility of P did not differ among soaked, fermented or phytase supplemented feeds. The level of P bound to $IP_6$ was affected by treatment ($P < 0.05$) and reduced by 10% in the soaked feed and 80% in fermented feed before feeding. NDF was also affected by treatment ($P < 0.05$), with a 4% reduction in soaked feed and 14% reduction in fermented feed before feeding. A lower ($P < 0.05$) content of $IP_6$ was found in ileal digesta from pigs fed the fermented feed compared with non-fermented feed, whereas content of $IP_6$ did not differ in the faeces. The degradation of the $IP_6$ before feeding was highest in the fermented treatment ($P < 0.05$), whereas the degradation of $IP_6$ in the gastrointestinal tract was lower ($P < 0.05$) at the ileal level in pigs fed the

fermentation feed compared with those fed non-fermented. Degradation of $IP_6$ at the total tract level was also affected by treatment ($P < 0.05$), with the lowest degradation in the fermentation treatment and no differences between the other feeds. Total P content in ileal digesta or in faeces did not differ between feeds.

## Performance

### *Growing-finishing pigs*

Soaking (as previously described) and P level were tested in a performance study (Paper **I**) with growing-finishing pigs (25-110 kg). Average daily weight gain was increased by both P level and soaking ($P < 0.01$). Soaking increased average daily weight gain in pigs fed low P diet ($P < 0.001$), and groups fed high and low P did not differ when the low P feed was soaked. The same results ($P < 0.01$) in daily weight gain were found for both growing (up to 60 kg) and finishing pigs (60-110 kg). Soaking increased both final weights and carcass weights only in pigs fed the low P diet ($P < 0.001$). The energy conversion ratio (ECR) for the growing-finishing period was affected by both P level and soaking ($P < 0.001$); soaking decreased ECR in the low P treatment ($P < 0.001$) and the same results were found for the growing period ($P < 0.01$). In the finishing period ECR was affected by soaking ($P < 0.01$), with the lowest ECR in the soaked low P treatment ($P < 0.05$). Daily energy consumption, meat percent and ham percent of carcass did not differ among treatments ($P > 0.05$) On the day before slaughter, dietary P level affected P ($P < 0.01$) and Ca serum levels ($P < 0.05$), with lower P levels in low P treatments and slightly lower Ca levels in high P treatments. Density of femur was affected by both P level ($P < 0.001$) and soaking ($P < 0.05$). Both low P treatments resulted in a lower density than the high P treatments ($P < 0.01$). Soaking of the low P diet did however result in higher ($P < 0.01$) density than low P diet given dry.

### *Pregnant sows*

P levels and supplementation of phytase was studied in pregnant sows in Paper **III**. Number of born piglets was higher in the low P treatments than in treatments with higher P ($P < 0.05$). However, mortality at birth was also highest in the low P diet without phytase supplementation, significantly higher than in the medium P diet ($P < 0.05$) and numerically higher than in the high P or low P phytase-supplemented diet. Birth mortality was lower in the medium P compared with non-supplemented low P ($P < 0.05$), it was also numerically lower in the medium P than in the supplemented low P and high P diet. Numbers of live-born piglets, piglet birth weight, sow weights, sow weight losses during lactation and piglet weight gains did not differ between treatments and there were no differences found between the two reproduction cycles. The P level in sow milk was affected by treatment ($P < 0.05$), and was highest in medium P. Treatment did not affect Ca levels in sow milk, or P and Ca levels in serum of sows and piglets. Treatment did not affect the contents of nitrogen, fat, P and Ca of the piglets.

### Properties of fermented feed (Paper IV)

During the first days of incubation, pH decreased to approximately 4 in all fermentation vessels and was then stable, but decreased more slowly to pH 4 10°C. However, pH in diet W increased slightly after feed exchange started (day 5) when fermented at 10°C. LAB grew in all feeds during fermentation. Yeast cfu values in diet WWDG and $H_2O$ were low during the first days, but increased when new yeast entered the feed system through cereals during feed exchange. Some moulds were in all fermentation vessels; however cfu values did not increase throughout the experiment. Moulds were only found in the cereals. *Enterobacteriaceae* were present in cereals and whey but were not detected in wet wheat distillers' grains. No Clostrida was found in any of the diets or feed components during the experiment. Microbiological and chemical compositions of the period, day 13 to 19 of fermentation, depended on temperature, liquid fractions and their interactions. Levels of cfu for both yeast and LAB were affected by temperature, liquid fraction and their interactions ($P < 0.001$), and were lowest in diet WWDG. Acid concentrations were mainly influenced by the liquid fraction ($P < 0.001$), with the highest concentrations of acetic, succinic and propionic acid in diet WWDG. Lactic acid concentrations were highest and at about the same levels in diets $H_2O$ and W, with higher concentrations at higher temperatures ($P < 0.05$). In vitro digestibility of OM was a result of liquid fraction ($P < 0.001$), with highest digestibility of organic matter in W and lowest in diet WWDG. $IP_6$ -bound P was 60% of the total P in the dry cereal mix. No IP-bound P were found in any of the fermented liquid diets.

# General discussion

## Liquid diets

*Soaking*

Soaking of a cereal-based feed in water can activate intrinsic phytase and result in reduction of $IP_6$ content (Fredlund et al., 1997; Skoglund et al., 1997b; Larsen et al., 1999), as we also found in Paper **I** and **II**. Carlson and Poulsen (2003) suggested that soaking might increase P digestibility for pigs, except by degradation of $IP_6$, due to a more optimal pH in soaked feed compared with ingested feed in the stomach since optimal pH for wheat and barley phytase is about 5 (Irving, 1980). They also found that the intrinsic phytase disappeared over time in correlation with pH dropping below 5, but not when soaked in 10°C, suggesting that soaking a diet at 10°C could result in higher P digestibility than soaking at higher temperatures. The soaking treatment in Paper **I** and **II** were set to 10°C and one hour, probably reducing a decline in phytase activity due to protelytic activity in the feed. A declining phytase activity in the feed was a possible explanation by Larsen et al. (1999) for not reaching significant increases in P digestibility despite $IP_6$ reductions (Skoglund et al., 1997b; Larsen et al., 1999). In the balance study (Paper **I**) the digestibility of P tended to be improved

by soaking, but did not reach significance, which may be explained by the small number of animals or possible co-precipitation of released orthophosphate with calcium. The performance study with soaking (Paper **I**) did however show an improved performance in slaughter pigs due to soaking of the low P diet, suggesting that P availability was increased despite the non significant improvements in digestibility in the balance study (Paper **I**). Soaking of the low P diet increased average daily gain, carcass weight and improved the energy conversion ratio, and an increased dietary P level (by inorganic P supplementation) resulted in no further improvement in performance. P levels in serum were however lower in the low P diets (Paper **I**) regardless of soaking, suggesting a main effect of P level on their P status (Teleni et al., 1976). On the other hand femur density was markedly increased by soaking the low P diet, especially when expressed in relation to the dietary content of digestible P. In contrast, a further increase in the dietary content of digestible P (high P diets) resulted in a considerably smaller increase in femur density. Although performance was unaffected by further increase in digestible P due to supplementation of inorganic P, the result indicates a marginal supply of P for bone mineralization in the soaked but not in P supplemented feed. Earlier data (Cromwell et al., 1970; Kornegay et al., 1981; Guéguen and Perez, 1981) also demonstrate that adequate mineral levels for maximum growth are insufficient for maximum bone mineralization. Pigs fed diets resulting in a low bone mineralization in the performance study (Paper **I**) were nevertheless not suffering from other health problems than pigs on other diets. The results of Paper **I** indicate that one hour of soaking in cold water may be sufficient to improve the digestibility of P in a barley-wheat-based diet, at least to the extent that the need of supplementation of feed phosphates or commercial microbial phytase may be reduced.

### *Fermentation*

Soaking of a diet starts a fermentation process (Canibe and Jensen, 2003) where microbial populations eventually develop, including LAB and yeast (Jensen and Mikkelsen, 1998) of which both have capacity to degrade $IP_6$ (Reale et al., 2004). Fermented feed (Paper **II** and diets $H_2O$ and W in Paper **IV**) had low pH (< 4.5) and prominent populations of LAB (> 9 log cfu $g^{-1}$) in agreement with Jensen and Mikkelsen (1998). LAB and yeast are not only capable of degrading $IP_6$. LAB contributes to the reduction of pH through lactic acid and can function as probiotics with positive effects on lower gut microflora (Vanbelle et al., 1990). Yeast can inhibit mould growth (Passoth and Schnürer, 2003) and be beneficial as a probiotic with positive effects in the gastrointestinal tract (Schroeder et al 2004). Nonetheless, yeast is sometimes considered undesirable in liquid diets because starch is turned into high alcohol levels and carbon dioxide representing energy losses. However, this was not the case in Paper **IV**. Some yeast can give bad flavours affecting palatability of diets (Brooks et al., 2003). Yeast was only counted in the study of Paper **IV**. When fresh feed mixes are added in the fermentation tanks daily (Paper **II** and **IV**), an $IP_6$ degradation of intrinsic phytase from the cereals, as well as microbial $IP_6$ degradation, is likely to occur before pH drops too low. $IP_6$ was much more degraded in fermented feed than in soaked or

dry feed (Paper **I**, **II** and **IV**). Apparent digestibility of P at the total tract level was nevertheless not significantly improved by either soaking or fermentation (Paper **II**) although treated feed (soaked, fermented or phytase supplemented) gave a higher P digestibility than untreated feed. In contrast, soaking did not affect ileal apparent digestibility of P whereas fermentation caused an increased ileal apparent digestibility. Most minerals are mainly absorbed by the end of the small intestine although some absorption of P may occur further down the gastrointestinal tract (Jongbled et al., 1992). Fermentation caused other positive effects as well, such as increased ileal apparent digestibility of OM and N, in agreement with earlier in vitro studies on fermented liquid feed (Pedersen and Lindberg, 2003). These improvements, and degradation of NDF in the feed, can be explained by microbial activities in the fermented feed affecting the degradation of both soluble and insoluble dietary cell wall carbohydrates (Pedersen and Lindberg, 2003). Degradation of $IP_6$ may be involved in increased digestibility of amino acids and other minerals (e.g. Ca and Mg) since $IP_6$ is capable of forming insoluble complexes with both cations and amino acids (Irving, 1980; Maga, 1982). Increases in Ca digestibility could be a result of released Ca from $IP_6$. The acidification of fermented feed could be sufficient to improve the ileal apparent digestiblity of amino acids (Kemme et al., 1999). The emptying rate of the stomach can be reduced by acidification of diets (Mayer, 1994) and thereby increase the time of digestion and stimulate secretion of proteolytic enzymes (Harada et al., 1986). The microbial and nutritional aspects of the cereal mix fermented with water, whey and wet wheat distillers' grains at 10, 15 and 20°C in Paper **IV** gave some further information of the specific properties involved in the formation of fermented liquid diets. The fermentations resulted in differences in both microbial growth and microbial populations developed, as well as pH and biochemical traits. The pH stabilised mainly around 4, whereas the acid compositions were different. Lactic acid levels were highest in diets $H_2O$ and W, with higher concentrations in higher temperatures, whereas acetic acid in diet WWDG was especially high, which could be negative for palatability (Beal et al., 2005). High concentrations of acetic acid and lactic acid as well as low pH are all found to prevent proliferation of spoilage organisms and food-borne pathogens (van Winsen et al., 2000; Beal et al., 2001; Beal et al., 2002; Brooks et al., 2003). *Enterobacteriaceae* counts are used as a general indicator of hygienic quality of diets and were not found when pH decreased in diets $H_2O$ and WWDG. *Enterobacteriaceae* were, however, present in diet W throughout the study in Paper **IV**. Some moulds were present during the experiment, most likely entering the system through spores added with the cereal mix. Both LAB and yeast were present in all fermentations although LAB in diet WWDG showed rather low growth compared to the other diets, possibly due to an adaptation of LAB in the wet wheat distillers' grains to grow at a much higher temperature in the production step. For a successful practical application of the wet-feeding technique, it would be desirable to be able to control the fermentation processes and biochemical traits to a higher degree.

## Phytase supplementation

Supplementation of commercial microbial phytase resulted in only slight effects on apparent digestibility of P in Paper **II**. Phytase supplementation has earlier been shown to degrade $IP_6$ and increase P digestibility (Jongbloed et al., 1992; Näsi et al., 1999; Kemme et al., 1999). The slight effects on P digestibility may depend on high levels of intrinsic phytase in the diets used in Paper **II**. High levels of intrinsic phytase reduce the effect of phytase supplementation on $IP_6$ degradation (Carlson and Poulsen, 2003) and Poulsen (1996) found more limited effects of phytase supplementation on P digestibility in high intrinsic phytase diets fed to growing pigs. Supplementing a diet with phytase did not significantly affect sow performance either (Paper **III**). A similar number of piglets were born on the phytase-supplemented diet as on the non-supplemented low P diet although the mortality at birth was numerically improved by phytase. The limited effects of phytase on sow performance were possibly an effect of relatively high level of intrinsic phytase in the diet, and that the P level provided may have been sufficient. Effects of phytase supplementation may also vary, depending on pig physiological status (Kemme et al., 1997) and origin of the supplemented phytase (Paditz et al., 2004). Kemme et al. (1997) found that the efficacy of supplemental phytase in generating digestible P decreased in the following order: lactating sows, growing-finishing pigs, sows at the end of pregnancy, piglets and sows at mid-pregnancy.

## P levels

### *Growing-finishing pigs*

The low P level in Paper **I** was most likely below the requirements of the pigs, which was manifested in restricted growth and higher energy conversion ratio. The seemingly higher level of digestible P manifested by the improved performance caused by soaking, and the impact it had on femur density (in contrast with the further smaller increase in density due to inorganic P supplementation), indicate that required digestible P level for a maximal bone mineralization probably were just above the achieved level at soaking. To have a margin for variations in P digestibility in available feedstuffs, one should probably aim for a recommended P level allowing maximal bone mineralization. Interestingly, the genetic background could influence the metabolic response to low dietary P levels (Hittmeier et al., 2005). The high P level in Paper **I** represented the current Swedish recommendations for growing-finishing pigs (6.0 g $kg^{-1}$ feed). Although management and genetic material differ, the Swedish recommendations can be reduced. Jongbloed et al. (1991) estimated requirements of P for pigs (30-50 kg LW) to be about 3.7 g digestible P per day and Poulsen (1994) found performance in pigs did not differ (25-50 kg LW) with an intake of 3.4 g and 4.7 g digestible P per day.

*Pregnant sows*

Overall performance of gestating sows was not impaired by feeding a cereal-based low P diet without supplementation of inorganic P. The low P diet positively affected the number of piglets born but negatively affected the piglet mortality at birth, resulting in no differences in number of live-born piglets between the low and high P diet. Higher piglet mortality could be related to a higher number of piglets born (Serenius et al., 2004). The performance data indicate that an adequate amount of available P was provided in the non-supplemented low P treatment. However, the study comprises only two parities, which may not be enough time to show the full effect of a marginal supply of P to gestating sows. Marginal P allowances may affect the sow skeleton in a longer perspective (Mahan, 1990). A high P lactation diet may partially have compensated for any possible marginal P supply during gestation. Total P content of the lactation diet was approximately three times the required amount of digestible P, as estimated based on requirements for growth and maintenance of piglets, sow maintenance (Jongbloed and Everts, 1992) and assumed P digestibility in milk (Jongbloed and Kemme, 1990). Estimated digestibility of P necessary to provide the estimated P required by the sows in the study are, however, reasonable and achievable in the type of diets fed with considerable phytase activity when compared with obtained levels in Paper **I** and **II** or in Liesegang et al. (2005). Still, Kemme et al. (1997) showed that digestibility of P was lower in sows of parity five or more than in growing pigs. Calculations of P requirements and estimated P digestibility necessary to fulfil the P needs in Paper **III** are based on previous published data on endogenous P losses, P content per LW (Jongbloed, 1987; Jongbloed and Everts, 1992), maternal tissues (Noblet et al., 1985; Jongbloed and Everts, 1992), P mobilisation from bones during lactation (Farries et al., 1984) and recorded production data in Paper **III** together with analysed P content in piglets. Obviously, there may be limitations in estimating the P requirements with such calculations. Estimated amounts of P mobilised in following lactation for example, based on Farries et al. (1984) and LW data from our study, may be somewhat too high. Farries et al. (1984) estimated the proportion of P loss from the skeleton on gilts with a 165 kg LW. That proportion may be different at a higher LW and parity. Furthermore, the usage of tissue reserves may depend on P supply (Mahan, 1990), where there is higher demand on P tissue reserves when more limited amounts of P are offered. The calculations still reasonably estimate the amount of P needed for different purposes in breeding sows. The estimated required level of digestible P per kg feed in our study (1.7 g $kg^{-1}$) appears reasonable although lower than the 2.2 g digestible P $kg^{-1}$ found by Jongbloed and Everts (1992). However, estimated requirements of digestible P for pregnant sows of 4.4 to 4.5 $day^{-1}$ for the entire pregnancy in our study were in concurrence with Everts et al. (1998) whose estimated requirements were 4.2 g $day^{-1}$ in mid-pregnancy (day 50-60) and 6.0 g $day^{-1}$ for late pregnancy (day 105-112). Similarly, Kemme et al. (1997) estimated P requirements to be 2.4 g $day^{-1}$ in mid pregnancy (at 60 days) and 5.0 g $day^{-1}$ for late pregnancy (at 100 days). One should, however, consider that requirements differ depending on factors such as diet, type of production, genotype and litter size (Jongbloed et al., 1991).

## Practical implications and future prospects

The present thesis has shown that the Swedish recommendations on total P levels in pig diets for both growing-finishing pigs and pregnant sows can be reduced without affecting performance. This has been acknowledged in the new Swedish recommendations (Simonsson, 2006). The new recommendations are given in digestible P, but with a minimum total P level. If following only the total P level is suggested, a digestibility between 50 and 60% is required. Digestibility levels achieved in the studies of this thesis were 51 to 54% with monocalcium phosphate supplementation and below 50% in diets without monocalcium phosphate supplementation. Recommendations in digestible P are easier to estimate than in total P, and are more accurate. Since there is a wide variation in digestibility of P in different diets depending on included feedstuffs and feed treatment, the total P recommendation should possibly be set according to feed treatment used (i.e. non-heat-treated meal or pellets) and feedstuffs used. One way to deal with the problem could be to recommend a minimum phytase level in the feed in connection with a recommended P level, possibly estimated with already measured phytase levels in different feedstuffs, at least for non-heat-treated meal feed. In wet-feed it could possibly be advisable to estimate the amount of $IP_6$ bound P since most $IP_6$ degradation seems to take place before ingestion (Paper **II**). New recommendations (Simonsson, 2006) for pregnant sows are 2.25 g digestible P $kg^{-1}$ feed and a minimum total P at about 4.2 g $kg^{-1}$, whereas the earlier recommendations were 6.5 g total P $kg^{-1}$ feed. A reduction to 4.5 g total P $kg^{-1}$ is a 30% reduction of P in the feed compared with earlier recommendations. As previously described, estimated requirements are made for different stages during gestation although the recommendations in Sweden are based on one diet for the entire gestation period. More information on P digestibility and long-term effects of a low dietary P content on maternal tissue P reserves and regulation of P absorption in reproductive sows would be of interest. Recommended levels for growing pigs is about 2.3 to 2.5 g digestible P $kg^{-1}$ feed, with a minimum total P level at 4.4 g $kg^{-1}$. Previous recommendations were of 6.0 g $kg^{-1}$ feed and a reduction to for example 4.5 g $kg^{-1}$ represents a 25% reduction. The use of digestible P in nutrient recommendations for pigs will positively affect Swedish pig production in times with strict legislations for manure disposal.

The higher performance due to soaking in Paper **I** indicates improved availability of P and a possibly reduced need for supplementation of feed phosphates or commercial microbial phytase. The extensive degradation of $IP_6$ (Paper **II** and **IV**) in fermented feed, which possibly affected the improved ileal digestibility of amino acids, P and Ca (Paper **II**), suggests a reduced need for supplementation of feed phosphates or commercial microbial phytase. Such feeding strategies would be of interest, nonetheless, for reduced feed costs. The positive effects of fermentation (apart from the high capacity to degrade $IP_6$) on gastrointestinal health, increased weight gains and improved feed conversion ratios (Jensen and Mikkelsen, 1998; Schloten et al., 1999; Brooks, 2003) make fermented feed very interesting. Biochemical properties and microbial populations developed in fermented feed affect hygienic quality, the intestinal health, performance as well as the nutritional aspects of the diet. To better control the fermentation processes

and biochemical traits in wet-feeding, more detailed information of the different microbial populations developed in different fermented liquid diets, dominant species, their properties as well as the effects of organic acid concentrations on palatability and nutrient utilisation, would be of interest.

# Conclusions

- One hour of soaking in cold water can reduce $IP_6$ content and may be sufficient to increase availability of P in non-heat-treated pig diets containing significant amounts of intrinsic phytase, to the extent that the need for supplementation of inorganic P or commercial phytase is reduced.

- Fermentation can efficiently degrade $IP_6$ and improve ileal apparent digestibility of organic matter, nitrogen, indispensable amino acids, P and Ca.

- Supplementation of commercial microbial phytase resulted in only slight effects on P digestibility, possibly due to already high intrinsic phytase levels in the diets.

- Under typical Swedish conditions of sow management and using normal pig genotypes, there were no negative effects on performance due to the reduced total P levels in gestation diets. Moreover, the estimations of P requirements in this thesis indicate that it should be possible to reduce the total P content in sow diets and thus reduce the environmental P pollution from the pig industry.

- Microbiological initial growth and biochemical content of stabilised fermented liquid diets are affected by temperature and involved feed components, with their substrates and original microflora.

# Populärvetenskaplig sammanfattning

I många gristäta områden i Europa har fofornivåer i gödseln blivit ett allt hetare ämne och en slaktsvinsuppfödare i Sverige som följer aktuella normer för hur mycket fosfor fodret bör innehålla, har ofta mer fosfor i gödseln än de har tillåtelse att sprida på sin åkermark. Spridning av fosfor i höga nivåer leder till ackumulering i markerna och urlakning till sjöar och vattendrag, där det bidrar till övergödning och algblomning. Fosfor är livsnödvändigt för djuren men fodret skulle inte behöva innehålla så höga mängder av fosfor i fall de kunde tillgodogöra sig all fosfor i fodret. Grisfoder består vanligen främst av spannmål där det mesta av fosforn uppträder bunden till en inositol ring, så kallad inositolfosfat eller fytat. För att den fosforn ska kunna utnyttjas av djuren krävs närvaro av enzymet fytas. Detta enzym finns natruligt i flertalet vegetabiliska fodermedel, men ofta i alltför låg koncentration för att positivt påverka fosforns tillgänglighet. Både andelen fosfor och fytasaktiviteten varierar i hög grad mellan olika fodermedel och bland annat vete och rågvete (triticale) har höga fytasaktiviteter. Hur mycket av fosforn i ett foder djuret kan utnyttja beror på mängden inositolbunden fosfor och dess fytasaktivitet. Olika processer vid tillverkning av foder som värmebehandling och pelletering, minskar den naturigt förekommande fytasaktiviteten. Eftersom grisarna endast kan utnyttja en begränsad del av fodrets fosfor tillsätts ofta oorganisk fosfor (mineralfoder) för att med säkerhet tillgodose djurens behov. Genom att blötlägga eller fermentera fodret alternativt tillsätta mikrobiellt fytas kan man istället öka utnyttjandet av den inositolbundna fosforn. Blötläggning av ett foder kan aktivera det naturligt förekommande fytasen i fodret och vid en fermentering tillväxer bland annat mjölksyrabakterier och jästsvamp som också kan bryta ned inositolfosfat-molekylen.

Denna avhandling bygger på fem olika studier som presenteras i fyra olika vetenskapliga artiklar. Två smältbarhetsstudier, där smältbarheten dvs upptaget av olika näringsämnen studerats. En studie där intaget av näringsämnen jämförs med det som återfinns vid en total uppsamling av träck och urin, och en studie på fistulerade grisar där intaget av näringsämnen samt mängden återfunnet i tarminnehåll (från fisteln) och i träck, ställs i relation till mängden av en osmältbar markör i foder, i tarminnehåll och träck. Smältbarhetsstudierna utfördes på institutionen för husdjurens utfodring och vård på Sveriges Lantbruks Universitet i Uppsala. Två produktionsstudier, ett på slaktsvin och ett på dräktiga suggor där effekter på produktionsresultat studerades genomfördes på Lantmännens forskningscenter i Svalöv. En laboratoriestudie på fermenterat foder där basala nutritionella och mikrobiella egenskaper studerades, utfördes på institutionen för mikrobiologi på Sveriges Lantbruks Universitet i Uppsala. Alla djurförsök var godkända av lokala etiska nämnder.

Effekten av blötläggning i relation till fosfornivå studerades i ett foder med relativt hög fytasaktivitet, med avseende på nedbrytning av inositolfosfat, smältbarheter samt produktionsresultat. Detta studerades i en smältbarhetstudie med total uppsamling av träck och urin, samt i ett produktionsförsök med 192 djur. De olika behandlingarna blötläggning, fermentering och tillsats av mikrobiellt fytas,

studerades därefter i ett smältbarhetsförsök med fistulerade grisar för att undersöka vad som händer i tunntarm respektive tjocktarm. Många olika varianter av fermenterat foder används i Europa idag och det finns olika uppfattningar om hälso- och näringsaspekter rörande fermenterat foder. I den laborativa studien studerades en spannmålsblandning som fermenterades med vatten, vassle (från Milko i Bollnäs) eller drank (från Absolut i Åhus). Blandningarna fermenterades alla i 10, 15 och 20°C, varpå innehållet studerades med avseende på nutritionella egenskaper och tillväxt av mikroorganismer. Olika fosfornivåer och mikrobiell fytas tillsats till fodret till dräktiga suggor undersöktes med en föreställning om att det vore möjligt att sänka nuvarande normer för fosfor i fodret till dräktiga suggor. Studien utfördes på 104 suggor över två dräktighetsperioder.

Blötläggning i vatten under en time av ett icke-värmebehandlat mjölfoder med låg totalfosfornivå, utan tillsatt oorganisk fosfor, resulterade i ett minskat innehåll av fytat i fodret men ingen signifikant ökning av smältbarheten av fosfor. Blötläggningen av lågfosforfodret resulterade dock i en ökad daglig tillväxt, slaktkroppsvikt och ett förbättrat energiutnyttjande och resulterade i samma produktionsresultat som med ett foder med hög totalfosfornivå och tillsatt oorganisk fosfor. Lågfosforfodret orsakade emellertid lägre densitet av lårbenen, även om blötläggningen gav en markant ökning i densitet. Detta antyder att den uppnådda nivån av tillgänglig fosfor i det blötlagda lågfosforfodret var strax under den nivå som behövdes för maximal benmineralisering, men tillräcklig för maximal tillväxt. När ett foder blötläggs under en lite längre tid så uppstår en spontan fermentering som kännetecknas av ett sänkt pH, en tillväxt av mikroorganismer och bildandet av organiska syror. Fermentering visade sig effektivt kunna reducera mängden fytat i fodret, och ökade den ileala smältbarheten av fosfor, organisk substans, kväve och många aminosyror. Vid jämförande av blötfoder fermenterade med vatten, vassle eller drank i 10, 15 och 20°C, visade det sig att utvecklingen av mikroorganismer och biokemiska egenskaper i fermenterat blötfoder starkt påverkas av de foderkomponenter som används samt temperatur. Tillsättningar av mikrobiellt fytas i både smältbarhetsförsök för växande grisar och för dräktiga suggor gav ganska måttliga effekter. Anledningen till dessa blygsamma effekter kan vara den redan höga fytas aktivitet som redan fanns i fodren, och för suggorna möjligen en indikation på att den låga fosfornivån de fick var tillräcklig. Det var tillsynes inga negativa effekter på suggornas produktionsresultat av en sänkt fosfornivå i fodret under dräktigheten. Mer långtida effekter är dock omöjliga att utesluta och mer information om suggornas förmåga att använda kroppsreserver och reglera absorption skulle vara till nytta.

Slutsatser:

- ✓ En timmes blötläggning av ett icke-värmebehandlat foder kan minska fytat innehållet i fodret, åtminstone till den grad att det minskar behovet av tillsatser i form av oorganisk fosfor eller mikrobiellt fytas.
- ✓ Fermentering kan effektivt minska innehållet av fytat i fodret och ökar den ileala smältbarheten av fosfor, kalcium, organisk substans, kväve och många aminosyror.

- ✓ Mikrobiell tillväxt och biokemiskt innehåll i fermenterat foder påverkas av temperatur och ingående foderkomponenter, samt den ursprungliga mikrofloran i foderkomponenterna.
- ✓ Blygsamma effekter på smältbarhet av mikrobiella fytastillsatser beror troligtvis på en redan hög fytasaktivitet i fodret
- ✓ Små effekter av mikrobiella fytastillsatser i foder till dräktiga suggor och avsaknad av synbara effekter på produktionen vid sänkta fosfornivåer till dräktiga suggor, tyder på att det är möjligt att sänka fosfortilldelningen.

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